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

Abdullah Qamar, Shahdab Ahmed Butt*, Toqeer Ahmad Iqbal**

CMHInstitute of Medical Sciences (CIMS) Bahawlpur/National University of Medical Sciences (NUMS) Pakistan, *Yusra Medical & Dental College Rawalpindi Pakistan, **Federal Medical & Dental College Islamabad Pakistan

ABSTRACT

Objective: To identify the histological effectsof 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[1]. Harper and Jacobson have shown that a 10 to 15 percent of statin users report with myopathies[2]. 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[3]. Atrophied muscles produce less force as there is a direct relation between the percent loss of muscle mass and percent decline in contractile force[4]. 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[5]. Skeletal muscle contains all the three β-adrenoceptor subtypes

Correspondence: Dr Abdullah Qamar, Assistant Professor of Anatomy, CMH Institute of Medical Sciences Bahawalpur Pakistan
Email: drabdullahqamar@gmail.com
Received: 15 Aug 2017; revised received: 14 Sep 2017; accepted: 03 Oct 2017

(β1, β2 and β3) with 10 fold greater proportion of the β2-adrenoceptor isoform[6,7]. 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[8]. 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 agonist[9].

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^{o}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) musclealong 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.

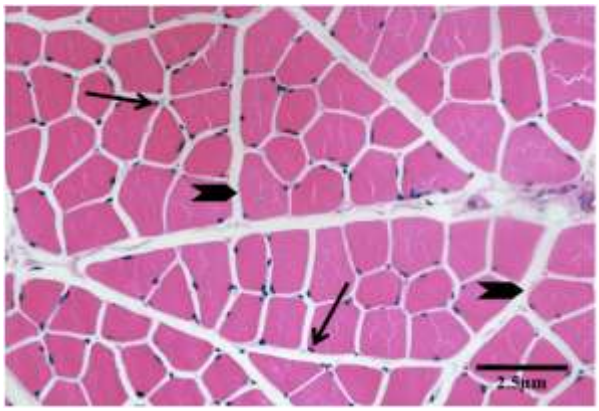

Figure-1: A photomicrograph of a transverse section of EDL muscle of a control rat (Group A) showing polygonal shaped skeletal muscle fibers and peripherally located nuclei (Arrow). The muscle fibers are separated by CT, endomysium (Arrow head).
H & E, Bar = 2.5 µm

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 μm^2. The number of myofibers was counted and its mean value was 316.63 ± 7.417. No central myonuclei was 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 μm^2. 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

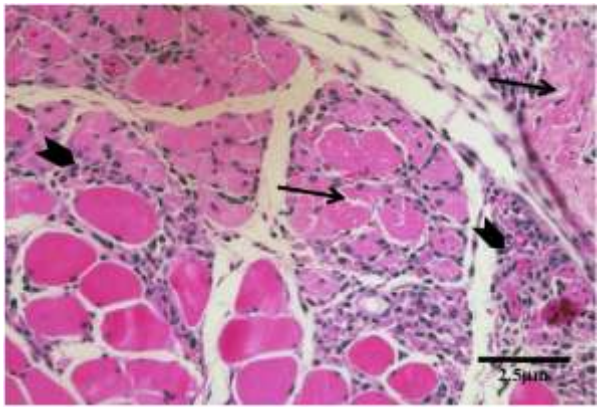

Figure-2: A photomicrograph of a transverse section of EDL muscle of a rat of (Group B) showing focal area containing necrotic fibers (Arrow) and showed multiple foci of mononuclear cell infiltration (Arrow head).
H & E, Bar = 2.5 µm

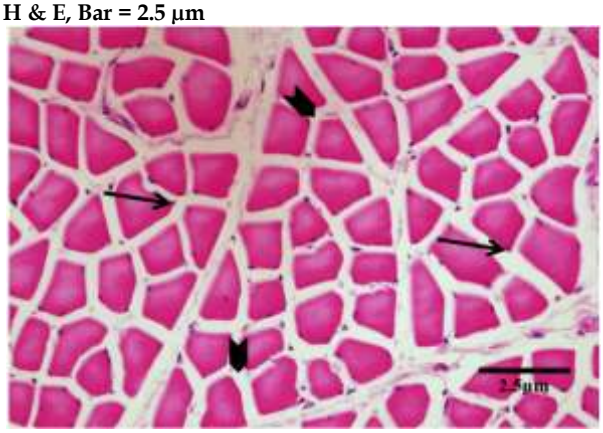

Figure-3: A photomicrograph of a transverse section of EDL muscle of a rat (Group C) showing skeletal muscle fibers with minimal variation in size and shape and peripherally located nuclei (Arrow). The muscle fibers are separated by CT, endomysium (Arrow head).
H & E, Bar = 2.5 µm

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 ± 1.296 μm^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 ± 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 but it was still lesser than those of control group A. The apparent variation of fibers size observed in this work is explained as a result of atrophy and compensatory hypertrophy of some fibers. Atrophy of myofibers leads to significant reduction in strength[10]. Muscle atrophy is brought about either by an enhancement of the normal rate of protein degradation or by a reduction in protein synthesis or by both processes occurring simultaneously[11]. Statins treatment inhibits HMG-CoA reductase pathway leading to impairment of protein synthesis with subsequent atrophy of muscle fibers[12]. Fiber hypertrophy causes increase in muscle cross section area. Hypertrophy facilitates the increase in the contractile material that is the number of cross-bridges and therefore increasing the force production. Synthetic β2-agonists stimulate skeletal muscle hypertrophy by triggering AMP dependent mechanisms that intensify protein synthesis and prevent protein degradation pathways[13,14]. Formoterol treated rats of group C showed significant increase in muscle fiber size thus β 2-agonists can accelerate muscle regeneration by increasing the numberof myofibers and there 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].

Table: Comparison of histological parameters between the groups.

Parameters	Groups	Mean ± SE	Statistical Significance		
			Group A/B	Group A/C	Group B/C
Fiber cross-sectional areas (μm^2)	A	66.07 ± 2.674	p<0.001	p<0.001	p<0.001
	B	28.89 ± 1.930			
	C	46.33 ± 1.296			
Number of fibers	A	616.63 ± 7.417	p<0.001	p<0.001	p<0.001
	B	473.47 ± 7.912			
	C	571.67 ± 8.215			
Number of Central myonuclei	A	0.00 ± 0.000	No statistics are computed because Location of myonuclei is a constant.		
	B	0.00 ± 0.000			
	C	0.00 ± 0.000			

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[19]. 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.

ACKNOWLEDGEMENT

Authors express gratitude to Prof Brig. Khadija Qamar, Col Shabnam Hamid & lt col Rizwan Bashir Kiani of Anatomy department, Army Medical College for their assistance and peef review.

CONFLICT OF INTEREST

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

REFERENCES

1. Valentino M, Al Danaf J, Panakos A, Ragupathi L, Duffy D, Whellan D. Impact of the 2013 American College of Cardiology/ American Heart Association cholesterol guidelines on the prescription of high-intensity statins in patients hospitalized for acute coronary syndrome or stroke. Am Heart J 2016; 181: 130-6.
2. Harper CR, Jacobson TA. Evidence-based management of statin myopathy. Current atherosclerosis reports 2010; 12(5): 322-30.
3. Ahmad Z. Statin Intolerance. Am J Cardiology 2014; 113(10): 1765-71.
4. Hutchinson KJ, Linderman JK, Basso DM. Skeletal muscle adaptations following spinal cord contusion injury in rat and the relationship to locomotor function: A time course study. J Neurotrauma 2001; 18(10): 1075-89.
5. Joassard OR, Durieux AC, Freyssenet DG. β2-Adrenergic agonists and the treatment of skeletal muscle wasting disorders. Int J Biochem Cell Biol 2013; 45(10): 2309-21.
6. Williams RS, Caron MG, Daniel K. Skeletal muscle beta-adrenergic receptors: variations due to fiber type and training. Am J Physiol 1984; 246(2 Pt 1): e160-7.
7. Kim YS, Sainz RD, Molenaar P, Summers RJ. Characterization of beta 1- and beta 2-adrenoceptors in rat skeletal muscles. Biochemical pharmacology 1991; 42(9): 1783-9.
8. Sato S, Shirato K, Mitsuhashi R, Suzuki H, Tachiyashiki K. Functional Roles of β2-Adrenergic Receptors in Skeletal Muscle Hypertrophy and Atrophy. In: Kanosue K, Oshima S, Cao Z-B, Oka K, editors. Physical Activity, Exercise, Sedentary Behavior and Health. Tokyo: Springer Japan 2015: 213-34.
9. Harcourt LJ, Schertzer JD, Ryall JG, Lynch GS. Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. Neuromuscular disorders: NMD 2007; 17(1): 47-55.
10. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. FEBS J 2013; 280(17): 4294-314.
11. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Disease Models &Amp; Mechanisms 2013; 6(1): 25-39.
12. du Souich P, Roederer G, Dufour R. Myotoxicity of statins: Mechanism of action. Pharmacology & therapeutics 2017; 1-16.
13. Lynch GS, Ryall JG. Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. Physiol Rev 2008; 88(2): 729-67.
14. Lynch GS, Schertzer JD, Ryall JG. Therapeutic approaches for muscle wasting disorders. Pharmacology & therapeutics 2007; 113(3): 461-87.
15. Beitzel F, Gregorevic P, Ryall JG, Plant DR, Sillence MN, Lynch GS. Beta2-adrenoceptor agonist fenoterol enhances functional repair of regenerating rat skeletal muscle after injury. J Applied Physiol 2004; 96(4): 1385-92.
16. Ryall JG, Plant DR, Gregorevic P, Sillence MN, Lynch GS. Beta 2-agonist administration reverses muscle wasting and improves muscle function in aged rats. J Physiol 2004; 555(Pt 1): 175-88.
17. Faber RM, Hall JK, Chamberlain JS, Banks GB. Myofiber branching rather than myofiber hyperplasia contributes to muscle hypertrophy in mdx mice. Skeletal Muscle 2014; 4(1): 10.
18. Silva MT, Wensing LA, Brum PC, Câmara NO, Miyabara EH. Impaired structural and functional regeneration of skeletal muscles from β2-adrenoceptor knockout mice. Acta Physiol 2014; 211(4): 617-33.
19. Yin H, Price F, Rudnicki MA. Satellite Cells and the Muscle Stem Cell Niche. Physiol Rev 2013; 93(1): 23-67.