The Beneficial Effect Of Steroid Therapy In DMD Is Mediated Through A Reduction In Stem Cell Exhaustion

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LB Qualifying Statement: Our study revealed the mechanism that steroids work on DMD, and will help the development of a new theory in treatment of DMD.

Introduction: Glucocorticoid corticosteroids have been use as standard medications for treating Duchenne muscular dystrophy (DMD) patients (1-2); however, except for the anti-inflammation effect (1), little is understood about the molecular mechanism(s) underlying the beneficial effects. In addition to the well-known myopathologic characteristics of DMD such as muscle wasting, degeneration and progressive fibrosis, stem cell exhaustion is also featured in the skeletal muscle of DMD patients (3). Stem cell exhaustion is now believed to be responsible for the impaired muscle regeneration capacity of dystrophic muscle, which occurs in DMD patients when they become symptomatic (3). Therefore, it would be of great interest for the treatment of DMD to determine whether corticosteroids may affect the progression of stem cell exhaustion in dystrophic muscle. This mechanistic understanding would also be helpful for developing improved corticosteroid therapies for treating DMD.

Methods: 1. Animals: Wild-type (WT, C57BL/10J) mice: Jackson Laboratory. mdx and dystrophin/utrophin double knockout (dKO) mice model of DMD were used in this project. Compared to mdx mice feature normal life span (~2 years), dKO mice feature early death (~8 weeks) and are more severe and more closely resembles the phenotype seen in DMD patients. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh. 2. In vivo prednisolone treatment of dKO mice: Prednisolone is one of the main Glucocorticoids used for DMD. Prednisolone dissolved in sterile phosphate-buffered saline (PBS) (1.6mg/ml) was injected intraperitoneally (1mg/kg) into dKO mice from 3-week old WT, mdx, and dKO mice showed that, β-gal+ senescent cells were generally undetectable in the WT MDCSs and mdx MDSCs but present in the dKO MDSCs (Figure 1A, C). Immunostaining of the hind-limb muscles (Gastrocnemius, GM) of 8-week old WT, mdx, and dKO mice revealed that, the number of Pax7+ satellite cells (muscle stem cells) was reduced in dKO compared to 2 years for mdx). Therefore, in the current study, the dKO mouse model was used to study the potential effects and mechanism of action that glucocorticoids have on the process of stem cell exhaustion and senescence.

Results: 1. Stem cell exhaustion and senescence occurs in skeletal muscle of dKO mice but not in WT and mdx mice. Single myofibers were isolated from Tibialis anterior (TA) muscle of 8-week old WT, mdx and dKO mice. Compared to the WT myofibers, more Pax7+ muscle progenitor cells were observed in the mdx myofibers, and far fewer Pax7+ cells were observed in the dKO myofibers (Figure 1A, B). Cell senescence assay with the muscle-derived stem cells (MDSCs) isolated from 6-week old WT, mdx and dKO mice showed that, β-galactosidase (β-gal)+ senescent cells were generally undetectable in the WT MDSCs and mdx MDSCs but present in the dKO MDSCs (Figure 1A, C). Immunostaining of the hind-limb muscles (Gastrocnemius, GM) of 8-week old WT, mdx, and dKO mice revealed that, the number of Pax7+ satellite cells (muscle stem cells) was reduced in dKO mice compared to WT or mdx mice (Figure 1D). The presence of β-gal+ senescent cells was exclusively observed in dKO mice but not in either the WT or mdx mice (Figure 1D).

Discussion: It is well-established that glucocorticoids such as prednisolone are effective at reducing inflammation and delaying disease progression in DMD patients. However little is known about the regulatory mechanisms that are involved. Stem cell...
exhaustion is now believed to be directly responsible for the impaired muscle regeneration capacity of dystrophic muscle, and our current results showed that, in addition to reducing inflammation, glucocorticoids could rescue the severely affected muscular dystrophic mice from stem cell exhaustion and senescence.

**Significance:** Our current data reveals for the first time the effect of glucocorticoids in repressing stem cell exhaustion and senescence in severely affected dystrophic muscle. This mechanistic understanding would be helpful for developing improved corticosteroid therapies or other more effective therapies for treating DMD.

**Acknowledgements:** This work was supported in part by the DOD and the Henry J. Mankin Endowed Chair at the University of Pittsburgh.

**References:**
Figure 1

A

Pax7
DAPI

WT
mdx
dKO

β-gal

B

Pax7+ cells

Percentage of WT

WT
mdx
dKO

C

β-gal+ cells

Percentage of cells

WT
mdx
dKO

Figure 2

A
dKO control
dKO-prednisolone

CD68
DAPI

B

β-Gal

C

Pax7
Col IV

ORS 2014 Annual Meeting
Poster No: 2019