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Potential Therapeutic Role of Hydrogen Sulfide-Releasing Molecule GYY4137 in a Rat Model of Acute Compartment Syndrome.

Moustafa Haddara, *The University of Western Ontario*

Supervisor: Dr. Abdel-Rahman Lawendy, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Surgery

POTENTIAL THERAPEUTIC ROLE OF HYDROGEN SULFIDE-RELEASING MOLECULE GYY4137 IN A RAT MODEL OF ACUTE COMPARTMENT SYNDROME

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by

Moustafa Haddara

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The University of Western Ontario

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ABSTRACT

Acute limb compartment syndrome (ACS) causes a unique form of limb ischaemia, which induces intense inflammatory response resulting in microcirculatory dysfunction, neutrophil activation and cell injury. Increased intracompartmental pressure is the hallmark of ACS. Decompression by fasciotomy is the gold standard treatment. While fasciotomy saves the limb from ischaemic threat, paradoxically, it causes further damage to the muscle by reperfusion injury. In addition, it does not address the inflammatory element purported to increase the tissue injury in ACS.

Recent evidence suggests that hydrogen sulfide (H_2S) can mitigate the damage associated with ischaemia-reperfusion injury. The purpose of this thesis was to examine the value of H_2S treatment in a rat model of ACS, using GYY4137 as H_2S-releasing molecule. We have demonstrated significant cytoprotective role of H_2S on the skeletal muscle following ACS.

These results suggest a potential therapeutic value of H_2S as an adjunctive to fasciotomy, for patients suffering ACS.

Keywords: *acute compartment syndrome; fasciotomy; tissue injury; inflammation; hydrogen sulfide; carbon monoxide; GYY4137; CORM-3.*

CO-AUTHORSHIP

While all of the co-authors listed below made important contributions to this work, I performed all the experiments, data collection, interpretation, and the statistical analysis. The manuscript was entirely written and prepared by me, with the assistance and critical review by the co-authors.

Dr. Abdel-Rahman Lawendy, MD, PhD, FRCSC, provided much leadership over the course of my project; his insight into the pathophysiology of compartment syndrome and knowledge of reperfusion injury mechanisms helped to direct the project towards the end product. He also critically reviewed this work.

Aurelia Bihari, MSc, provided invaluable technical support, taught me all the necessary skills to perform this project, and offered guidance and support on data collection, analysis, and interpretation. She trained me on the rat model of compartment syndrome that had been developed by this lab. She also kindly assisted in the manuscript editing.

DEDICATION

I dedicate this work to the memory of my father, the first scholar I had ever met. He was a pivotal figure in the Egyptian academic culture, though humble and conscientious. He taught me to love the knowledge, not merely to learn, and encouraged me to delight in the adventures of intellectual curiosity.

To my mother, it shall be difficult to express my gratitude towards you. Heaven truly is at the feet of our mothers.

To my brothers and sisters: my life's biggest strength and the beautiful memories of my childhood.

To my wife Sahar: through all of our life's curvy roads, you have been my heavenly abode.

To Raneem and Mohammad, whom I'll forever hold close to my heart.

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3-MST	3-mercaptopyruvate sulfurtransferase
ACS	acute compartment syndrome
AP-1	activator protein-1
ATP	adenosine 5′-triphosphate
BB	bisbenzimide
CAT	cysteine aminotransferase
CBS	cystathionine-β-synthase
CO	carbon monoxide
CORM-3	carbon monoxide-releasing molecule-3
CPC	continuously perfused capillaries
CSE	cystathionine-γ-lyase
EB	ethidium bromide
EDL	extensor digitorum longus muscle
EDRF	endothelial-derived relaxing factor
cNOS	constitutive nitric oxide synthase
eNOS	endothelial nitric oxide synthase
GSH	glutathione
GYY4137	morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate
H+	hydrogen ion
H_2O_2	hydrogen peroxide
H_2S	hydrogen sulfide

HO	heme oxygenase
HO-1	inducible heme oxygenase
HO-2	constitutive heme oxygenase
HOCl	hypochlorous acid
HS^-	hydrosulfide ion
ICAM-1	intercellular adhesion molecule 1
iCORM-3	inactive CORM-3
ICP	intracompartmental pressure
IL-1	interleukin-1
IL-4	interleukin-4
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
IL-13	interleukin-13
iNOS	inducible nitric oxide synthase
IP	intraperitoneal
IPC	intermittently perfused capillaries
IR	ischaemia-reperfusion
IV	intravenous
IVVM	intravital video microscopy
LFA-1	leukocyte function-associated antigen-1
KC	neutrophil chemoattractant protein
NAC	N-acetyl cysteine

NF-kB	nuclear factor kappa-B
NH_3	ammonia
NO	nitric oxide
NOS	nitric oxide synthase
NPC	non-perfused capillaries
NSAIDs	non-steroidal anti-inflammatory drugs
O_2^-	superoxide anion
$^{\bullet}OH$	hydroxyl radical
RISK	reperfusion injury salvage kinases
ROS	reactive oxygen species
SOD	superoxide dismutase
TA	tibialis anterior
TF	transcription factors
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
XO	xanthine oxidase
ΔP	differential pressure

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. COMPARTMENT SYNDROME

1.1.1. Overview

Acute compartment syndrome (ACS) is a unique form of ischemia affecting group of muscles and nerves enclosed within a relatively noncompliant fascial sheath (Matsen, Winquist, and Krugmire 1980; Sadasivan *et al*. 1997). The hallmark of ACS is elevation of the intracompartmental pressure (ICP), which results in progressive microvascular ischemia and, if left untreated, leads to muscle cell death, loss of motor function, or loss of limb (Matsen 1975; Rorabeck 1984). The severity of cellular deterioration observed in ACS appears to be more than that seen in ischemia alone (Heppenstall *et al*. 1988; Heppenstall *et al*. 1989).

ACS is a critical clinical episode that typically occurs after trauma, and represents a diagnostic and therapeutic challenge to orthopaedic surgeons. Emergency surgical decompression by fasciotomy remains the only proven treatment that mitigates the high pressure, thus saving the tissues from progressive damage (Matsen, Winquist, and Krugmire 1980; Shaw and Spencer 1995). However, fasciotomy is not an entirely benign procedure and is associated with substantial morbidity, as well as potential mortality (Shaw and Spencer 1995; McQueen and Court-Brown 1996). The awareness of ACS has increased significantly in the recent years due to serious complications that

resulted in medicolegal issues (Taylor, Sullivan, and Mehta 2012; Shadgan *et al.* 2010; Giannoudis *et al*. 2002).

Solid evidence suggests that intense inflammatory response and release of reactive oxygen metabolites are critically involved in the development of the cellular damage in ACS (Perler, Tohmeh, and Bulkley 1990; Sadasivan *et al*. 1997; Kearns *et al*. 2004; McGarr 2009; Lawendy, Sanders, Bihari, and Badhwar 2011; Lawendy 2014a). Despite the high morbidity and litigation associated with ACS, there is no medical treatment available for routine use, as an adjunctive to surgical decompression to improve the prognosis. Conversely, with the current available knowledge of the pathophysiology of ACS and ischaemia reperfusion injury, there could be a chance to discover a therapeutic agent that may improve the tissue tolerance to ischaemia, or reduce its deleterious effects.

1.1.2. Sequelae Of ACS

The outcome of untreated ACS is catastrophic. Ischaemic insult to the nerve and muscle may lead to muscle contractures, joint stiffness and neurological deficits, resulting in severe functional disabilities. Amputation may be necessary in some cases, particularly if opportunistic infection flares up in the dead muscles (Styf 2003). The deleterious effects of ACS do not occur only locally in the affected limb, but may also happen systemically, resulting in remote organ involvement and severe metabolic disturbance (Heemskerk and Kitslaar 2003; Finkelstein, Hunter, and Hu 1996; Bocca *et al*. 2002; McQueen and Duckworth 2014; Whitesides and Heckman 1996; Malik *et al.* 2009). Acute renal

failure, and even death from the ‘crush syndrome’ are known results of massive muscle necrosis seen in ACS (Reis and Michaelson 1986). The complications are strongly related to the duration of ACS (Pearse 2002). Many researchers reported good clinical recovery if fasciotomy was performed within 6 hours (Rorabeck and Clarke 1978). However, irreversible damage has been reported in 37% of patients after only 3 hours of ACS in a large clinical study (Vaillancourt *et al.* 2004). The 3-hour window was found to be comparable with a tourniquet-induced ischemia in an animal experimental model (Heppenstall *et al*. 1986). It was reported that only 8% of patients regained useful function when surgery had been done after 12 hours of ACS (Sheridan and Matsen 1976).

1.1.3. Historical Perspective

Richard Volkmann was one of the first to realise that ACS resulted from an ischaemic insult to the muscles rather than just nerve paralysis, as it had been believed. He named the condition “*ischaemic muscle paralysis and contracture”* pointing to both, the known pathology and the common end result (Volkmann 1881; Volkmann 2007). This term was then adopted by several authors (Huntington 1907; Hildebrand 1906; Düben 1969; Botte *et al*. 1996). In 1908, the term “*Volkmann's ischemic paralysis and contracture*” appeared in the literature (Sayre 1908), which described the pathology, the end-point, and accredited Volkmann for his scientific contribution. The new term became very popular with many authors (Thomas 1909) and was occasionally shortened to “Volkmann’s

paralysis" (Brooks 1922), or "Volkmann's contracture" (Clarke 1946; Griffiths 1940).

By the middle of the last century, there had been increasing reports of cases of ACS, but with unusual presentations and different clinical scenarios, which was thought to be entities different from ACS. Therefore, special names were given to those conditions: e.g. "*march gangrene*" (Blandy and Fuller 1957) and e*xercise ischaemia"* (Kirby 1970). Thus, the term "syndrome" started to appear in the literature reflecting the varied clinical picture, complexity of pathophysiology and systemic, as well as local consequences of ACS.

In the English literature, the term "*anterior tibial syndrome*" (Carter 1949) appeared in a study analysing previous publication of two cases that had suffered necrosis of the anterior compartment muscles without any fractures: one had developed following a repair of muscular hernia, while the second one had been a soldier who had developed bilateral anterior tibial muscle necrosis following marching (Sirbu 1944). Mortez suggested the term "*compartment ischemia syndrome*" (Mortez 1953). Kunkel and Lynn, the Saskatoon surgeons, simplified the term to "compartment syndrome" (Kunkel and Lynn 1958). Matsen then successfully put forth all the published literature, attempting to understand the principal mechanisms behind the clinical presentations, and outlining the diagnostic and therapeutic rationale. The result was the unified concept of ACS (Matsen 1975).

1.1.4. Clinical Anatomy

A compartment is a closed osseofascial anatomical space packed with muscles, nerves and vessels. The muscles in the extremities are arranged into groups, each enclosed within a fascial envelope (Ellis 2006). Each compartment has a limited capacity to accommodate a sudden increase in the volume of the contents, as happens when acute severe oedema or bleeding inside the compartment follow trauma (Matsen 1975). Knowledge of the surgical anatomy of the compartments is a paramount necessity for a safe and complete surgical decompression. In the leg, there are four distinct anatomical compartments: anterior, lateral, superficial posterior and deep posterior (Figure 1.1).

1.1.5. Aetiology

Fractures cause 69% of cases of ACS, with tibial shaft fractures being the most common of all (McQueen, Gaston, and Court-Brown 2000). Soft tissue trauma is the second most common (23.2%) (McQueen, Gaston, and Court-Brown 2000). Other causes of ACS include arterial injury, reperfusion injury, constrictive dressings and casts, bleeding disorders, injection injuries, burns, and infection (Donaldson, Haddad, and Khan 2014). The overall incidence of ACS following extremity trauma is 2.8% (Branco *et al.* 2011).

1.1.6. Epidemiology

ACS affects mainly young adults, but it has been reported in all ages, including children and neonates (Willis 2012; Ragland *et al.* 2005; Goubier, .

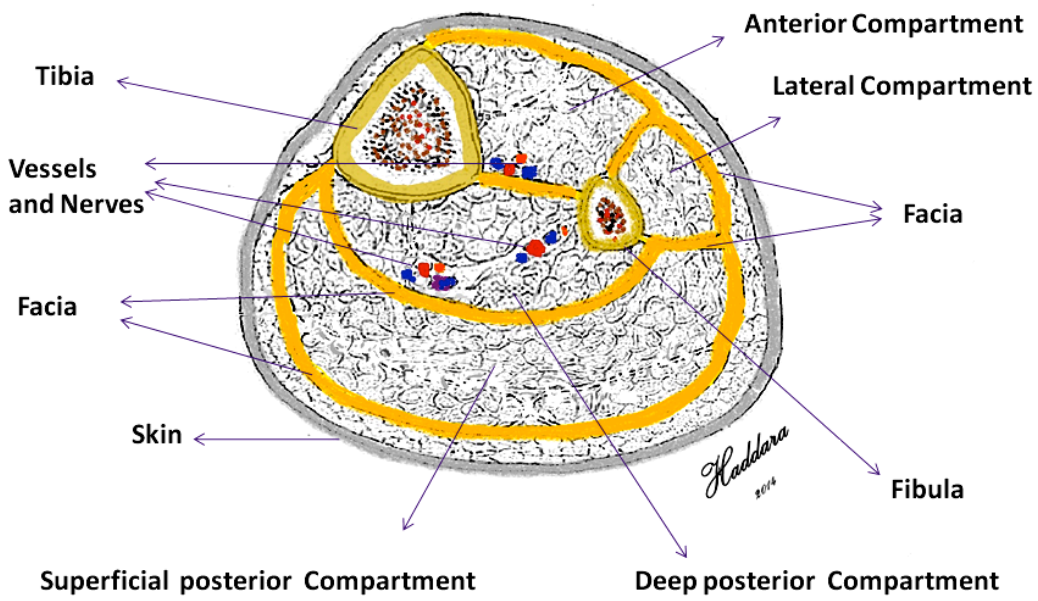

Figure 1.1. Anatomical compartments of the leg, shown at the level of the mid calf.

Romaña, and Molina 2005).

The average age for males is 32 years, while that for females is 44 years (McQueen, Gaston, and Court-Brown 2000). It is estimated that 3.1 per 100,000 of the population suffer compartment syndrome per year, men representing 91 % of cases (McQueen 2000). In the extremities, 75% of ACS affects leg compartments, 16% the forearm, 4% the hand, 3% the thigh, while only 1% is reported in the arm or the foot (Vaillancourt *et al*. 2004).

1.1.7. Diagnosis

The diagnosis of ACS is, essentially, a clinical skill; diagnosis can be confirmed by the measurement of intracompartmental pressure (Elliott and Johnstone 2003; Hargens *et al*. 1989; Matsen, Winquist, and Krugmire 1980). A high index of clinical suspicion helps in detecting early signs of ACS, before the irreversible damage occurs. ICP measurement is currently done using sophisticated digital pressure probes.

Clinical findings include a swollen, tense, and tender compartment. Sensory deficits and motor weakness can be detected early, while paralysis occurs late in the presentation. The classical symptom is pain out of proportion to what would normally be expected from the existing injury, and is characteristically accentuated by passive stretch of the afflicted muscles (Hargens and Mubarak 1998). The most striking sign is the presence of palpable distal arterial pulsation in most patients, contrary to cases of complete ischaemia (Whitesides and Heckman 1996).

1.1.8. Intracompartmental Pressure (ICP)

The pathognomonic factor in ACS is elevated ICP (Matsen 1975). The critical pressure threshold beyond which muscle cannot survive had been thoroughly investigated: an absolute compartment pressure of 30 mmHg had been suggested by some authors (Hargens et al. 1978; Mubarak *et al*. 1978; Mubarak, Pedowitz, and Hargens 1989; Hargens *et al*. 1981), while 45 mmHg had been suggested by others (Rorabeck and Clarke 1978). Some also suggested calculating the difference between the mean arterial pressure and compartmental pressure, calling it the differential pressure (ΔP) (Heckman *et al*. 1993; Hartsock *et al.* 1998; Mars and Hadley 1998; Heppenstall *et al*. 1988). A ΔP of 30 mmHg was found to impair muscle metabolism significantly at normal conditions, while a ΔP of 40 mmHg was found to cause similar effect in an injured muscle (Heppenstall et al. 1989).

1.1.9. Fasciotomy

The treatment in the late 1800s and early 1900s mainly addressed the complications of ACS rather than termination or prevention of the disease (Rowlands 1905; Sayre 1908). It was not until the mid 1970s that the literature showed general consensus on fasciotomy as the standard treatment for ACS (Willhoite and Moll 1970; Sheridan and Matsen 1976).

Fasciotomy is a surgical procedure in which the fascial wall of the compartment is opened longitudinally, through a substantial surgical exposure, to fully decompress the contents (Mubarak and Owen 1977; Finkelstein, Hunter,

and Hu 1996) (Figure 1.2). Fasciotomy is the gold standard treatment of ACS and should be done as soon as the diagnosis is reached.

The most important predictor of outcome after ACS is time to fasciotomy. A significant progression of tissue injury and irreversible damage are very likely if elevated ICP persists for more than 6 hours (Rorabeck 1984; Sheridan and Matsen 1976; Elliott and Johnstone 2003). In clinical practice, this critical period is called a 'surgical window'.

Although fasciotomy is a limb saving procedure, it comes with its own complications. Even when carried out in a timely manner, the sequelae of ACS cannot be fully avoided in every case (Echtermeyer and Horst 1997; Giannoudis *et al.* 2002). Thus, there is a great need to find an adjunctive therapy, or alternative treatment to surgery, that can reduce complications, prolong the surgical window and improve the overall clinical outcome of the ACS.

1.2. PATHOPHYSIOLOGY OF ACS

Pathophysiology of ACS appears to be very complex. Although there have been many research studies, explanations, and theories, it is not yet fully understood. The hallmark of compartment syndrome is the elevated ICP, with oedema as an essential cause.

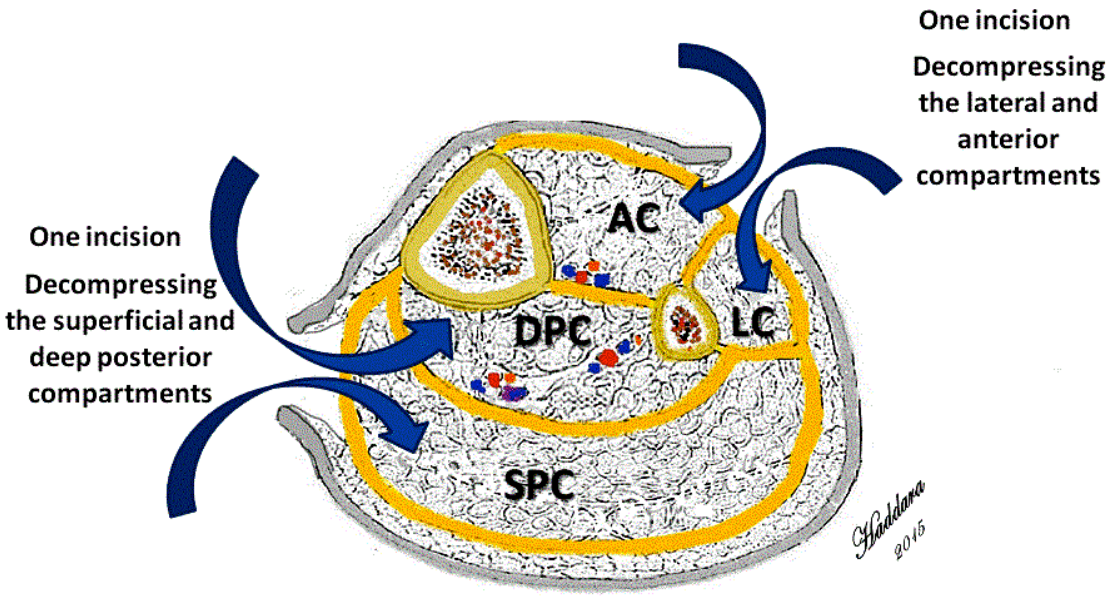

Figure 1.2. Fasciotomy of the leg compartments. For a successful fasciotomy, all affected compartments should be decompressed by complete opening of the fascial envelope, including the deep ones, through a skin incision along the length of the compartment, taking care not to injure nerves and vessels. Following fasciotomy, all wounds should be left open to allow expansion of the oedematous muscles. Wound care protocols should be initiated, until delayed closure.

AC, anterior compartment; *LC*, lateral compartment; *DPC*, deep posterior compartment; *SPC*, superficial posterior compartment.

1.2.1. Elevated ICP and Ischaemia

While the precise mechanism of muscle ischemia in ACS is elusive, it is widely accepted that it occurs mainly at the level of the microcirculation (Hartsock *et al.* 1998). Theories on the pathophysiology of ACS tried to explain how the elevated ICP would lead to ischaemia. Most of these considered only the mechanical force of elevated hydrostatic pressure as the cause of damage. The theories postulated that elevated ICP may result in ischaemia through an induction of arterial insufficiency, venous stasis, collapse of the capillaries, or a combination of these mechanisms (Murphy 1914; Brooks 1922; Jepson 1926; Griffiths 1940; Burton and Yamada 1951; Kinmonth 1952; Eichler and Lipscomb 1967). Once ICP reaches a critical level above the perfusion pressure, it will lead to severe hypo-perfusion (Hartsock et al. 1998; Hargens and Mubarak 1998).

1.2.2. Oedema and The Vicious Circle Concept

Oedema commonly occurs following trauma, but it is particularly severe and rapidly progressive in ACS. While it is not known why oedema occasionally goes beyond the limits in certain cases, it is well-known that it leads to increased ICP to a level that triggers the occurrence of ACS.

The self-perpetuating cycle is a plausible concept that could explain the relation between oedema and ICP. An increase in oedema would cause an increase in ICP, which would further increase the oedema and so on, leading to the establishment of ACS (Matsen and Clawson 1975) (Figure 1.3).

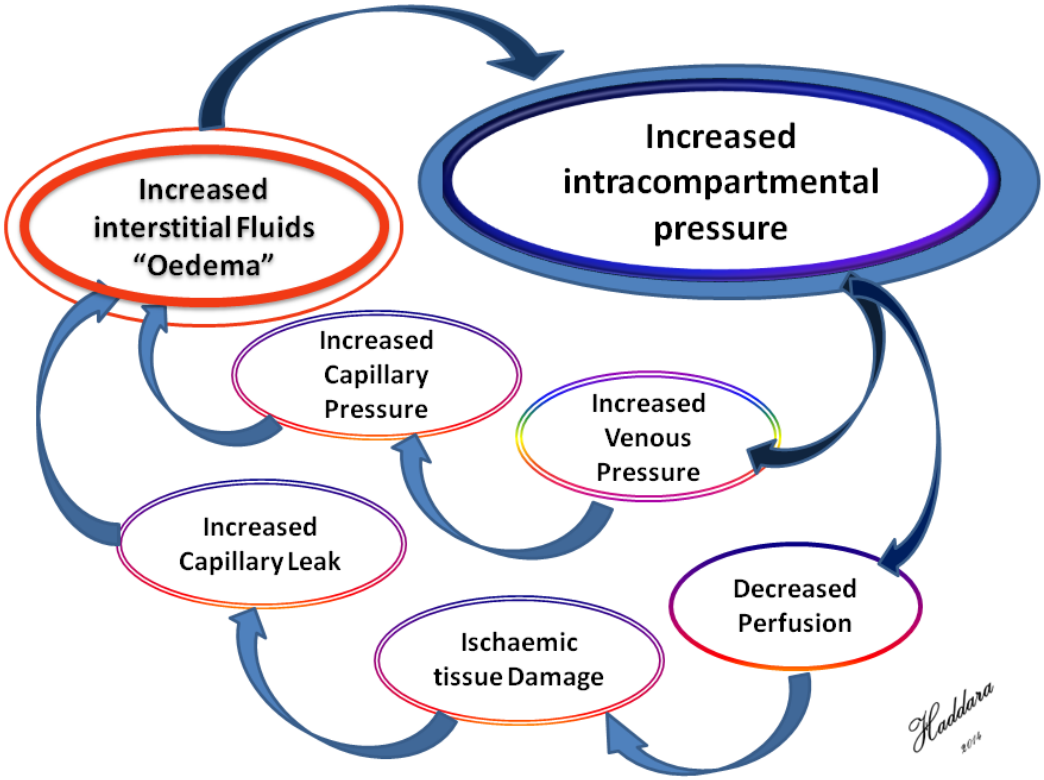

Figure 1.3. The vicious circle concept. Increased compartment pressure leads to an increase in tissue oedema, which, in turn, leads to increased compartmental pressure and so on. The process would be self perpetuating. *Adapted from (Matsen and Clawson 1975).*

1.2.3. Inflammation

Echtermeyer questioned if the pathophysiology of ACS could only be explained by the high ICP (Echtermeyer and Horst 1997). Experimental studies had suggested that cellular hypoxia during the phase of elevated ICP would induce a cascade of inflammatory reactions resulting in microcirculatory changes and tissue damage (Perler, Tohmeh, and Bulkley 1990; Sadasivan *et al*. 1997). Recently, inflammation was shown to play a major role in the induction and augmentation of cellular damage seen in ACS (Manjoo *et al*. 2010; Wang, Baynosa, and Zamboni 2011a; Gillani *et al*. 2012; Blaisdell 2002; Lawendy 2014a). Leukocytes (predominantly neutrophils) coupled with the production of pro-inflammatory mediators were found significantly contributing to the cellular damage (Lawendy, Sanders, Bihari, and Badhwar 2011; Lawendy, Sanders, Bihari, Parry, *et al.* 2011).

It was not only local inflammation that had been observed in ACS, but also a systemic inflammatory response (Harvey *et al.* 2012; Lawendy *et al*. 2011; McGarr 2009; Lawendy 2014a). Our laboratory had previously shown that elevation of ICP led to a progressive sustained elevation in the serum tumor necrosis factor alpha (TNF-α), which further continued to increase, even at 45 minutes after fasciotomy (Lawendy, Sanders, Bihari, Parry, *et al*. 2011; Lawendy *et al*. 2011; Lawendy *et al*. 2014). Lawendy *et al* also reported 7-fold increase in hepatocellular injury in an animal model of limb compartment syndrome (Lawendy 2014a, chap. 4).

1.3. ISCHAEMIA AND COMPARTMENT SYNDROME

1.3.1. Compartment Syndrome versus Complete Ischaemia

While tissue ischaemia is the common denominator of ACS and complete ischaemia, the elevated ICP creates a different pathophysiological state. ACS develops and progresses while arterial pulsation is still present, reflecting a unique ischaemic insult compared to complete ischaemia.

Heppenstall *et al* compared the pathological effects of complete ischaemia and compartment syndrome in a canine model (Heppenstall *et al*. 1986). They elevated ICP for 3 hours, followed by 2 hours of reperfusion. The structural and metabolic deterioration of the skeletal muscle was then assessed. ACS was induced by autologous plasma infusion in the anterior compartment, while acute ischaemia was induced by a pneumatic tourniquet. The researchers reported a marked ultrastructural deterioration of skeletal muscle in ACS group compared to that observed in complete ischemia. They also demonstrated striking dissimilarities in the metabolic response between the two groups. The two most notable observations in the ACS group were the rapid depletion of adenosine triphosphate (ATP) levels and the marked intracellular acidosis compared the tourniquet group. In the complete ischemia group, ATP did not change at all throughout the experiment. Surprisingly, neither the acidosis nor the ATP levels recovered at 2 hours post-fasciotomy in the ACS group, while pH levels immediately recovered in the complete ischemia group following the release of tourniquet. The authors stressed the fact that in cases of ACS, there is always some degree of perfusion present (Heppenstall *et al*. 1986).

1.3.2. Cellular Effects of Ischaemia

Oxygen is fundamental to human physiology. While it is essential for mitochondrial ATP generation, ATP production is associated with the risk of oxidative damage; thus, oxygen concentrations must be tightly regulated (Semenza 1999).

Oxygen deficiency during ischaemia results in the reduction of ATP production and acidosis due to accumulation of lactic acid from glycolysis. Sodium-potassium pumps are ATP dependent; therefore, during ischaemia the intracellular sodium and calcium are increased. This attracts water into the intracellular environment, causing cellular oedema.

Ischaemia also promotes the production of pro-inflammatory substances, like leukocyte adhesion molecules, TNF-α and thromboxane A2. Additionally, ischaemia suppresses some ‘protective’ substances, like constitutive nitric oxide synthase (cNOS) and prostacyclin (Carden and Granger 2000).

If ischaemia persists beyond the critical point of tissue tolerance, cellular necrosis becomes inevitable. While reperfusion is the only way to minimize ischaemic necrosis, it initiates tissue injury beyond that caused by the ischaemic insult alone.

1.3.3. Limb Ischaemia-Reperfusion (IR) Injury

Restoration of blood flow (reperfusion) to ischemic tissue is vital, but sudden reperfusion after a prolonged period of ischaemia is also deleterious (Lejay *et al.* 2014). The tissue damage caused by reperfusion was found to be

progressively proportionate to the time of reperfusion (Carmo-Araújo et al. 2007), and was estimated to be more than that caused by ischemia alone in the rat skeletal muscle (Loerakker et al. 2011). The main factors responsible for local and systemic damage caused by IR appear to be the reactive oxygen species (ROS) and activated neutrophils (Gillani *et al.* 2012).

Sudden supply of hypoxic tissue with oxygen leads to rapid formation of large quantities of ROS in the mitochondria, which exacerbates the mitochondrial dysfunction that had already started during ischaemia, by damaging the mitochondrial membrane. While mitochondrial function is to necessitate preservation of membrane potential (Wang *et al*. 2011), the IR damage leads to loss of this, leading to cessation of ATP production and producing cell necrosis. It also results in matrix swelling and eventual rupture of the mitochondrial membrane, releasing cytochrome c into the cytosol. This initiates apoptosis (Wang, Baynosa, and Zamboni 2011b).

1.3.4. Low-Flow Ischemia

It has been known that the distal pulsation is intact in most cases of compartment syndrome. This may be interpreted as the ischaemia in ACS is not complete. Heppenstall *et al* stressed the fact that there was always some degree of perfusion in cases of ACS (Heppenstall *et al.* 1986). Lawendy *et al* have termed it ‘low-flow ischaemia’ (Lawendy 2014a).

Conrad *et al* compared complete and incomplete ischaemia in a murine model. They reported marked early increase of a pro-inflammatory cytokine KC,

which is analogous to human Interleukin-8 (IL-8), in the partial ischaemia group (Conrad *et al.* 2005). Its early presence in partial ischaemia elucidated difference in pathophysiological sequences compared to complete ischaemia (Ghasemi *et al*. 2011). Lawendy et al also demonstrated significant early increase in the pro-inflammatory TNF-α during the course of ACS, in contrast to complete ischaemia (Lawendy 2014a).

1.3.5. ACS and IR Injury

The severity of cellular damage reported in ACS appears to be more than that seen in complete ischaemia alone (Heppenstall *et al*. 1988; Heppenstall *et al*. 1989). This fact created a general impression that partial ischaemia is more damaging than complete ischaemia. However, recent studies reported that complete ischaemia causes more muscle damage than that caused by partial ischemia, contrary to what was generally believed (Conrad *et al*. 2005; Conrad *et al.* 2006). This new insight does not explain the extensive damage in ACS compared to complete ischaemia based on blood flow rate. Thus, there must be another mechanism.

There are several pathological similarities between IR and ACS-induced striated muscle injury (Heppenstall *et al*. 1986; Sadasivan *et al*. 1997; Tollens, Janzing, and Broos 1998). The understanding that the reperfusion injury is more damaging than ischaemia itself may be the key to how to explain this puzzling fact (Loerakker *et al*. 2011). This compelled researchers to consider ACS as one of the clinical situations associated with skeletal muscle IR injury (Gillani *et al*.

2012), in which the reperfusion resulted in marked oxidative stress and an intense inflammatory response, thereby maximizing the local damage already caused by ischaemia, Additionally, it also induced a systemic inflammatory response (Carden and Granger 2000; Girn *et al*. 2007; Gillani *et al*. 2012).

It should be remembered that ACS also results in a much greater level of metabolic strain and cellular deterioration than IR injury alone (Stephen R Kearns *et al*. 2010). Therefore, both ischaemia and IR may be concomitantly occurring in ACS.

1.3.6. Fasciotomy and Reperfusion Injury

Upon release of elevated ICP, Sadasivan *et al* reported no-reflow phenomenon, similar to that of IR injury (Sadasivan *et al.* 1997). Surgical decompression by fasciotomy promptly aborts the self-perpetuating cycle of oedema/elevated ICP by normalising the tissue pressure, thus saving the muscle by regaining blood supply. However, this leads to a sudden reperfusion of previously hypoxic tissues with oxygen-rich blood, producing an intense IR injury, mainly by the liberated ROS. This effect is called a 'second hit' (Sellei, Hildebrand, and Pape 2014) which, in conjunction with the activated neutrophils, leads to an increase in the microvascular dysfunction and the net tissue damage (Gillani *et al.* 2012).

Reperfusion injury normally occurs only after allowing normal blood flow to resume (for example, rearterization), which, theoretically, is what fasciotomy does in ACS. Still, this does not explain the early changes and damages seen

immediately following normalization of compartment pressure after 2 hours of experimental ACS (Sadasivan *et al.* 1997; Lawendy, Sanders, Bihari, Parry, *et al*. 2011).

1.4. PATHOLOGICAL FINDINGS IN ACS

Apart from the myonecrosis, reported in ACS by most authors (Volkmann 1881; Brooks 1922; Mabee and Bostwick 1993; Sheridan, Matsen, and Krugmire 1977; Zimmerman and Shen 2013), recent researchers were able to visualise the *in vivo* changes occurring within the muscle immediately following fasciotomy in animal models.

1.4.1. Microvascular Dysfunction

Microcirculation is the part of blood circulatory system that provides skeletal muscles with oxygen and various nutrients, and removes the noxious waste products of metabolism (Hudlicka 2011). The vitality of the muscle, or any living tissue, depends on adequate perfusion through the capillary circulation. In normal physiological conditions, most capillaries are continuously perfused (CPC), with few intermittently perfused (IPC) or non-perfused (NPC) capillaries.

Microcirculation is considered a ‘battlefield’ in inflammation and reperfusion (Wang, Baynosa, and Zamboni 2011a). Using the modern intravital video microscopy (IVVM) technology, researchers in our laboratory were able to elucidate the microvascular dysfunction associated with ACS, using a rat model (Lawendy, Sanders, Bihari, Parry, *et al*. 2011; Manjoo *et al*. 2010; McGarr 2009).

They reported significant reduction in the CPC, coupled with a significant increase in the NPC; in other words, there was a failure to restore the normal physiological micro flow. The microvascular dysfunction noticed in ACS was, to a great extent, similar to that reported in reperfusion injury following complete limb ischaemia, where the vascular bed behaves contrary to what is expected following re-establishment of the blood flow (Potter *et al*. 1993; Menger and Messmer 1993; Lam *et al*. 1994; Blaisdell 2002; Galiuto and Crea 2006; Maksimenko and Turashev 2012). A significant increase in the number of NPC causes an overall microvascular blood flow failure. This characteristic scenario is known as the 'no-reflow phenomenon' (Nanobashvili *et al*. 2003; Reffelmann and Kloner 2006). The main players responsible for the microvascular dysfunction appear to be ROS and activated leukocytes (Sadasivan *et al*. 1997; Gillani *et al*. 2012; Blaisdell 2002).

It worth mentioning that there are two distinct types of no-reflow: structural or otherwise irreversible, and functional (Galiuto and Crea 2006). The severity is related to the pathogenesis and duration of ischaemic insult (Reffelmann and Kloner 2006; Galiuto and Crea 2006).

1.4.2. Tissue Injury

Cellular injury is one of the earliest recognized sequelae of ACS. Studies have shown that tissue damage could be reverted by therapeutic modalities, implying that some damage may have been reversible (Lawendy *et al.* 2014; Manjoo *et al.* 2010). *In vivo* experiments in leukocyte-deplete rats have

demonstrated 50% reduction in cellular injury after 90 minutes of induced ACS (Lawendy 2014b), highlighting the important role of activated leukocytes in producing the cell damage.

Recent studies have shown that cell damage in IR injury is not just necrotic but, to a great extent, apoptotic, and appears to be induced by the effect of TNF-α (Sherwood and Toliver-Kinsky 2004). Apoptosis, in contrast to necrosis, is a programmed cell death process that can be triggered by several factors, but needs the caspases enzymatic pathway for apoptosis execution (de Nigris *et al*. 2003; Haddad 2004; Riedl and Shi 2004; Nikoletopoulou *et al*. 2013).

1.4.3. Leukocyte Activation

Activated leukocytes tend to roll and adhere to the endothelium prior to transmigration through the vessel wall (Ley 2008). Significant increase in leukocyte rolling and adhesion was reported in the post capillary venules of the skeletal muscle in a rat model of ACS (Lawendy *et al.* 2014; Manjoo *et al.* 2010; Lawendy 2014a; McGarr 2009). Leukocyte activation (rolling and adhesion) is controlled by differential expression of various adhesion molecules.

1.5. MECHANISM OF INFLAMMATION IN ACS

The literature reports of studies exploring the mechanism(s) of inflammation and tissue damage in ACS are scarce. However, mechanisms and pathways elucidated in IR injury can provide valuable insight on the possible

damaging and/or protective factors in ACS. There are enough similarities between IR injury and ACS to assume that both may be sharing some of these.

1.5.1. Oxidative Stress

1.5.1.1. Overview

At normal conditions, aerobic metabolism within the mitochondria produces very active and potentially damaging ROS. These are potent oxidizing agents capable of cellular membrane lipid peroxidation and activation of neutrophils (Granger 1988). ROS are normally controlled by the natural antioxidant activity within the system. Oxidative stress is a state of imbalance between the pro-oxidants and antioxidants, in favour of the pro-oxidant (Ma, Qi, and Chen 2008; Ogura and Shimosawa 2014; Bar-Or *et al*. 2015). Several ROS may be involved in IR injury and ACS: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), hypochlorous acid (HOCl), and nitric oxide-derived peroxynitrite (Perler, Tohmeh, and Bulkley 1990; Hancock, Desikan, and Neill 2001; C. Li and Jackson 2002).

1.5.1.2. ROS in IR Injury

ROS play an integral role in the progression of reperfusion injury, by inducing cell necrosis, apoptosis and activation of leukocytes (Hancock, Desikan, and Neill 2001; P. H. Chan 2001; C. Li and Jackson 2002; Wang *et al*. 2006; Arató *et al*. 2008; Wang, Baynosa, and Zamboni 2011b). NADPH oxidase and xanthine oxidase are perhaps two of the most important enzymes involved in the

generation of ROS (Hancock, Desikan, and Neill 2001), whereas superoxide dismutase (SOD) and glutathione peroxidase appear as two of very important defensive mechanisms to ameliorate the harmful effects of ROS.

1.5.1.2.1. ROS in ACS

ROS also appear to be important players contributing to the cellular damage in ACS. Ricci *et al* were able to markedly reduce the muscle damage of post-ischaemic ACS by administration of free radical scavengers in a canine model (Ricci *et al*. 1989). Likewise, Perler *et al* were able to inhibit the formation of compartment syndrome by ablation of free radical-mediated reperfusion injury (Perler, Tohmeh, and Bulkley 1990).

1.5.2. Neutrophil Activation

During reperfusion, activated leukocytes exhibit rolling behaviour, followed by firm adhesion to the endothelium of the post capillary venules. This creates favourable microenvironment permitting transmigration to the interstitial space (Weiss 1989). Upon activation, neutrophils release the contents of their intracellular granules containing ROS, cytokines, chemokines and proteolytic enzymes that mediate the injurious effects in reperfusion injury (Welbourn *et al.* 1991; Hampton, Kettle, and Winterbourn 1998; Arnhold 2004; Klebanoff 2005; Robinson 2009).

1.5.3. Endothelial Dysfunction

Endothelial dysfunction is one of the characteristics of IR injury. Upon exposure to ischaemia or reperfusion, the endothelium gets activated, creating a pro-inflammatory and pro-thrombotic surface, accompanied with loss of local vasodilation mechanisms. These changes collectively are termed endothelial dysfunction (Girn *et al*. 2007). The resulting vasoconstriction of the muscle microcirculation creates the known capillary no-reflow phenomenon (Wang et al. 2005; de With *et al*. 2005). Reperfusion-induced vasoconstriction has been attributed to several factors, but recently was found to be caused by deficiency of endothelial nitric oxide synthase (eNOS) (but not the inducible nitric oxide synthase iNOS) (Wang, Baynosa, and Zamboni 2011b).

1.5.4. Cytokines

Cytokines are a group of signalling molecules that play an important role in modulation of the immune response (Cannon 2000; Zhang and An 2007). Almost all nucleated cells are capable of synthesizing cytokines and, in turn, respond to them (Dinarello 2000). Cytokines can regulate the expression of genes at the transcriptional level through selective alterations of expression of various transcription factors, thereby inducing the production of other cytokines and cell surface receptors. Additionally, they can also down-regulate themselves by a negative feedback loop (Grivennikov *et al*. 2006).

1.5.4.1. Pro-Inflammatory Cytokines

Pro-inflammatory cytokines are involved in the upregulation of inflammatory mediators. TNF-α and Interleukin-1 (IL-1) are probably the most studied cytokines in IR injury. Both are produced at the site of local inflammation and their net effect is considered synergistic (Dinarello 2000). Interestingly, both exert similar roles but through different pathways and different receptors, showing a certain degree of redundancy (Dinarello 2000). TNF-α and IL-1 play important roles in downstream cytokine/chemokine-triggered leukocyte activation, production of endothelial adhesion molecules, stimulation of phagocytosis, and induction of apoptosis (Khan 2008). They also upregulate iNOS and induce transcription of cyclooxygenase-2 (COX-2), leading to an increase in prostaglandin-2 (PGE-2) synthesis (Zhang and An 2007; Dinarello 2000).

In ACS, a sustained elevation in TNF-α has been demonstrated during the period of elevated ICP, with a further, more pronounced increase following decompression (Lawendy *et al*. 2014).

1.5.4.2. Anti-Inflammatory Cytokines

These are immunoregulatory molecules that limit the potentially injurious inflammatory reaction due to the induction of the proinflammatory cytokines, if it becomes persistent or excessive (Opal and DePalo 2000). Their physiologic and pathologic roles are increasingly recognized in determining the outcome of disease by timely restoration of the proper balance. Molecules like IL-4, IL-10, IL-

13, and transforming growth factor-β (TGF-β) can suppress the production of IL-1, TNF-α, and vascular adhesion molecules; therefore, they are considered anti-inflammatory cytokines (Dinarello 2000).

1.5.4.3. Chemokines

Chemokines are cytokines that possess chemotactic properties attracting the circulating leukocytes towards the site of inflammation or injury (Sherwood and Toliver-Kinsky 2004). IL-8 is an example of a potent chemokine that specifically attracts neutrophils. Additionally, it is capable of stimulating their adherence to the endothelium of post capillary venule (Kobayashi 2006; Bickel 1993). Neutrophil chemoattractant protein (KC) is the murine equivalent of human IL-8 (Conrad *et al.* 2005).

1.5.5. Transcription Factors

Transcription factors (TF) are proteins that control the copying of genetic material from DNA to the messenger RNA (Tjian 1996). This is done by the TF binding to a specific DNA region adjacent to the target gene. TF may increase or decrease the gene expression by activating or blocking RNA polymerase, the enzyme responsible for converting the DNA to messenger RNA (Schwabe, Rybakova, and Bruggeman 2012).

Nuclear factor kappa-B (NF-κB) is a TF induced in response to a wide range of stimuli during IR injury, particularly oxidative stress and proinflammatory

cytokines (Nichols 2004; Lille *et al*. 2001). Activator protein-1 (AP-1) is another TF induced in a similar fashion (Lefler *et al*. 2002). Activation of NF-κB and AP-1 in IR results in the upregulation of expression of various inflammatory molecules, including cytokines, adhesion molecules, and iNOS (Lefler *et al*. 2002; Nichols 2004).

1.5.6. Xanthine Oxidase

Xanthine oxidase (XO) is an enzyme known to generate superoxide and hydrogen peroxide (J. K. Smith, Carden, and Korthuis 1989). Interactions with these two ROS can yield a range of cytotoxic agents, including hydroxyl radicals (Granger, Höllwarth, and Parks 1986; B. J. Zimmerman, Grisham, and Granger 1990). XO-derived ROS are known to activate NF-κB leading to an upregulation of various inflammatory molecules.

XO is produced by many tissues when subjected to IR insult, and is released in the systemic circulation (Tan *et al*. 1995). The circulating XO binds to the surface of endothelial cells and continues to produce oxidants. This, in turn, can trigger endothelial dysfunction, thereby contribute to the local damage, and cause remote organ injury (Pacher, Nivorozhkin, and Szabó 2006).

NO has been shown to act as an endogenous suppressor of XO activity; thus, reduced level of the NO-mediated suppression of XO may result in increased superoxide generation (Ichimori *et al*. 1999; Pacher, Nivorozhkin, and Szabó 2006).

1.5.7. Caspases

Caspases are a family of endoproteases that play a critical role in controlling inflammation and cell death. Activation of apoptotic caspases results in the generation of a cascade of signalling events that allows a controlled demolition of cellular components (McIlwain, Berger, and Mak 2013). This is how some caspases were given the nickname of ‘executioner’ proteins (Riedl and Shi 2004). On the other hand, activation of inflammatory caspases results in the stimulation of innate immune responses, and the subsequent production of active proinflammatory cytokines (McIlwain, Berger, and Mak 2013).

Recent evidence from experimental models of IR has shown that apoptosis doubled necrosis in skeletal muscle after 4 hours of ischemia and 24 hours of reperfusion (Wang *et al.* 2008).

1.5.8. Heme Oxygenase

Heme oxygenase (HO) is an enzyme that degrades heme to biliverdin, iron and carbon monoxide (CO). There are two isoforms: the inducible HO-1 and constitutively-expressed HO-2. HO-1 functions as a ‘sensor’ of cellular stress, thus increasing cellular adaptation to stress and limiting tissue damage (Motterlini and Foresti 2014).

The HO system has been shown to play important cytoprotective functions through the effects of its by-products. CO has been reported to diminish the severity of microvascular dysfunction and inflammation caused by ACS

(Lawendy *et al* 2014), while biliverdin and iron were found to be potent antioxidants (Ryter *et al* 2006).

1.6. POTENTIAL NON-OPERATIVE TREATMENTS FOR ACS

Apart from emergency surgical decompression and standard supportive treatment to prevent other systemic sequelae during the recovery period, few other treatment options exist for routine use in ACS (S. A. Olson and Glasgow 2005).

Adjunctive therapy is used together with the primary treatment to increase the chance of a cure, or to increase the efficacy of the primary treatment. In ACS, the primary treatment is the surgical decompression, and any medical or physical treatment given in conjunction with fasciotomy is considered adjunctive treatment.

Since the cellular damage in ACS appears to be a result of intense ischaemia-induced inflammatory reaction coupled with oxidative stress, therapeutic modalities specifically targeting these factors may be beneficial in ACS as adjunctive treatment.

1.6.1. Hyperbaric Oxygen

Hyperbaric oxygen therapy showed promising results as an adjunct to fasciotomy, by decreasing complications from ACS. It has been shown to have the ability to reach ischaemic tissues suffering low perfusion during elevated ICP

state. Previously, it was found to be effective in reducing amputation rate and improving wound healing (Greensmith 2004; Strauss 2012). It has also been reported to reduce swelling, improve fasciotomy wound healing and fight infection (Weiland 2007). There is no large scale clinical study available on hyperbaric oxygen therapy in ACS, possibly because the very specialised equipment needed for it is not available in most trauma centres.

1.6.2. Antioxidants

Inhibition of ROS by strong specific antioxidants has proven to be successful experimentally in ablation of free radical-mediated reperfusion injury. Perler *et al* demonstrated that by scavenging the superoxide radical at reperfusion with SOD or blocking the hydroxyl radical formation with deferoxamine, a significant amelioration of the rise in compartment pressure could be achieved (Perler, Tohmeh, and Bulkley 1990). Vitamin C is also a powerful general antioxidant that concentrates preferentially in leukocytes. It has been tried experimentally in an animal model of ACS, and has been reported to attenuate the muscle injury (Kearns *et al*. 2004).

1.6.3. Non-Steroidal Anti-inflammatory Drugs

Anti-inflammatory drugs, in theory, may have therapeutic effect in ACS. Indomethacin, a strong anti-inflammatory drug, demonstrated protective effects in an experimental animal model of ACS (Manjoo *et al*. 2010).

1.6.4. N-Acetylcysteine

N-acetylcysteine (NAC) has been shown to reduce the cellular damage due to production of free radicals from neutrophils, in an experimental animal model. It has been suggested that its activity may be due to the combined antioxidant and glutathione-replenishing activities (Kearns *et al*. 2010).

1.6.5. Gasotransmitters

All cells in the living organisms are constantly receiving information about their surrounding environment necessary for the control and regulation of their activities, mediated by signalling molecules. Examples of these include hormones, neurotransmitters, pheromones, and cytokines. A class of small gaseous molecules have recently been discovered to function as signalling molecules; hence, they were termed ‘gasotransmitters’. There are three known gasotransmitters synthesized by mammalian cells: nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) (Farrugia and Szurszewski 2014). There appears to be a considerable interplay among the members of the gasotransmitter family (Ling Li, Hsu, and Moore 2009; Whiteman and Moore 2009). While signalling molecules usually require specialised receptors to evoke a response, gasotransmitters do not (Moody and Calvert 2011). Instead, they can freely permeate the plasma membrane and pass the message directly to an intracellular target (Rui Wang 2003). The use of these transmitters as a pharmacological agent in IR injury has gained a lot of attention in the last two decades (Moody and Calvert 2011).

1.6.5.1. Nitric Oxide (NO)

NO was the very first gasotransmitter to be recognized. Its signalling role in the cardiovascular function as the endothelial-derived relaxing factor (EDRF) had been discovered by the 1998 Nobel Prize-winning work of Furchgott, Murad and Ignarro (The Nobel Foundation 2015). Since its discovery, NO has emerged as a fundamental signalling molecule responsible for the regulation of several cellular functions, as well as a potent mediator of cellular damage in a wide range of conditions (Pacher, Beckman, and Liaudet 2007).

NO is synthesized from L-arginine by the enzyme NOS (Michel and Feron 1997). It was identified as a strong vascular smooth muscle relaxant (Furchgott and Zawadzki 1980). Endothelial NOS (eNOS) is the isoform that plays a main role in vasorelaxation (Lamas *et al.* 1992). NO produced inside endothelial cells diffuses directly from the endothelium to the smooth muscle layer within the vascular wall, where it exerts its relaxing action (Dudzinski *et al*. 2006).

NO has been reported to protect against ischaemic injury in the heart, brain, liver, and other tissues, when administered as a therapeutic agent (Siegfried *et al*. 1992; Hataishi *et al*. 2006).

1.6.5.2. Carbon Monoxide (CO)

CO is a well-known toxic gas, recently identified as another gasotransmitter produced in humans (Marks *et al*. 1991). CO is produced endogenously through the activity of HO (Ryter and Otterbein 2004; Knauert *et*

al. 2013). Synthesis of CO has been shown to occur in almost all tissues (Maines 1997).

CO, like NO, is also a known vasodilator, with direct effect on vascular smooth muscle (Kharitonov *et al*. 1995) and the central nervous system (Wiesel *et al*. 2001). It has been demonstrated to be involved in cell proliferation, inflammation, neurotransmission, mitochondrial biogenesis, autophagy and apoptosis (Brouard *et al*. 2000; Suliman *et al.* 2007; Knauert *et al*. 2013).

Cytoprotective effects of CO have been reported in several IR conditions, as well as in an animal model of ACS (Hangaishi *et al*. 2000; Yet *et al.* 2001; Tulis *et al.* 2001; Sato *et al.* 2001; Nakao *et al*. 2005; Ott *et al.* 2005; Scott *et al*. 2009; Patel *et al.* 2012; Lawendy *et al.* 2014). It appears to act through different cytoprotective pathways, although the precise molecular interactions have not yet been fully elucidated (Knauert *et al*. 2013).

Recent studies have demonstrated strong anti-inflammatory properties of CO. One of the proposed pathways appears to be related to the activation of HO expression, which itself can decrease inflammation, and thrombosis (Patel *et al*. 2012). CO also appears to inhibit the activity of NADPH oxidase, thus reducing the production of ROS. Finally, it is also a potent vasodilator, providing benefit by improving the microcirculation in IR injury (Motterlini and Otterbein 2010).

Previously, our laboratory demonstrated that carbon monoxide, liberated from carbon monoxide-releasing molecule-3 (CORM-3), was able to mitigate the microvascular dysfunction and cellular injury in a rat model of ACS (Lawendy *et al.* 2014).

1.7. HYDROGEN SULFIDE

Hydrogen sulfide gas (H_2S) has long been known for its bad smell and toxic nature (Reiffenstein, Hulbert, and Roth 1992). Recently, it had been discovered that mammalian body can generate H_2S endogenously, at cellular level, in many tissues and vital organs (Stipanuk and Beck 1982; Goodwin *et al*. 1989; Hosoki, Matsuki, and Kimura 1997). The role of endogenously produced H_2S has been suggested as an important signalling molecule, with invaluable cytoprotective functions (Abe and Kimura 1996; Snijder *et al*. 2013; Kimura 2014a; Hancock and Whiteman 2014).

1.7.1. History of H_2S

Bacterial activity has long been known to result in the production of hydrogen sulfide in the colon, producing gases with offensive smell (Suarez *et al.* 1997), and, occasionally, in the oral cavity, resulting in halitosis (Suarez *et al*. 2000). In contrast, the capacity of mammalian tissues to endogenously produce H_2S became known only within the last century (Sluiter 1930; Meister, Fraser, and Tice 1954; Koj, Frendo, and Janik 1967). Surprisingly, H_2S was considered as a by-product, rather than a physiologically active molecule, and thus has been largely ignored.

In the 1980s, the endogenous production of H_2S started to grab the attention, after an *in vivo* rat study demonstrating mammalian tissue production under physiological conditions (Stipanuk and Beck 1982). Stipanuk and Beck

measured the concentration of H_2S in different tissues and found it to be the highest in the liver, followed by the kidney; they also proved its presence in the heart, brain and skeletal muscle (Stipanuk and Beck 1982). While their work did not uncover the biological value of H_2S, it was enough to hypothesize that there must be a physiological role for this compound.

After analysing different biological processes in the brain, Abe and Kimura suggested that the endogenous H_2S acts as a neuromodulator (Abe and Kimura 1996). This observation was succeeded by several studies demonstrating the presence and biological value of H_2S, in many organs and tissues. Soon after, H_2S was reintroduced to the medical literature as a third gasotransmitter.

Canadian scientists at the University of Saskatchewan then demonstrated that H_2S significantly decreased blood pressure of rats, most likely through its relaxant effect on the vascular smooth muscle, and as an opener of K_{ATP} channels (Zhao *et al.* 2001). Such observations quickly made H_2S a hot topic for active research, and highly raised its therapeutic expectations.

1.7.2. Description

H_2S is an inorganic gas. It is colourless, flammable and heavier than air. The gas emits odour of rotten eggs at very low concentration, and is poisonous at high concentrations (Hughes, Centelles, and Moore 2009; Wedmann *et al*. 2014).

1.7.3. Solution Chemistry of H_2S

H_2S can freely penetrate the cell membranes of all tissues, without using any specific transporting system. It is soluble in water and plasma, and readily dissociates into hydrogen (H^+) and hydrosulfide (HS^-) ions (Fiorucci *et al.* 2006). Under normal physiological conditions, two thirds of H_2S are dissociated (Kashfi and Olson 2013; Li and Moore 2008) as follows:

$$H_2S \Leftrightarrow HS^- + H^+$$

H_2S can reduce other compounds by simply giving away its H^+; this property gives H_2S the ability to directly reduce ROS, which would provide great value in cell protection. It is this property that gives it one of the most important proposed biological roles (Nagy *et al*. 2014).

1.7.4. Toxicity

The concentration of H_2S in unpolluted air is very low, only 0.03 – 0.1μg/m^3. The human nose is capable of detecting H_2S when its concentration reaches 11μg/m^3. However, when the concentration reaches 140mg/m^3, the olfactory mucosa adapts, subjecting the person to inhalational toxicity. A few breaths at a concentration of 700mg/m^3 can be fatal (Chou *et al*. 2006). The cellular toxicity of H_2S is attributed to its capacity to inhibit cytochrome *c* oxidase, the terminal enzyme of oxidative phosphorylation, producing cellular hypoxia (Koj, Frendo, and Janik 1967; Epithelium *et al*. 2002).

1.7.5. Endogenous Production of H_2S

H_2S biosynthesis can occur via enzymatic and non-enzymatic pathways (Kolluru *et al.* 2013).

1.7.5.1. Enzymatic Pathway

In mammals, H_2S is synthesized from the sulfur-containing amino acids, cysteine and homocysteine, through the activity of four enzymes of three enzymatic pathways (Kashfi and Olson 2013; Kuksis, Smith, and Ferguson 2014) (Figure 1.4). Two pathways, believed to produce most of the endogenous H_2S, are active in the cytosol: the cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). Both uses pyridoxal 5′-phosphate (vitamin B6) as a cofactor (Kolluru et al. 2013; Hughes, Centelles, and Moore 2009; Kimura 2014b).

The third pathway is mainly localized in the mitochondrial matrix, and occurs in a two-step reaction utilising 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT) enzymes (Kodela, Chattopadhyay, and Kashfi 2012; Kimura 2014b). All three pathways are distributed across many tissues, and are often jointly present in many organs. CBS, highly expressed in the brain, produces H_2S from cysteine (Abe and Kimura 1996). CSE is expressed in the liver, pancreas, aorta, ileum, portal vein, and uterus (Kolluru *et al.* 2013; Kuksis, Smith, and Ferguson 2014), and also produces H_2S from cysteine. The 3-MST is active in the brain and vascular system.

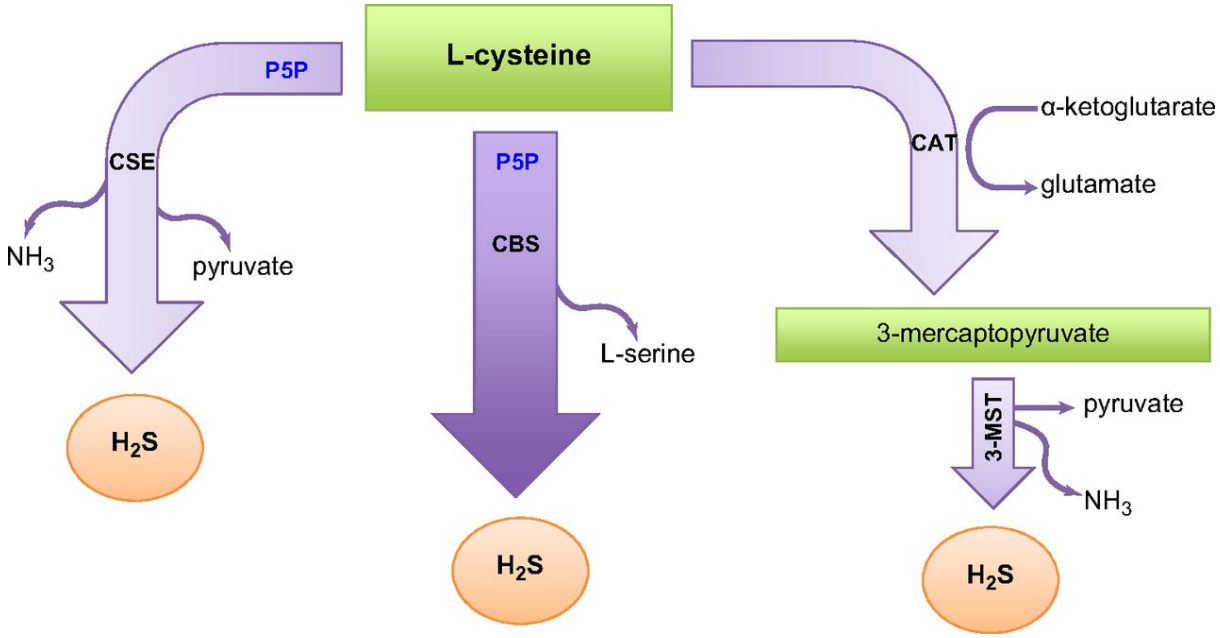

Figure 1.4. Enzymatic pathways of hydrogen sulfide production..

L-Cysteine can be converted to H_2S via two P5P-dependent pathways. H_2S synthesis via CSE also generates NH_3 and pyruvate; synthesis of H_2S via CBS generates L-serine. The P5P-independent pathway (occurring primarily in mitochondria) is a 2-step process: CAT converts L-cysteine to the intermediary, 3-mercaptopyruvate, (depends on α-ketoglutarate). 3-mercaptopyruvate is then converted 3-MST into H_2S.

CSE, cystathionine γ-lyase; *CBS*, cystathionine β-synthase; *P5P*, pyroxidal-5′-phosphate; *CAT*, cysteine (aspartate) aminotransferase; *3-MST*, 3-mercaptopyruvate sulfurtransferase.

Adapted from (Chan and Wallace 2013)*, with permission.*

1.7.5.2. Non-Enzymatic Pathway

The non-enzymatic production of H_2S is less understood, and there is no general agreement about its value in the production of endogenous H_2S. It occurs through direct reduction of elemental sulfur, and from dietary inorganic and organic sulfides (Papapetropoulos, Whiteman, and Cirino 2014; Kolluru *et al*. 2013). Plants rich in dietary sulfides include *Allium* family; these have characteristic taste and smell (Munday 2012; Kuo, Chien, and Ho 1990).

Allium vegetables (garlic, onion and shallots) have long been felt beneficial to health as an antioxidant. Recent evidence suggests that a number of beneficial effects of garlic may be derived from H_2S production (Sener, Sakarcan, and Yegen 2007; Benavides *et al*. 2007; Y. Zhang *et al*. 2008; Namazi 2008; Tsubura *et al*. 2011; Padiya *et al*. 2011). The beneficial effects of garlic in the prevention of IR injury have been reported in several organs (Namazi 2008). Garlic extract was also shown to vasodilate rat aorta via H_2S production (Benavides *et al*. 2007).

Allicin, diallyl disulfide and diallyl trisulfide in garlic extract are all considered H_2S-donor compounds (Benavides *et al*. 2007; Kaschula *et al*. 2011). Under anoxic conditions, red blood cells were shown to produce H_2S from the garlic extract within minutes (Benavides *et al*. 2007). Also, an increase in exhaled H_2S levels was reported after infusion of diallyl disulfide in rats, thus providing an *in vivo* support for H_2S production from garlic (Insko *et al*. 2009).

1.7.6. Release and Storage

Despite the well-known enzymatic production pathways of H_2S, the mechanisms of its release are not well understood. It is assumed that either H_2S is released as soon as it is produced, or it is kept at intracellular stores, to be released in response to a physiologic signal (Ishigami *et al*. 2009).

1.7.7. Metabolic degradation

Degradation of H_2S occurs in almost all tissues by mitochondrial oxidation; the process produces inactive sulphate, which is then excreted in urine.

1.7.8. Plasma Level

The normal, physiologic level of H_2S in plasma is controversial (Olson 2009). Some studies have reported values of 10 – 300μM, while others have concluded that circulating levels of H_2S are negligible (Sparatore *et al.* 2009); thus physiological H_2S may only act by means of autocrine or paracrine mechanisms (Whitfield *et al.* 2008).

1.7.9. Physiologic Role

H_2S appears to plays a wide and varied physiological role (Kolluru et al. 2013). It has been shown to regulate blood pressure (Yang *et al.* 2008), inflammation (Hu *et al.* 2007; Ling Li, Hsu, and Moore 2009; Dufton *et al.* 2012), and to act as a cell protector in oxidative stress (Kimura and Kimura 2004; Whiteman *et al.* 2005; Kimura *et al.* 2006; Wu *et al.* 2014). H_2S has also been

reported as a neuromodulator (Whiteman *et al.* 2004), smooth muscle relaxant (Kimura 2010), learning and memory enhancer (Kimura 2002), aid in insulin secretion (Ali *et al*. 2007; W. Yang *et al.* 2005), and may even be involved in longevity (Kimura 2010). It may play a physiological role in many organ systems, including cardiovascular (Bucci and Cirino 2011; Al-Magableh, Kemp-Harper, and Hart 2015), respiratory (Kubo *et al*. 2007), digestive (Farrugia and Szurszewski 2014), brain and nervous system (B. H. Tan, Wong, and Bian 2010), kidney (Lobb, Sonke, *et al.* 2014), liver (Mani *et al.* 2014), endocrine, reproductive (X.-Y. Zhu, Gu, and Ni 2011), and muscle (DU *et al.* 2013).

H_2S has been shown to be capable of exerting analgesic effects (Distrutti *et al.* 2006; Smith 2009; Lin *et al.* 2014; Donatti *et al.* 2014). Combining H_2S with NSAIDs has opened new horizons for a safer and more effective results (McCarberg and Cryer 2014; Baskar *et al.* 2008; Chattopadhyay *et al.* 2012; Fiorucci, Santucci, and Distrutti 2007; Fomenko *et al.* 2014).

One of the most interesting effects of H_2S is the induction of a hibernation-like state in mice, where the metabolic rate is dramatically reduced to less than 10% of its normal hypothermic state. This has been termed ‘suspended animation’ (Blackstone, Morrison, and Roth 2005). The effect appears with the administration of sub-toxic concentration of inhalational H_2S. The suspended animation state was shown to be highly protective against ischemic damage to cells by preserving mitochondria (Elrod *et al*. 2007; Blackstone and Roth 2007; Bos *et al.* 2009).

1.8. H_2S AND IR INJURY

The protective ability of H_2S to reduce the extent of cellular damage in response to IR insult has been well documented by many researchers (Jha *et al*. 2008; Calvert, Coetzee, and Lefer 2010; Henderson *et al*. 2010; King and Lefer 2011; Lobb *et al*. 2012; Zhu *et al*. 2012; Lobb, Zhu, *et al*. 2014; Lobb, Sonke, *et al.* 2014).

It appears that multiple pathways may be involved, as there is no known single mechanism that could explain all the cytoprotective effects of H_2S: it was not possible to formulate a hypothesis of 'one size fits all' (Bos *et al*. 2014).

1.8.1. Mitochondrial Protection

H_2S cytoprotective role appears to start at the level of mitochondria, through the inhibition of cytochrome *c* oxidase function. This would reduce respiration at mitochondrial level during IR, thus reducing the overproduction of ROS (Kimura *et al*. 2005; Wagner *et al*. 2009; Veeranki and Tyagi 2014). Mitochondrial protection may also be mediated by reducing membrane potential depolarization (Calvert, Coetzee, and Lefer 2010; Tang *et al*. 2013).

1.8.2. Antioxidant Properties

The anti-oxidant properties of H_2S have been demonstrated by many researchers. H_2S has been shown to diminish the production of ROS when administered at therapeutic doses (Calvert, Coetzee, and Lefer 2010; Chen *et al*. 2006; Nicholson and Calvert 2010). The effect appeared to be mediated either by

directly scavenging ROS, or through indirect means, by upregulating intracellular antioxidants.

H_2S has been shown to induce the production of GSH, known for its potent antioxidant role (Kimura, Goto, and Kimura 2010; Bos *et al*. 2014). Alternatively, Sun *et al* observed reduction in the levels of ROS through inhibition of mitochondrial cytochrome *c* oxidase, and increasing activation of SOD (Sun *et al*. 2012). H_2S also reported to reduce the oxidative stress by directly scavenging free radicals like superoxide and hydrogen peroxide (Geng *et al*. 2004), as well as the cytotoxic lipid oxidation products (Schreier *et al.* 2010; Calvert, Coetzee, and Lefer 2010), and the highly reactive peroxynitrite (Whiteman *et al*. 2004; Pacher, Beckman, and Liaudet 2007).

1.8.3. Anti-Inflammatory Effects

While the exact role of H_2S in inflammation is not known, it has been a subject of several recent studies (Li and Moore 2008; R. Wang 2012). Whereas some have demonstrated clear anti-inflammatory effects of H_2S, others also reported that it may be pro-inflammatory. Most of the evidence, however, points towards the anti-inflammatory properties of H_2S (Chan and Wallace 2013).

The results of Fiorucci *et al* have shown that exogenous application of H_2S was capable of inhibiting leukocyte adherence to the vascular endothelium by suppressing the expression of intercellular adhesion molecule-1 (ICAM-1) and leukocyte function-associated antigen-1 (LFA-1) (Fiorucci *et al*. 2005). H_2S has also been shown to reduce production of pro-inflammatory cytokines and

chemokines, and assist the shift of macrophages to an anti-inflammatory phenotype (Wallace, Ferraz, and Muscara 2012; Dufton *et al*. 2012). In a rat model of colitis, H_2S was found to reduce the levels of mucosal TNF-α (Bai, Ouyang, and Hu 2005). Inhibition of liver TNF-α has also been reported (Fiorucci *et al*. 2006). Moreover, H_2S was shown to suppress NF-κB activation in the colon of patients suffering from ulcerative colitis (Bai, Ouyang, and Hu 2005). Suppressing NF-κB would, in turn, suppress the production of other inflammatory substances including cytokines, adhesion molecules, and iNOS (Lefler *et al*. 2002; Nichols 2004). H_2S also appears to reduce the progression of inflammation by enhancing the apoptotic death of polymorphonuclear leukocytes, thus limiting the neutrophil-mediated tissue injury (Mariggiò *et al.* 1998; Zanardo *et al*. 2006).

Finally, H_2S may exert some of its anti-inflammatory effects through upregulation of HO, which is known to reduce inflammation and exhibits cytoprotection by producing CO and bilirubin (Oh *et al.* 2006; Qingyou *et al*. 2004; Ryter, Alam, and Choi 2006).

1.8.4. Vasorelaxation

H_2S has been shown to exhibit potent vasodilator activity, both *in vitro* and *in vivo* (Bhatia 2005; Jackson-Weaver 2012; Stubbert *et al*. 2014). The effect appears to be through opening vascular smooth muscle K_{ATP} channels (Bhatia 2005; Mustafa *et al*. 2011), and the inhibition of vascular tone (Jackson-Weaver 2012). The vasorelaxation can be by both nitric oxide-dependent and independent means (Altaany *et al*. 2014; Holwerda, Karumanchi, and Lely 2015).

Like nitric oxide, the function of H_2S is now considered as that of yet another major EDRF-like molecule, perhaps an endothelium-derived hyperpolarising factor (EDHF) (Mustafa *et al*. 2011; Tang *et al*. 2013).

1.9. H_2S IN EXPERIMENTATION

1.9.1. Stimulation of Endogenous Production

H_2S is naturally produced in mammalian tissues; perhaps, for therapeutic or experimental examinations, one can attempt to boost its endogenous production instead of applying it exogenously. Garlic or allyl disulfide were used in several experiments as H_2S-boosting substances, particularly in IR injury within the heart, kidney or the brain (Sener, Sakarcan, and Yegen 2007; Benavides *et al.* 2007). NAC and L-cysteine also function as precursors for the endogenous H_2S synthesis (Caliendo *et al*. 2010).

1.9.2. Inhibition of Endogenous Production

Inhibition of endogenous production of H_2S has been useful in studies examining its physiological role. Currently, pharmacological inhibitors of the CSE enzyme are available, and genetically targeted CSE inhibition in mice is another available option (G. Yang et al. 2008; Sha et al. 2014).

1.9.3. Exogenous Application of H_2S

There are several options available for the administration of H_2S in experimental settings. Apart from H_2S gas inhalation, H_2S-donor compounds can

be used. These may come in the form of sulfide salts, synthetic moieties (slow-releasing H_2S donors) or hybrids of H_2S donors.

One major drawback in using sulfide salts (e.g. NaSH or Na_2S) is the very rapid release and subsequent rapid decrease in H_2S levels upon systemic administration; this makes it difficult to achieve controlled, stable and therapeutic levels *in vitro* or *in vivo* (Bos *et al*. 2014).

On the contrary, slow-releasing H_2S donors (the synthetic moieties), were developed to achieve stable release of H_2S. These have been shown to release H_2S in a slower and steady level following their administration (Ling Li *et al*. 2007; Ling Li *et al*. 2008; Y. Zhao, Wang, and Xian 2011; Marutani *et al*. 2012; Predmore *et al*. 2012). GYY4137 (morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate) is a water-soluble, slow-releasing H_2S donor (Ling Li *et al*. 2008). One millimole of GYY4137 releases approximately 40 micromoles of H_2S within the first 10 min of activation, and then steadily continues to release H_2S over a period of 3 hours. Systemic application of 133μmol/kg, either intraperitoneally (IP) or intravenously (IV), in rats was reported to increase plasma concentration of H_2S from 32 μmol/l to 80μmol/l within 30 min; H_2S remained elevated, at 50μmol/l, for further 3 hours (Kashfi and Olson 2013). In one *in vitro* study using human breast adenocarcinoma, a constant release of H_2S for up to 7 days was noticed following addition of GYY4137 to cell culture media. They postulated that GYY4137 may accumulate inside cancer cells and thereby release larger amounts of H_2S intracellularly (Lee *et al.* 2011).

Hybrids of H_2S donors are novel anti-inflammatory agents where the traditional NSAIDs are chemically modified to release H_2S. Thus, these combine the cyclooxygenase (COX)-inhibitory activity with COX-independent anti-inflammatory actions (Sidhapuriwala *et al*. 2007; Baskar *et al*. 2008; Zhang *et al*. 2012; Chattopadhyay *et al*. 2013; Lee *et al*. 2013).

1.10. STUDY MODELS FOR ACS

1.10.1. Human Volunteers

Studying ACS on humans is quite difficult, as the condition is potentially dangerous and permanent disabilities cannot be excluded. Moreover, it would be impossible to do certain *in vivo* studies on human the way it is done on laboratory animals like the intravital video microscopy (IVVM). The literature on ACS is scarce of experimental studies done on living human volunteers. We are aware of only two published experiments; the first was performed using cylindrical air splints applied to the legs of three enthusiastic investigators namely Frederick A. Matsen III, Keith A Mayo, and Richard B. Krugmire, JR. (Matsen *et al.* 1977), while the second was performed on 10 volunteers by wearing anti-shock trousers (Willy *et al*. 2001).

1.10.2. Animal Use

Using animals for experimental studies on ACS was reported in the 1800s when Volkmann published his insight on the pathology of ACS pointing out to the data he got from animal studies (Volkmann 1881). Dogs, pigs, rabbits, mice and

rats are the common animals utilized by different researchers over the past century (Jepson 1926; Bardenheuer 1910; Heppenstall *et al*. 1986). Research studies on animals are just tools that may indicate a potential therapeutic benefit of any drug. Recognising and addressing the limitations of pre-clinical animal studies is a paramount for a successful translation of therapy from the bench to the bedside (Lotfi *et al*. 2013).

1.10.2.1. ACS Rat Model

Lawendy and his group have developed a clinically relevant model for ACS (Lawendy, Sanders, Bihari, Parry, *et al*. 2011). The model is relatively simple, in which normal saline infusion is used to elevate the ICP of the anterior compartment. It has been validated in several studies (Lawendy, Sanders, Bihari, Parry, *et al*. 2011; Lawendy *et al*. 2011; Lawendy, Sanders, Bihari, and Badhwar 2011; Lawendy 2014a; Lawendy et al. 2015).

Rats are good models of human disease with easy to monitor physiology (Iannaccone and Jacob 2009; Clause 1998). They are also easy to house and handle, relatively inexpensive, and readily available. The suitable size of the rat, compared to the mouse, allows easy surgical fasciotomy and dissection of the skeletal muscle. The rat size also is quite suitable to be moved on the microscope for direct real-time visualization of the microcirculatory dysfunctions using the IVVM technology.

1.11. THESIS RATIONALE

The ultimate goal of this study was to test a potential pharmacological therapy for ACS: a therapeutic option that could be used as an adjunctive treatment to the standard surgical decompression, capable of reducing the extent of tissue damage resulting from the ACS-induced ischaemic insult, or the subsequent reperfusion injury.

1.11.1 Hypotheses

1.11.1.1. The Effect of H_2S on ACS

The main objective of the thesis was to assess the therapeutic potentials of H_2S in acute compartment syndrome. Based on previous studies in transplantation research or IR injury, it was hypothesized that H_2S would also have a cytoprotective effect on the skeletal muscle in ACS.

1.11.1.2. *Combined CO/H_2S Effect*

The second objective of this thesis was to explore the possible therapeutic effect of combining CO and H_2S, as a possible adjunctive treatment of ACS.

It has been shown previously, in a rat model of ACS, that CORM-3-derived CO could be beneficial in minimising the ACS-induced microvascular perfusion derangements, tissue injury and inflammation (Lawendy et al. 2014). However, to our knowledge, there have been no studies examining the combined effects of CO and H_2S, whether in IR injury or transplantation surgery (Siriussawakul, Chen, and Lang 2012).

Both H_2S and CO are lately-discovered gaseous signalling molecules. Their cytoprotective roles in IR injury has been documented in various organs and tissues (Ryter, Alam, and Choi 2006; Nicholson and Calvert 2010; Calvert, Coetzee, and Lefer 2010; Mancardi et al. 2009; Ozaki, Kimura, and Murase 2012). Therefore, we hypothesised that combination of H_2S and CO may have added protective benefit in ACS treatment, perhaps providing a synergistic therapeutic effect. In this study, we undertook to evaluate the cytoprotective potential of H_2S in ACS in reference to the established results of CORM-3.

CHAPTER 2. MATERIALS AND METHODS

2.1. ANIMAL CARE

All experimental protocols conducted in this study were approved by the Animal Use Subcommittee at the University of Western Ontario, London, Canada. The daily animal care, handling and housing were performed in accordance with the guidelines set out by the Canadian Council on Animal Care. All rats used for the experiments were housed in pairs, in clear plastic cages, under 12:12 light:dark cycle, and had access to food and water *ad libitum*.

2.2. ANIMAL DESCRIPTION

Twenty-five male Wistar rats, body weight 170–290 g, were used in the study.

2.3. EXPERIMENTAL TREATMENT DRUGS

Two treatment drugs were used in these experiments, plus an inert vehicle: hydrogen sulfide (H_2S) and carbon monoxide (CO).

2.3.1. Hydrogen Sulfide

Hydrogen sulfide-releasing molecule, GYY4137 (Cayman Chemicals, Ann Arbor, Michigan, USA), was used at a dose of 50 mg/kg (Li *et al*. 2009). It was diluted in normal saline just prior to injection, and administered intraperitoneally to the designated group**.** Normal saline served as an inert vehicle (control).

2.3.2. Carbon Monoxide

Carbon monoxide-releasing molecule-3 (CORM-3) was used at a dose of 10 mg/kg (Lawendy *et al*. 2014). CORM-3 was synthesized in our lab, in accordance with the previously published method (Motterlini & Otterbein 2010), and was diluted it in normal saline just prior to injection to be administered intraperitoneally to the designated groups.

Inactive CORM-3 (iCORM-3) served as an inert vehicle control; iCORM-3 was prepared in the same manner as CORM-3, but was allowed to release all CO from the solution prior to being administered to the animals.

2.4. EXPERIMENTAL SETUP

2.4.1. General Overview

Acute compartment syndrome (ACS) was experimentally generated in the rat hind limb under general anaesthesia. Elevated intracompartmental pressure was maintained at 30 - 40 mmHg for 2 hours, followed by fasciotomy and 30 minutes of reperfusion time. Intravital video microscopy (IVVM) was then performed. Experimental drug Injections were administered to all animals, as per the appropriate group, just before fasciotomy. Sham animals underwent all procedures, but the compartment pressure was maintained at a baseline level of 0mmHg.

2.4.2. Anaesthesia

Inhalational isoflurane was used to anaesthetize all animals. Induction of general anaesthesia was carried out by 5% isoflurane in a 1:1 mixture of oxygen and nitrogen gas, at a flow rate of 2L/min. Anaesthesia was maintained at 2% isoflurane, at a flow rate of 0.5L/min (Flecknell 1996), and adjusted as required to maintain the adequate anaesthetic depth. Left carotid artery was cannulated for the continuous monitoring of blood pressure, fluid replacement and arterial blood sampling. Rectal digital thermometer probe was used to continuously monitor the core body temperature; a constant core temperature of 37°C was maintained by the means of heating lamp.

2.4.3. Acute Compartment Syndrome

ACS was induced as previously described (Lawendy *et al*. 2011). Briefly, sterile isotonic saline solution (Baxter, Deerfield, IL) was continuously infused into the anterior compartment or the rat hind limb, elevating ICP via a 25-gauge needle (BD, Franklin Lakes, NJ). ICP was measured by an electronic compartmental pressure monitoring system (Synthes, West Chester, PA), placed in the anterior compartment via a 14-gauge angiocatheter (BD). Saline infusion was carried out by gravity-feed system, with the saline bag elevated 120 cm above the working bench level. ICP was maintained at an average of 30 - 40 mmHg for 2 hours (Figure 2.1).

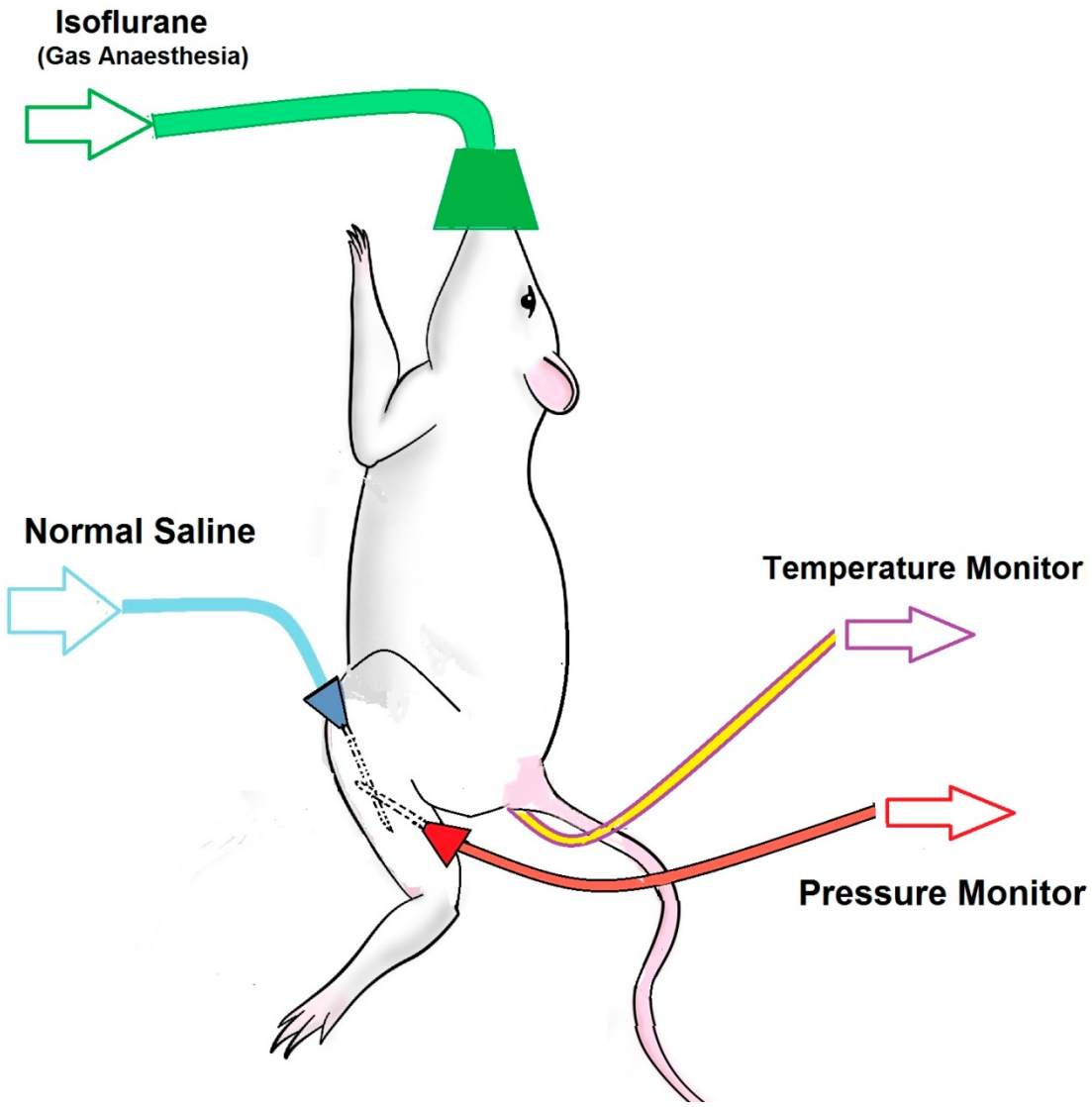

Figure 2.1. Experimental Setup in ACS. Saline is infused into the anterior compartment of the rat hind limb, elevating ICP to 30mmHg. ICP is continuously monitored by the use of electronic compartment pressure monitor, also inserted into the anterior compartment.

2.4.4. Fasciotomy

Following 120 minutes of elevated ICP, fasciotomy and exposure of the Extensor Digitorum Longus muscle (EDL) were undertaken, as previously described (Tyml and Budreau 1991; Lawendy *et al*. 2011). Briefly, the skin overthe lateral aspect of the leg was carefully incised, exposing the anterior and posterior compartments. The underlying biceps femoris muscle was partially incised to fully expose the tibialis anterior (TA), peroneus longus (PL) and lateral gastrocnemius muscles. Fasciotomy of the anterior compartment was carefully performed, taking care to avoid injury to the underlying muscles. EDL muscle was exposed by gentle separation of TA and PL, without physically touching its fibers. EDL tendon, identified at the ankle level, was tied using 3-0 silk ligature and cut as close as possible to the insertion. Following EDL preparation, animal was moved onto the stage of IVVM microscope, and the, EDL belly was partially reflected anteriorly into saline bath (Potter *et al*. 1993).

2.5 EXPERIMENTAL GROUPS

Rats were randomly assigned to one of the five experimental groups: (1) Sham, (2) ACS, (3) H_2S, (4) CO, (5) Combo.

2.5.1 Sham

All animals in this group (n=6) underwent all procedures, but the ICP was maintained at a baseline level of 0mmHg.

2.5.2 ACS

Animals in this group (n=5) underwent ACS, followed by inert vehicle injection at fasciotomy.

2.5.3 H_2S

Rats (n=4) were subjected to ACS, and received GYY4137 injection at fasciotomy. GYY4137 served as a pharmacological means of hydrogen sulfide delivery.

2.5.4 CO

Animals (n=4) were subjected to ACS, followed by CORM-3 administration at fasciotomy. CORM-3 served as a pharmacological means of CO delivery, as previously described (Lawendy *et al*. 2014).

2.5.5 Combo

Animals in the combo group (n=6) were subjected to ACS, and received a combined treatment of both GYY4137 and CORM-3 at fasciotomy.

2.6 INTRAVITAL VIDEO MICROSCOPY

Rats under anaesthesia were carefully moved from the surgical bench to the stage of IVVM microscope. Extreme care was given to the dissected EDL, avoiding its being traumatised, twisted, stretched, or even touched by anything.

The muscle was then reflected onto a glass slide, into saline bath containing vital dyes. The EDL was then stabilized to the microscope stage using the attached silk ligatures, and covered with glass coverslip.

2.6.1 Vital Dye Staining

Two fluorescent vital dyes were used at a concentration of 5μg/mL each: ethidium bromide (EB) (Sigma Aldrich, Mississauga, ON), and bisbenzimide (BB) (Sigma Aldrich, Mississauga, ON). BB stains the nuclei of all cells, as it is a small enough molecule to readily permeate across the cell membrane. EB stains only the nuclei of injured/damaged cells, as its larger size requires compromised cellular membrane (Potter *et al*. 1995). Cellular injury was expressed as the ratio of ethidium bromide-labelled nuclei to bisbenzimide labelled nuclei (EB/BB).

2.6.2 Microscopy

Microvascular perfusion and leukocyte behaviour within the post-capillary venules were recorded by transillumination in five adjacent fields of view with 20x and 40x objectives, respectively (final magnification of 700x and 1400x). Under 20x objective, each field of view contained a complete capillary bed, and was recorded for 60 seconds. Additional 10 seconds from the same fields of view were then recorded under the appropriate epifluorescence illumination, with the application of appropriate filters for BB (excitation wavelength=343nm, emission wavelength=483 nm) and EB (excitation wavelength=482 nm; emission wavelength=610nm).

Under the 40x objective, leukocytes were recorded within five randomly chosen post-capillary venules, with the approximate diameter of at least 20μm, for 45 seconds. All videos were captured into the computer using Adobe Premiere software for offline video analysis.

2.7 OFFLINE VIDEO ANALYSIS

2.7.1 Microvascular Perfusion

Microvascular perfusion was quantified by counting the number of capillaries crossing three equidistant parallel lines drawn on the computer monitor, perpendicular to the capillary axis (Lawendy *et al*. 2014). Each capillary was categorized into one of three functional states: continuously-perfused (CPC), intermittently-perfused (IPC) and non-perfused (NPC). The results were expressed as percentage of total capillary count in the capillary bed, as per their functional state (De Backer et al. 2007)

2.7.2 Injury Analysis

Tissue injury was expressed as a ratio of EB-stained to BB-stained cells (EB/BB) (Potter *et al*. 1995).

2.7.3 Analysis of Leukocytes

Leukocyte behaviour was classified as the number of rolling or adherent leukocytes per 30 seconds in each capillary venule. An adherent leukocyte was defined as a cell that remained stationary for a minimum of 30 seconds (Granger

et al. 1989). The area of venules was measured using ImageJ software (NIH, Bethesda, MD). The results were expressed as number of leukocyte/30s/1000μm^2 (Lawendy *et al*. 2011).

2.8 TNF-α ELISA

Serum from animals was used to quantify systemic TNF-α levels at the following points: baseline (pre-ACS), 1 hour into ACS, 2 hours into ACS (just prior to injection and fasciotomy), 10 minutes post-fasciotomy, 20 minutes post-fasciotomy, 30 minutes post-fasciotomy and 45 minutes post-fasciotomy. Serum was obtained by allowing the whole blood to coagulate at room temperature for 30 minutes, followed by centrifugation at 1500xg for 20 minutes at 4°C. The supernatants (serum) were collected and stored at -80°C.

Levels of TNF-α were assessed using enzyme-linked immunosorbent assay (ELISA) kit (Pierce Biotechnology, c/o Thermo Scientific, Rockford, IL), as per manufacturer's instructions. The assay was sensitive to less than 5pg/ml.

All samples were run in duplicate. Standard curve was obtained by performing serial dilutions of reconstituted lyophilized TNF-α standard; 2,500pg/ml, 833pg/ml, 500pg/ml, 278pg/ml, 93pg/ml, 31pg/ml and 0pg/ml were used to obtain the standard curve. Serum samples were diluted at 1:1 ratio with sample diluent buffer. All plate incubations were carried out at room temperature. Fifty μl of each sample or standard were added to the appropriate designated wells on a 96-well plate, and incubated for 1 hour. After three washes, 50μl biotinylated TNF-α antibody was added to each well and incubated for 1 hour.

Following three washes, 100µl streptavidin-HRP reagent was added to each well and incubated for 30 minutes. After the final three washes, 100µl tetramethyl benzidine (TMB) substrate was added to each well and the plate was incubated for 10 minutes in the dark. 100µl stop solution was used to stop the reaction. The absorbance was read on microplate reader (model 680, BioRad) at 450nm. The results were calculated by 4-point logistic curve fitting software against TNF-α standard.

2.9 STATISTICAL ANALYSIS

All data was analyzed using analysis of variance (ANOVA), with Newman-Keuls multiple comparison post-hoc test where appropriate. Two-way ANOVA was used to analyse the systemic TNF-α levels (pre- and post-treatment), while one-way ANOVA was used to analyse the rest of the results. Statistical significance was defined as $p<0.05$. Data analysis was performed using Prism version 4.0c for Mac (GraphPad Software Inc., San Diego, CA).

CHAPTER 3. RESULTS

3.1. MICROVASCULAR PERFUSION

3.1.1. Continuously-Perfused Capillaries (CPC)

Elevation of ICP resulted in a significant decrease in the CPC, from 75±2.6% in the sham to 48±5% in the ACS group ($p<0.01$). Hydrogen sulfide treatment (H_2S group) led to a significant increase in CPC from ACS, to 66.4±13.3% ($p<0.05$). The CPC in both the CO and Combo groups also significantly increased, compared to ACS group (62.7±4.8%, $p<0.05$ and 69.8±11%, $p<0.01$, respectively). There was no significant difference in the CPC between H_2S, CO and Combo groups compared to sham (Figure 3.1).

3.1.2. Intermittently-Perfused Capillaries (IPC)

There were no significant differences in IPC in any of the groups, although there was a trend towards an increase in the ACS group

3.1.3. Non-Perfused Capillaries (NPC)

Elevation of ICP led to a significant increase in NPC, from 10.6±1.3% in sham to 29.9%±5.3% in ACS group ($p<0.001$). Hydrogen sulfide treatment (H_2S group) led to a significant decrease in NPC to 16.5±2.7% ($p<0.05$) when compared to ACS group. Both CO and Combo treatments resulted in a significant reduction in NPC, to 16.4±1.1% ($p<0.05$) and 18±1.9% ($p<0.01$), respectively, compared to ACS group (Figure 3.1). There were no significant differences in NPC between H_2S, CO, Combo and sham groups (Figure 3.1).

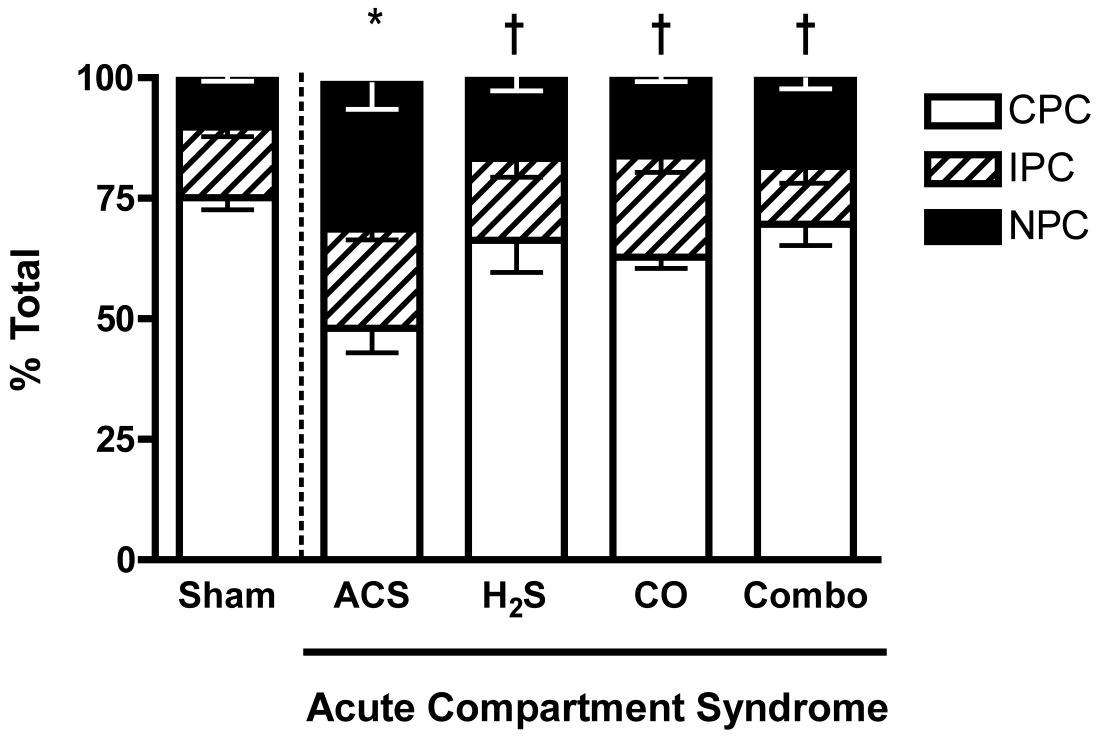

Figure 3.1 The Effect of H_2S on Skeletal Muscle Perfusion Following ACS. ACS resulted in a significant microvascular dysfunction; H_2S partially restored the perfusion, similar to that of CO or combination of H_2S and CO (Combo). *CPC*, continuously perfused capillaries; *IPC*, intermittently perfused capillaries; *NPC*, non-perfused capillaries (*$p<0.001$ from sham; †$p<0.05$ from ACS).

3.2. TISSUE INJURY

Elevation of ICP caused a significant increase in tissue injury, from 0.09±0.02 in sham to 0.33±0.02 in ACS group ($p<0.001$). Hydrogen sulfide treatment (H_2S group) resulted in a significant decrease to 0.14±0.07 ($p<0.001$), compared to ACS group. Both CO and Combo groups also displayed a significant reduction in the tissue injury (0.16±0.07, $p<0.01$ and 0.13±0.01, $p<0.001$, respectively), compared to ACS group (Figure 3.2). There were no significant differences in the tissue injury between H_2S, CO, Combo or sham groups (Figure 3.2).

3.3. LEUKOCYTE BEHAVIOUR

3.3.1. Leukocyte Rolling

Elevation of ICP resulted in a significant increase in leukocyte rolling, from 1.33±0.29 leukocytes/30s/1000μm^2 in sham to 4.55±1.49 leukocytes/30s/1000μm^2 in ACS group ($p<0.05$). Hydrogen sulfide treatment (H_2S group) led to a significant reduction in leukocyte rolling to 1.82±0.51 leukocytes/30s/1000μm^2 ($p<0.05$), compared to ACS group. Both CO and Combo treatments also led to a significant decrease in leukocyte rolling (0.82±0.12 leukocytes/30s/1000μm^2 and 1.17±0.3 leukocytes/30s/1000μm^2; $p<0.05$, respectively) when compared to ACS group. There were no significant differences in leukocyte rolling between H_2S, CO, Combo, and sham groups (Figure 3.3).

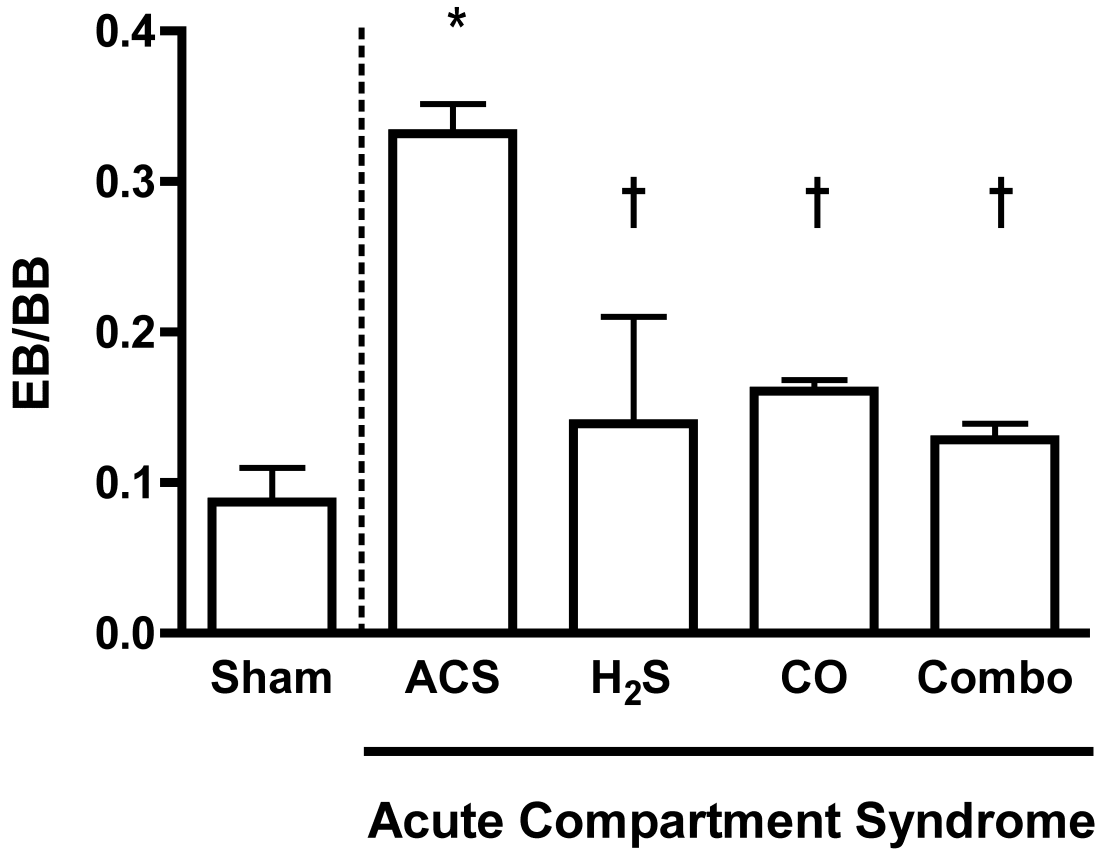

Figure 3.2 The Effect of H_2S on Skeletal Muscle Tissue Injury Following ACS. ACS resulted in a significant increase in tissue injury; H_2S significantly diminished the ACS-induced injury, similar to that of CO or combination of H_2S and CO (Combo) (*$p<0.001$ from sham; †$p<0.01$ from ACS).

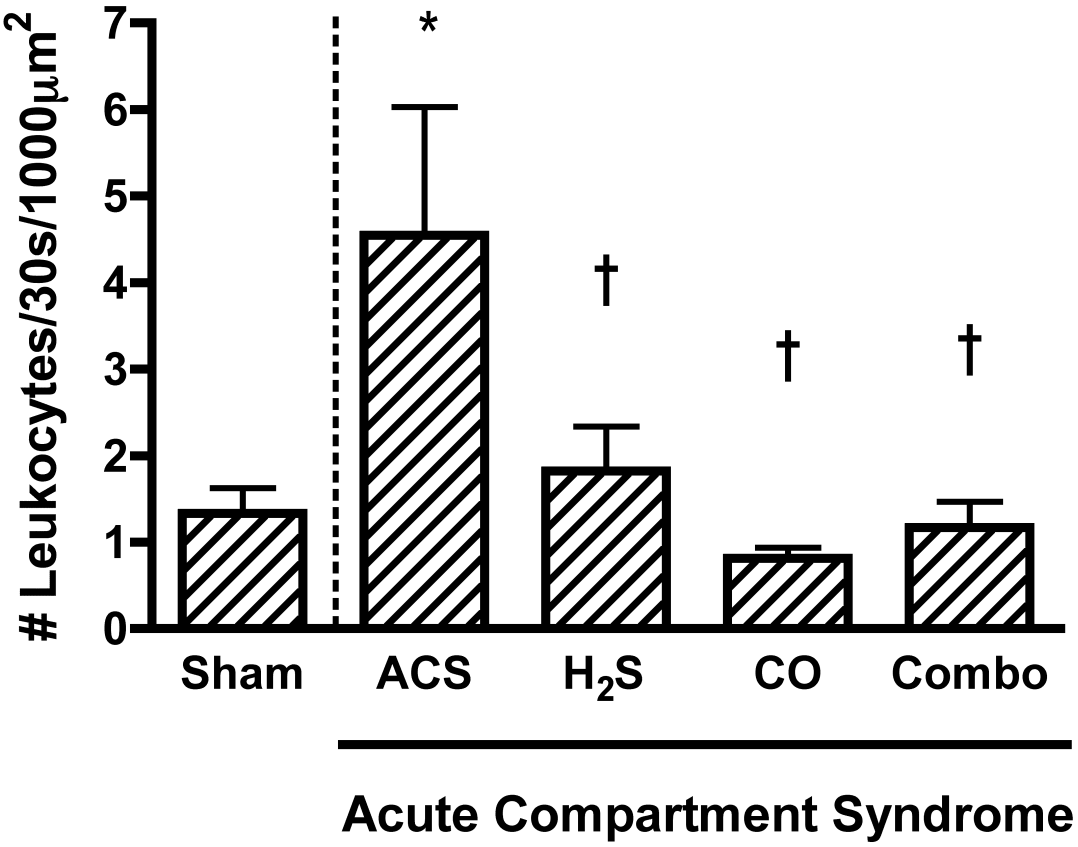

Figure 3.3. The Effect of H_2S on Leukocyte Activation Following ACS: Leukocyte Rolling. ACS led to a significant increase in leukocyte rolling; H_2S attenuated this response, similar to that of CO or combination of H_2S and CO (Combo) (*$p<0.05$ from sham; †$p<0.05$ from ACS).

3.3.2. Leukocyte Adhesion

Elevation of ICP resulted in a significant increase in leukocyte adhesion, from 0.83±0.16 leukocytes/30s/1000μm^2 in sham to 4.49±0.79 leukocytes/30s/1000μm^2 in ACS group ($p<0.001$). Hydrogen sulfide treatment (H_2S group) led to a significant decrease to 0.89±0.20 leukocytes/30s/1000 μm^2 ($p<0.001$), compared to ACS group. Both CO and Combo groups also displayed significantly lower leukocyte adherence compared to ACS group (1.29 ± 0.25 leukocytes/30s/1000 μm^2 and 0.67±0.16 leukocytes/30s/1000 μm^2, $p<0.001$, respectively). There were no significant differences in leukocyte adhesion between H_2S, CO, Combo, and sham groups (Figure 3.4).

3.4 SYSTEMIC TNF-α LEVELS

Elevation of ICP resulted in a continuous, sustained increase in systemic levels of cytokine TNF-α, from the baseline of 1.3±1.5pg/ml to 558.8±202.6pg/ml and 1222.9±591.5pg/ml at 1hr and 2hr ACS, respectively (Figure 3.5). Following fasciotomy, TNF-α levels continued to increase to 2834.38±730.1pg/ml at 45 minutes post-fasciotomy. Hydrogen sulfide treatment (H_2S group) led to a significant reduction in systemic TNF-α levels to 893.7±450.4pg/ml, 588.7±398.5pg/ml, 454.7±253.4pg/ml and 205.3±112.7pg/ml at 10min, 20min, 30min and 45min post-fasciotomy, respectively ($p<0.05$ from ACS alone). Combo treatment resulted in an almost immediate significant reduction in systemic TNF-α levels to 31.8±26.84pg/ml, 17.2±10.9pg/ml, 8.9±10.8pg/ml and 7.0±4.9pg/ml at

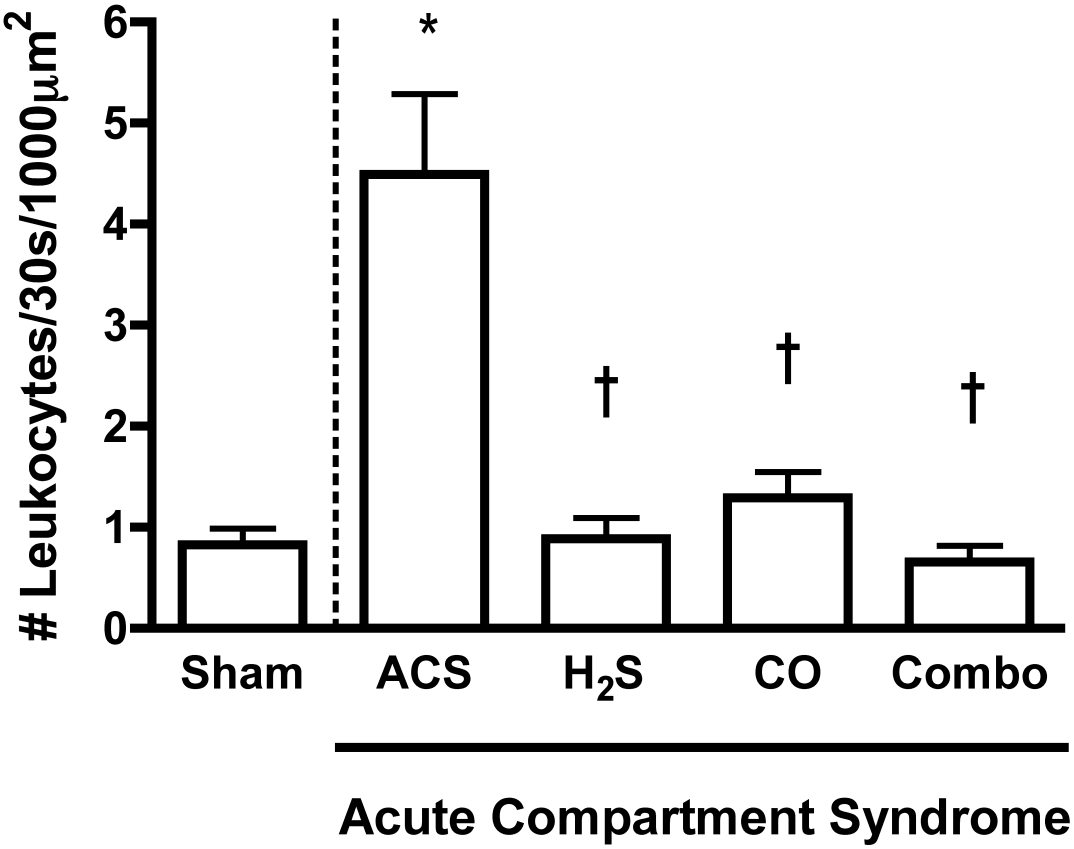

Figure 3.4. The Effect of H_2S on Leukocyte Activation Following ACS: Leukocyte Adhesion. ACS led to a significant increase in leukocyte adhesion; H_2S attenuated this response, similar to that of CO or combination of H_2S and CO (Combo) (*$p<0.05$ from sham; †$p<0.05$ from ACS).

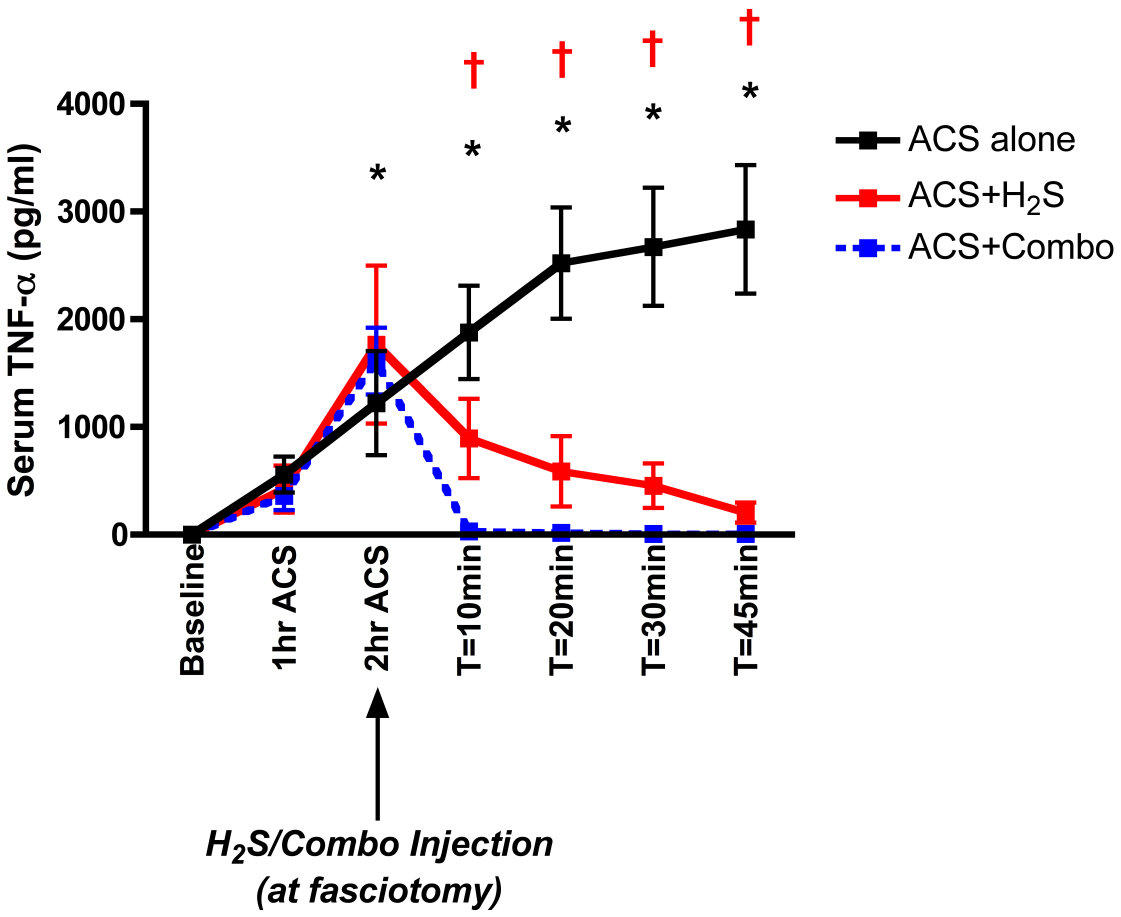

Figure 3.5. Effect of H_2S on Systemic TNF-α levels in ACS. ACS led to significant increase in systemic TNF-a levels; H_2S injection at fasciotomy diminished this response, similar to that of combination of H_2S and CO (Combo) (*p<0.05 from baseline; †p<0.05 from ACS alone).

10min, 20min, 30min and 45min post-fasciotomy, respectively ($p<0.001$ from ACS alone). There was no statistical difference between H_2S and combo groups (Figure 3.5).

CHAPTER 4. DISCUSSION

4.1 GENERAL OVERVIEW OF COMPARTMENT SYNDROME

ACS is a known surgical emergency in which the viability of the compartment contents are endangered by the persistence of elevated ICP. The local and systemic outcome of untreated ACS may be catastrophic (Defraigne & Pincemail 1998; Bocca *et al.* 2002; Ritenour *et al*. 2008; Hershey 2013; Bonnaig *et al*. 2014). Fasciotomy remains the only gold standard treatment, and should be performed as soon as the diagnosis is reached; yet, it comes with its own complications, and the sequelae of ACS cannot be fully avoided (Echtermeyer & Horst 1997; Giannoudis *et al*. 2002).

The pathophysiology of ACS appears to be complex and still not yet fully understood. While there has been general agreement that the cellular damage occurs at the microcirculation level, there has been disagreement on the pathomechanisms of this damage (Ashton 1962; Matsen 1975; Whitesides *et al*. 1975; Ashton 1975; Matsen *et al*. 1979; Reneman *et al*. 1980; Heppenstall *et al*. 1988; Matsen & Rorabeck 1989; Perler *et al*. 1990; Hartsock *et al*. 1998).

4.1.1 ACS as a Low-Flow Ischaemia

Elevation of ICP in ACS does not appear to cause complete ischaemia (Heppenstall et al. 1986), but rather a low-flow ischaemia (Lawendy 2014). Even though, ACS causes more ultrastructural damage in skeletal muscle compared to complete ischaemia (Heppenstall *et al*. 1986; Heppenstall *et al.* 1988;

Heppenstall *et al.* 1989). While ACS and partial ischaemia share a unique temporal and qualitative patterns of cytokines expression compared to complete ischaemia (Conrad *et al.* 2006; Lawendy *et al*. 2014), partial ischaemia causes less muscle damage than complete ischaemia (Conrad *et al*. 2006). These puzzling observations raised the question if the damage in ACS could only be explained based on the magnitude of ischaemia resulting from high ICP (Echtermeyer and Horst 1997). On the other hand, several pathological and biochemical similarities have been found between ACS and IR injury including the induced microvascular dysfunctions in the skeletal muscle and early intense inflammatory response (Heppenstall *et al*. 1986; Sadasivan *et al*. 1997; Tollens *et al*. 1998; Loerakker *et al*. 2011; Lawendy, Sanders, *et al*. 2011; Lawendy *et al*. 2014; Zhao *et al*. 2014), and thus compelled researchers to consider ACS as one of the clinical situations associated with skeletal muscle IR injury (Gillani *et al*. 2012).

This new insight, considering both ischaemia and IR injury are occurring in ACS, could explain the mystifying fact that the observed damage in ACS is usually beyond that of ischaemic one. In orthodox thinking, the expected scenario would be an ischaemic insult during the phase of elevated ICP and IR injury after fasciotomy, as normalizing the ICP by fasciotomy allows the oxygenated blood to flow to the ischaemic muscles. Whereas this sounds a logical scenario, it does not explain the IR-like microvascular dysfunctional changes observed immediately following fasciotomy (Sadasivan *et al*. 1997; Lawendy, Sanders, *et al*. 2011) compared to 3 hours of reperfusion following

complete ischaemia (Olivas *et al*. 2001). These aforementioned observations led us to assume that IR injury could be happening concomitantly during the course of elevated ICP and become maximized after fasciotomy. The mechanism of occurrence of IR injury during the phase of elevated ICP may need to be evaluated experimentally.

Hargens *et al* (1981) stated that it was unknown how much microvascular occlusion and reduced flow exist at a tissue pressure of 30 mmHg or greater after studying pressurized canine leg compartments using technetium-99m (Hargens *et al*. 1981). Hartsock *et al* (1998) have shown significant decreases in the number of perfused capillaries as tissue pressure became progressively elevated during *in vivo* video microscopy of the rat cremaster muscle (Hartsock et al. 1998). From these findings, it was clear that microcirculation dose not cease completely or uniformly in the muscle as the ICP rises.

The low-flow ischaemia occurring in ACS should not be considered as a low-degree of global muscle ischemia, but rather a heterogeneous or mal-distributed blood flow pattern in the muscle, as described by Sadasivan *et al* (1997). Elevated ICP may be creating a differential state of microvascular dysfunctional where some micro areas within the muscle are suffering severe ischaemia, while others are better perfused. Endothelium and leukocytes would quickly become activated in the ischaemic milieu. The non-perfused and perfused areas may physiologically alternate by selective arteriolar response, as a protective mechanism against ischaemic necrosis, or due to changes in interstitial fluid distribution. Allowing blood to flow in the previously non-perfused

micro areas would then create a limited micro-reperfusion injury. Occurrence of multiple micro-reperfusions would release the pro-inflammatory cytokines and ROS that are known to cause capillary leak and tissue oedema, leading to an increase in tissue pressure, resulting in more micro areas of non-perfused muscle, thus initiating the known theory of vicious cycle.

While the sudden supply of acutely ischaemic tissue by oxygenated blood is the common known cause to induce IR injury, non-acute hypoperfusion situations also display a form of continuous and recurrent IR injuries. For example, in peripheral arterial disease, where there is a long standing hypoperfusion of the limb muscles, IR injuries with release of ROS have been reported on walking (Ciuffetti *et al*. 1991; Hickman *et al.* 1994; Dopheide *et al*. 2013; Hiatt *et al*. 2015). IR injury has even been blamed also for chronic non-healing wounds (Mustoe *et al*. 2006), and the pathogenesis and progression of diabetic ulcers (Alavi *et al*. 2014). These findings strengthen our assumption of concomitant occurrence of ischaemia and IR in ACS during elevated ICP phase.

Recently, experimental studies have shown that ACS induces a cascade of inflammatory reactions, resulting in microcirculatory changes and tissue damage (Perler, Tohmeh, and Bulkley 1990; Sadasivan *et al*. 1997; Manjoo *et al*. 2010; W. Z. Wang, Baynosa, and Zamboni 2011a; Gillani *et al*. 2012; Blaisdell 2002; Lawendy 2014; Lawendy *et al*. 2015). Several pro-inflammatory mediators and ROS are known to be released during this process, resulting in microcirculatory dysfunction, leukocyte activation and cell damage (Menger *et al*. 1992; Sadasivan *et al*. 1997; Tollens, Janzing, and Broos 1998; Lawendy,

McGarr, *et al*. 2011; Gillani *et al*. 2012). Although timely fasciotomy saves limbs from further ischaemic insult, it does not address the inflammatory element resulting from the ischaemia itself or from the IR injury. Moreover, the sudden reperfusion, induced by fasciotomy, probably causes the so called 'second hit' (Sellei et al. 2014), where ROS increase the microvascular dysfunction and maximizes the net tissue damage (Gillani *et al*. 2012).

4.1.2 The Search for the Magic Bullet

Although ACS contributes significantly to morbidity and mortality by inducing an array of ischaemic and intense inflammatory response, there has been little progress made towards development of translatable therapeutic interventions that might ameliorate ACS sequelae. A therapeutic intervention capable of changing the biochemical environment during the elevated ICP and/or following fasciotomy would be of significant benefit (Henderson *et al*. 2010).

Cellular damage in ACS appears to be a result of intense ischaemia-induced inflammatory reaction, coupled with oxidative stress; thus, targeting these factors may be beneficial. The ideal adjunctive therapy should be able to either prevent the progress of ACS (perhaps by enhancing ischemic tolerance of the tissue), or at least reduce the damage caused by oxidative stress and inflammation. The final outcome would be a reduction in the need for surgery, improvement in function, and fewer systemic complications. Additionally, the post-fasciotomy muscle oedema would be diminished, allowing for an easier delayed wound closure, without the need for skin grafting.

There is a marathon race toward discovering pharmacological agents capable of blocking or ameliorating the damages in ACS. Research in transplantation surgery and ischaemic heart disease has enriched the body of knowledge in the quest for the potential therapeutic agents in IR. Our laboratory has previously shown the efficacy of carbon monoxide (CO) and indomethacin in mitigating the damaging effects of ACS on skeletal muscle (Lawendy *et al*. 2014; Manjoo *et al*. 2010). This led us to the current work, the purpose of which was to evaluate the role of H_2S in ACS.

4.2 EFFECTS OF ELEVATED ICP

We found that elevation of ICP resulted in a significant local effects on skeletal muscle, as demonstrated by the microvascular perfusion changes (Figure 3.1), tissue injury (Figure 3.2) and leukocyte activation (Figures 3.3. and 3.4). We also demonstrated systemic effects of elevated ICP as demonstrated by increased TNF-α level (Figure 4.5).

4.2.1. Perfusion Changes

Significant increase in the number of non-perfused capillaries appears to be a consistent finding in ACS (Lawendy, Sanders, *et al*. 2011; Manjoo *et al.* 2010; McGarr 2009). This observation resembles the no-reflow phenomenon associated with the IR injury.

Using the modern technology of IVVM, direct visualisation of the skeletal muscle microcirculation has demonstrated a failure to restore the normal physiological micro-flow following fasciotomy (Figure 3.1). Consistent with previous studies, this hypoperfusion was evidenced by significant increase of NPC coupled with significant decrease in CPC (Manjoo *et al.* 2010; Lawendy, Sanders, *et al*. 2011; Lawendy *et al*. 2014; Lawendy 2014; Lawendy *et al*. 2015).

Microcirculatory studies of IR described this failure of capillary perfusion as 'no-reflow'. It had been demonstrated in several organs, such as the brain (Ames *et al.* 1968), skeletal muscle (Blaisdell 2002), kidney (Summers & Jamison 1971), and even skin (Chait *et al*. 1978). The occurrence of no-reflow would lead to focal ischemia and hypoxia, and despite normalization of compartment pressures, it would reduce recovery in muscle and increase the damage. The decrease in CPC was reported to correlate well with the increase in tissue necrosis in IR injury (Olivas *et al*. 2001).

The complexity of the pathogenesis of no-reflow is not completely understood (Durante & Camici 2015). No-reflow could be either a functional disturbance of microcirculation caused by vasoconstriction (Galiuto & Crea 2006; Maksimenko & Turashev 2012), or structural change caused by luminal blocking by aggregates of erythrocytes or leukocytes with platelets (Jaffe *et al*. 2008; Niccoli *et al*. 2008). While the functional no-reflow is a potentially reversible process, tissue injury may become irreversible after the 7- 8 hour interval (Olivas *et al*. 2001).

Our data displayed considerable deterioration of the perfusion following ACS, as seen at 30 minutes post-fasciotomy (Figure 3.1). There was a significant increase in the non-perfused capillaries, and a significant reduction in continuously perfused capillaries, consistent with what had been reported in previous publications. Thus or data confirmed the hypoperfusion state/the no-reflow phenomenon seen after fasciotomy (Lawendy 2014; Lawendy, Sanders, *et al*. 2011). The decrease in CPC was found to correlate well with the increase in percentage of tissue damage (Figure 3.2), consistent with what previously found (Olivas *et al*. 2001).

Although there are great similarities noticed between the no-reflow phenomenon reported in ACS and that reported in IR injury following complete ischaemia, there are clear temporal and qualitative differences. While we reported significant decrease in CPC after only 30 minutes of fasciotomy, a similar significant decreases was reported after 3 hours of reperfusion after complete ischaemia (Olivas *et al*. 2001).

4.2.2 Tissue Injury

Our data demonstrated a significant cellular injury after 2 hours of elevated ICP (Figure 3.2). We reported 33% cellular injury, compared to 9% seen in sham. This was consistent with the previously reported cellular injury ratio (Manjoo *et al*. 2010; Lawendy, Sanders, *et al*. 2011; Lawendy *et al*. 2014).

These data are also consistent with previous studies demonstrating an increase in tissue necrosis due to IR but with different temporal and qualitative

patterns, as we reported significant tissue injure after 30 minutes of fasciotomy, while it required 7 hours of reperfusion to show significant tissue injury after 3 hours of complete ischaemia (Olivas *et al*. 2001).

Cellular death appears to be the net result of all destructive mechanisms involved in pathophysiology of ACS. Cell necrosis has long been considered to be the natural fate of ischaemic insult. Recent evidence from experimental models indicates that apoptosis is a major contributor to IR-induced cell death (Genescà *et al*. 2002; Zhang *et al*. 2008; Wang *et al*. 2013). However, there are several confounding factors that determine the extent and degree of the damage, like the duration of ischemia and the tissue type (Gillani *et al*. 2012). In IR studies, apoptosis has been shown to exceed necrosis in skeletal muscle (Wang *et al*. 2008), as well as the heart (McCully *et al*. 2004), liver (Rüdiger & Clavien 2002), kidney (Du *et al*. 2003), and brain (Vogt *et al*. 1998). Kajstura *et al* demonstrated that cardiomyocyte apoptosis began after 2 hours of ischemia *in vivo* (Kajstura *et al*. 1996). It was estimated that the ratio of apoptotic and necrotic cell death is 31:1 at 2 hours after rat coronary artery occlusion (Anversa *et al*. 1998). While in skeletal muscle the difference did not appear to be that extensive, it was estimated to be 2:1 after 4 hours of ischemia and 24 hours of reperfusion in an *in vitro* rat study (Wang *et al.* 2008).

Apoptosis is a programmed cell death triggered by stimuli from outside or within the cell, leading to the activation of caspases and subsequent cell death (Primeau *et al*. 2002). The apoptotic program is complex, involving pro-apoptotic and protective proteins. Controlling these factors can modify the outcome of the

response to an apoptotic stimulus (Scarabelli *et al*. 2006). The knowledge that IR could induce apoptosis in skeletal muscle is clinically relevant, since it could allow for a therapeutic design aimed at apoptosis inhibition. Caspases are the executioners of the apoptotic process (Fischer *et al.* 2003). TNF-α is one of the most important external stimuli capable of inducing cell apoptosis in response to IR (de Nigris *et al*. 2003; Haddad 2004; Wu *et al.* 2014). Mitochondria appear as the main source of internal apoptotic stimuli, due to apoptotic factors present in the mitochondrial intermembrane space (Saelens *et al*. 2004). Cytochrome *c*, a component of the electron transport chain, is generally the earliest and most critical initiating factor for mitochondrial-mediated apoptosis when released into the cytosol from the inner mitochondrial membrane (Jiang & Wang 2004).

While ischaemia itself may be an expected cause, inflammation and microvascular dysfunction are also strongly involved in the cellular damage seen in IR injury, and, to a great extent, in ACS. ROS in particular are considered the main factor responsible for cellular damage caused by both IR and ACS (Perler *et al*. 1990; Sadasivan *et al.* 1997; Hancock *et al.* 2001; Chan 2001; Li & Jackson 2002; Kearns *et al.* 2004; Wang et al. 2006; Arató *et al*. 2008; Kearns *et al*. 2010; Wang *et al*. 2011b; Gillani *et al*. 2012) Both ischemia and IR are known to induce defects in the electron transport chain, leading to increased production of ROS (Kalogeris *et al*. 2014). While there are several sources of ROS, xanthine oxidase enzyme and activated neutrophils are the most common source. ROS tend to oxidize biomolecules, such as membrane-bound polyunsaturated fatty acids and DNA, damaging their structural or functional roles (Chen *et al.* 2012).

In addition, they also activate cyclooxygenase and lipoxygenase pathways (Toyokuni 1999), which, in turn, are responsible for neutrophil activation. Furthermore, ROS impair endothelium-dependent vasodilator mechanisms, exacerbating the microcirculatory dysfunction. Thus, the destructive effects of ROS may lead to either necrosis or apoptosis. The intense inflammatory reaction also contributes to tissue death in several ways. While activated leukocytes are responsible for tissue injury by the capillary plugging and production of proteolytic enzymes, inflammatory mediators play a major additional role. Necroptosis, another type of cell death induced mainly by TNF-α and NF-κB signalling, is a form of regulated cell death not mediated by caspases (Sun & Wang 2014). Inflammation is known to contribute to this process (de Almagro & Vucic 2015).

Previously, several experimental therapies targeting ROS have demonstrated reduction of ACS-associated cell injury, reflecting the reversible nature of cell damage. Kearns *et al* (2004) successfully diminished the cell injury in experimental ACS using vitamin C, while Perler *et al* (1990) did so by the ablation of ROS. In a more recent study, a potent antioxidant, N-acetylcysteine, demonstrated significant reduction of cell injury due to ACS (Kearns *et al.* 2010).

4.2.3 Leukocyte Activation

Involvement of leukocytes in the pathophysiology of ACS has been one of the earliest observations where the differences between ACS and complete ischaemia were clearly highlighted.

Leukocytes, predominantly neutrophils, significantly contribute to the pathogenesis of IR injury (Korthuis *et al*. 1988; Carden *et al.* 1990; Schofield *et al*. 2013; Sabido *et al*. 1994). Similar role for neutrophils has been demonstrated in ACS (Sadasivan *et al*. 1997; Lawendy *et al*. 2011; Lawendy *et al*. 2015). Ischaemic insult leads to upregulation of chemical mediators, which, in turn, activate neutrophils. TNF-α, IL-1β, IL-6 and IL-8 are all major chemical mediators involved in the neutrophil activation process (Ball *et al*. 2013). Activated neutrophils further exacerbate the tissue damage by the release of ROS, proteolytic enzymes, and physical blockage of the capillaries thereby preventing reperfusion of the tissue (Schofield *et al*. 2013).

Endothelium is very sensitive to any change in the internal milieu of the blood vessel. In IR, the endothelium becomes activated and creates a proinflammatory and prothrombotic surface to which leukocytes and other blood components adhere, resulting in endothelial dysfunction (Girn *et al*. 2007). Integrins and selectins are adhesion molecules that are generated in the activation process of endothelium and leukocytes, leading to neutrophil recruitment via a multistep cascade. Activated leukocytes exhibit a characteristic sequence pattern within the post capillary venules, namely rolling, adhesion, and transendothelial migration (Schofield *et al*. 2013).

Using IVVM, we were able to provide direct evidence of significant neutrophil activation in ACS. Both rolling (Figure 3.3) and adherence (Figure 3.4) were significantly increased after 2 hours of elevated ICP and 30 minutes of reperfusion. These findings are in agreement with previous observations of many

other investigators (Sadasivan *et al.* 1997; Manjoo *et al*. 2010; Lawendy, Sanders, *et al*. 2011; Lawendy *et al*. 2014; Lawendy 2014; Lawendy *et al*. 2015)

4.2.4 TNF-α Levels

In this study, we observed a marked increase in the levels of circulating TNF-α in ACS-challenged animals. TNF-α escalated as ICP increased and continued to do so after fasciotomy. This observation had previously been reported by Lawendy *et al* (2014).

4.3 THE EFFECT OF EXPERIMENTAL DRUGS ON ACS

4.3.1 Hydrogen Sulfide

Recently, H_2S has demonstrated considerable protective role in IR injury. The cytoprotective role of H_2S has been well documented in different tissues and organs. While the mechanism(s) remain largely unknown, it is thought to be multifactorial: reduction of the oxidative stress directly, upregulation of antioxidant enzymes, promotion of vasorelaxation, and modulation of mitochondrial respiration (King & Lefer 2011). H_2S has also been shown to attenuate leukocyte-mediated inflammation and inhibit apoptosis (Henderson *et al.* 2011; Jackson-Weaver 2012).

In this study, we examined the potential protective effect of H_2S on skeletal muscle microcirculation in a rat model of ACS. While H_2S has been used experimentally as an inhalational gas and as a parenteral preparation via H_2S-

donor molecules, we preferred the parenteral route. We avoided the use of gaseous H_2S, not only because of its offensive smell and the inherent hazard of being a highly toxic gas, but also because of the practical difficulties in standardizing the dose, each subject would receive by inhalation. Additionally, lung inflammatory reactions have been reported following inhalational H_2S application (Trevisani *et al*. 2005), which may have altered our outcomes of the inflammatory response due to ACS. Parenteral compounds are safer to store and handle, easy to prepare, and permit weight-dependent calculation of administered amount, thus ensuring consistency of dosing. Whereas both the intraperitoneal and intravenous routes have almost identical pharmacokinetic effects (Li *et al*. 2008), we opted for the intraperitoneal route because of ease of administration.

Using our rat model of ACS, we were able to demonstrate the cytoprotective value of H_2S. We found that H_2S markedly improved the microvascular perfusion (as evidenced by a significant increase in the number of continuously perfused capillaries and a reduction in the non-perfused capillaries) (Figure 3.1), reduced the overall cell injury (Figure 3.2) and leukocyte activation (Figures 3.3. and 3.4) within the post capillary venules.

4.3.1.1 H_2S and Microvascular Perfusion

Systemic administration of H_2S resulted in a noteworthy improvement in the muscle perfusion (Figure 3.1). We found a strong inhibitory effect of H_2S on the no-reflow phenomenon, with substantial increase in the number of

continuously perfused capillaries. The net effect of H_2S was the re-establishment of the microvascular perfusion to levels near those seen in the sham. This suggests that GYY4137-derived H_2S may have a great potential, not only in restoration/maintenance of adequate perfusion, but a subsequent protective role against further damage as well.

The mechanism(s) by which H_2S improved the microcirculation are not fully understood, but many pathways could play a role. Perhaps it works through re-establishing the redox balance between the pro-oxidants and anti-oxidants by directly scavenging ROS, enhancing the innate antioxidant mechanisms, and/or reducing the production of ROS themselves (Henderson *et al*. 2010; Bos *et al.* 2014).

The direct scavenging power of H_2S has not escaped a notice by many observers. It has been suggested that H_2S is capable of directly scavenging superoxide and hydrogen peroxide (Geng *et al*. 2004), cytotoxic lipid peroxidation products (Schreier *et al*. 2010; Calvert *et al*. 2010), the highly reactive peroxynitrite (Whiteman *et al*. 2004; Pacher *et al.* 2007), as well as hypochlorous acid (Whiteman *et al.* 2005; Laggner *et al.* 2007). The damaging power of ROS is due to their extreme reactivity, stemming from their chemical structure: these molecules have unpaired electrons. The readiness of H_2S to reduce other compounds by simply giving away a proton H^+ may explain its ability to directly reduce ROS, without the need of any enzymatic activity (Nagy *et al.* 2014).

H_2S appears to play a very important role in augmenting the body's innate antioxidant response (Calvert *et al*. 2010; Nishida *et al*. 2012). It has been

reported to increase the synthesis of GSH (Jha *et al*. 2008), and to increase activation of SOD (Sun *et al*. 2012). Both SOD and GSH are well-known important defensive mechanisms against the harmful effects of ROS. SOD is the major scavenger of superoxide (Fang *et al*. 2009). H_2S has consistently been reported to increase the expression of SOD under oxidative stress (Kimura & Kimura 2004; Kimura *et al.* 2006; Wu *et al*. 2014). On the other hand, GSH is known for its potent antioxidant role by reducing hydrogen peroxide to water (Albrecht *et al*. 2011).

H_2S has been shown to reduce oxidative stress by diminishing the production of ROS when administered at therapeutic doses (Calvert *et al*. 2010; Chen *et al*. 2006; Nicholson & Calvert 2010). This effect appears to be mediated through inactivating ROS-producing enzymes. Sun *et al* (2012) observed reduction in the levels of ROS through reversible inhibition of mitochondrial cytochrome *c* oxidase through partial inhibition of oxidative phosphorylation. In addition, H_2S has also been reported to inhibit the expression of NADPH oxidase (Tyagi *et al*. 2009; Muzaffar *et al.* 2008) and xanthine oxidase, most likely by activation of SOD (Bar-Or *et al*. 2015).

H_2S may have inhibited the no-reflow phenomenon by its potent vasodilator effect. It has been proposed that H_2S-mediated vasorelaxation is mediated through the opening of the mitochondrial K_{ATP} channel (Zhao *et al*. 2001; Cheng *et al*. 2004), a property that may be the key factor in reducing the infarct size in cardiac muscles following IR injury (Zhao *et al*. 2001; Johansen *et al*. 2006; Osipov *et al*. 2009). Interestingly, the vasorelaxing power of H_2S

appears to be directly correlated to the severity of the local hypoxia (Koenitzer *et al*. 2007; Dombkowski *et al*. 2011).

While NO is considered to be the most potent endothelium-derived relaxing factor in the vasculature, H_2S is capable of increasing NO levels by upregulation of eNOS in ischemic tissue. Conversely, NO has been shown to upregulate H_2S production by increasing the expression of CSE. Thus, H_2S and NO appear to be mutually dependent in controlling vascular relaxation (Coletta *et al.* 2012).

4.3.1.2 H_2S and Cellular Injury

We have demonstrated that H_2S administration in ACS led to a significant reduction in the cellular injury (Figure 3.2). Our findings are consistent with those of others, who had demonstrated the cytoprotective potential of H_2S in a rat model of myocardial IR (Sivarajah *et al*. 2009; Yao *et al*. 2012; Wu *et al*. 2014), liver (Mani *et al*. 2014), and kidney (Lobb *et al.* 2012) . Henderson *et al* had shown that not only was H_2S protective in skeletal muscle IR, but this protection was long lasting, for up to 4 weeks after the initial IR insult (Henderson *et al.* 2010). This would suggest that the therapeutic effect of H_2S does not merely shift the cellular damage several hours ahead, but is truly protective to the tissue.

While the exact mechanism of cellular protection is most likely related to the effect on vasodilation and anti-oxidant properties, there is another possibility: inhibition of apoptosis through the direct effect of H_2S on mitochondria. H_2S has been reported to preserve the mitochondrial function and membrane integrity

upon administration at the commencement of reperfusion in the ischaemic heart (Elrod *et al*. 2007). Through its ability to inhibit of cytochrome *c* oxidase function, H_2S is capable of inhibiting the electron transport chain during early phases of IR, thus reducing the overproduction of ROS (Kimura *et al.* 2005; Wagner *et al.* 2009; Veeranki & Tyagi 2014). Mitochondrial protection may also be mediated by maintaining membrane potentials. As an active opener of mitochondrial K_{ATP} channels, H_2S would prevent mitochondrial hyperpolarization, calcium overload and deleterious opening of the mitochondrial permeability transition pore (Calvert *et al*. 2010; Tang *et al.* 2013).

Previously, it has been demonstrated that the administration of H_2S at reperfusion was capable of reduction of cardiomyocyte apoptosis, both *in vitro* and *in vivo* (Elrod *et al*. 2007). This effect is most likely mediated by the ability of H_2S to inhibit the activation of caspases (Rinaldi *et al*. 2006).

H_2S could also inhibit apoptosis through inhibition of TNF-α, which is a major extracellular stimulus to induce apoptosis. We demonstrated significant reduction in the systemic levels of TNF- α, in our experiment, following H_2S injection (Figure 3.5).

Given the fact that we administered H_2S upon fasciotomy, it would appear that the protective effect of H_2S was most likely due to its modulation of the severity of reperfusion injury that follows fasciotomy. This kind of therapeutic application is considered a ‘pharmacological postconditioning’. H_2S-mediated pharmacological postconditioning had been successfully tested in completely

ischaemic rat skeletal muscle, and shown to significantly limit IR-induced damage (Henderson *et al*. 2011).

4.3.1.3 H_2S and Leukocyte Activation

In the current study, H_2S demonstrated a strong anti-inflammatory role, evidenced by the inhibition of leukocyte activation. H_2S administration resulted in a significant reduction in the number of both adherent and rolling leukocytes in the post-capillary venules (Figures 3.3 and 3.4). This anti-inflammatory property of H_2S has an added value in the cytoprotective power of H_2S, knowing that leukocyte activation amplifies the extent of damage through its role in inflammatory response.

H_2S appears to play an important role in inhibiting leukocyte activation in normal physiological conditions. For example, inhibition of H_2S synthesis in healthy animals led to a rapid increase in leukocyte adherence to the vascular endothelium (Zanardo *et al.* 2006). Rats with a diet-induced vitamin B deficiency (a co-factor in H_2S synthesis) exhibited significantly enhanced accumulation of leukocytes in the affected tissues (Flannigan *et al*. 2014). Heterozygous mice deficient in cystathionine-β-synthase (i.e. diminished synthesis of H_2S) also exhibit increased levels of leukocyte adherence to the vascular endothelium and an increase in vascular permeability (Kamath *et al*. 2006).

Just like in our study, administration of a therapeutic dose of H_2S has been shown to produce a marked suppression of inflammatory response in

several other animal models, including that of endotoxic shock (Fiorucci *et al.* 2007; Li *et al.* 2007). While the mechanism of the effect of H_2S on leukocyte adhesion is yet to be elucidated, Ball *et al* had demonstrated that H_2S suppressed leukocytes recruitment due to downregulation of L-selectin expression on activated human neutrophils (Ball *et al*. 2013). Additionally, H_2S has been shown to inhibit myeloperoxidase activity within neutrophils (Pálinkás *et al.* 2014), promote the shift of macrophages to an anti-inflammatory phenotype (Dufton *et al*. 2012), and induce apoptosis of neutrophils (Serhan et al. 2007). Finally, H_2S has also been shown to inhibit endothelial ICAM-1 expression, triggered by hyperglycaemia (Guan *et al*. 2013).

It has been demonstrated that exogenous administration of H_2S is capable of reducing the production of a number of proinflammatory cytokines, by suppressing NFκB activity (Sen *et al*. 2012). While H_2S appears to suppress the proinflammatory cytokines, it does not affect the expression of IL-10, which is anti-inflammatory (Dinarello 2000; Li *et al*. 2007). Recently, an interesting regulatory interaction between IL-10 and H_2S has been described. IL-10-deficient mice that spontaneously developed colitis were found to exhibit impaired H_2S synthesis in the colon. Administration of H_2S to these animals increased IL-10 expression and restored normal colonic H_2S synthesis (Gemici *et al.* 2015).

H_2S also appears to lead to an upregulation of HO, which is known to reduce inflammation and exhibit cytoprotection by producing CO and bilirubin (Oh *et al*. 2006; Qingyou *et al.* 2004; Ryter *et al.* 2006).

4.3.1.3 H_2S and TNF-α

We reported dramatic reduction of TNF-α levels following injection of H_2S, reaching baseline values in 45 minutes (Figure 3.5). Inhibition of TNF-α (one of the most potent pro-inflammatory cytokines) reflects the compelling role of H_2S as an anti-inflammatory agent, not only protecting tissues locally at the site of ischaemia, but also in reducing the systemic sequelae of ACS.

Interestingly, the TNF-α level pattern was mirrored by leukocyte activation in the ACS-challenged muscle, as demonstrated by changes in adherent and rolling leukocytes within the post capillary venules (Figures 3.3 and 3.4).

4.3.2 Combination of Hydrogen Sulfide and Carbon Monoxide

Both H_2S and CO are gaseous signalling molecules with documented cytoprotective roles in IR injury (Ryter *et al*. 2006; Nicholson & Calvert 2010; Calvert *et al.* 2010; Mancardi *et al*. 2009; Ozaki *et al*. 2012). Our data demonstrated that the improvement in the microvascular perfusion and tissue injury, as well as inhibition of leukocyte activation and TNF-α, induced by GYY4137-derived H_2S, was very comparable to that induced by CORM-3-derived CO, as reported by Lawendy *et al* (2014).

Though we hypothesised that combination of H_2S and CO may have an added protective benefit in ACS, the combined effect of H_2S and CO, in our experiments, was not significantly different from that of either of them, to our surprise.

There could be several possibilities explaining the failure of combination treatment. First, perhaps the improvements we had observed could represent the maximum improvement that could ever be achieved by any drug. Thus, combining two or more agents would not be of value. For example, it is possible that capillary damage produced structural no-reflow phenomenon, thus it would not respond to any treatment (Galiuto & Crea 2006).

Another possibility is that both H_2S and CO may be exhibiting their protective effects by influencing one common pathway, albeit from different starting points. For example, H_2S may exert some of its anti-inflammatory effects through upregulation of HO, while CO is the final product of this pathway (Oh *et al*. 2006; Qingyou *et al*. 2004; Ryter *et al*. 2006). HO-derived CO has been shown to control vascular tone in NO-dependent manner (Ishikawa *et al*. 2005); H_2S has been found to increase NOS expression in endothelial cells (Bir *et al*. 2012). The actions of both H_2S and CO converge at cGMP, although H_2S does not directly activate soluble guanylate cyclase (Coletta *et al*. 2012; Schallner *et al.* 2013).

Finally, there is a possibility that a much more complex interplay between the two molecules occurs. Some effects may be synergistic, while and others antagonistic; the final outcome would be dictated by which effect would be more pronounced. For example, CBS has been proposed to be a specific sensor for CO (Omura 2005). Thus, CO would have the potential to inhibit CBS activity and, therefore, the generation of endogenous H_2S (Taoka & Banerjee 2001).

4.4 STUDY LIMITATIONS

While our results demonstrated significant potential role of H_2S as a novel pharmacological therapeutic agent in ACS, we also recognize that there were certain limitations to our study. We used a small animal model of simulated acute compartment syndrome, and although there are some similarities between rat and human physiology, there are also significant differences between the two species (Radermacher & Haouzi 2013). Thus, the observed data may not necessarily reflect the human physiologic responses to an extremity ACS.

In our experiment, we chose to induce ACS for two hours, based on our previous publication demonstrating the beneficial effects of CO (Lawendy *et al*. 2014). The equivalence of two hours of ACS in rats is questionable when compared to humans. Many studies exist that compare the physiological processes among living organisms (basal metabolic rate, life span, body mass and other parameters), and how they mathematically relate to one another. Different equations applicable to all living organisms are available for comparative physiology (Marquet *et al*. 2005; Hulbert *et al.* 2007; Hoppeler & Weibel 2005; Radermacher & Haouzi 2013). Based on these, it was extrapolated that one hour of ischaemia in a rat muscle would be the equivalent of about 4 hours in humans (McGarr 2009; Lawendy 2014), which means that the 2 hours we used in the rat model could be equal to 8 hours of ACS in humans. While the design of our experiment was not geared to prove this theory, it would be interesting to validate it in future studies.

Another limitation is the type of tissue examined. Our study was designed to determine the potential of H_2S in attenuation of the ACS-associated damaging effects in the striated muscle. Although our data suggests skeletal muscle protection in terms of reduced cell damage, reduced leukocyte activation and improved microcirculation, the study design did not address the protective effects on the nerves or the antinociceptive effects of H_2S. Success in reducing muscle damage does not necessarily mean associated nerve protection, as it was noticed previously that ischaemic preconditioning may prevent skeletal muscle tissue injury, but not nerve lesion upon tourniquet-induced ischaemia (Schoen *et al*. 2007)

While we demonstrated the immediate protective effects of H_2S, we did not study the overall long-term changes, sequelae, and recovery of muscle function. Thus, any functional clinical outcomes cannot be directly inferred from our experiments.

Finally, while we assessed one dose of H_2S delivered intraperitoneally just before fasciotomy, we did not investigate the dose–response relationship. Knowing that timing of the medication may change the results, additional studies would be required to determine the optimum time to administer H_2S, and to examine the possibility that more than one dose may be needed.

4.5 FUTURE DIRECTIONS

In the future, there are several possible experiments that could further elucidate the role of hydrogen sulfide in ACS.

1. H_2S dose-time optimization: Considering that H_2S was recently shown to serve as a “fuel” for mitochondria during anoxia (Chan & Wallace 2013; Szabo *et al*. 2014), this opens a new horizon for trials during the state of elevated ICP, as this mechanism may ameliorate the ischaemic insult itself. Adding to its known cytoprotective role, H_2S may be able to reduce the demand for fasciotomy.

2. The next logical step for this line of work is to evaluate functional outcomes of the treatment in recovery studies. Gait analysis testing a few days following the ACS, and even microscopic examination of skeletal muscle should provide the necessary functional as well as pathological data to support the use of H_2S in clinical trials.

3. Evaluation of the effects of H_2S on Large animal model would be of more clinical relevance than the rat model, and thus would be the next logical step forwards clinical trials.

4.6 SUMMARY AND CONCLUSIONS

Although ACS remains a significant source of morbidity and mortality, there has been little progress made toward the development of an adjunctive therapeutic intervention, beside surgery, that might ameliorate the damaging inflammatory response and associated oxidative stress. The ideal intervention would simultaneously target interacting inflammatory mechanisms, abort the build up of oedema/increased ICP cycle, and protect the cells from IR injury.

We were able to demonstrate that H_2S may have a considerable cytoprotective role on the skeletal muscle in ACS, acting through several possible mechanisms (Figure 4.1). We also established that combining H_2S and CO was not superior to using either one of the substances individually. Hydrogen sulfide shows potential as an adjunctive therapy for compartment syndrome and warrants additional assessment to make it a translatable therapy for clinical applications.

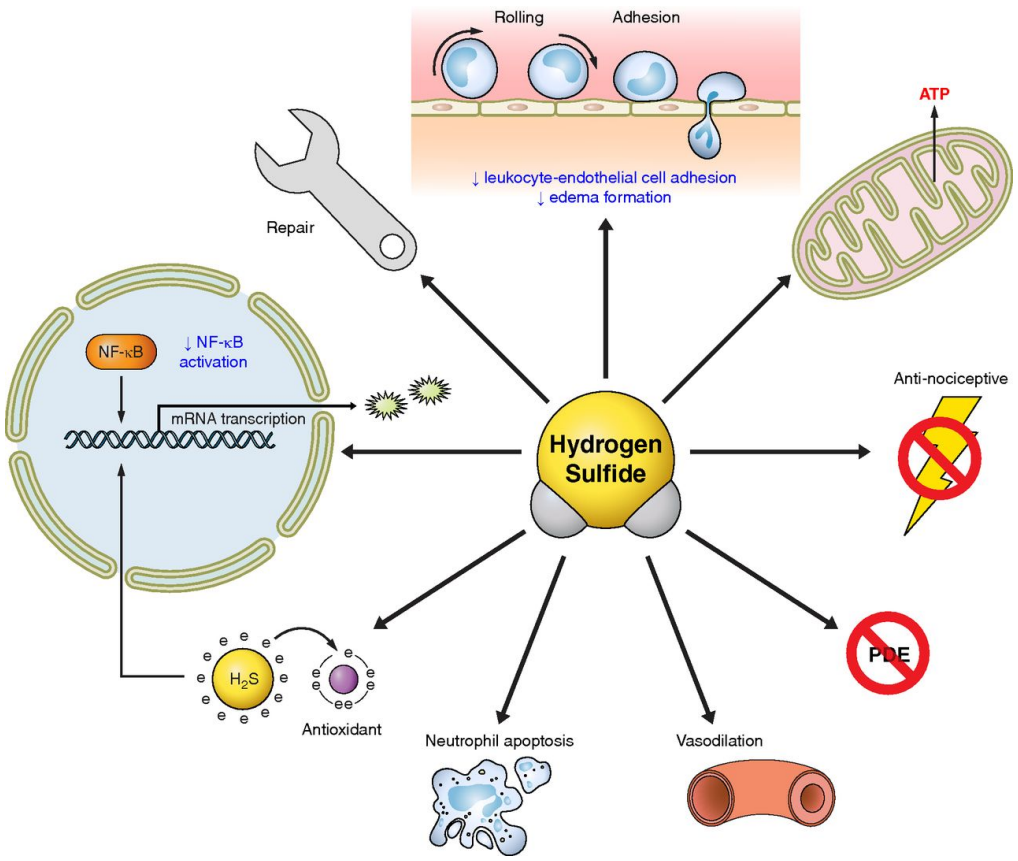

Figure 4.1. Anti-inflammatory actions of H_2S. H_2S inhibits leukocyte adherence to the vascular endothelium, and promotes neutrophil apoptosis. Mitochondria can utilize H_2S as a fuel in ATP production, particularly during anoxia/hypoxia, thus reduce generation of ROS. By inhibiting phosphodiesterases (PDE), H_2S can elevate tissue cGMP levels, which contributes to vasodilation. The antioxidant actions of H_2S further reduce tissue injury. Several anti-inflammatory and antioxidant systems are activated by H_2S through its effects on transcription factors. H_2S can stimulate angiogenesis, thus promote repair of damaged tissue.

Adapted from (Chan & Wallace 2013)*, with permission.*

REFERENCES

Abe K, Kimura H (1996). The Possible Role of Hydrogen Sulfide as an Endogenous Neuromodulator. J Neurosci 16(3): 1066–71.

Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG (2014). Diabetic Foot Ulcers: Part I. Pathophysiology And Prevention. J Am Acad Dermatol 70(1): 1.E1–18; Quiz 19–20.

Albrecht SC, Barata AG, Grosshans J, Teleman AA, Dick TP (2011). *In vivo* Mapping of Hydrogen Peroxide and Oxidized Glutathione Reveals Chemical and Regional Specificity of Redox Homeostasis. Cell Metab 14(6):819–29.

Ali MY, Whiteman M, Low C-M, Moore PK (2007). Hydrogen Sulphide Reduces Insulin Secretion From HIT-T15 Cells by a KATP Channel-Dependent Pathway. J Endocrinol 195(1): 105–12.

Al-Magableh MR, Kemp-Harper BK, Hart JL (2015). Hydrogen Sulfide Treatment Reduces Blood Pressure and Oxidative Stress in Angiotensin II-Induced Hypertensive Mice. Hypertens Res38(1): 13–20.

Altaany Z, Moccia F, Munaron L, Mancardi D, Wang R (2014). Hydrogen Sulfide And Endothelial Dysfunction: Relationship With Nitric Oxide. Curr Med Chem 21(32): 3646–61.

Ames A, Wright RL, Kowada M, Thurston JM, Majno G (1968). Cerebral Ischemia. II. The No-Reflow Phenomenon. Am J Pathol 52(2): 437–53.

Anversa P, Cheng W, Liu Y, Leri A, Redaelli G, Kajstura J (1998). Apoptosis and Myocardial Infarction. Basic Res Cardiol 93 Suppl 3:8–12.

Arató E, Jancsó G, Sínay L, Kürthy M, Lantos J, Ferencz S, Horváth, S, Shafiei, M, Kasza, G, Verzár, Z, Kollár, L, Roth, E, Wéber, G, Menyhei, G (2008). Reperfusion Injury and Inflammatory Responses Following Acute Lower Limb Revascularization Surgery. Clin Hemorheol Microcirc 39(1-4): 79–85.

Arnhold J (2004). Properties, Functions, and Secretion of Human Myeloperoxidase. Biochem (Mosc) 69(1): 4–9.

Ashton H (1962). Critical Closing Pressure in Human Peripheral Vascular Beds. Clin Sci 22: 79–87.

Ashton H (1975). The Effect of Increased Tissue Pressure on Blood Flow. Clin Orthop Relat Res 113:15–26.

Bai A-P, Ouyang Q, Hu R-W (2005). Diallyl Trisulfide Inhibits Tumor Necrosis Factor-Alpha Expression In Inflammed Mucosa Of Ulcerative Colitis. Dig Dis Sci 50(8): 1426–31.

Ball CJ, Reiffel AJ, Chintalapani S, Kim M, Spector JA, King MR (2013). Hydrogen Sulfide Reduces Neutrophil Recruitment in Hind-Limb Ischemia-Reperfusion Injury in an L-Selectin and ADAM-17-Dependent Manner. Plast Reconstr Surg 131(3): 487–97.

Bardenheuer (1910). Die Entstehung Und Behandlung der Ischämischen Muskelkontraktur und Gangrän. Dtsch Zeitschrift für Chir 108(1-2): 44–201.

Bar-Or D, Bar-Or R, Rael LT, Brody EN (2015). Oxidative Stress in Severe Acute Illness. Redox Biol 4C: 340–5.

Baskar R, Sparatore A, Del Soldato P, Moore PK (2008). Effect Of S-Diclofenac, A Novel Hydrogen Sulfide Releasing Derivative Inhibit Rat Vascular Smooth Muscle Cell Proliferation. Eur J Pharmacol 594(1-3): 1–8.

Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley-Usmar VM, Doeller JE, Kraus DW, (2007). Hydrogen Sulfide Mediates the Vasoactivity of Garlic. Proc Natl Acad Sci USA 104(46): 17977–82.

Bhatia M (2005). Hydrogen sulfide as a vasodilator. IUBMB Life 57(9): 603–6.

Bickel M (1993). The Role of Interleukin-8 In Inflammation and Mechanisms of Regulation. J Periodontol 64(5 Suppl): 456–60.

Bir SC, Kolluru GK, Mccarthy P, Shen X, Pardue S, Pattillo CB, Kevil CG, (2012). Hydrogen Sulfide Stimulates Ischemic Vascular Remodeling Through Nitric Oxide Synthase and Nitrite Reduction Activity Regulating Hypoxia-Inducible Factor-1α and Vascular Endothelial Growth Factor-Dependent Angiogenesis. J Am Heart Assoc 1(5): E004093.

Blackstone E, Morrison M, Roth MB (2005). H_2S Induces a Suspended Animation-Like State in Mice. Science 308(5721): 518.

Blackstone E, Roth MB (2007). Suspended Animation-Like State Protects Mice From Lethal Hypoxia. Shock 27(4): 370–2.

Blaisdell FW (2002). The Pathophysiology Of Skeletal Muscle Ischemia And The Reperfusion Syndrome: A Review. Cardiovasc Surg 10(6):620–30.

Blandy JP, Fuller R (1957). March Gangrene; Ischaemic Myositis of the Leg Muscle From Exercise. J Bone Joint Surg Br 39-B(4): 679–93.

Bocca G, van Moorselaar JA, Feitz WFJ, van der Staak FHJM, Monnens LAH (2002). Compartment Syndrome, Rhabdomyolysis and Risk of Acute Renal Failure as Complications of the Lithotomy Position. J Nephrol 15(2): 183–5.

Bonnaig N, Dailey S, Archdeacon M (2014). Proper Patient Positioning and Complication Prevention in Orthopaedic Surgery. J Bone Joint Surg Am [96(13): 1135–40.

Bos EM, Leuvenink HGD, Snijder PM, Kloosterhuis NJ, Hillebrands J-L, Leemans JC, Florquin S, van Goor H (2009). Hydrogen Sulfide-Induced Hypometabolism Prevents Renal Ischemia/Reperfusion Injury. J Am Soc Nephrol 20(9): 1901–5.

Bos EM, van Goor H, Joles JA, Whiteman M, Leuvenink HGD (2014). Hydrogen Sulfide: Physiological Properties and Therapeutic Potential in Ischaemia. Br J Pharmacol 172(6): 1479–93.

Botte MJ, Santi MD, Prestianni CA, Abrams RA (1996). Ischemic Contracture Of The Foot And Ankle: Principles Of Management And Prevention. Orthopedics 19(3): 235–44.

Branco BC, Inaba K, Barmparas G, Schnüriger B, Lustenberger T, Talving P, Lam L, Demetriades D (2011). Incidence and Predictors For the Need For Fasciotomy After Extremity Trauma: A 10-Year Review In A Mature Level I Trauma Centre. Injury 42(10): 1157–63.

Brooks B (1922). Pathologic Changes in Muscle as a Result of Disturbances Of Circulation; an Experimental Study of Volkmann's Ischemic Paralysis. Arch Surg 5(1): 188–216.

Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP (2000). Carbon Monoxide Generated by heme Oxygenase 1 Suppresses Endothelial Cell Apoptosis. J Exp Med 192(7): 1015–26.

Bucci M, Cirino G (2011). Hydrogen Sulphide in Heart and Systemic Circulation. Inflamm Allergy Drug Targets 10(2): 103–8.

Burton AC, Yamada S (1951). Relation Between Blood Pressure and Flow in The Human Forearm. J Appl Physiol 4(5): 329–39.

Caliendo G, Cirino G, Santagada V, Wallace JL (2010). Synthesis and Biological Effects of Hydrogen Sulfide (H_2S): Development of H2S-Releasing Drugs as Pharmaceuticals. J Med Chem 53(17): 6275–86.

Calvert JW, Coetzee W a, Lefer DJ (2010). Novel Insights Into Hydrogen Sulfide--Mediated Cytoprotection. Antioxid Redox Signal 12(10): 1203–17.

Cannon JG (2000). Inflammatory Cytokines in Nonpathological States. News Physiol Sci 15: 98–303.

Carden DL, Granger DN (2000). Pathophysiology of Ischaemia-Reperfusion Injury. J Pathol 190(3): 255–66.

Carden DL, Smith JK, Korthuis RJ (1990). Neutrophil-Mediated Microvascular Dysfunction in Postischemic Canine Skeletal Muscle. Role of Granulocyte Adherence. Circ Res 66(5): 1436–44.

Carmo-Araújo EM, Dal-Pai-Silva M, Dal-Pai V, Cecchini R, Anjos Ferreira AL (2007). Ischaemia And Reperfusion Effects On Skeletal Muscle Tissue: Morphological And Histochemical Studies. Int J Exp Pathol 88(3): 147–54.

Carter AB, Richards RL, Zachary RB (1949). The Anterior Tibial Syndrome. Lancet 2(6586): 928–34.

Chait LA, May JW, O'Brien BM, Hurley J V (1978). The Effects of the Perfusion of Various Solutions on the No-Reflow Phenomenon in Experimental Free Flaps. Plast Reconstr Surg 61(3): 421–30.

Chan M V, Wallace JL (2013). Hydrogen Sulfide-Based Therapeutics And Gastrointestinal Diseases: Translating Physiology to Treatments. Am J Physiol Gastrointest Liver Physiol 305(7): G467–73.

Chan PH (2001). Reactive Oxygen Radicals in Signaling and Damage in the Ischemic Brain. J Cereb Blood Flow Metab 21(1): 2–14.

Chattopadhyay M, Kodela R, Nath N, Dastagirzada YM, Velázquez-Martínez CA, Boring D, Kashfi K (2012). Hydrogen Sulfide-Releasing NSAIDs Inhibit the Growth Of Human Cancer Cells: A General Property And Evidence Of A Tissue Type-Independent Effect. Biochem Pharmacol 83(6): 715–22.

Chattopadhyay M, Nath N, Kodela R, Sobocki T, Metkar S, Gan ZY, Kashfi K (2013). Hydrogen Sulfide-Releasing Aspirin Inhibits The Growth of Leukemic Jurkat Cells And Modulates B-Catenin Expression. Leuk Res 37(10): 1302–8.

Chen AF, Chen D-D, Daiber A, Faraci FM, Li H, Rembold CM, Laher I. (2012). Free Radical Biology of the Cardiovascular System. Clin Sci (Lond) 123(2): 73–91.

Chen Q, Moghaddas S, Hoppel CL, Lesnefsky EJ (2006). Reversible Blockade of Electron Transport During Ischemia Protects Mitochondria and Decreases Myocardial Injury Following Reperfusion. J Pharmacol Exp Ther 319(3): 1405–12.

Cheng Y, Ndisang JF, Tang G, Cao K, Wang R (2004). Hydrogen Sulfide-Induced Relaxation of Resistance Mesenteric Artery Beds of Rats. Am J Physiol Heart Circ Physiol 287(5): H2316–23.

Chou S, Fay M, Keith S, Ingerman L, Chappell L (2006). Toxicological Profile for Hydrogen Sulfide. Agency for Toxic Substances and Disease Registry; Atlanta, Georgia. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp114.pdf

Ciuffetti G, Mercuri M, Mannarino E, Ott C, Lombardini R, Pasqualini L, Lupatelli G, Santambrogio L. (1991). Free Radical Production in Peripheral Vascular Disease. A Risk for Critical Ischaemia? Int Angiol 10(2): 81–7.

Clarke WT (1946). Volkmann's Contracture. Can Med Assoc J 54(4): 339–41.

Clause BT (1998). Rats (Not Mice) and History. Mendel News l (7):2–7.

Coletta C, Papapetropoulos A, Erdelyi K, Olah G, Módis K, Panopoulos P, Asimakopoulou A, Gerö D, Sharina I, Martin E, Szabo C (2012). Hydrogen Sulfide and Nitric Oxide are Mutually Dependent in the Regulation of Angiogenesis and Endothelium-Dependent Vasorelaxation. Proc Natl Acad Sci USA 109(23): 9161–6.

Conrad MF, Albadawi H, Stone DH, Crawford RS, Entabi F, Watkins MT (2006). Local Administration of the Poly ADP-Ribose Polymerase (PARP) Inhibitor, PJ34 During Hindlimb Ischemia Modulates Skeletal Muscle Reperfusion Injury. J Surg Res 135(2): 233–7.

Conrad MF, Stone DH, Albadawi H, Hua HT, Entabi F, Stoner MC, Watkins, Michael T (2005). Local Inflammatory and Thrombotic Responses Differ in a Murine Model of Partial and Complete Hindlimb Ischemia/Reperfusion. Surgery 138(2): 375–81.

De Almagro MC, Vucic D (2015). Necroptosis: Pathway Diversity and Characteristics. Semin Cell Dev Biol 39: 56–62.

De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince S (2007). How to Evaluate the Microcirculation: Report of a Round Table Conference. Crit Care 11(5): R101.

De Nigris F, Lerman A, Ignarro LJ, Williams-Ignarro S, Sica V, Baker AH, Lerman LO, Geng YJ, Napoli C (2003). Oxidation-Sensitive Mechanisms, Vascular Apoptosis and Atherosclerosis. Trends Mol Med9(8): 351–9.

De With MCJ, Haug SJ, Brigitte van der Heijden EPA, Segal SS (2005). Ischemia-Reperfusion Impairs Ascending Vasodilation in Feed Arteries of Hamster Skeletal Muscle. Microcirculation 12(7): 551–61.

Defraigne JO, Pincemail J (1998). Local and Systemic Consequences of Severe Ischemia and Reperfusion Of The Skeletal Muscle. Physiopathology and Prevention. Acta Chir Belg 98(4): 176–86.

Dinarello CA (2000). Proinflammatory Cytokines. Chest J 118(2): 503.

Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, Antonelli E, Roviezzo F, Morelli A, Cirino G, Wallace JL, Fiorucci S, (2006). Evidence That Hydrogen Sulfide Exerts Antinociceptive Effects In The Gastrointestinal Tract By Activating K_{ATP} Channels. J Pharmacol Exp Ther 316(1):325–35.

Dombkowski RA, Naylor MG, Shoemaker E, Smith M, Deleon ER, Stoy GF, Gao Y, Olson K R (2011). Hydrogen Sulfide (H_2S) and Hypoxia Inhibit Salmonid Gastrointestinal Motility: Evidence for H_2S As An Oxygen Sensor. J Exp Biol 214(Pt 23): 4030–40.

Donaldson J, Haddad B, Khan WS (2014). The Pathophysiology, Diagnosis And Current Management Of Acute Compartment Syndrome. Open Orthop J 8: 185–93.

Donatti AF, Araujo RM, Soriano RN, Azevedo LU, Leite-Panissi CA, Branco LGS (2014). Role of Hydrogen Sulfide in the Formalin-Induced Orofacial Pain in Rats. Eur J Pharmacol 738: 49–56.

Dopheide JF, Doppler C, Scheer M, Obst V, Radmacher M-C, Radsak MP, Gori T, Warnholtz A, Fottner C, Münzel T, Daiber A, Espinola-Klein C (2013). Critical Limb Ischaemia is Characterised by an Increased Production of Whole Blood Reactive Oxygen Species and Expression of TREM-1 on Neutrophils. Atherosclerosis 229(2): 396–403.

Du C, Guan Q, Yin Z, Masterson M, Zhong R, Jevnikar AM (2003). Renal Tubular Epithelial Cell Apoptosis by Fas-Fasl-Dependent Self-Injury Can Augment Renal Allograft Injury. Transplant Proc 35(7):2481–2.

DU J, Li W, Yang J, Tang C, Li Q, Jin H (2013). Hydrogen Sulfide Is Endogenously Generated In Rat Skeletal Muscle And Exerts A Protective Effect Against Oxidative Stress. Chin Med J (Engl) 126(5):930–6.

Düben W (1969). [Etiology and treatment of ischemic contractures of the forearm and hand]. Handchirurgie 1(2):63–9.

Dudzinski DM, Igarashi J, Greif D, Michel T (2006). The Regulation And Pharmacology Of Endothelial Nitric Oxide Synthase. Annu Rev Pharmacol Toxicol 46: 235–76.

Dufton N, Natividad J, Verdu EF, Wallace JL (2012). Hydrogen Sulfide and Resolution of Acute Inflammation: A Comparative Study Utilizing a Novel Fluorescent Probe. Sci Rep 2: 499

Durante A, Camici PG (2015). Novel Insights into an “Old” Phenomenon: The No-Reflow. Int J Cardiol 187: 273–80.

Echtermeyer V, Horst P (1997). Compartment Syndrome. Exclusively The Result Of Increased Pressure In The Muscular Compartment? Unfallchirurg 100(12): 924–37.

Eichler GR, Lipscomb PR (1967). The changing treatment of Volkmann’s ischemic contractures from 1955 to 1965 at the Mayo Clinic. Clin Orthop Relat Res 50: 215–23.

Elliott KGB, Johnstone AJ (2003). Diagnosing Acute Compartment Syndrome. J Bone Joint Surg Br 85-B(5): 625–32.

Ellis H (2006). Clinical Anatomy : A Revision and Applied Anatomy for Clinical Students [Internet]. Eleventh E. Oxford: Blackwell Publishing Ltd.

Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus D*W, Tao L,* Jiao X, Scalia R, Kiss L, Szabo Csaba, Kimura H, Chow C-W, Lefer DJ (2007). Hydrogen Sulfide Attenuates Myocardial Ischemia-Reperfusion Injury By Preservation Of Mitochondrial Function. Proc Natl Acad Sci USA 104(39): 15560–5.

Epithelium N, Dorman DC, Moulin FJ, Mcmanus BE, Mahle KC, James RA, James RA, Struve MF (2002). Cytochrome Oxidase Inhibition Induced by Acute Hydrogen Sulfide Inhalation : Correlation with Tissue Sulfide Concentrations in the Rat. Toxicol Sci 65(1): 18-25.

Fang J, Seki T, Maeda H (2009). Therapeutic Strategies by Modulating Oxygen Stress in Cancer and Inflammation. Adv Drug Deliv Rev 61(4): 290–302.

Farrugia G, Szurszewski JH (2014). Carbon Monoxide, Hydrogen Sulfide, and Nitric Oxide as Signaling Molecules in the Gastrointestinal Tract. Gastroenterology 147(2): 303–13.

Finkelstein JA, Hunter GA, Hu RW (1996). Lower Limb Compartment Syndrome: Course After Delayed Fasciotomy. J Trauma 40(3): 342–4.

Fiorucci S, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di Sante M, Morelli A, Cirino G, Wallace JL (2005). Inhibition of Hydrogen Sulfide Generation Contributes to Gastric Injury Caused by Anti-Inflammatory Nonsteroidal Drugs. Gastroenterology 129(4): 1210–24.

Fiorucci S, Orlandi S, Mencarelli A, Caliendo G, Santagada V, Distrutti E, Santucci L, Cirino G, Wallace JL. (2007). Enhanced Activity of a Hydrogen Sulphide-Releasing Derivative of Mesalamine (ATB-429) in a Mouse Model of Colitis. Br J Pharmacol 150(8): 996–1002.

Fiorucci S, Santucci L, Distrutti E (2007). NSAIDs, Coxibs, CINOD and H_2S-Releasing NSAIDs: What Lies Beyond the Horizon. Dig Liver Dis 39(12): 1043–51.

Fiorucci, Distrutti E, Cirino G, Wallace JL (2006). The Emerging Roles of Hydrogen Sulfide in the Gastrointestinal Tract and Liver. Gastroenterology 131(1): 259–71.

Fischer U, Jänicke RU, Schulze-Osthoff K (2003). Many Cuts to Ruin: A Comprehensive Update of Caspase Substrates. Cell Death Differ 10(1): 76–100.

Flannigan KL, Agbor TA, Blackler RW, Kim JJ, Khan WI, Verdu EF, Ferraz JGP, Wallace JL (2014). Impaired Hydrogen Sulfide Synthesis and IL-10 Signaling Underlie Hyperhomocysteinemia-Associated Exacerbation of Colitis. Proc Natl Acad Sci USA 111(37): 13559–64.

Flecknell, P.A. 1996. “Anaesthesia of Common Laboratory Species.” In Laboratory Animal Anaesthesia, 159–223. Elsevier.

Fomenko I, Sklyarov A, Bondarchuk T, Biletska L, Panasyuk N, Wallace JL (2014). Effects Of Conventional And Hydrogen Sulfide-Releasing Non-Steroidal Anti-Inflammatory Drugs In Rats With Stress-Induced And Epinephrine-Induced Gastric Damage. Stress 17(6): 528–37.

Furchgott RF, Zawadzki J V (1980). The Obligatory Role of Endothelial Cells in the Relaxation of Arterial Smooth Muscle by Acetylcholine. Nature 288(5789): 373–6.

Galiuto L, Crea F (2006). No-reflow: a Heterogeneous Clinical Phenomenon With Multiple Therapeutic Strategies. Curr Pharm Des 12(29): 3807–15.

Gemici B, Elsheikh W, Feitosa KB, Costa SKP, Muscara MN, Wallace JL (2015). H_2S-Releasing Drugs: Anti-Inflammatory, Cytoprotective and Chemopreventative Potential. Nitric Oxide 46: 25–31.

Genescà M, Sola A, Miquel R, Pi F, Xaus C, Alfaro V, Hotter G (2002). Role of Changes in Tissular Nucleotides on the Development of Apoptosis During Ischemia/Reperfusion in Rat Small Bowel. Am J Pathol 161(5): 1839–47.

Geng B, Chang L, Pan C, Qi Y, Zhao J, Pang Y, Du J, Tang C (2004). Endogenous Hydrogen Sulfide Regulation of Myocardial Injury Induced by Isoproterenol. Biochem Biophys Res Commun 318(3): 756–63.

Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J, Tang C (2004). H_2S Generated by Heart in Rat and its Effects on Cardiac Function. Biochem Biophys Res Commun 313(2): 362–8.

Ghasemi H, Ghazanfari T, Yaraee R, Faghihzadeh S, Hassan ZM (2011). Roles of IL-8 in Ocular Inflammations: a Review. Ocul Immunol Inflamm 19(6): 401–12.
Giannoudis P V, Nicolopoulos C, Dinopoulos H, Ng A, Adedapo S, Kind P (2002). The impact of lower leg compartment syndrome on health related quality of life. Injury 33(2): 117–21.

Gillani S, Cao J, Suzuki T, Hak DJ (2012). The Effect Of Ischemia Reperfusion Injury On Skeletal Muscle. Injury 43(6): 670–5.

Girn HRS, Ahilathirunayagam S, Mavor AID, Homer-Vanniasinkam S (2007). Reperfusion Syndrome: Cellular Mechanisms Of Microvascular Dysfunction And Potential Therapeutic Strategies. Vasc Endovascular Surg 41(4): 277–93.

Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia MW, Reiffenstein RJ, Dowling G (1989). Determination of Sulfide in Brain Tissue by Gas Dialysis/Ion Chromatography: Postmortem Studies and Two Case Reports. Journal of analytical toxicology 13(2): 105-9

Goubier JNN, Romaña C, Molina V (2005). Le Syndrome De Volkmann Néonatal. À Propos De Deux Cas. Neonatal Volkmann's Compartment Syndrome. A Report of Two Cases. Chirurgie de la main 24(1):45-7

Granger DN (1988). Role of Xanthine Oxidase and Granulocytes in Ischemia-Reperfusion Injury. Am J Physiol 255(6): H1269–75.

Granger DN, Benoit JN, Suzuki M, Grisham MB (1989). Leukocyte Adherence to Venular Endothelium during Ischemia-Reperfusion. Am J Physiol 257(5 Pt 1): G683–8.

Granger DN, Höllwarth ME, Parks DA (1986). Ischemia-Reperfusion Injury: Role of Oxygen-Derived Free Radicals. Acta Physiol Scand Suppl 548: 47–63.

Greensmith JE (2004). Hyperbaric Oxygen Therapy In Extremity Trauma. J Am Acad Orthop Surg 12(6): 376–84.

Griffiths DL (1940). Volkmann's Ischaemic Contracture. Br J Surg 28(110): 239–60.

Grivennikov SI, Kuprash D V, Liu Z-G, Nedospasov SA (2006). Intracellular Signals and Events Activated by Cytokines of the Tumor Necrosis Factor Superfamily: From Simple Paradigms to Complex Mechanisms. Int Rev Cytol 252: 129–61.

Guan Q, Wang X, Gao L, Chen J, Liu Y, Yu C, Zhang N, Zhang X, Zhao J (2013). Hydrogen Sulfide Suppresses High Glucose-Induced Expression of Intercellular Adhesion Molecule-1 in Endothelial Cells. J Cardiovasc Pharmacol 62(3): 278–84.

Haddad JJ (2004). Redox and Oxidant-Mediated Regulation of Apoptosis

Signaling Pathways: Immuno-Pharmaco-Redox Conception of Oxidative Siege versus Cell Death Commitment. Int Immunopharmacol 4(4): 475–93.

Hamam AW (2014). Functional Assessment and Potential Therapeutic Role of Carbon Monoxide Releasing Molecule-3 in A Rodent Model of Compartment Syndrome. MSc. University of Western Ontario, Canada

Hampton MB, Kettle AJ, Winterbourn CC (1998). Inside the Neutrophil Phagosome: Oxidants, Myeloperoxidase, and Bacterial Killing. Blood 92(9): 3007–17.

Hancock JT, Desikan R, Neill SJ (2001). Role of Reactive Oxygen Species in Cell Signalling Pathways. Biochem Soc Trans 29(2): 345–50.

Hancock JT, Whiteman M (2014). Hydrogen Sulfide And Cell Signaling: Team Player or Referee? Plant Physiol Biochem 78: 37–42.

Hangaishi M, Ishizaka N, Aizawa T, Kurihara Y, Taguchi J, Nagai R, Kimura S, Ohno M (2000). Induction of Heme Oxygenase-1 Can Act Protectively against Cardiac Ischemia/Reperfusion *in Vivo*. Biochem Biophys Res Commun 279(2): 582–8.

Hargens AR, Akeson WH, Mubarak SJ, Owen CA, Evans KL, Garetto LP, Gonsalves MR, Schmidt DA (1978) Fluid Balance Within the Canine Anterolateral Compartment and its Relationship to Compartment Syndromes. J Bone Joint Surg Am 60(4): 499–505.

Hargens AR, Akeson WH, Mubarak SJ, Owen CA, Gershuni DH, Garfin SR, Lieber RL, Danzig LA, Botte MJ, Gelberman RH (1989). Kappa Delta Award Paper. Tissue Fluid Pressures: From Basic Research Tools To Clinical Applications. J Orthop Res 7(6): 902–9.

Hargens AR, Mubarak SJ (1998). Current Concepts in the Pathophysiology, Evaluation, and Diagnosis of Compartment Syndrome. Hand Clin 14(3): 371–83.

Hargens AR, Schmidt DA, Evans KL, Gonsalves MR, Cologne JB, Garfin SR, Mubarak SJ, Hagan PL, Akeson WH (1981). Quantitation of Skeletal-Muscle Necrosis in a Model Compartment Syndrome. J Bone Joint Surg Am 63(4): 631–6.

Hartsock LA, O'Farrell D, Seaber A V, Urbaniak JR (1998). Effect Of Increased Compartment Pressure On The Microcirculation Of Skeletal Muscle. Microsurgery 18(2): 67–71.

Harvey EJ, Sanders DW, Shuler MS, Lawendy A-R, Cole AL, Alqahtani SM, Schmidt AH (2012) What's New In Acute Compartment Syndrome? J Orthop Trauma 26(12): 699–702.

Hataishi R, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raher MJ, Furutani E, Ichinose F, Gladwin MT, Rosenzweig A, Zapol WM, Picard MH, Bloch KD, Scherrer-Crosbie M (2006). Inhaled Nitric Oxide Decreases Infarction Size and Improves Left Ventricular Function In A Murine Model of Myocardial Ischemia-Reperfusion Injury. Am J Physiol Heart Circ Physiol 291(1): H379–84.

Heckman MM, Whitesides TE, Grewe SR, Judd RL, Miller M, Lawrence JH (1993). Histologic Determination of The Ischemic Threshold of Muscle in the Canine Compartment Syndrome Model. J Orthop Trauma 7(3): 199–210.

Heemskerk J, Kitslaar P (2003). Acute Compartment Syndrome of the Lower Leg: Retrospective Study on Prevalence, Technique, and Outcome Of Fasciotomies. World J Surg 27(6): 744–7.

Henderson PW, Jimenez N, Ruffino J, Sohn AM, Weinstein AL, Krijgh DD, Reiffel AJ, Spector JA (2011). Therapeutic Delivery of Hydrogen Sulfide for Salvage of Ischemic Skeletal Muscle after the Onset of Critical Ischemia. J Vasc Surg 53(3): 785–91.

Henderson PW, Singh SP, Belkin D, Nagineni V, Weinstein AL, Weissich J, Spector JA (2010).Hydrogen Sulfide Protects Against Ischemia-Reperfusion Injury In an *in vitro* Model Of Cutaneous Tissue Transplantation. J Surg Res 159(1): 451–5.

Henderson PW, Singh SP, Weinstein AL, Nagineni V, Rafii DC, Kadouch D, Krijgh D, Spector J (2010). Therapeutic Metabolic Inhibition: Hydrogen Sulfide Significantly Mitigates Skeletal Muscle Ischemia Reperfusion Injury *in vitro and in vivo*. Plast Reconstr Surg 126(6): 1890–8.

Heppenstall RB, Sapega AA, Izant T, Fallon R, Shenton D, Park YS Chance B (1989). Compartment syndrome: a quantitative study of high-energy phosphorus compounds using 31P-magnetic resonance spectroscopy. J Trauma 29(8):1113–9.

Heppenstall RB, Sapega AA, Scott R, Shenton D, Park YS, Maris J, Chance B (1988). The compartment syndrome. An experimental and clinical study of muscular energy metabolism using phosphorus nuclear magnetic resonance spectroscopy. Clin Orthop Relat Res (226): 138–55.

Heppenstall RB, Scott R, Sapega A, Park YS, Chance B. A (1986). Comparative Study Of The Tolerance Of Skeletal Muscle To Ischemia. Tourniquet Application Compared With Acute Compartment Syndrome. J Bone Joint Surg Am 68(6): 820–8.

Hershey K. Fracture Complications (2013). Crit Care Nurs Clin North Am 25(2): 321–31.

Hiatt WR, Armstrong EJ, Larson CJ, Brass EP (2015). Pathogenesis of the Limb Manifestations and Exercise Limitations in Peripheral Artery Disease. Circ Res116(9): 1527–39.

Hickman P, Harrison DK, Hill A, Mclaren M, Tamei H, Mccollum PT, Belch JJ Exercise in Patients with Intermittent Claudication Results in the Generation of Oxygen Derived Free Radicals and Endothelial Damage. Adv Exp Med Biol 361: 565–70.

Hildebrand O (1906). Die Lehre von den ischämischen Muskellähmungen und Kontrakturen [Ischemic Muscle Paralysis and Contractures]. Samml Klin Vor 4337: 559–84.

Holwerda KM, Karumanchi SA, Lely AT (2015). Hydrogen Sulfide: Role in Vascular Physiology and Pathology. Curr Opin Nephrol Hypertens 24(2): 170–6.
Hoppeler H, Weibel ER (2005). Scaling Functions to Body Size: Theories and Facts. J Exp Biol 208(Pt 9): 1573–4.

Hosoki R, Matsuki N, Kimura H (1997). The Possible Role of Hydrogen Sulfide as an Endogenous Smooth Muscle Relaxant in Synergy with Nitric Oxide. Biochem Biophys Res Commun 237(3): 527–31.

Hu L-F, Wong PT-H, Moore PK, Bian J-S (2007). Hydrogen Sulfide Attenuates Lipopolysaccharide-Induced Inflammation By Inhibition Of P38 Mitogen-Activated Protein Kinase In Microglia. J Neurochem 100(4): 1121–8.

Hudlicka O (2011). Microcirculation in Skeletal Muscle. Muscles Ligaments Tendons J 1(1): 3–11.

Hughes MN, Centelles MN, Moore KP (2009). Making and Working with Hydrogen Sulfide: The Chemistry and Generation of Hydrogen Sulfide *in vitro* and its Measurement *in vivo*: A Review. Free Radic Biol Med 47(10): 1346–53.

Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA (2007). Life and Death: Metabolic Rate, Membrane Composition, and Life Span of Animals. Physiol Rev 87(4): 1175–213.

Huntington TW (1907). Ischemic Paralysis and Contracture. Cal State J Med (7):160–3.

Iannaccone PM, Jacob HJ (2009). Rats! Dis Model Mech 2(5-6): 206–10.

Ichimori K, Fukahori M, Nakazawa H, Okamoto K, Nishino T (1999). Inhibition of Xanthine Oxidase and Xanthine Dehydrogenase by Nitric Oxide. Nitric Oxide Converts Reduced Xanthine-Oxidizing Enzymes Into The Desulfo-Type Inactive Form. J Biol Chem 274(12): 7763–8.

Insko MA, Deckwerth TL, Hill P, Toombs CF, Szabo C (2009). Detection of Exhaled Hydrogen Sulphide Gas in Rats Exposed to Intravenous Sodium Sulphide. Br J Pharmacol 157(6): 944–51.

Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H (2009). A Source of Hydrogen Sulfide and a Mechanism of Its Release in the Brain. Antioxid Redox Signal 11(2):205–14.

Ishikawa M, Kajimura M, Adachi T, Maruyama K, Makino N, Goda N, Yamaguchi T, Sekizuka E, Suematsu M (2005). Carbon Monoxide From Heme Oxygenase-2 is a Tonic Regulator Against NO-Dependent Vasodilatation in The Adult Rat Cerebral Microcirculation. Circ Res 97(12): E104–14.

Jackson-Weaver O (2012). Mechanisms of Hydrogen Sulfide Induced Vasodilation. PhD University of New Mexico, Mexico

Jaffe R, Charron T, Puley G, Dick A, Strauss BH (2008). Microvascular Obstruction and the No-Reflow Phenomenon after Percutaneous Coronary Intervention. Circulation 117(24): 3152–6.

Jepson PN (1926). Ischaemic Contracture: Experimental Study. Ann Surg 84(6): 785–95.

Jha S, Calvert JW, Duranski MR, Ramachandran A, Lefer DJ (2008). Hydrogen Sulfide Attenuates Hepatic Ischemia-Reperfusion Injury: Role Of Antioxidant And Antiapoptotic Signaling. Am J Physiol Heart Circ Physiol 295(2): H801–6.

Jiang X, Wang X (2004). Cytochrome C-Mediated Apoptosis. Annu Rev Biochem 73: 87–106.

Johansen D, Ytrehus K, Baxter GF (2006).Exogenous Hydrogen Sulfide (H_2S) Protects Against Regional Myocardial Ischemia-Reperfusion Injury--Evidence for a Role Of K_{ATP} Channels. Basic Res Cardiol 101(1): 53–60.

Johnson EE, Srikanth C V, Sandgren A, Harrington L, Trebicka E, Wang L, Borregaard N, Murray M, Cherayil BJ (2010). Siderocalin Inhibits the Intracellular Replication of Mycobacterium Tuberculosis in Macrophages. FEMS Immunol Med Microbiol 58(1): 138–45.

Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P(1996). Apoptotic and Necrotic Myocyte Cell Deaths are Independent Contributing Variables of Infarct Size in Rats. Lab Invest 74(1):86–107.

Kalogeris T, Bao Y, Korthuis RJ (2014). Mitochondrial Reactive Oxygen Species: A Double Edged Sword in Ischemia/Reperfusion vs Preconditioning. Redox Biol 2: 702–14.

Kamath AF, Chauhan AK, Kisucka J, Dole VS, Loscalzo J, Handy DE, Wagner DD (2006). Elevated Levels of Homocysteine Compromise Blood-Brain Barrier Integrity in Mice. Blood 107(2): 591–3.

Kaschula CH, Hunter R, Hassan HT, Stellenboom N, Cotton J, Zhai XQ, Parker MI (2011). Anti-Proliferation Activity of Synthetic Ajoene Analogues on Cancer Cell-Lines. Anticancer Agents Med Chem 11(3): 260–6.

Kashfi K, Olson KR (2014). Biology and Therapeutic Potential of Hydrogen Sulfide and Hydrogen Sulfide-Releasing Chimeras. Biochem Pharmacol 85(5): 689–703.

Kearns SR, Daly AF, Sheehan K, Murray P, Kelly C, Bouchier-Hayes D. Oral vitamin C reduces the injury to skeletal muscle caused by compartment syndrome (2004). J Bone Joint Surg Br 86(6): 906–11.

Kearns SR, O'Briain DE, Sheehan KM, Kelly C, Bouchier-Hayes D (2010). N-Acetylcysteine Protects Striated Muscle in a Model of Compartment Syndrome. Clin Orthop Relat Res 468(8): 2251–9.

Khan MM (2008). Role of Cytokines. Immunopharmacology. Boston, MA: Springer USA.

Kharitonov VG, Sharma VS, Pilz RB, Magde D, Koesling D (1995). Basis of Guanylate Cyclase Activation by Carbon Monoxide. Proc Natl Acad Sci USA 92(7): 2568–71.

Kimura H (2002). Hydrogen Sulfide as a Neuromodulator. Mol Neurobiol 26(1): 13–9.

Kimura H (2010). Hydrogen Sulfide: From Brain To Gut. Antioxid Redox Signal 12(9): 1111–23.

Kimura H (2014). Production and Physiological Effects of Hydrogen Sulfide. Antioxid Redox Signal 20(5): 783–93.

Kimura H (2014). The Physiological Role of Hydrogen Sulfide and Beyond. Nitric Oxide [41: 1–7.

Kimura H, Nagai Y, Umemura K, Kimura Y (2005). Physiological Roles of Hydrogen Sulfide: Synaptic Modulation, Neuroprotection, and Smooth Muscle Relaxation. Antioxid Redox Signal 7(5-6): 795–803.

Kimura Y, Dargusch R, Schubert D, Kimura H (2006). Hydrogen Sulfide Protects HT22 Neuronal Cells From Oxidative Stress. Antioxid Redox Signal 8(3-4): 661–70.

Kimura Y, Goto Y-I, Kimura H (2010). Hydrogen Sulfide Increases Glutathione Production and Suppresses Oxidative Stress in Mitochondria. Antioxid Redox Signal 12(1): 1–13.

Kimura Y, Kimura H (2004). Hydrogen Sulfide Protects Neurons From Oxidative Stress. FASEB J 18(10):1165–7.

King AL, Lefer DJ (2011). Cytoprotective Actions Of Hydrogen Sulfide In Ischaemia-Reperfusion Injury. Exp Physiol 96(9): 840–6.

Kinmonth JB (1952). The Physiology and Relief of Traumatic Arterial Spasm. Br Med J 1(4749): 59–64.

Kirby NG (1970). Exercise Ischaemia In The Fascial Compartment Of Soleus. Report of a Case. J Bone Joint Surg Br 52(4): 738–40.

Klebanoff SJ (2005). Myeloperoxidase: Friend and Foe. J Leukoc Biol 77(5): 598–625.

Knauert M, Vangala S, Haslip M, Lee PJ (2013). Therapeutic Applications of Carbon Monoxide. Oxid Med Cell Longev 2013: 360815.

Kobayashi Y (2006). Neutrophil Infiltration and Chemokines. Crit Rev Immunol 26(4): 307–16.

Kodela R, Chattopadhyay M, Kashfi K (2012). NOSH-Aspirin: A Novel Nitric Oxide-Hydrogen Sulfide-Releasing Hybrid: A New Class of Anti-inflammatory Pharmaceuticals. ACS Med Chem Lett 3(3): 257–62.

Koenitzer JR, Isbell TS, Patel HD, Benavides GA, Dickinson DA, Patel RP, Darley-Usmar VM, Lancaster JR, Doeller JE, Kraus DW (2007).Hydrogen Sulfide Mediates Vasoactivity in an O_2-Dependent Manner. Am J Physiol Heart Circ Physiol 292(4): H1953–60.

Koj A, Frendo J, Janik Z (1967). [^{35}S]Thiosulphate Oxidation by Rat Liver Mitochondria in the Presence of Glutathione. Biochem J 103(3): 791–5.

Kolluru GK, Shen X, Bir SC, Kevil CG (2013). Hydrogen Sulfide Chemical Biology: Pathophysiological Roles and Detection. Nitric oxide 35: 5–20.

Korthuis RJ, Grisham MB, Granger DN (1988). Leukocyte Depletion Attenuates Vascular Injury in Postischemic Skeletal Muscle. Am J Physiol 254(5 Pt 2): H823–7.

Kubo S, Doe I, Kurokawa Y, Kawabata A (2007). Hydrogen Sulfide Causes Relaxation in Mouse Bronchial Smooth Muscle. J Pharmacol Sci 104(4):392–6.

Kuksis M, Smith PM, Ferguson A V (2014). Hydrogen Sulfide Regulates Cardiovascular Function by Influencing the Excitability of Subfornical Organ Neurons. PLoS One 9(8): e105772.

Kunkel MG, Lynn RB (1958). The Anterior Tibial Compartment Syndrome. Can J Surg 1(3): 212–7.

Kuo MC, Chien M, Ho CT (1990). Novel Polysulfides Identified in the Volatile Components from Welsh Onions (Allium Fistulosum L. Var. Maichuon) and Scallions (Allium Fistulosum L. Var. Caespitosum). J Agric Food Chem 38(6): 1378–81.

Laggner H, Muellner MK, Schreier S, Sturm B, Hermann M, Exner M, Gmeiner BMK, Kapiotis S (2007). Hydrogen Sulphide: A Novel Physiological Inhibitor of LDL Atherogenic Modification by Hocl. Free Radic Res 41(7):741–7.

Lam C, Tyml K, Martin C, Sibbald W (1994). Microvascular Perfusion Is Impaired In A Rat Model Of Normotensive Sepsis. J Clin Invest 94(5): 2077–83.

Lamas S, Marsden PA, Li GK, Tempst P, Michel T (1992). Endothelial Nitric Oxide Synthase: Molecular Cloning and Characterization of A Distinct Constitutive Enzyme Isoform. Proc Natl Acad Sci U S A 89(14): 6348–52.

Lawendy A-R (2014). Inflammatory Contribution to Cellular Injury in Compartment Syndrome; In Pathophysiology Of Compartment Syndrome. PhD. University of Western Ontario, Canada.

Lawendy A-R (2014). Pathophysiology of Compartment Syndrome. PhD. University of Western Ontario, Canada.

Lawendy A-R, Bihari A, Sanders DW, McGarr G, Badhwar A, Cepinskas G (2015). Contribution of Inflammation to Cellular Injury in Compartment Syndrome in an Experimental Rodent Model. Bone Joint J 97-B(4): 539–43.

Lawendy A-R, Bihari A, Sanders DW, Potter RF, Cepinskas G (2014). The Severity of Microvascular Dysfunction Due to Compartment Syndrome Is Diminished by the Systemic Application of CO-Releasing Molecule-3. J Orthop Trauma 28(11): e263–8.

Lawendy A-R, McGarr G, Phillips J, Sanders DW, Bihari A, Badhwar A (2011). Compartment Syndrome Causes A Systemic Inflammatory Response And Remote Organ Injury. J Bone Joint Surgery Br 93-B(SUPP III): 280.

Lawendy A-R, Sanders DW, Bihari A, Badhwar A (2011). Inflammation Causes Muscle Injury In Compartment Syndrome: An Experimental Study. J Bone Joint Surg Br 93-B(SUPP III): 243.

Lawendy A-R, Sanders DW, Bihari A, Parry N, Gray D, Badhwar A (2011). Compartment Syndrome-Induced Microvascular Dysfunction: An Experimental Rodent Model. Can J Surg 54(3): 194–200.

Lee M, McGeer E, Kodela R, Kashfi K, McGeer PL (2013). NOSH-Aspirin (NBS-1120), A Novel Nitric Oxide And Hydrogen Sulfide Releasing Hybrid, Attenuates Neuroinflammation Induced by Microglial and Astrocytic Activation: A New Candidate For Treatment of Neurodegenerative Disorders. Glia 61(10): 1724–34.

Lee Z, Zhou J, Chen C-S, Zhao Y, Tan C-H, Li L, Moore PK, Deng, L-W (2011).The Slow-Releasing Hydrogen Sulfide Donor, GYY4137, Exhibits Novel Anti-Cancer Effects *in vitro and in vivo*. Plos One 6(6): E21077.

Lefler SR, Lille ST, Huemer GM, Tucker R, Murray T, Schoeller T, Mulligan DC (2002). Activation Time Course of Activator Protein-1 and Effect of Proline Dithiocarbamate During Ischemia-Reperfusion in Rat Skeletal Muscle. Ann Plast Surg 49(6): 654–9.

Lejay A, Meyer A, Schlagowski A-I, Charles A-L, Singh F, Bouitbir J, Pottecher J, Chakfé N, Zoll, Joffrey, Geny B (2014). Mitochondria: Mitochondrial Participation In Ischemia-Reperfusion Injury In Skeletal Muscle. Int J Biochem Cell Biol 50: 101–5.

Ley K (2008). The Microcirculation in Inflammation. In: Tuma R, Durán W, Ley K, editors. Microcirculation, Second Edition, Elsevier.

Li C, Jackson RM (2002). Reactive Species Mechanisms Of Cellular Hypoxia-Reoxygenation Injury. Am J Physiol Cell Physiol 282(2): C227–41.

Li L, Hsu A, Moore PK (2009). Actions and Interactions of Nitric Oxide, Carbon Monoxide and Hydrogen Sulphide in the Cardiovascular System and In Inflammation--A Tale of Three Gases! Pharmacol Ther 123(3): 386–400.

Li L, Moore PK (2008). Putative Biological Roles of Hydrogen Sulfide in Health and Disease: A Breath of Not So Fresh Air? Trends Pharmacol Sci 29(2): 84–90.

Li L, Rossoni G, Sparatore A, Lee LC, Del Soldato P, Moore PK (2007). Anti-Inflammatory and Gastrointestinal Effects of a Novel Diclofenac Derivative. Free Radic Biol Med 42(5): 706–19.

Li L, Salto-Tellez M, Tan C-H, Whiteman M, Moore PK (2009). GYY4137, A Novel Hydrogen Sulfide-Releasing Molecule, Protects Against Endotoxic Shock in the Rat. Free Radic Biol Med 47(1): 103–13.

Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, Zhao Y, Baskar R, Tan C-H, Moore PK (2008). Characterization of a Novel, Water-Soluble Hydrogen Sulfide-Releasing Molecule (GYY4137): New Insights into the Biology of Hydrogen Sulfide. Circulation 117(18): 2351–60.

Lille ST, Lefler SR, Mowlavi A, Suchy H, Boyle EM, Farr AL, Su CY, Frank N, Mulligan DC (2001). Inhibition of the Initial Wave of NF-kappaB Activity in Rat Muscle Reduces Ischemia/Reperfusion Injury. Muscle Nerve24(4): 534–41.

Lin J-Q, Luo H-Q, Lin C-Z, Chen J-Z, Lin X-Z (2014). Sodium Hydrosulfide Relieves Neuropathic Pain In Chronic Constriction Injured Rats. Evid Based Complement Alternat Med 2014: 514898.

Lobb I, Mok A, Lan Z, Liu W, Garcia B, Sener A (2012). Supplemental Hydrogen Sulphide Protects Transplant Kidney Function And Prolongs Recipient Survival After Prolonged Cold Ischaemia-Reperfusion Injury By Mitigating Renal Graft Apoptosis And Inflammation. BJU Int 110(11 Pt C): E1187–95.

Lobb I, Sonke E, Aboalsamh G, Sener A (2014). Hydrogen Sulphide and the Kidney: Important Roles in Renal Physiology and Pathogenesis and Treatment of Kidney Injury and Disease. Nitric Oxide 46(46): 55–65.

Lobb I, Zhu J, Liu W, Haig A, Lan Z, Sener A (2014). Hydrogen Sulfide Treatment Ameliorates Long-Term Renal Dysfunction Resulting From Prolonged Warm Renal Ischemia-Reperfusion Injury. Can Urol Assoc J 8(5-6): E413–8.

Loerakker S, Oomens CWJ, Manders E, Schakel T, Bader DL, Baaijens FPT, Nicolay K, Strijkers GJ (2011). Ischemia-Reperfusion Injury in Rat Skeletal Muscle Assessed With T2-Weighted and Dynamic Contrast-Enhanced MRI. Magn Reson Med 66(2): 528–37.

Lotfi S, Patel AS, Mattock K, Egginton S, Smith A, Modarai B (2013). Towards a More Relevant Hind Limb Model of Muscle Ischaemia. Atherosclerosis 227(1): 1–8.

Ma A, Qi S, Chen H (2008). Antioxidant Therapy for Prevention of Inflammation, Ischemic Reperfusion Injuries And Allograft Rejection. Cardiovasc Hematol Agents Med Chem 6(1): 20–43.

Mabee JR, Bostwick TL (1993). Pathophysiology and Mechanisms of Compartment Syndrome. Orthop Rev 22(2): 175–81.

Maines MD (1997). The Heme Oxygenase System: A Regulator of Second Messenger Gases. Annu Rev Pharmacol Toxicol 37: 517–54.

Maksimenko A V, Turashev AD (2012). No-Reflow Phenomenon and Endothelial Glycocalyx of Microcirculation. Biochem Res Int 2012: 859231.

Malik AA, Khan WSA, Chaudhry A, Ihsan M, Cullen NP (2009). Acute Compartment Syndrome--A Life and Limb Threatening Surgical Emergency. J Perioper Pract 19(5):137–42.

Mancardi D, Penna C, Merlino A, Del Soldato P, Wink D a, Pagliaro P (2009). Physiological and Pharmacological Features of the Novel Gasotransmitter: Hydrogen Sulfide. Biochim Biophys Acta 1787(7): 864–72.

Mani S, Cao W, Wu L, Wang R (2014). Hydrogen Sulfide and the Liver. Nitric Oxide 41:62–71.

Manjoo A, Sanders D, Lawendy A, Gladwell M, Gray D, Parry N, Badhwar A (2010). Indomethacin Reduces Cell Damage: Shedding New Light On Compartment Syndrome. J Orthop Trauma 24(9): 526–9.

Mariggiò MA, Minunno V, Riccardi S, Santacroce R, De Rinaldis P, Fumarulo R (1998). Sulfide Enhancement of PMN Apoptosis. Immunopharmacol Immunotoxicol 20(3): 399–408.

Marks GS, Brien JF, Nakatsu K, McLaughlin BE (1991). Does Carbon Monoxide Have a Physiological Function? Trends Pharmacol Sci 12(5): 185–8.

Marquet PA, Quiñones RA, Abades S, Labra F, Tognelli M, Arim M, Rivadeneira M (2005). Scaling and Power-Laws in Ecological Systems. J Exp Biol 208(Pt 9): 1749–69.

Mars M, Hadley GP (1998). Raised Intracompartmental Pressure And Compartment Syndromes. Injury 29(6): 403–11.

Marutani E, Kosugi S, Tokuda K, Khatri A, Nguyen R, Atochin DN, Kida K,
Van Leyen K, Arai K, Ichinose F (2012). A Novel Hydrogen Sulfide-Releasing N-Methyl-D-Aspartate Receptor Antagonist Prevents Ischemic Neuronal Death. J Biol Chem 287(38): 32124–35.

Matsen FA (1975). Compartmental syndrome. An unified concept. Clin Orthop Relat Res (113): 8–14.

Matsen FA, Clawson DK (1975). The Deep Posterior Compartmental Syndrome of The Leg. J Bone Joint Surg Am 57(1):34–9.

Matsen FA, Krugmire RB, King R V. Nicolas A (1979). Increased Tissue Pressure and its Effects on Muscle Oxygenation in Level and Elevated Human Limbs. Clin Orthop Relat Res 144: 311–20.

Matsen FA, Mayo KA, Krugmire RB, Sheridan GW, Kraft GH (1977). A Model Compartmental Syndrome in Man with Particular Reference to the Quantification of Nerve Function. J Bone Joint Surg Am 59(5): 648–53.

Matsen FA, Rorabeck CH (1989). Compartment Syndromes. Instr Course Lect 38: 463–72.

Matsen FA, Winquist RA, Krugmire RB (1980). Diagnosis and management of compartmental syndromes. J Bone Joint Surg Am 62(2): 286–91.

McCarberg BH, Cryer B (2014). Evolving Therapeutic Strategies to Improve Nonsteroidal Anti-inflammatory Drug Safety. Am J Ther (Epub ahead of print).

Mccully JD, Wakiyama H, Hsieh Y-J, Jones M, Levitsky S (2004). Differential Contribution of Necrosis and Apoptosis in Myocardial Ischemia-Reperfusion Injury. Am J Physiol Heart Circ Physiol 286(5): H1923–35.

McGarr GW (2009). Mechanisms of Inflammation in Compartment Syndrome. MSc. University of Western Ontario, Canada.

McIlwain DR, Berger T, Mak TW (2013). Caspase Functions in Cell Death and Disease. Cold Spring Harb Perspect Biol 5(4): a008656.

McQueen MM, Court-Brown CM. Compartment monitoring in tibial fractures (1996). The pressure threshold for decompression. J Bone Joint Surg Br 78(1):99–104.

McQueen MM, Duckworth AD (2014). The Diagnosis Of Acute Compartment Syndrome: A Review. Eur J Trauma Emerg Surg 40(5): 521–8.

McQueen MM, Gaston P, Court-Brown CM (2000). Acute Compartment Syndrome: Who Is At Risk? J Bone Joint Surg Br 82-B(2): 200–3.

Meister A, Fraser PE, Tice S V (1954). Enzymatic Desulfuration of Beta-Mercaptopyruvate to Pyruvate. J Biol Chem 206(2): 561–75.

Menger MD, Messmer K (1993). [Microcirculation Of Skeletal Muscle After Ischemia And Reperfusion]. Wiener medizinische Wochenschrift 143(7-8): 148–58.

Menger MD, Pelikan S, Steiner D, Messmer K (1992). Microvascular Ischemia-Reperfusion Injury in Striated Muscle: Significance of “Reflow Paradox.” Am J Physiol Hear Circ Physiol 263(6): H1901–6.

Michel T, Feron O (1997). Nitric Oxide Synthases: Which, Where, How, and Why? J Clin Invest 100(9): 2146–52.

Moody BF, Calvert JW (2011). Emergent Role of Gasotransmitters in Ischemia-Reperfusion Injury. Med Gas Res 1(1): 3.

Mortez WH (1953). The Anterior Compartment (Anterior Tibial) Ischemia Syndrome. Am Surg 19(8): 728–49.

Motterlini R, Foresti R (2014). Heme Oxygenase-1 as a Target for Drug Discovery. Antioxid Redox Signal 20(11): 1810–26.

Motterlini R, Otterbein LE (2010). The Therapeutic Potential of Carbon Monoxide. Nat Rev Drug Discov 9(9): 728–43.

Mubarak SJ, Owen CA (1977). Double-Incision Fasciotomy Of The Leg For Decompression In Compartment Syndromes. J Bone Joint Surg Am 59(2): 184–7.

Mubarak SJ, Owen CA, Hargens AR, Garetto LP, Akeson WH (1978). Acute Compartment Syndromes: Diagnosis and Treatment With the Aid of The Wick Catheter. J Bone Joint Surg Am 60(8): 1091–5.

Mubarak SJJ, Pedowitz RAA, Hargens ARR (1989). Compartment Syndromes. Curr Orthop 3(1): 36–40.

Munday R (2012). Harmful and beneficial effects of organic monosulfides, disulfides, and polysulfides in animals and humans. Chem Res Toxicol 25(1): 47–60.

Murphy J (1914). Myositis. JAMA 63(15): 1249–55.

Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, Amzel, LM, Berkowitz DE, Snyder SH (2011) Hydrogen Sulfide As Endothelium-Derived Hyperpolarizing Factor Sulfhydrates Potassium Channels. Circ Res 109(11): 1259–68.

Mustoe TA, O'Shaughnessy K, Kloeters O (2006). Chronic Wound Pathogenesis and Current Treatment Strategies: A Unifying Hypothesis. Plast Reconstr Surg 117(7 Suppl): 35S – 41S.

Muzaffar S, Shukla N, Bond M, Newby AC, Angelini GD, Sparatore A, *Del* Soldato P, Jeremy, JY (2008). Exogenous Hydrogen Sulfide Inhibits Superoxide Formation, NOX-1 Expression And Rac1 Activity in Human Vascular Smooth Muscle Cells. J Vasc Res 45(6): 521–8.

Nagy P, Pálinkás Z, Nagy A, Budai B, Tóth I, Vasas A (2014). Chemical Aspects of Hydrogen Sulfide Measurements In Physiological Samples. Biochim Biophys Acta 1840(2): 876–91.

Nakao A, Neto JS, Kanno S, Stolz DB, Kimizuka K, Liu F, Bach FH,
Billiar TR, Choi AMk, Otterbein LE, Murase N (2005). Protection Against Ischemia/Reperfusion Injury In Cardiac And Renal Transplantation With Carbon Monoxide, Biliverdin And Both. Am J Transplant 5(2): 282–91.

Namazi H (2008). The Role of Garlic in the Prevention of Ischemia-Reperfusion Injury: A New Mechanism. Mol Nutr Food Res 52(6): 739; author reply 740.

Nanobashvili J, Neumayer C, Fuegl A, Blumer R, Prager M, Sporn E, Polterauer P, Malinski T, Huk I (2003). Development of "No-Reflow" Phenomenon in Ischemia/Reperfusion Injury: Failure of Active Vasomotility and Not Simply Passive Vasoconstriction. Eur Surg Res 35(5): 417–24.

Niccoli G, Giubilato S, Russo E, Spaziani C, Leo A, Porto I, Leone AM, Burzotta F, Riondino S, Pulcinelli F, Biasucci LM, Crea F (2008). Plasma Levels of Thromboxane A2 on Admission are Associated With No-Reflow after Primary Percutaneous Coronary Intervention. Eur Heart J 29(15): 1843–50.

Nichols TC (2004). NF-kappaB and Reperfusion Injury. Drug News Perspect 17(2): 99–104.

Nicholson CK, Calvert JW (2010). Hydrogen Sulfide and Ischemia-Reperfusion Injury. Pharmacol Res 62(4): 289–97.

Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N (2013). Crosstalk Between Apoptosis, Necrosis And Autophagy. Biochim Biophys Acta 1833(12): 3448–59.

Nishida M, Sawa T, Kitajima N, Ono K, Inoue H, Ihara H, Leone AM, Burzotta F, Riondino S, Pulcinelli F, Biasucci LM, Crea F (2012). Hydrogen Sulfide Anion Regulates Redox Signaling Via Electrophile Sulfhydration. Nat Chem Biol 8(8): 714–24.

Ogura S, Shimosawa T (2014). Oxidative Stress and Organ Damages. Curr Hypertens Rep 16(8):452.

Oh G-S, Pae H-O, Lee B-S, Kim B-N, Kim J-M, Kim H-R, Jeon SB, Jeon WK, Chae H-J, Chung H-T (2006). Hydrogen Sulfide Inhibits Nitric Oxide Production and Nuclear Factor-Kappa-b Via Heme Oxygenase-1 Expression in RAW264.7 Macrophages Stimulated With Lipopolysaccharide. Free Radic Biol Med 41(1): 106–19.

Olivas TP, Saylor TF, Wong HP, Stephenson LL, Zamboni WA (2001). Timing of Microcirculatory Injury from Ischemia Reperfusion. Plast Reconstr Surg 107(3): 785–8.

Olson KR (2009). Is Hydrogen Sulfide A Circulating “Gasotransmitter” In Vertebrate Blood? Biochim Biophys Acta 1787(7): 856–63.

Olson SA, Glasgow RR (2005). Acute Compartment Syndrome In Lower Extremity Musculoskeletal Trauma. J Am Acad Orthop Surg 13(7): 436–44.

Omura T (2005). Heme-Thiolate Proteins. Biochem Biophys Res Commun 338(1): 404–9.

Opal SM, DePalo VA (2000). Anti-Inflammatory Cytokines. Chest 117(4): 1162–72.

Osipov RM, Robich MP, Feng J, Liu Y, Clements RT, Glazer HP, Sodha N R, Szabo C, Bianchi C, Sellke FW (2009). Effect of Hydrogen Sulfide in A Porcine Model of Myocardial Ischemia-Reperfusion: Comparison of Different Administration Regimens and Characterization of the Cellular Mechanisms of Protection. J Cardiovasc Pharmacol 54(4): 287–97.

Ott MC, Scott JR, Bihari A, Badhwar A, Otterbein LE, Gray DK, Harris K A, Potter RF (2005). Inhalation of Carbon Monoxide Prevents Liver Injury and Inflammation Following Hind Limb Ischemia/Reperfusion. FASEB J 19(1): 106–8.

Ozaki KS, Kimura S, Murase N (2012). Use of Carbon Monoxide in Minimizing Ischemia/Reperfusion Injury in Transplantation. Transplant Rev (Orlando) 26(2): 125–39.

Pacher P, Beckman JS, Liaudet L (2007). Nitric Oxide and Peroxynitrite in Health and Disease. Physiol Rev 87(1): 315–424.

Pacher P, Nivorozhkin A, Szabó C (2006). Therapeutic Effects of Xanthine Oxidase Inhibitors: Renaissance Half A Century After the Discovery of Allopurinol. Pharmacol Rev 58(1): 87–114.

Padiya R, Khatua TN, Bagul PK, Kuncha M, Banerjee SK (2011). Garlic Improves Insulin Sensitivity and Associated Metabolic Syndromes in Fructose Fed Rats. Nutr Metab (Lond) 8(1): 53.

Pálinkás Z, Furtmüller PG, Nagy A, Jakopitsch C, Pirker KF, Magierowski M, Jasnos K, Wallace JL, Obinger C, Nagy P (2014). Interactions of Hydrogen Sulfide with Myeloperoxidase. Br J Pharmacol 172(6): 1516–32.

Papapetropoulos A, Whiteman M, Cirino G (2014). Pharmacological Tools for Hydrogen Sulphide Research: A Brief, Introductory Guide for Beginners. Br J Pharmacol 14: 1–5.

Patel R, Albadawi H, Steudel W, Hashmi FF, Kang J, Yoo H-J, Watkins MT (2012). Inhalation of Carbon Monoxide Reduces Skeletal Muscle Injury After Hind Limb Ischemia-Reperfusion Injury in Mice. Am J Surg 203(4): 488–95.

Pearse MF (2002). Acute Compartment Syndrome Of The Leg: Fasciotomies Must Be Performed Early, But Good Surgical Technique Is Important. BMJ 325(7364): 557–8.

Perler BA, Tohmeh AG, Bulkley GB (1990). Inhibition of the compartment syndrome by the ablation of free radical-mediated reperfusion injury. Surgery 108(1): 40–7.

Potter RF, Dietrich HH, Tyml K, Ellis CG, Cronkwright J, Groom AC (1993). Ischemia-Reperfusion Induced Microvascular Dysfunction In Skeletal Muscle: Application Of Intravital Video Microscopy. Int J Microcirc Clin Exp 13(3): 173–86.

Potter RF, Peters G, Carson M, Forbes T, Ellis CG, Harris KA, DeRose G, Jamieson W G (1995). Measurement of Tissue Viability Using Intravital Microscopy and Fluorescent Nuclear Dyes. J Surg Res59(5):521–6.

Predmore BL, Kondo K, Bhushan S, Zlatopolsky MA, King AL, Aragon JP, Grinsfelder DB, Condit ME, Lefer DJ (2012).The Polysulfide Diallyl Trisulfide Protects the Ischemic Myocardium by Preservation of Endogenous Hydrogen Sulfide and Increasing Nitric Oxide Bioavailability. Am J Physiol Heart Circ Physiol 302(11): H2410–8.

Primeau AJ, Adhihetty PJ, Hood DA (2002). Apoptosis in Heart and Skeletal Muscle. Can J Appl Physiol 27(4): 349–95.

Qingyou Z, Junbao D, Weijin Z, Hui Y, Chaoshu T, Chunyu Z (2004). Impact of Hydrogen Sulfide on Carbon Monoxide/Heme Oxygenase Pathway in the Pathogenesis Of Hypoxic Pulmonary Hypertension. Biochem Biophys Res Commun 317(1): 30–7.

Radermacher P, Haouzi P (2013). A Mouse is Not a Rat is Not a Man: Species-Specific Metabolic Responses to Sepsis - A Nail in the Coffin of Murine Models for Critical Care Research? Intensive Care Med Exp 1(1): 7.

Ragland R, Moukoko D, Ezaki M, Carter PR, Mills J (2005). Forearm compartment syndrome in the newborn: report of 24 cases. J Hand Surg Am 30(5): 997–1003.

Reffelmann T, Kloner RA (2006). The No-Reflow Phenomenon: A Basic Mechanism of Myocardial Ischemia and Reperfusion. Basic Res Cardiol 101(5): 359–72.

Reiffenstein RJ, Hulbert WC, Roth SH (1992). Toxicology of Hydrogen Sulfide. Annu Rev Pharmacol Toxicol 32(5): 109–34.

Reis ND, Michaelson M. Crush injury to the lower limbs (1986). Treatment of The Local Injury. J Bone Joint Surg Am 68(3): 414–8.

Reneman RS, Slaaf DW, Lindbom L, Tangelder GJ, Arfors KE (1980). Muscle Blood Flow Disturbances Produced by Simultaneously Elevated Venous and Total Muscle Tissue Pressure. Microvasc Res 20(3): 307–18.

Ricci MA, Graham AM, Corbisiero R, Baffour R, Mohamed F, Symes JF (1989). Are Free Radical Scavengers Beneficial in the Treatment Of Compartment Syndrome After Acute Arterial Ischemia? J Vasc Surg 9(2): 244–50.

Riedl SJ, Shi Y (2004). Molecular Mechanisms Of Caspase Regulation During Apoptosis. Nat Rev Mol Cell Biol 5(11): 897–907.

Rinaldi L, Gobbi G, Pambianco M, Micheloni C, Mirandola P, Vitale M (2006). Hydrogen Sulfide Prevents Apoptosis of Human PMN Via Inhibition of P38 and Caspase 3. Lab Invest 86(4): 391–7.

Ritenour AE, Dorlac WC, Fang R, Woods T, Jenkins DH, Flaherty SF, Wade CE, Holcomb JB (2008). Complications after Fasciotomy Revision and Delayed Compartment Release in Combat Patients. J Trauma 64(2 Suppl): S153–61; Discussion S161–2.

Robinson JM (2009). Phagocytic Leukocytes and Reactive Oxygen Species. Histochem Cell Biol 131(4): 465–9.

Rorabeck CH, Clarke KM (1978). The Pathophysiology Of The Anterior Tibial Compartment Syndrome: An Experimental Investigation. J Trauma 18(5): 299–304.

Rorabeck CH. The treatment of compartment syndromes of the leg (1984). J Bone Joint Surg Br 66(1): 93–7.

Rowlands R (1905). A Case Of Volkmann's Contracture Treated By Shortening The Radius And Ulna. Lancet 166(4286): 1168–71.

Rüdiger HA, Clavien P-A (2002). Tumor Necrosis Factor Alpha, but not Fas, Mediates Hepatocellular Apoptosis in the Murine Ischemic Liver. Gastroenterology 122(1): 202–10.

Ryter SW, Alam J, Choi AMK (2006). Heme Oxygenase-1/Carbon Monoxide: From Basic Science To Therapeutic Applications. Physiol Rev 86(2): 583–650.

Ryter SW, Otterbein LE (2004). Carbon Monoxide in Biology and Medicine. Bioessays 26(3): 270–80.

Sabido F, Milazzo VJ, Hobson RW, Duran WN (1994). Skeletal Muscle Ischemia-Reperfusion Injury: A Review of Endothelial Cell-Leukocyte Interactions. J Invest Surg 7(1): 39–47.

Sadasivan KK, Carden DL, Moore MB, Korthuis RJ (1997). Neutrophil mediated microvascular injury in acute, experimental compartment syndrome. Clin Orthop Relat Res (339): 206–15.

Saelens X, Festjens N, Vande Walle L, Van Gurp M, Van Loo G, Vandenabeele P (2004). Toxic Proteins Released from Mitochondria in Cell Death. Oncogene 23(16): 2861–74.

Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AM, Bach FH, Soares MP (2001). Carbon Monoxide Generated by Heme Oxygenase-1 Suppresses the Rejection of Mouse-to-Rat Cardiac Transplants. J Immunol 166(6): 4185–94.

Sayre RH (1908). “Volkmann’s Ischemic Paralysis and Contracture. Am J Orthop Surg 26(2): 221–33.

Scarabelli TM, Knight R, Stephanou A, Townsend P, Chen-Scarabelli C, Lawrence K, Gottlieb R, Latchman D, Narula J (2006). Clinical Implications of Apoptosis in Ischemic Myocardium. Curr Probl Cardiol 31(3): 181–264.

Schallner N, Romão CC, Biermann J, Lagrèze WA, Otterbein LE, Buerkle H, Loop T, Goebe lU (2013). Carbon Monoxide Abrogates Ischemic Insult To Neuronal Cells Via The Soluble Guanylate Cyclase-Cgmp Pathway. Plos One 8(4): E60672.

Schoen M, Rotter R, Gierer P, Gradl G, Strauss U, Jonas L, Mittlmeier T, Vollmar B (2007). Ischemic Preconditioning Prevents Skeletal Muscle Tissue Injury, but Not Nerve Lesion Upon Tourniquet-Induced Ischemia. J Trauma 63(4): 788–97.

Schofield ZV, Woodruff TM, Halai R, Wu MC-L, Cooper MA (2013). Neutrophils: A Key Component of Ischemia-Reperfusion Injury. Shock 40(6): 463–70.

Schreier SM, Muellner MK, Steinkellner H, Hermann M, Esterbauer H, Exner M, Gmeiner BMK, Kapiotis S, Laggner H (2010). Hydrogen Sulfide Scavenges the Cytotoxic Lipid Oxidation Product 4-HNE. Neurotox Res17(3): 249–56.

Schwabe A, Rybakova KN, Bruggeman FJ (2012). Transcription Stochasticity of Complex Gene Regulation Models. Biophys J 103(6): 1152–61.

Scott JR, Cukiernik MA, Ott MC, Bihari A, Badhwar A, Gray DK, Harris KA, Parry NG, Potter RF (2009). Low-Dose Inhaled Carbon Monoxide Attenuates the Remote Intestinal Inflammatory Response Elicited by Hindlimb Ischemia-Reperfusion. Am J Physiol Gastrointest Liver Physiol 296(1): G9–14.

Sellei RM, Hildebrand F, Pape H-C (2014). [Acute extremity compartment syndrome: current concepts in diagnostics and therapy]. Unfallchirurg 117(7): 633–49.

Semenza GL (1999). Perspectives on oxygen sensing. Cell 281–4.

Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, Kim S, Snyder SH (2012). Hydrogen Sulfide-Linked Sulfhydration of NF-Kb Mediates Its Antiapoptotic Actions. Mol Cell 45(1): 13–24.

Sener G, Sakarcan A, Yegen BC (2007). Role of Garlic in the Prevention of Ischemia- Reperfusion Injury. Mol Nutr Food Res 51(11): 1345–52.

Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LAJ, Perretti M, Rossi AG, Wallace JL (2007). Resolution of Inflammation: State Of The Art, Definitions and Terms. FASEB J 21(2): 325–32.

Sha L, Linden DR, Farrugia G, Szurszewski JH (2014). Effect of Endogenous Hydrogen Sulfide on the Transwall Gradient of the Mouse Colon Circular Smooth Muscle. J Physiol [592(Pt 5): 1077–89.

Shadgan B, Menon M, Sanders D, Berry G, Martin C, Duffy P, Stephen D, O'Brien PJ (2010). Current thinking about acute compartment syndrome of the lower extremity. Can J Surg 53(5): 329–34.

Shaw CJ, Spencer JD (1995). Late management of compartment syndromes. Injury 26(9):633–5.

Sheridan GW, Matsen FA (1976). Fasciotomy in the Treatment of the Acute Compartment Syndrome. J Bone Joint Surg Am 58(1): 112–5.

Sheridan GW, Matsen FA, Krugmire RB (1977). Further Investigations on the Pathophysiology of the Compartmental Syndrome. Clin Orthop Relat Res (123): 266–70.

Sherwood ER, Toliver-Kinsky T (2004). Mechanisms of the Inflammatory Response. Best Pract Res Clin Anaesthesiol 18(3): 385–405.

Sidhapuriwala J, Li L, Sparatore A, Bhatia M, Moore PK (2007). Effect of S-Diclofenac, a Novel Hydrogen Sulfide Releasing Derivative, on Carrageenan-Induced Hindpaw Oedema Formation in The Rat. Eur J Pharmacol 569(1-2): 149–54.

Siegfried MR, Carey C, Ma XL, Lefer AM (1992). Beneficial Effects of SPM-5185, a Cysteine-Containing NO Donor in Myocardial Ischemia-Reperfusion. Am J Physiol 263(3 Pt 2): H771–7.

Sirbu AB, Murphy MJ, White AS (1944). Soft Tissue Complications of Fractures of the Leg. Cal West Med 60(2): 53–6.

Siriussawakul A, Chen LI, Lang JD (2012). Medical Gases: A Novel Strategy for Attenuating Ischemia-Reperfusion Injury in Organ Transplantation? J Transplant 2012: 819382.

Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, Cuzzocrea S, Fantozzi R, Thiemermann C (2009). Anti-Apoptotic and Anti-Inflammatory Effects of Hydrogen Sulfide in a Rat Model of Regional Myocardial I/R. Shock 31(3): 267–74.

Sluiter E (1930). The Production of Hydrogen Sulphide by Animal Tissues. Biochem J.;24(2):549–63.

Smith HS (2009). Hydrogen Sulfide's Involvement In Modulating Nociception. Pain Physician 12(5): 901–10.

Smith JK, Carden DL, Korthuis RJ (1989). Role of Xanthine Oxidase in Postischemic Microvascular Injury in Skeletal Muscle. Am J Physiol 257(6 Pt 2): H1782–9.

Snijder PM, van den Berg E, Whiteman M, Bakker SJL, Leuvenink HGD, van Goor H (2013). Emerging Role of Gasotransmitters In Renal Transplantation. Am J Transplant 13(12): 3067–75.

Sparatore A, Perrino E, Tazzari V, Giustarini D, Rossi R, Rossoni G, Erdmann K, Erdman K, Schröder H, Del Soldato P (2009). Pharmacological Profile of a Novel H(2)S-Releasing Aspirin. Free Radic Biol Med 46(5): 586–92.

Stipanuk MH, Beck PW (1982). Characterization of the Enzymic Capacity for Cysteine Desulphhydration in Liver and Kidney of the Rat. Biochem J 206(2): 267–77.

Strauss MB (2012). The Effect of Hyperbaric Oxygen in Crush Injuries and Skeletal Muscle-Compartment Syndromes. Undersea Hyperb Med 39(4): 847–55.

Stubbert D, Prysyazhna O, Rudyk O, Scotcher J, Burgoyne JR, Eaton P (2014). Protein Kinase G Iα Oxidation Paradoxically Underlies Blood Pressure Lowering By The Reductant Hydrogen Sulfide. Hypertension 64(6): 1344–51.

Styf J (2003). Compartment Syndromes : Diagnosis, Treatment, and Complications. Bosa Roca: Taylor & Francis Inc. United States.

Suarez F, Furne J, Springfield J, Levitt M (1997). Insights Into Human Colonic Physiology Obtained from the Study of Flatus Composition. Am J Physiol 272(5 Pt 1): G1028–33.

Suarez FL, Furne JK, Springfield J, Levitt MD (2000). Morning Breath Odor: Influence of Treatments on Sulfur Gases. J Dent Res 79(10): 1773–7.

Suliman HB, Carraway MS, Tatro LG, Piantadosi CA (2007). A New Activating Role for CO in Cardiac Mitochondrial Biogenesis. J Cell Sci 120(Pt 2): 299–308.

Summers WK, Jamison RL (1971). The No-Reflow Phenomenon in Renal Ischemia. Lab Invest 25(6): 635–43.

Sun L, Wang X (2014). A New Kind of Cell Suicide: Mechanisms and Functions of Programmed Necrosis. Trends Biochem Sci (12): 587–93.

Sun W-H, Liu F, Chen Y, Zhu Y-C (2012). Hydrogen Sulfide Decreases The Levels of ROS by Inhibiting Mitochondrial Complex IV and Increasing SOD Activities in Cardiomyocytes Under Ischemia/Reperfusion. Biochem Biophys Res Commun 421(2):164–9.

Szabo C, Ransy C, Módis K, Andriamihaja M, Murghes B, Coletta C, Olah G, Yanagi K, Bouillaud F (2014). Regulation of Mitochondrial Bioenergetic Function by Hydrogen Sulfide. Part I. Biochemical and Physiological Mechanisms. Br J Pharmacol 171(8): 2099–122.

Tan BH, Wong PT-H, Bian J-S (2010). Hydrogen Sulfide: A Novel Signaling Molecule in the Central Nervous System. Neurochem Int 56(1): 3–10.

Tan S, Gelman S, Wheat JK, Parks DA (1995). Circulating xanthine oxidase in human ischemia reperfusion. South Med J 88(4): 479–82.

Tang G, Yang G, Jiang B, Ju Y, Wu L, Wang R (2013). H_2S is an Endothelium-Derived Hyperpolarizing Factor. Antioxid Redox Signal 19(14): 1634–46.

Taoka S, Banerjee R (2001). Characterization of NO Binding to Human Cystathionine B-Synthase: J Inorg Biochem 87(4): 245–51.

Taylor RM, Sullivan MP, Mehta S (2012). Acute compartment syndrome: obtaining diagnosis, providing treatment, and minimizing medicolegal risk. Curr Rev Musculoskelet Med 5(3): 206–13.

The Nobel Foundation (2015). Nobel Prizes in Physiology or Medicine 1998. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1998/

Thomas JJ (1909). III. Nerve Involvement in the Ischaemic Paralysis and Contracture of Volkmann. Ann Surg 49(3): 330–70.

Tjian R (1996). The Biochemistry of Transcription in Eukaryotes: A Paradigm for Multisubunit Regulatory Complexes. Philos Trans R Soc Lond B Biol Sci 351(1339): 491–9.

Tollens T, Janzing H, Broos P (1998). The Pathophysiology of the Acute Compartment Syndrome. Acta Chir Belg 98(4): 171–5.

Toyokuni S (1999). Reactive Oxygen Species-Induced Molecular Damage and its Application in Pathology. Pathol Int 49(2): 91–102.

Trevisani M, Patacchini R, Nicoletti P, Gatti R, Gazzieri D, Lissi N, Zagli G, Creminon C, Geppetti P, Harrison S (2005). Hydrogen Sulfide Causes Vanilloid Receptor 1-Mediated Neurogenic Inflammation in The Airways. Br J Pharmacol 145(8): 1123–31.

Tsubura A, Lai Y-C, Kuwata M, Uehara N, Yoshizawa K (2011). Anticancer Effects of Garlic and Garlic-Derived Compounds for Breast Cancer Control. Anticancer Agents Med Chem 11(3):249–53.

Tulis DA, Durante W, Peyton KJ, Evans AJ, Schafer AI (2001). Heme Oxygenase-1 Attenuates Vascular Remodeling Following Balloon Injury in Rat Carotid Arteries. Atherosclerosis 155(1): 113–22.

Tyagi N, Moshal KS, Sen U, Vacek TP, Kumar M, Hughes WM,
Kundu S, Tyagi SC (2009). H_2S Protects Against Methionine-Induced Oxidative Stress in Brain Endothelial Cells. Antioxid Redox Signal 11(1): 25–33.

Tyml K, Budreau CH (1991). A New Preparation of Rat Extensor Digitorum Longus Muscle For Intravital Investigation of the Microcirculation. Int J Microcirc Clin Exp 10(4): 335–43.

Vaillancourt C, Shrier I, Vandal A, Falk M, Rossignol M, Vernec A, Somogyi D (2004). Acute Compartment Syndrome: How Long Before Muscle Necrosis Occurs? CJEM 6(3): 147–54.

Veeranki S, Tyagi SC (2014). Role of Hydrogen Sulfide in Skeletal Muscle Biology And Metabolism. Nitric Oxide 46:66–71.

Vogt M, Bauer MK, Ferrari D, Schulze-Osthoff K (1998). Oxidative Stress and Hypoxia/Reoxygenation Trigger CD95 (APO-1/Fas) Ligand Expression in Microglial Cells. FEBS Lett 429(1): 67–72.

Volkmann R (1881). Die Ischaemischen Muskellähmungen Und-Kontrakturen.[Ischemic Muscle Paralysis and Contractures]. Centrabl f Chir 8(51): 801–3.

Volkmann R (2007). Ischaemic Muscle Paralyses and Contractures 1881. Clin Orthop Relat Res 456: 20–1.

Wagner F, Asfar P, Calzia E, Radermacher P, Szabó C (2009). Bench-to-Bedside Review: Hydrogen Sulfide--the third Gaseous Transmitter: Applications for Critical Care. Crit Care 13(3):213.

Wallace JL, Ferraz JGP, Muscara MN (2012). Hydrogen Sulfide: An Endogenous Mediator Of Resolution Of Inflammation And Injury. Antioxid Redox Signal 17(1): 58–67.

Wang J, Chen S, Ma X, Cheng C, Xiao X, Chen J, Liu S, Zhao B, Chen Y (2013). Effects of Endothelial Progenitor Cell-Derived Microvesicles on Hypoxia/Reoxygenation-Induced Endothelial Dysfunction and Apoptosis. Oxid Med Cell Longev 2013: 572729.

Wang R (2003). The Gasotransmitter Role of Hydrogen Sulfide. Antioxid Redox Signal 5(4): 493–501.

Wang R (2012). Physiological Implications of Hydrogen Sulfide: A Whiff Exploration That Blossomed [Internet]. Physiological Reviews 92 (2): 791-896

Wang WZ, Baynosa RC, Zamboni WA (2011). Therapeutic Interventions Against Reperfusion Injury In Skeletal Muscle. J Surg Res 171(1): 175–82.

Wang WZ, Baynosa RC, Zamboni WA (2011). Update on Ischemia-Reperfusion Injury for the Plastic Surgeon. Plast Reconstr Surg 128(6): 685e-92e

Wang WZ, Fang X-H, Stephenson LL, Khiabani KT, Zamboni WA (2006). Melatonin Reduces Ischemia/Reperfusion-Induced Superoxide Generation in Arterial Wall and Cell Death in Skeletal Muscle. J Pineal Res 41(3): 255–60.

Wang WZ, Fang X-H, Stephenson LL, Khiabani KT, Zamboni WA (2008). Ischemia/Reperfusion-Induced Necrosis and Apoptosis in the Cells Isolated from Rat Skeletal Muscle. J Orthop Res 26(3): 351–6.

Wang WZ, Fang X-H, Stephenson LL, Zhang X, Khiabani KT, Zamboni WA (2011). Melatonin attenuates I/R-induced mitochondrial dysfunction in skeletal muscle. J Surg Res 171(1): 108–13.

Wang WZ, Fang X-H, Stepheson LL, Khiabani KT, Zamboni WA (2005). Acute Microvascular Action of Vascular Endothelial Growth Factor in Skeletal Muscle Ischemia/Reperfusion Injury. Plast Reconstr Surg 115(5)1355–65.

Wedmann R, Bertlein S, Macinkovic I, Böltz S, Miljkovic JL, Muñoz LE, Herrmann M, Filipovic MR (2014). Working with “H_2S”: Facts and Apparent Artifacts. Nitric Oxide 41:85–96.

Weiland DE (2007). Fasciotomy Closure Using Simultaneous Vacuum-Assisted Closure And Hyperbaric Oxygen. Am Surg 73(3): 261–6.

Weiss SJ (1989). Tissue Destruction by Neutrophils. N Engl J Med 320(6): 365–76.

Welbourn CR, Goldman G, Paterson IS, Valeri CR, Shepro D, Hechtman HB (1991). Pathophysiology of Ischaemia Reperfusion Injury: Central Role of the Neutrophil. Br J Surg 78(6): 651–5.

Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong B-S, Cheung NS, Halliwell B, Moore PK (2004). The Novel Neuromodulator Hydrogen Sulfide: An Endogenous Peroxynitrite “Scavenger”? J Neurochem 90(3): 765–8.

Whiteman M, Cheung NS, Zhu Y-Z, Chu SH, Siau JL, Wong BS, Armstrong JS, Moore PK (2005). Hydrogen Sulphide: A Novel Inhibitor of Hypochlorous Acid-Mediated Oxidative Damage in The Brain? Biochem Biophys Res Commun 326(4):794–8.

Whiteman M, Moore PK (2009). Hydrogen Sulfide and the Vasculature: A Novel Vasculoprotective Entity and Regulator of Nitric Oxide Bioavailability? J Cell Mol Med 13(3): 488–507.

Whitesides T, Heckman M (1996). Acute Compartment Syndrome: Update on Diagnosis and Treatment. J Am Acad Orthop Surg 4(4): 209–18.

Whitesides TE, Haney TC, Morimoto K, Harada H (1975). Tissue Pressure Measurements as a Determinant for the Need of Fasciotomy. Clin Orthop Relat Res 113: 43–51.

Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR (2008). Reappraisal of H2S/Sulfide Concentration in Vertebrate Blood and its Potential Significance in Ischemic Preconditioning and Vascular Signaling. Am J Physiol Regul Integr Comp Physiol 294(6): R1930–7.

Wiesel P, Patel AP, Carvajal IM, Wang ZY, Pellacani A, Maemura K, DiFonzo N, Rennke HG, Layne MD, Yet SF, Lee ME (2001). Exacerbation of Chronic Renovascular Hypertension and Acute Renal Failure in Heme Oxygenase-1-Deficient Mice. Circ Res 88(10): 1088–94.

Willhoite DR, Moll JH (1970). Early Recognition And Treatment Of Impending Volkmann's Ischemia In The Lower Extremity. Arch Surg 100(1): 11–6.

Willis B (2012). Compartment Syndrome. In: Abzug JM, Herman MJ, editors. Pediatric Orthopedic Surgical Emergencies. New York: Springer, NY; p. 29–42.

Willy C, Sterk J, Völker H-UU, Sommer C, Weber F, Trentz O, Gerngross H, Trenz O, Gerngroß H (2001).[Acute Compartment Syndrome. Results of a Clinico-Experimental Study of Pressure and Time Limits for Emergency Fasciotomy]. Unfallchirurg 104(5): 381–91.

Wu D, Hu Q, Liu X, Pan L, Xiong Q, Zhu YZ. Hydrogen Sulfide Protects Against Apoptosis Under Oxidative Stress Through SIRT1 Pathway in H9c2 Cardiomyocytes. Nitric Oxide 46: 204-12

Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R (2008). H_2S as a Physiologic Vasorelaxant: Hypertension in Mice with Deletion of Cystathionine Gamma-Lyase. Science 322(5901): 587–90.

Yang W, Yang G, Jia X, Wu L, Wang R (2005). Activation of KATP Channels by H2S in Rat insulin-Secreting Cells and the Underlying Mechanisms. J Physiol 569(Pt 2): 519–31.

Yao X, Tan G, He C, Gao Y, Pan S, Jiang H, Zhang Y, Sun X (2012). Hydrogen Sulfide Protects Cardiomyocytes from Myocardial Ischemia-Reperfusion Injury by Enhancing Phosphorylation of Apoptosis Repressor with Caspase Recruitment Domain. Tohoku J Exp Med 226(4): 275–85.

Yet SF, Tian R, Layne MD, Wang ZY, Maemura K, Solovyeva M, Ith B, Melo LG, Zhang L, Ingwall JS, Dzau VJ, Lee ME, Perrella MA (2001). Cardiac-Specific Expression of Heme Oxygenase-1 Protects Against Ischemia and Reperfusion Injury in Transgenic Mice. Circ Res 89(2): 168–73.

Zanardo RCO, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL (2006). Hydrogen Sulfide is an Endogenous Modulator of Leukocyte-Mediated Inflammation. FASEB J 20(12): 2118–20.

Zhang H, Guo C, Zhang A, Fan Y, Gu T, Wu D, Sparatore A, Wang C (2012). Effect of S-Aspirin, a Novel Hydrogen-Sulfide-Releasing Aspirin (ACS14), on Atherosclerosis in Apoe-Deficient Mice. Eur J Pharmacol 697(1-3): 106–16.

Zhang J, An J (2007). Cytokines, Inflammation, and Pain. Int Anesthesiol Clin 45(2): 27–37.

Zhang Y, Yao H-P, Huang F-F, Wu W, Gao Y, Chen Z-B, Liang Z-Y,
Liang T-B (2008). Allicin, a Major Component Of Garlic, Inhibits Apoptosis In Vital Organs In Rats With Trauma/Hemorrhagic Shock. Crit Care Med 36(12): 3226–32.

Zhao W, Zhang J, Lu Y, Wang R (2001). The Vasorelaxant Effect of H(2)S as a Novel Endogenous Gaseous K(ATP) Channel Opener. EMBO J 20(21): 6008–16.

Zhao Y, Feng Q, Huang Z, Li W, Chen B, Jiang L, Wu B, Ding W, Xu G, Pan H, Wei W, Luo W, Luo Q (2014). Simvastatin Inhibits Inflammation in Ischemia-Reperfusion Injury. Inflammation 37(5): 1865–75.

Zhao Y, Wang H, Xian M (2011). Cysteine-Activated Hydrogen Sulfide (H_2S) Donors. J Am Chem Soc 133(1): 15–7.

Zhu JXG, Kalbfleisch M, Yang YX, Bihari R, Lobb I, Davison M, Mok A, Cepinskas G, Lawendy AR, Sener A (2012). Detrimental Effects Of Prolonged Warm Renal Ischaemia-Reperfusion Injury Are Abrogated By Supplemental Hydrogen Sulphide: An Analysis Using Real-Time Intravital Microscopy And Polymerase Chain Reaction. BJU Int 110(11 Pt C): E1218–27.

Zhu X-Y, Gu H, Ni X (2011). Hydrogen Sulfide in the Endocrine and Reproductive Systems. Expert Rev Clin Pharmacol 4(1): 75–82.

Zimmerman BJ, Grisham MB, Granger DN (1990). Role of Oxidants in Ischemia/Reperfusion-Induced Granulocyte Infiltration. Am J Physiol 258(2 Pt 1): G185–90.

Zimmerman JL, Shen MC (2013). Rhabdomyolysis. Chest 144(3): 1058–65.

APPENDICES

APPENDIX I. Copy of the Animal Protocol Approval

11.01.13
This is the original approval for this protocol
A full protocol submission will be required in 2017

Dear Dr. Lawendy:

Your animal use protocol form entitled:

Direct and Remote Organ Injury Following Hind Limb Compartment Syndrome

Funding agency Orthopaedic Trauma Association – Direct and Remote Organ Injury Following Hind Limb Compartment Syndrome – Grant #R4889A04 has been approved by the University Council on Animal Care.

This approval is valid from **11.01.13 to 11.30.17** with yearly renewal required.

The protocol number for this project is **2009-083**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 YEARS

Species	Strain	Other Detail	Pain Level	Animal # Total for 4 years
Rat	Wistar	150-350 g	C	680
Pig	Yorkshire-Landrace	50-60 kg	B	30

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. R Bihari, T Carter, K Bothwell, P Coakwell

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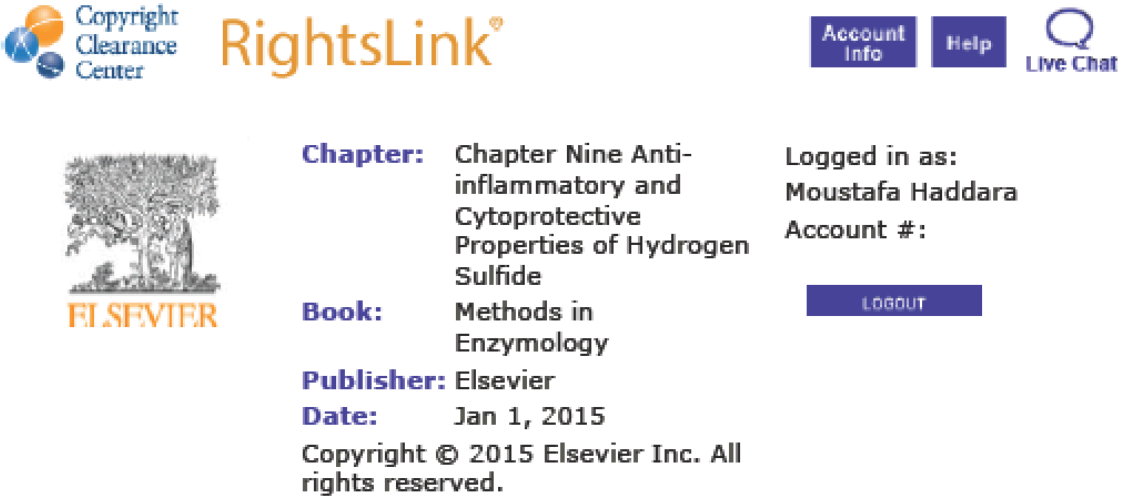

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VITA

Name: Moustafa Mohamed Haddara

Post-secondary Education and Degrees:

Alexandria University
Alexandria, Egypt
1976-83, Bachelor of Medicine and Surgery (MBBCh)

Alexandria University
Alexandria, Egypt
1984-87, Master of Orthopaedics (MChOrth)

Royal College of Surgeons
Dublin, Ireland
1995, Fellow of Royal College of Surgeons (FRCS)

Strathclyde University
Glasgow, UK
1998-99, Diploma in Orthotics and Biomechanics

Honours and Awards:

Award of Excellence, Egyptian Ministry of Education
1976-82

Dean's Honour List, Faculty of Medicine, Alexandria
1977-83

AO Fellowship, University of Basel, Switzerland
2001

Work Experience:

Orthopaedic Surgeon
Alexandria, Egypt
1988-2014

Publications:

Haddara, M. (2015). Cytoprotective Effects of Hydrogen Sulfide in Acute Limb Compartment Syndrome. London Health Research Day, London, ON, Canada.

Haddara, M. (2006). *Reconstructive Limb Surgery in a Developing Country*. Second international Course of Skeletal Deformities Correction; Cairo, Egypt.

Haddara, M. (2003). *Limb Salvage in Mangled Extremity Injuries.* Almana Medical Proceedings, Vol 4, pp. 22- 25.