CCN1 LEVELS IN DUCHENNE MODELLED MICE

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a genetic disorder that arises from the loss of or mutations in dystrophin, a cytoskeletal protein, resulting in progressive muscle degeneration. The lack of this protein leads to chronic inflammation. CCN1 is an extracellular protein that plays a variety of roles, including being associated with areas of inflammation (Jun and Lau, 2010). In tissues such as the skin, CCN1 works to limit the deposition of extracellular matrix, reducing the risk of fibrosis during wound healing (Wynn, 2008). Thus I predict that CCN1 levels increase as DMD progress in mice.

OBJECTIVE

To investigate the levels of CCN1 as DMD mice progress (4-5 and 8-9 weeks).

METHODOLOGY

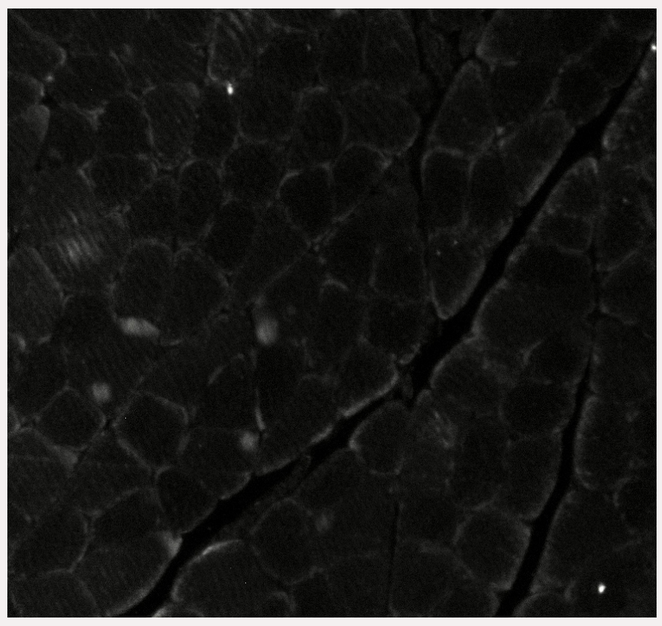
The tissue used in this experiment was the gastrocnemius of mice. Wild type (WT) and Mdx/Utrn +/- (Het) mice were used.

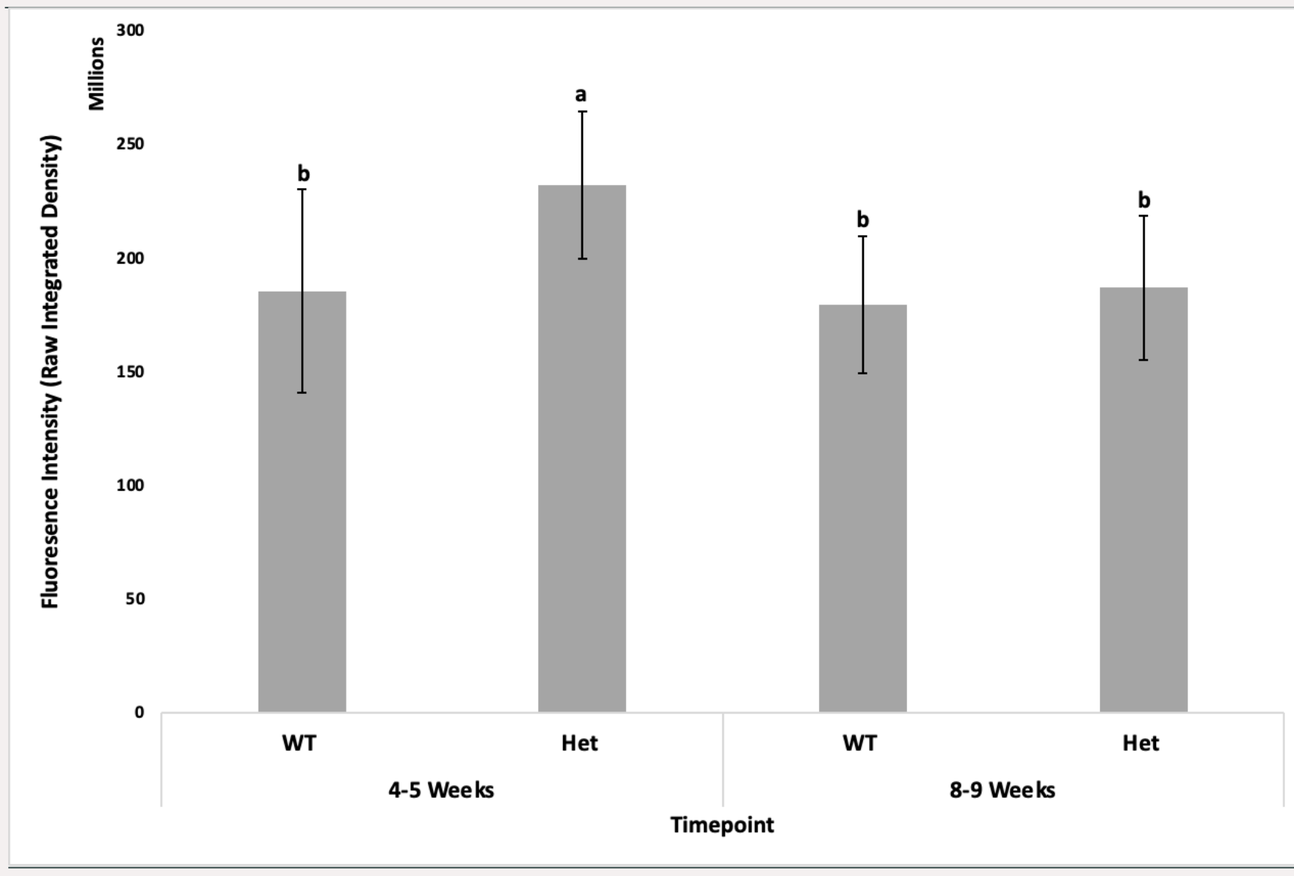
Immunohistochemistry (IHC) was used to stain the tissues. Tissues were first deparaffinized then washed three times with PBS. Antigen retrieval was then done with citrate buffer and 0.05% tween followed by 3x PBS wash. A 5 min background sniper wash was completed with another 3x PBS wash after. The anti CCN1 primary antibody in 1:100 dilution in 1% BSA-PBS and incubated overnight at 4°C. After a 3x PBS wash, the goat anti-rabbit (CY5) secondary antibody (1:200) was incubated at room temperature for 2 hours. DAPI with prolong gold was mounted on the tissue.

IMAGING

Imaging was done by the Nikon Eclipse Ts2R. The levels of CCN1 were measured by the intensity of fluorescence determined by the raw integrated density obtained from Imagej.

The image to the right is of 4-5 week Het mice





RESULTS

For the Wildtype group, there was no significant difference in the level of fluorescence of CCN1 from the 4-5 week and 8-9 week group. For the Het group, the level of fluorescence of CCN1 significantly decreased in the 8-9 week group compared to the 4-5 week group ($p<0.05$). As well the Het group for 4-5 weeks was significantly higher in fluorescence than the WT group.

Fig 1. Effect of time (4-5W and 8-9W) on the mean fluorescence of CCN1 (+/- SD) of WT and Het mice Means followed by the same letter are not significantly different according to the T-test

DISCUSSION

This experiment investigated how CCN1 levels changed as DMD mice progressed. It was observed that the levels of CCN1 decreased as time progressed for DMD mice and that the level of CCN1 is higher than WT mice only in the 4-5W group. CCN1 was expected to be linked to inflammation and increase as DMD progressed in mice. A possible reason for this is that CCN1 can be heavily influenced by the microenvironment (Jun and Lau, 2011). The changing environment due to the progression of DMD may alter its activity.

LITERATURE CITED

Jun JI, Lau LF (2010). "The Matricellular Protein CCN1/CYR61 Induces Fibroblast Senescence and Restricts Fibrosis in Cutaneous Wound Healing". Nat. Cell Biol. 12 (7): 676–685.

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