SIMVASTATIN INDUCED HISTOMORPHOLOGICAL CHANGES IN SKELETAL MUSCLE FIBER OF RATS AND PROTECTIVE EFFECT OF FORMOTEROL CO-ADMINISTRATION

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ABSTRACT

Objective: To study Simvastatin induced, qualitative histomorphological changes in skeletal muscle fibers of rats and find protective effect of Formoterol co-administration in Simvastatin induced myopathy.

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 with weight as 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 Hematoxylin and Eosin staining. Fiber variability, inflammation, necrosis, shape of nuclei and location of nuclei were assessed per high power field in each specimen of all the groups.

Results: Examination of H&E stained sections of the extensor digitorum longus muscle of the control group revealed the normal structure of skeletal muscle. Fiber variability, inflammation and necrosis were significantly higher in group B as compared to the control group A. Group C showed significantly decrease in myofiber variability, inflammation and necrosis in myofibers as compared to group B.

Conclusion: Simvastatin induced the histomorphological changes in the skeletal muscle of experimental rats by increasing myofiber variability, inflammation and necrosis. Formoterol co-administration minimized the simvastatin induced myopathy by significantly decreasing myofiber variability, inflammation and necrosis.

Keywords: Formoterol, Myopathy, Skeletal muscle, Statin.

INTRODUCTION

Elevated blood cholesterol level, obesity and atherosclerosis are important health problems in our society. They stimulate cardiovascular disorders as coronary atherosclerosis, myocardial infarction and stroke. An effective way to decrease the related morbidity and mortality is to treat the high blood cholesterol level with Statins[1]. Statins are used worldwide as an effective treatment for hypercholesterolemia. Generally, they are well tolerated. Important adverse effect associated with statin therapy is the myotoxicity that ranges from mild myopathy i.e. muscle pain, cramps, and weakness to rhabdomyolysis[2]. These complications are observed in 10.5% of the statintreated patients[3]. Investigations have been performed to understand the mechanism of statininduced changes in muscle. Although, these studies provide some detail on the nature of the changes in muscle but they do not provide a complete picture; and thus, the precise mechanism is still unknown[4].

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Synthetic β2-adrenoceptor agonists are derived from the chemical structure of adrenaline, and are traditionally used for the treatment of bronchospasm associated with asthma and the treatment of symptomatic patients with COPD. At higher doses, β2 -agonists have an anabolic effect on skeletal muscle[5]. This increase in muscle mass is due to β2-adrenoceptor mediated protein addition via increase in intracellular cAMP, which promotes both an increase in protein synthesis and a decrease in degradation[6].

In this study, we desired to contribute to the understanding of Simvastatin induced muscle changes by studying the precise histopathological changes in rat model, 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 in recommending the Formoterol to COPD patients 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, from Jan 2015 to Jun 2018. Approval from the ethical review committee was taken. Adult male Sprague-dawley rats were procured from the NIH, Islamabad. Their approximate average age was 70-80 days and weight 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^{o}C for 3 months. The animals were selected non-probability convenient sampling. They were equally divided into three groups. 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 piece of 0.5 cm^3 from the mid belly region of the muscle were obtained for histological processing, microcutting and staining with Hematoxylin and Eosin. One cross and one longitudinal histological sections were selected from each of the specimen for study. Five qualitative parameters were assessed. Eight microscopic fields per slide were selected with the help of 40x objective magnification to assess the muscle fiber variability, inflammation, necrosis, shape of nuclei and location of nuclei.

Data were analyzed using computer software IBM SPSS (Statistical Package for Social Sciences) version 21. These qualitative variables were expressed as frequency and percentages followed by Chi square test. A *p*-value of <0.05 was considered statistically significant.

RESULTS

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). Skeletal muscle fibers were multinucleated. Oval shaped nuclei were peripherally located just beneath the cell membrane. Muscle was devoid of any inflammatory cells. Necrosis, inflammation, degeneration or regeneration were not found (table).

The histological sections of skeletal muscle of group B, showed muscle fibers not extending the entire length of the cell depicting the splitting of myofibers. Splitting of some fibers was also observed which appeared as a transverse invagination or complete separation. Myofibers showed variability in orientation being united in 10 (33.3%) specimens and split in 20 (67.7%) specimens (fig-2). This variability was significant as compared to the control group A (p<0.001). Skeletal muscle fibers were multinucleated. Oval shaped nuclei were seen in 11 (36.7%) whereas round shaped nuclei were seen in 19 (63.3%) specimen, being statistically significant from control group A (p<0.001). Nuclei were peripherally located just beneath the cell membrane in all 30 (100%) specimens and compared to control group A, it was statistically insignificant (table). Inflammation was significantly higher as compared to control group A (p<0.001); present in 21 (70%) specimens and absent in 9 (30%) specimens. Inflammatory infiltrates were diffuse and focal, usually interspersed between the individual muscle fibers. The infiltrates consisted largely of small mononuclear cells. Necrosis was seen in most specimens. A necrotic fiber is pale stained on H&E and infiltrated by phagocytes (fig-2). It was significantly higher as compared to control group A (p<0.001); being present in 23 (77%) and absent in 7 (23%) specimens (table). Some muscle fibers were completely degenerated and replaced by debris of the damaged tissue.

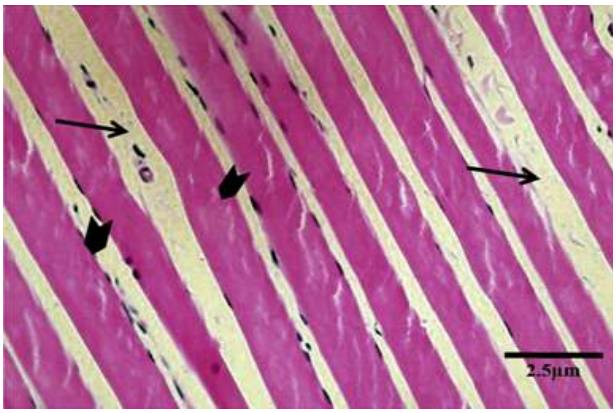

Figure-1: A photomicrograph of a longitudinal section of EDL muscle of a control rat (Group A) showing parallel muscle fibers (Arrow) and multiple elongated nuclei peripherally located beneath the sarcolemma (Arrow head).
H & E, Bar = 2.5 µm

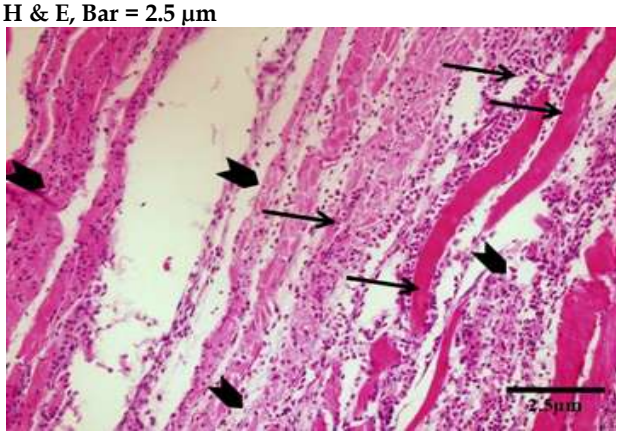

Figure-2: A photomicrograph of a longitudinal section of EDL muscle of a rat of (Group B) showing necrotic fibers (Arrow head) and splitting of muscle fiber (Arrow). H & E.

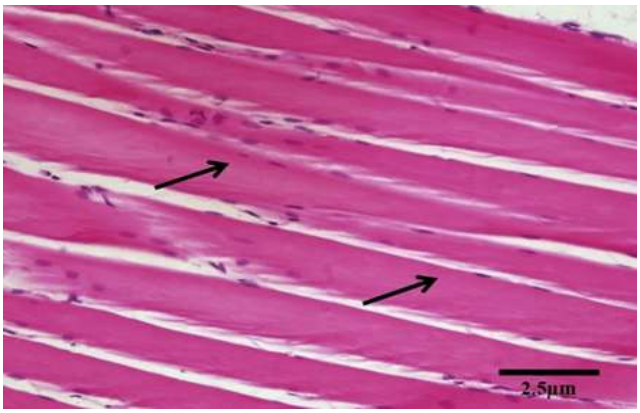

Figure-3: A photomicrograph of a longitudinal section of EDL muscle of a rat (Group C) showing parallel muscle fibers and multiple elongated nuclei (Arrow).
H & E, Bar = 2.5 µm

Histological sections of skeletal muscle of group C showed all of its components with the light microscope and these was more or less similar to control group A. Muscle cells were darkly stained and the myocytes were clearly revealed. Skeletal muscle consisted of longitudinal arrays of cylindrical myofibers, mostly extending the length of the cell. Muscle fibers were arranged parallel to one another (fig-3). Myofibers showed slight variability in orientation as compared to group B; being united in 25 (83.3%) specimen and split in 5 (16.7%) specimens. Skeletal muscle fibers were multi-

nucleated cells. Frequency and percentage of oval shaped nuclei was significantly more as compared to group B; seen in 18 (60%) whereas round shaped nuclei were seen in 12 (40%) specimens. Nuclei were peripherally located just beneath the cell membrane in all 30 (100%) specimens similar to groups A and group B (table). Inflammation was less and statistically significant (p<0.001) when compared to group B; being present in 1 (3.3%) specimen and absent in 29 (96.7%) specimens. Necrosis was absent in 24 (80%) specimens and present in 6 (20%). Necrosis was significantly lesser when compared to group B (p<0.001) (table).

Table: Comparison of fiber orientation, inflammation, shape of nuclei, location of nuclei, cross striations and necrosis between control group A, experimental groups B and group C.

Parameters	Groups			Statistical Significance		
	A	B	C	A/B	B/C	A/C
Fiber Orientation						
United	30 (100%)	10 (33.3%)	25 (83.3%)	p<0.001	p<0.001	p<0.02
Split	0 (0%)	20 (66.7%)	5 (16.7%)			
Inflammation						
Present	0 (0%)	21 (70%)	1(3.3%)	p<0.001	p<0.001	p=1
Absent	30 (100%)	9 (30%)	29 (96.7%)			
Shape of nuclei						
Oval	30 (100%)	11 (36.7%)	18 (60%)	p<0.001	p=0.07	p<0.001
Round	0 (0%)	19 (63.3%)	12 (40%)			
Location of nuclei						
Peripheral	30 (100%)	30 (100%)	30 (100%)	No statistics are computed because Location of Nuclei is a constant.		
Central	0 (0%)	0 (0%)	0 (0%)			
Necrosis						
Absent	30 (100%)	7 (23%)	24 (80%)	p<0.001	p<0.001	p=0.01
Present	0 (0%)	23 (77%)	6 (20%)			

DISCUSSION

The light microscopic structure of the skeletal muscle studied in control group A was similar to that described in earlier studies[7,8]. Muscle fibres were darkly stained as compared to less densely stained connective tissue and the myocytes were clearly demonstrated. Myofibers were multinucleated. Haematoxylin and eosin stained muscle cross sections showed nuclei positioned at periphery. Myofibers in group B showed marked variability in orientation; being united in 33.3% specimens and split in 67.7% specimens. This variability was significant as compared to the control group A which may be a consequence of the muscle undergoing degeneration. Fiber splitting is a distinguishing feature of muscle degeneration. This has been commonly observed in muscles of patients suffering from muscular diseases as well as in aging mouse muscles. It has been mentioned that splitting of muscle fibers is an adaptive response, which occurs when the fiber reaches a certain critical size where metabolism is disturbed as the supply of oxygen and exchange of metabolites are no longer efficient[9]. It may be suggested that myofibers in group B couldn't preserve their orientation because of potential mechanisms underlying the statin induced myopathy. Myofibers of group C showed slight variability in orientation as compared to group B; being united in 83.3% specimen and split in 6.7% specimens. This may be a result of the muscle undergoing repeated cycles of degeneration or regeneration. Slight fiber splitting or branching seen in group C is perhaps due to the partial fusion of myofibers regenerating inside the same basal lamina. This phenomenon has been mentioned in earlier study as well[9]. Skeletal muscle fibers are multinucleated cells. Percentage of oval shaped nuclei of group C was significantly more as compared to group B.

This can be accredited to repair capacity of formoterol illustrated by restoration of normal histological pattern of shape of myonuclei. No significant differences in location of nuclei were noted in myofibers from all groups, indicating that there was no effect on location of nuclei.

Inflammation was greater and significantly increased in group B when compared to control group A. Focal areas with mononuclear cellular infiltration observed in the connective tissue of muscle and in perivascular areas in group B could be explained by the release of certain mediators during degeneration of myocytes[10]. Histological characteristics in inflammatory myopathy include mononuclear cell infiltration followed by muscle fiber degeneration[11]. It is established that whenever skeletal muscle undergoes continuous injury and repair, it causes release of pro inflammatory cytokines and chemokines eg TNF-α and NF-kB. TNF-α can directly bring about the death of muscle cells, whereas NF-kB blocks MyoD to prevent development of the new myofibers. This pathway not only enhances the death of existing muscle fibers but also inhibits formation of new muscle fibers leading to the loss of skeletal muscle mass and weakness[12]. Inflammation was lesser and significantly reduced in group C when compared to group B and this was comparable to previous works which demonstrated that Formoterol has anti-inflammatory effects in vitro[13]. Beta 2 agonists trigger their anti-inflammatory effects by inhibiting mediator release from eosinophils, macrophages T-lymphocytes, and neutrophils[14].

Necrosis of the myofibers is the preliminary event of muscle degeneration. Necrosis was seen in most of the specimens of group B. Necrosis of the myofibers is usually activated by distraction of the myofiber sarcolemma. This leads to amplified myofiber permeability. Disrupted muscle fibers undergo focal or total necrosis depending upon the magnitude of the damage[15]. Necrosis was absent in 80% of specimens of group C and was significantly lesser when compared to group B. β2-agonist in higher doses (5 mg/kg) can initiate apoptosis and necrosis in skeletal muscle[16-18]. Our results suggest that lower micromolar doses of β2-agonist can be used to elicit skeletal muscle growth and development with minimum potential for necrosis or apoptosis.

CONCLUSION

Simvastatin induced the histomorphological changes in the skeletal muscle of experimental rats by increasing myofiber variability, inflammation and necrosis. Formoterol co-administration minimized the simvastatin induced myopathy by significantly decreasing myofiber variability, inflammation and necrosis.

CONFLICT OF INTEREST

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

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