EFFECT OF LONG ACTING β2-AGONISTS ON GROSS MORPHOLOGY OF SKELETAL MUSCLES AND CREATINE PHOSPHOKINASE LEVEL IN SIMVASTATIN INDUCED MYOPATHIES IN RATS

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ABSTRACT

Objective: To study the gross morphology of skeletal muscles and plasma Creatinine phosphokinase levels of rats and find effect of long acting β2-agonists co-administration in Statin induced myopathies.

Study Design: Quasi experimental study.

Place and Duration of Study: 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.

Methodology: Adult male Sprague-Dawley rats were procured from NIH Islamabad. Their average approximate age was 70-80 days with weight range 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 for dissolved in distilled water (3µg/kg/day) once daily for 12 weeks. 5ml sample of blood was taken in a plain tube directly from the heart for the quantitative measurement of creatine phosphokinase (CPK) levels before sacrificing of the animals. The animals were sacrificed after three months of the experimental period.

Results: Examination of plasma levels of CPK of the control group revealed the normal values. It was significantly higher in group B as compared to the control group A. The serum creatine phosphokinase levels showed a decrease in the mean levels after treatment with Formoterol in group C

Conclusion: Simvastatin induced the myopathic changes in the skeletal muscle of experimental rats which was shown by increased CPK levels. Formoterol co-administration decreased CPK levels in simvastatin induced myopathies.

Keywords: Creatine phosphokinase, Formoterol, Myopathy, Skeletal muscle, Statin.

INTRODUCTION

Statins represent the first line of treatment for high blood cholesterol and they decrease the risk of atherosclerotic disorders, heart attacks and strokes[1]. Despite their good safety profile, statins can be associated with muscle-related symptoms. Myopathy is a well-documented side effect of statins ranging from simple myalgia to myositis with or without CPK elevation. These symptoms are usually mild and can be tolerated. These complaints rarely lead to severe muscle damage that they can cause kidney failure and even death[2]. These myopathies are potentially reversible; Hence a prompt recognition is quite helpful for the early diagnosis and treatment. Clinical approach to treat these myopathies is to stop the statin therapy and monitor CPK levels till they are resolved[3]. Muscle biopsies are not recommended in most of the patients experiencing myopathies due to statin use[4]. Myalgia has infrequently been scrutinized in statin users. CPK elevation signifying muscle damage, can occur even in the absence of myalgias or weakness[5].

Skeletal muscle adrenoceptors population consists predominantly of β2-adrenoceptors[6]. β2 agonists augment muscle repair and restore muscle function after myotrauma. They reverse

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the muscle wasting[7]. Formoterol is a highly selective β2-adrenoceptor agonist with prolonged duration of action and increased safety profile[8]. Formoterol administration improves the functional and morphological properties in skeletal muscle of dystrophic mice[9]. In this study; Simvastatin induced plasma creatine kinase levels in rats were determined, followed by evaluation of Formoterol co-administration.

Atherosclerosis, hypercholesterolemia 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[10]. This study will help us recommending Formoterol to COPD patient who are also having hyperlipidemias and taking Statins. This would control bronchospasm and also prevent myopathies.

The objective of the present work is to assess muscle related alterations induced by Simvastatin treatment in male Sprague Dawely rats. Myopathy was evaluated with the following indices; body weight change, muscle weight change, muscle length change and CPK levels.

METHODOLOGY

The study design was quasi experimental study. 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. Duration of study was 18 months 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 normal 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 and divided into three groups. The animals were randomly selected by computer generated numbering. They were divided into three groups. It was non probability consecutive 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)[11] 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)[12] by oral gavage once daily for 12 weeks.

The body weight of all the animals was recorded at the start of the study and before sacrificing the animals. 5ml sample of blood was taken in a plain tube directly from the heart for the quantitative measurement of creatine phosphokinase (CPK) levels before sacrificing of the animals. The samples were labeled according to the groups. The animals were sacrificed after three months of the experimental period. Extensor digitorum longus (EDL) muscle was isolated and dissected out along with muscle (fig-1). The tendon to tendon length of the dissected muscle was measured with the scale (fig-2). The dissected muscle was weighed on a digital balance. All adhering fat and connective tissue were removed from the muscle prior to weighing.

Figure-1: Showing dissection of rat hind limb and isolation of extensor digitorum longus.

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. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

The body weight of all the animals was recorded both at the commencement and at the end of the study. The mean initial animal weight in control group A was 266.27 ± 2.738 gm (table–I). At the end of the study, animal weight in group A was increased to the value of 297.10 ± 2.987 gm (table-II). The Extensor digitorum longus was weighed after the dissection, the mean weight of the muscle was 203.37 ± 0.523mg (table-III). The tendon to tendon length of the dissected muscle was measured with the scale; the mean value was 3.60 ± 0.024 cm (table-III). Measurement of creatine phosphokinase (CPK) levels was done at the end of 3 months. Mean value was 277.40 ± 5.344 IU/L (table-IV).

Figure-2: Showing measurement of muscle length.

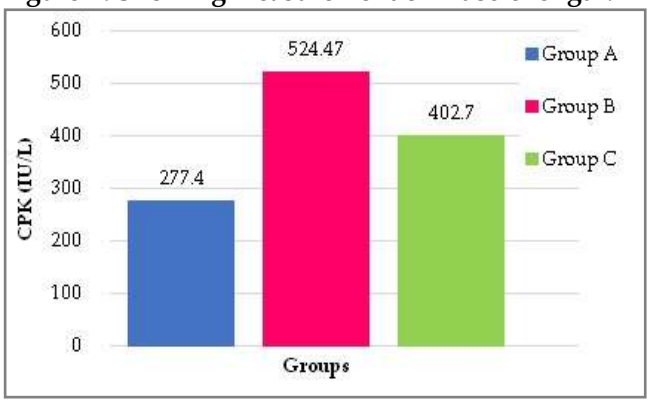

Figure-3: CPK (IU/L) levels in control group A and experimental groups B and C.

The mean initial animal weight in control group B was 264.97 ± 3.350 gm. It was statistically insignificant when compared to both B and C groups (*p*>0.79) (table-I). At the end of the study, animal weight in group B was increased to the value of 278.43 ± 3.428 gm. Final weight was significantly lesser as compared to the control group A (*p*< 0.001) (table-II). The Extensor digitorum longus was weighed after the dissection. The mean weight of the muscle was 184.82 ± 0.993mg. Mean muscle weight was significantly lower as compared to the control group A (*p*< 0.001) (table-III). The tendon to tendon length of the dissected muscle was measured with the scale; The mean value was 3.48 ± 0.021 cm. It was significantly lower as compared to the control group A (*p*-value 0.001) (table-III). Measurement of creatine phosphokinase (CPK) levels was done at the end of 3 months. Mean value was 524.47 ± 15.943 IU/L. It was significantly higher when compared to control group A (*p*<0.001) (table-IV).

Table-I: Comparison of initial weight of animals in control group A, experimental groups B & C.

Weight of Animal	Groups	Mean ± SD	*p*-value
Initial weight (gm)	A	266.27 ± 2.738	0.79
	B	264.97 ± 3.350	
	C	265.57 ± 3.368	

The mean initial animal weight in control group C was 265.57 ± 3.368 gm. It was statistically insignificant when compared to both A and B groups (*p*=0.79) (table-I). At the end of the study, animal weight in group C was increased to the value of 290.73 ± 3.570 gm. It was higher and statistically significant when compared to group B (*p*<0.001) but insignificant from control Group A (*p*=0.372) (table-II). The Extensor digitorum longus was weighed after the dissection, the mean weight of the muscle was 199.51 ± 0.935 mg. It was significantly higher from group B but lesser than group A (*p*<0.001) (table- III). The tendon to tendon length of the dissected muscle was measured with the scale; the mean value was 3.56 ± 0.013 cm. It was significantly higher from group B but lesser than group A (*p*<0.001) (table-III). Measurement of creatine phosphokinase (CPK) levels was done at the end of 3 months.

Mean value was 402.70 ± 8.397 IU/L. It was significantly lesser when compared to group B but significantly more when compared to control group A (p<0.001) (fig-3 and table-IV).

DISCUSSION

In this study, the animals were weighed both at the start as well as at the end of the experiment. Weight gain was compared between each group and it was found that the change in weight was significant. This shows that the medications in the experimental groups have affected the body weight of the animals (table-I). It was seen that Simvastatin treated rats had significantly lesser weight gain than healthy control rats. Studies have shown that statins cause significant muscular weakness, atrophy and lower body mass[13]. The results of this study are in agreement with previous works. Simvastatin decreases cholesterol content in skeletal muscle membranes triggering myocyte membrane instability and apoptosis. This results in increased protein breakdown in muscles which may lead to muscles atrophy and resultantly lowering body mass[14]. However, a significant difference in weight gain between Simvastatin treated and Formoterol treated rats was also noted. Formoterol treated rats were weighing more than those of the Simvastatin treated group at the time of dissection. Studies of β2-agonists in rats have shown to increase protein synthesis, decrease protein degradation and a net increase in myofibrillar protein content[15,16]. Thus formoterol treated rats were better able to maintain weight gain as compared to simvastatin treated rats.

The skeletal muscles of simvastatin treated rats exhibited significant weight and length

Table-II: Comparison of final weight of animals in control group A, experimental groups B and group C.

Weight of Animal	Groups	Mean ± SE	p-value ANOVA	Post Hoc Statistical Significance		
				Group A/B	Group A/C	Group B/C
Final Weight (gm)	A	297.10 ± 2.987	0.001	p<0.001	p=0.372	p=0.029
	B	278.43 ± 3.428				
	C	290.73 ± 3.570				

Table–III: Comparison of weight and length of the muscle in control group A, experimental groups B and group C.

Parameters	Groups	Mean ± SE	p-value ANOVA	Post Hoc Statistical Significance		
				Group A/B	Group A/C	Group B/C
Weight of muscle (mg)	A	203.37 ± 0.523	0.01	p<0.001	p<0.001	p<0.001
	B	184.82 ± 0.993				
	C	199.51 ± 0.935				
Length of the muscle (cm)	A	3.60 ± 0.024	0.01	p<0.001	p=0.477	p=0.034
	B	3.48 ± 0.021				
	C	3.56 ± 0.013				

Table–IV: Comparison of CPK in control group A, experimental groups B and group C.

Parameter	Groups	Mean ± SE	p-value ANOVA	Post Hoc Statistical Significance		
				Group A/B	Group B/C	Group A/C
CPK (IU/L)	A	277.40 ± 5.344	0.01	p<0.001	p<0.001	p<0.001
	B	524.47 ± 15.493				
	C	402.70 ± 8.397				

reduction as compared to healthy and formoterol treated rats (table-III). Skeletal muscle atrophy or hypertrophy is determined by a fine balance between protein degradation and protein synthesis. It has been shown previously that the EDL in simvastatin treated rats shows significant atrophy[17]. Furthermore, we found a significant decrease in fiber diameters of the EDL, allowing us to determine that alterations in muscle weight are partially due to atrophy of the skeletal muscle fiber. This data allows us to conclude that the change in body weight is a direct result of skeletal muscle atrophy. Formoterol treated group showed significantly more muscle weight and length when compared to group B. Muscle fibers gain length by the addition of new sarcomeres and diameter by the addition of the myofibrils. These two influences are alleged explanation for the increases in total muscle mass[18]. With significant increase in total body weight, along with an increase in the muscle weight and length, we can infer that Formoterol increases muscle weight and length in Simvastatin treated rats thus enhancing the growth. Our results are in agreement with previous studies showing that β2-agonists increase the weight of the muscle and enhance skeletal muscle growth and development after injury[15,16,19]. Formoterol increases absolute EDL mass and has considerable therapeutic potential for muscular dystrophies[20,21].

Serum creatine kinase levels are often used as an indicator of damage to skeletal muscle in myopathies. We measured Creatine phosphokinase (CPK) levels at the end of 3 months. It was significantly lesser when compared to group B but significantly more when compared to control group A (table-IV). This indicates that Formoterol reduces CPK level by restoring skeletal muscle structure of degenerated muscle. This restoration was not complete as mean CPK level was still higher in group C when compared to group A. Statin treatment was related to greater muscle damage as assessed by CPK levels. CPK level was more pronounced in those animals who displayed more deranged histomorphological changes, Similarly the levels reduced in those animals that showed better restoration of muscle architecture. Our results were contrary to the results of Sykes *et al* 1991. In his study CPK levels were raised with infused beta 2 agonist terbutaline, probably due to tremor which is a common side effect of large doses of terbutaline[22]. However, there is lack of research on monitoring CPK levels with newer generation B2 agonists like Formoterol.

CONCLUSION

Simvastatin induced the myopathic changes in the skeletal muscle of experimental rats. Formoterol co-administration minimized and has a role in reversing gross morphological changes in Simvastatin induced myopathies. Serum creatine phosphokinase levels were raised by simvastatin in the skeletal muscle of experimental rats. Formoterol co-administration decreased CPK levels in simvastatin induced myopathies

CONFLICT OF INTEREST

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

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