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Role of Formoterol on Myogenic Regulatory Factor to Ameliorate Statin-Induced Myopathy

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ABSTRACT

Objective: To investigate the effects of β2-adrenergic agonist formoterol on expression of myogenic regulatory factor myogenin in statin-induced myopathies in rats

Methodology: Adult male Sprague-Dawley rats were randomized into control (A), statin-only (B), and statin + formoterol groups (C) (n=30 per group). The control group A received no treatment. B group received simvastatin at 60 mg/kg/day by oral gavage for 12 weeks to induce myotoxicity. C group received simvastatin at 60 mg/kg/day plus formoterol at 3 µg/kg/day by oral gavage for 12 weeks. After 12 weeks, extensor digitorum longus muscles were dissected and 5 µm transverse sections were immunostained for myogenin expression. One cross section was selected from each of the specimen for study. Myogenin-positive nuclei were quantified in 8 random 40x magnification fields per muscle section by a blinded investigator. Data was analyzed using IBM SPSS.

Results: Myogenin-positive nuclei were minimal in the control (A) group and statin-only (B) groups. In contrast, the statin + formoterol (C) group exhibited a significant increase in myogenin-positive nuclei compared to both control and statin-only groups ($p<0.001$), indicating enhanced muscle regeneration.

Conclusion: Formoterol treatment augmented skeletal muscle repair pathways in a rat model of statin-induced myopathy, potentially via activation of quiescent muscle satellite cells and upregulation of myogenic regulatory factor myogenin in group C.

Keywords: Statin, Formoterol, Myopathy, Myogenin, Muscle regeneration

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,3] Critical analysis and manuscript review; [5,6] Data analysis; Manuscript Editing.

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Introduction

Statins are extensively prescribed cholesterol-lowering agents and first-line therapy for hypercholesterolemia and prevention of coronary heart disease (CHD).[1] However, statin-induced myopathy is a common adverse effect and the leading cause of statin intolerance and withdrawal.[2] Statin myopathy manifests as muscle fatigue, weakness, pain, tenderness, and cramping exacerbated by exercise.[3] Proposed mechanisms underlying statin myotoxicity include depletion of mevalonate pathway products and isoprenoids, mitochondrial dysfunction, and apoptosis induction.[4] These muscle side effects are potentially reversible.[5] Skeletal muscle repair and regeneration relies on satellite cells (SCs), quiescent mononuclear progenitors responsible for postnatal muscle growth and injury repair. Upon muscle damage, SCs are activated and express myogenic regulatory factors (MRFs) controlling proliferation, differentiation, and

maturation.[6] Myogenin is a critical MRF for myogenic commitment expressed in proliferating myoblasts and differentiating myocytes. Myogenin regulates skeletal muscle cell division, fusion, and fiber maturation.[7] Stimulating β-adrenergic receptors promotes skeletal muscle repair in myopathic states[8]. β-agonists like formoterol increase muscle protein synthesis and reduce degradation.[9] Formoterol exhibits greater efficacy versus older β2-agonists like clenbuterol.[10]

This study investigated formoterol, a long-acting β2-agonist, on myogenic regulatory factor myogenin in statin-induced myopathy. This study aims to understand the molecular and cellular factors that control skeletal muscle repair after injury. Learning more about these natural regeneration pathways could help develop new treatments to improve muscle healing in muscle diseases and injuries.

Methodology

This randomized controlled experimental trial was conducted at Army Medical College Rawalpindi in collaboration with National Institute of Health, Islamabad and Armed Forces Institute of Pathology, Rawalpindi. Adult male Sprague-Dawley rats (n=90, age 70-80 days, weight 250±50g) were acquired from NIH Islamabad. Rats were housed at 18-26°C on a 12-hour light/dark cycle with ad libitum access to standard rodent diet and water.

Rats were randomly assigned to three groups (n=30 per group). Group A received no treatment. Group B received simvastatin (60 mg/kg/day) dissolved in distilled water via oral gavage for 12 weeks to induce myopathy[11]. Group C received simvastatin (60 mg/kg/day) plus formoterol (3 µg/kg/day) dissolved in distilled water via oral gavage for 12 weeks[8].

After 12 weeks, rats were euthanized by isoflurane overdose and cervical dislocation. Extensor digitorum longus (EDL) muscles were harvested, and 0.5 cm transverse sections obtained from mid-belly were fixed in 10% neutral-buffered formalin. Sections were paraffin-embedded, and 5 µm thick slices immunostained for myogenic regulatory factor myogenin.[12]

Myogenin+ nuclei were identified by dark brown immunostaining and counted in 8 random 40x fields per section by a blinded investigator.[4] Data were analyzed using ANOVA and Tukey's post-hoc tests in SPSS v21, with $p<0.05$ indicating statistical significance.

Results

Male Light microscopic analysis of extensor digitorum longus (EDL) muscles from the control group showed the expected normal histological architecture of skeletal muscle tissue. Myogenin+ myonuclei were counted in separate slides. They appeared dark brown in positive specimen. The number of Myogenin+ myonuclei was counted and its mean value was 14.17 ± 0.538 (Table 1).

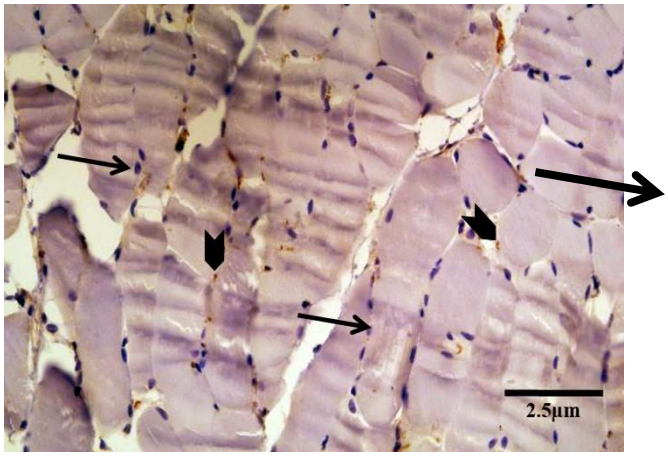

Figure 1: Transverse section of Extensor Digitorum Longus of a rat from group B. There are occasional Myogenin positive satellite nuclei (Arrow head). IHC anti- Myogenin antibody, Bar = 2.5 µm

Cross section of skeletal muscle of group B showed polygonal shaped bundles of skeletal muscle fibers having peripherally located nuclei. The number of Myogenin+ myonuclei was counted (Figure 1) and its mean value was 14.20 ± 0.397. This difference was statistically insignificant from control group A ($p>1.0$) (Table 1).

Table 1: Comparison of Immunohistochemical marker Myogenin+ myonuclei in three groups

Parameter	Group	Mean ±SE	Statistical Significance		
			Group A/B	Group A/C	Group B/C
Number of Myogenin + myonuclei	A	14.17 ± 0.538	p > 1.0	p < 0.001	p < 0.001
	B	14.20 ± 0.397			
	C	184.87 ± 10.845			

Cross section of EDL of group C showed polygonal shaped bundles of skeletal muscle fibers having peripherally located nuclei. The number of Myogenin+ myonuclei was counted (Figure 2) and its mean value was 184.87 ± 10.845. It was significantly higher when compared to groups A and B ($p < 0.001$) (Table 1).

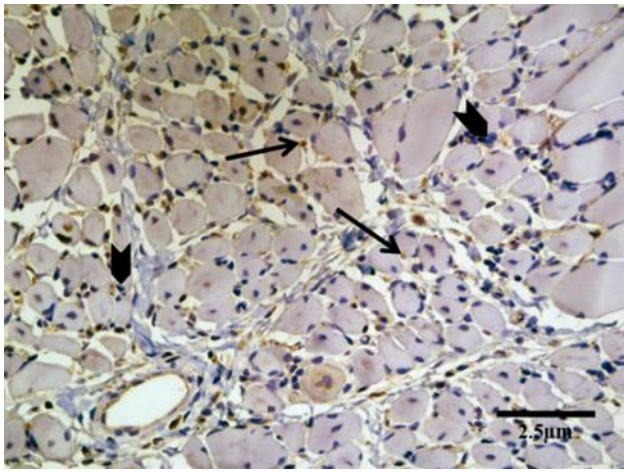

Figure 2: Transverse section of Extensor Digitorum Longus of a rat from group C. There are numerous Myogenin positive satellite nuclei (Arrow).
IHC anti- Myogenin antibody, Bar = 2.5 µm

Discussion

Skeletal muscle has a remarkable capacity to regenerate after injury through activation of muscle stem cells called satellite cells. Satellite cells are normally quiescent but rapidly activate upon damage, initiating the muscle repair cascade.[12] After activation, satellite cells proliferate and express myogenic regulatory factors (MRFs) including MyoD, Myf5, and myogenin to differentiate and fuse into mature myofibers.[13] Understanding and harnessing these endogenous myogenic programs is an attractive therapeutic strategy for strengthening muscle regeneration in various neuromuscular disorders.

In this study, we found that the β2-adrenergic agonist formoterol significantly increased expression of the MRF myogenin in a rat model of statin-induced myopathy. Myogenin plays a critical role in muscle development by stimulating myoblast differentiation and fusion.[14] The robust upregulation of myogenin indicates that formoterol augments satellite cell activation and engagement of muscle regeneration pathways.

Regenerating skeletal muscle displays heightened β2-adrenoceptor density compared to undamaged muscle.[15] This provides a rationale for using β2-agonists like formoterol, which raise intracellular cAMP levels leading to activation of muscle growth signaling cascades.[16] The precise mechanisms underlying formoterol's enhancement of myogenin expression and satellite cell activity require further elucidation but may involve increased myogenin gene transcription mediated by CREB binding to the myogenin promoter.[17]

In addition to stimulating muscle regeneration, formoterol has also been shown to reduce muscular oxidative stress and stabilize components of the dystrophin glycoprotein complex disrupted by statins.[18] The dual actions of stimulating satellite cell myogenic programs while mitigating damage processes likely underlie formoterol's efficacy in our model.

Overall, targeted modulation of regulatory factors governing muscle repair represents a promising approach for managing muscle diseases. Clinical trials are warranted to determine if formoterol safely improves statin tolerability in patients by boosting muscle regeneration. If efficacious, β2-

agonist therapy could help more individuals benefit from cholesterol lowering treatment.
findings.

Conclusion

Formoterol increased the regenerative capacity of skeletal muscle fibers of rats after Statin induced myopathy. Formoterol caused the activation of dormant satellite cells and is the possible cause of the increased Myogenin expression in group C which were demonstrated by immunohistochemistry.

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