PREVENTIVE EFFECT OF LONG ACTING B2-AGONISTS ON STATIN INDUCED MYOPATHIES

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ABSTRACT

Objective: To identify the histological effects of statin-induced skeletal muscle myopathy in a Rat model and to find protective effect of long acting β2-agonists.

Study Design: Laboratory based experimental randomized controlled trial.

Place and Duration of Study: Study was conducted at the department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad and Armed forces institute of Pathology (AFIP) Rawalpindi, from Jan 2015 to Jun 2016.

Material and Methods: Adult male Sprague-Dawley rats were procured from NIH Islamabad. Their average approximate age was 70-80 days and weight range was 250 ± 50 grams. The animals were randomly selected and divided into three groups. Group A was the control. Each rat of group B received Simvastatin dissolved in distilled water, by oral gavage (60mg/kg/day) once daily, for 12 weeks. Animals of group C received simvastatin dissolved in distilled water, (60mg/kg/day) once daily plus formoterol dissolved in distilled water (3µg/kg/day) once daily for 12 weeks. Both were administered with the help of oral gavage. The animals were sacrificed after three months of the experimental period. Extensor digitorum longus (EDL) tendon was isolated and dissected out. Tissue processing was done on the EDL muscle followed by Haematoxylin and Eosin staining. Fiber cross-sectional areas, Number of myofibers and Central Myonuclei were counted per high power field in each specimen of all three groups.

Results: Examination of H&E stained sections of the extensor digitorum longus muscle of the control group revealed the histological structure of skeletal muscle. Cross sectional area of myofibers and number of myofibers were significantly lower in group B as compared to the control group A. Group C showed significant increase in cross sectional area of myofibers and number of myofibers as compared to group B. No central myonuclei was seen in any section.

Conclusion: Simvastatin induced the histomorphological changes in the skeletal muscle of experimental rats by reducing myofiber size and number. Formoterol co-administration minimized simvastatin induced myopathy by significantly increasing myofiber size and number.

Keywords: Formoterol, Myopathy, Skeletal muscle, Statin.

INTRODUCTION

Statins are essential in the prevention and treatment of patients with hyperlipidemia, ischemic heart disease and nonhemorrhagic stroke. Harper and Jacobson have shown that a 10 to 15 percent of statin users report with myopathies. Statin related myopathy is a clinically important reason of statin intolerance and discontinuation. It comprises muscular pains, weakness, cramps, stiffness, myalgias, myositis, and rare life threatening rhabdomyolysis. Atrophied muscles produce less force as there is a direct relation between the percent loss of muscle mass and percent decline in contractile force. Adult skeletal muscle has the significant ability for repair and regeneration after myotrauma. β2-agonists have significant role in skeletal muscle growth, development, and repair after injury in rats. They increase protein synthesis, decrease protein degradation and cause a net increase in myofibrillar protein content. Skeletal muscle contains all the three β-adrenoceptor subtypes.
(β1, β2 and β3) with 10 fold greater proportion of the β2-adrenoceptor isoform. β-adrenergic signaling is an important regulator of skeletal muscle regeneration. Stimulation of skeletal muscle β-adrenoceptors can accelerate the structural and functional recovery of myotoxic injury. Formoterol is a highly selective β2-adrenoceptor used in bronchial asthma. Formoterol has more pronounced anabolic effects on skeletal muscle, unlike older generations of β2-adrenoceptor agonists.

In this study; Simvastatin induced histomorphological changes in skeletal muscle fibers of rats were determined, followed by evaluation of protective effect of Formoterol co-administration.

Atherosclerosis, coronary heart disease and chronic obstructive pulmonary disease are common entities, which often coexist due to common risk factors like smoking, old age and decrease in physical activity. This study will help us recommending Formoterol to COPD patient who are also having hyperlipidemias and taking Statins. This will control bronchospasm and will also prevent myopathies.

**MATERIAL AND METHODS**

The study design was laboratory based experimental randomized controlled trial. It was conducted at the department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad and Armed forces institute of Pathology (AFIP) Rawalpindi. Adult male Sprague-Dawley rats were procured from the NIH Islamabad. Their approximate average age was 70-80 days and weight range was 250 ± 50 grams. All rats received the standard animal house diet. The food and water were available ad libitum. They were kept in cages at the room temperature of 18-26°C for 3 months. The animals were randomly selected by computer generated numbering. They were divided into three groups. It was non probability convenient sampling. Group A was the control and it comprised of thirty rats, numbered from A1 to A30. They were kept without any medication. The group B comprised of thirty rats numbered from B1 to B30. Each rat of group B received Simvastatin dissolved in distilled water, by oral gavage (60mg/kg/day) once daily, for 12 weeks. Group C included thirty rats and they were numbered as C1 to C30. Each rat of group C received simvastatin dissolved in distilled water, by oral gavage (60mg/kg/day) once daily plus formoterol dissolved in distilled water (3µg/kg/day) by oral gavage once daily for 12 weeks.

The animals were sacrificed after three months of the experimental period. The anesthetized rats were placed on a dissection board and Extensor Digitorum Longus (EDL) muscle along with tendon was dissected out.

A transverse and a longitudinal section were obtained from each muscle. A 0.5cm thick section from the mid belly region of the muscle was cut for processing. The sections were stained with Haematoxylin and Eosin for routine histological study.

One cross and one longitudinal section were selected from each of the specimen for histological study. Three quantitative parameters were determined. Eight microscopic fields per slide were selected in unit area under the 40x objective magnification to calculate fiber cross-sectional areas (CSA), to count number of myofiber and central myonuclei. CSA of muscle fibers in each
microscopic field was measured by the morphometric computer software “Image J” and observations on each were recorded in a proforma followed by calculating the mean. The criteria used in the selection of muscle fibers to measure the CSA included an intact, distinct endomysium without significant signs of distortion or folding. Myofibers were counted and recorded in proforma. Criteria to be included in counting number of fibers consisted of the ability to distinguish a single fiber with distinct endomysium. Nucleus not lining the endomysium in a normal, degenerating, or regenerating fiber was regarded as a central myonucleus.

Data was analyzed using computer software IBM SPSS (Statistical package for social sciences) version 21. Quantitative variables were expressed as mean ± standard error. Analysis of variance (ANOVA) test was used to determine difference among various groups for quantitative variables followed by Tukey’s Post Hoc test. A p-value of <0.05 was considered statistically significant.

RESULTS

Results were studied on all 90 rats of the study. Thirty rats of group A remained active and healthy throughout the study period. There were no detrimental effects on the behavioral and general health of the animals during entire period of study. Examination of H&E stained sections of the extensor digitorum longus muscle of the control group revealed the normal structure of skeletal muscle (fig-1). CSA of myofibers was measured and its mean value was 66.077 ± 2.674 µm². The number of myofibers was counted and its mean value was 316.63 ± 7.417. No central myonuclei were seen (table).

Thirty rats of group B tolerated the drug well and no untoward side effects were observed during the experiment. The treatment protocol caused no mortality in this experimental group. Histological sections of skeletal muscle of group B stained with H&E showed muscle fibers of variable CSA (fig-2), CSA of myofibers was measured and its mean value was 28.89 ± 1.93 µm². It was significantly lower as compared to the control group A (p<0.001). The number of myofibers was counted and its mean value was 173.47 ± 7.912. It was significantly lower as compared to the control group A (p<0.001). No central myonuclei were seen (table).

Thirty rats of group C tolerated both the drugs well and there were no obvious signs of ill health. The animals remained active during the study period. The treatment protocol caused no mortality in this experimental group. Histological sections of skeletal muscle of group C stained with H&E showed all of its components with the...
light microscope and it was more or less similar to control group A (fig-3). CSA of myofibers was measured and its mean value was $46.33 \pm 1.296 \, \mu m^2$. It was significantly lesser than control group A but significantly higher than group B ($p<0.001$). The number of myofibers was counted and its mean value was $271.67 \pm 8.251$. It was significantly lesser than control group A but significantly higher than group B ($p<0.001$). No central myonuclei were seen (table).

**DISCUSSION**

Decreased size of myofiber is an initial feature of diseased and atrophied muscle. The degree of atrophy suffered by the muscle fibers was determined from their cross-sectional areas (CSA) of the individual myofibers. It was statistically different in all groups. CSA of myofibers in group B was significantly reduced as compared to control group A. Formoterol treated rats of group C showed significant increase in muscle fiber size thus β2-agonists can accelerate muscle regeneration by increasing the number of myofibers and thereby by improving muscle function in myopathic conditions. This is comparable to previous work which concluded that β2 agonists can be used to improve muscle function if administered at appropriate times during the repair process.$^{15, 16}$

The number of myofibers in Simvastatin treated B group was significantly lesser than control group A. Group C showed significant increase in number of muscle fiber as compared to group B. It was comparable to previous studies. Once fusion of myogenic cells is accomplished, newly formed muscle fibers grow in size. Thus the hyperplasia, of myofibers, as seen in our study could result from fiber branching with subsequent hypertrophy of new fibers or myogenesis. It has been hypothesized and seen in earlier studies as well$^{17}$. It has been demonstrated that β2 agonists increase protein content in regenerating muscles which is important substrate required for muscle repair/regeneration$^{18}$.

No significant differences in location of nuclei were noted in myofibers from all groups.
Nuclei were peripherally located just beneath the cell membrane in all specimens of the 3 groups and the overall distribution was uniform. It was however contrary to the previous study which showed central myonuclei in regenerating myofibers. It could be explained on basis of time course of observations. Rats of group C didn't show central nuclei as they were almost completely regenerated at the time of dissection after 90 days. It is known that regenerated muscle is morphologically in distinguishable from undamaged muscles. Moreover, earlier studies showing central myonuclei were spanned on duration of 14 to 30 days whereas our study spanned on duration of 90 days, probably yielding more time to damaged muscle to regain its normal morphological characteristics. One more explanation can be attributed to quicker repair of damaged muscle by Formoterol depicted by restoration of normal histological pattern. Our study shows that Formoterol has significant potential of treating muscle wasting disorders and myopathies.

CONCLUSION
In this study, the histomorphological changes induced by simvastatin in the skeletal muscle of experimental rats and its reversal by formoterol were proved. Formoterol co-administration minimized simvastatin induced myopathy by significantly increasing myofiber size and number.

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CONFLICT OF INTEREST
This study has no conflict of interest to declare by any author.

REFERENCES