Archives of Razi Institute, Vol. 77, No. 1 (2022) 285-291

DOI: 10.22092/ARI.2021.356683.1895

Original Article

Investigation of the Effect of Atorvastatin on Skeletal Muscles in Male Rats and the Involved Mechanisms

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Received 11 November 2021; Accepted 4 December 2021
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Abstract

It has been approved that atrovastatin is a preferred treatment for hyperlipidemia. One of the atrovastatin drawbacks would be the detrimental effects on skeletal muscles. Therefore, the current study was designed to evaluate all the skeletal muscles alteration in rats' after administration of atrovastatin and identification the mechanisms involved in these structural alterations in the skeletal muscles. A total of 12 healthy adult male rats (*Rattus norvegicus)* were randomly divided into two groups (n=6). The control group (G1) included rats that received distilled water as the placebo, and the treatment group (G2) included animals that were treated with atorvastatin (80 mg/kg/day) dissolved in distilled water and administrated by a gastric tube for eight weeks. At the end of the experiment, trapezius and vastus medialis muscle tissues were sampled and fixed with 10% formalin for histopathological studies. Atorvastatin administration gave rise to morphological changes in the skeletal muscle fibers and the nerve fibers, including atrophied myofibers, infarction, irregular arrangement of myonuclei, disappearance of nuclei from their normal peripheral position with acute skeletal muscular infarction, and infiltration of accumulated inflammatory cells. Atorvastatin has been revealed to have several adverse effects on the skeletal muscle and the nerve supply. Based on the data in the current study, it is evident that atorvastatin administration for less than two months resulted in some sorts of myotoxic structural changes and apoptosis as evident by deformity and lack of striation degeneration of nuclei, as well as splitting of the muscle fibers in the adult male rats' skeletal muscle.

Keywords: Hyperlipidemia, Low-density lipoprotein cholesterol, Myofibers

1. Introduction

It has been well documented that one of the most important risk factor for developing cardiovascular disease in the developed and developing countries would be the Hyperlipidemia which lead to the serious death rate in the populations. In fact, elevated concentration of low-density lipoprotein cholesterol (LDL-C) has been detected in nearly about 30% of adults in the United States. Therefore, the risk for heart attack has been doubled by these elevated levels of LDL-C. The HMG-CoA reductase is a pivotal enzyme as a rate limiting enzyme in the cholesterol biosynthesis. Statins are drugs which have been used to cure hypercholesterolemia by inhibiting HMG-CoA reductase. Although the statins drug could be effective in the prevention of cardiovascular disorders, but the consumption of this drug is frequently associated with statin-induced myopathy (SIM). The SIM includes myalgia, muscle cramps, weakness, and even rhabdomyolysis (1, 2). Myalgias in most case allude to symptoms such as pain, tenderness, or weakness without correlated increase in serum creatine kinase (CK) in the course of statin use and are reported to happen at frequencies ranging from 1% to 10%. Myopathy usually refers to myalgias correlated with increment in serum CK concentration and occurs at a

lower frequency (<5%) (3, 4). For prevention of cardiovascular disorders statins are widely used. Although some of these cardiovascular disorders generally well tolerated, but different grades of myopathy, ranging from mild myalgia to fatal rhabdomyolysis has been reported (1, 5-6).

It has been well documented that the rhabdomyolysis is the most severe detrimental effects characterizing by the structural damages on skeletal muscle. The muscle proteins released to the blood by these damages. The rhabdomyolysis caused by Statin has divided into three different classes as the following: acute, sub acute, or chronic, and may be permanent despite dose reduction or substitutions. The renal failure which is one of the most complications in the persons who consume the statin is triggered by the Myoglobin release from the damaged muscle to the blood stream. In the several previously published studies the researchers showed that adverse muscle events have been associated with consumption of all kinds of statins (7-9). In the patients who suffered from higher levels of triglycerides (TG), for reducing the elevated levels of TG statins considered as the best option which can lead to reduction in the level of LDL-C and VLDL-C. The lower doses of statins cannot be effective in many patients who suffered from higher level of TG to reduce TG, and cholesterol. Administration of the higher doses of statins may lead to a dramatic increase in the risk of myopathy. The mechanisms of statin-induced myopathy may include the acute depletion of secondary metabolic intermediates and induction of apoptosis (8, 10, 11).

Therefore, current research was aimed to evaluate detrimental effect of consumption of atrovastatin at a dose of 80 mg/kg on structure of skeletal muscles in adult male rats.

2. Materials and Methods

2.1. Animals and Experimental Design

A total of 12 healthy adult male rats (*Rattus norvegicus*), with a mean age of 10-12 weeks and a mean weight of 200±25 g, were selected and housed at the Animal House of the Science College, Misan University, Maysan, Iraq. The animals were housed beneath a controlled standard situation (at 20-23°C and a controlled room on a 12:12 light: dark cycle). The rats (n=12) were then randomly divided into two equal groups, housed individually in polycarbonate cages with hygienic beddings, and fed with laboratory food. In the control group (G1), the animals received distilled water as the placebo, and the treatment group (G2) included rats that were treated with atorvastatin (80 mg/kg/day) dissolved in distilled water and administrated by a gastric tube for eight weeks.

Atorvastatin tablets (40 mg) were purchased from Sobhan Company, Iran. Before use the tablets were thoroughly ground and then the atorvastatin powedered used to formulate a dose of 80 mg/kg B.W. Subsequently, the drug was dissolved in distilled water (DW) at the time of use. At the end of the experiment, the rats were anesthetized utilizing an overdose of chloroform. For histopathological studies the trapezius and vastus medialis muscles were fixed by use of 10% formalin (2).

A light microscopic examination was conducted as follows: the slides were stained with Hematoxylin & Eosin, Iron hematoxylin, and P.A.S, stains; following that, they were examined using a light microscope.

2.2. Statistical Analysis

The included statistical data were expressed as mean, standard error (±SE), and standard deviation (±SD). Moreover, the student t-test was employed to elucidate the differences between the treatment and control groups. The obtained data were analyzed using SPSS software (version 15).

3. Results

Light microscopic observations on sections from trapezius and vastus medialis skeletal muscles related to the control group showed normal parameters, such as cylindrical long multinucleated tubes; preserved myofibers extended and arranged as longitudinal, regular, and parallel fibers; vesicular and peripherally situated myonuclei; and perimysium connective tissues fill with intercellular spaces and separated muscle fibers.

On the other hand, the cross-sections from normal skeletal muscle showed normally preserved myofibrils

with clear striation, peripheral nuclei, and small capillaries. Moreover, the recorded data obtained from the cross-sections showed the strands of collagenous fibers and fibrocytes extended between muscles bundles. The regular muscle bundles were surrounded by dense connective tissue known as the epimysium. The septa were extended from epimysium to surround the fibers bundle and blood capillaries (Figures 1- 3).

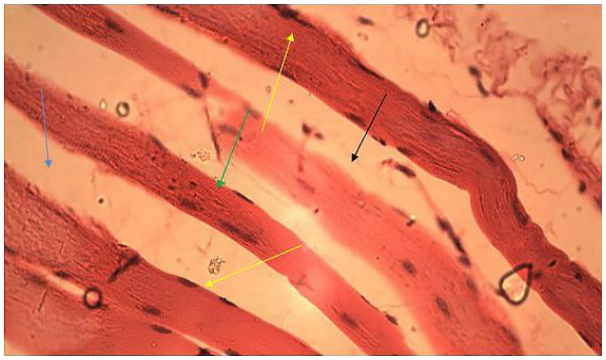

Figure 1. A longitudinal section on the muscle from the control rats showing the bundles of non-branching cylindrical-shaped muscle fiber with acidophilic sarcoplasm (⟶) and clear striation (⟶) separated from these fibers by spaces (⟶) and multiple elongated vesicular nuclei peripherally located beneath the sarcolemma (⟶) (H&E stain; 40×)

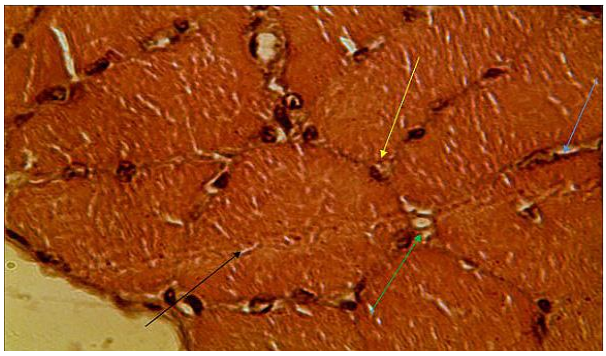

Figure 2. A transverse section of the control skeletal muscle tissue showing polygonal-shaped skeletal muscle fibers with acidophilic cytoplasm (⟶) separated by normal intercellular space (⟶), peripherally located nuclei (⟶), and normal blood vessels (⟶) (H&E stain; 40×)

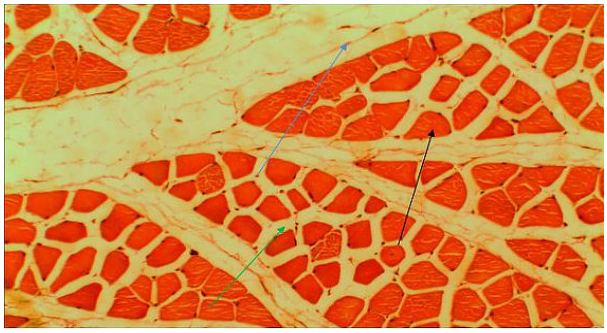

Figure 3. A transverse section on the control skeletal muscle showing a regular section of the muscle fibers (⟶) separated by normal endomysium (⟶) and fine strands of collagen fibers (⟶) (H&E stain; 40×)

The sections from the rats' skeletal muscles, tissue trapezius, and vastus medialis, obtained from the treatment group showed divers changes, including atrophied myofibers, infarction, irregularly arranged myonuclei, disappearance of nuclei from their normal peripheral position with acute skeletal muscular infarction, and infiltration of accumulated inflammatory cells. Most muscle fibers in the treatment group had wavy shapes, and the others had taper endings and barely surface; moreover, their striation was less obvious. On the other hand, the sections showed the extension of dense perimysium among muscle fibers with large intramuscular nerve trunk surrounded by epineurium and branches of myelinated nerve fibers to supply the skeletal muscle fibers. Moreover, the section depicts the dense collagenous fibers and congested blood capillaries with heavy inflammatory cells around them. Furthermore, the cross-sections from the treatment group showed fibers with completely myonecrosis, as well as a significant increase in the spaces in the connective tissues that separate muscle fibers. In the connective tissues that separate the muscle fibers, pale areas were clearly distinguishable which revealed the damage of myofibrils and discontinuity. In addition, muscle fragments were noticed, and the cross-sections obtained

from the treated animals showed that the nerve trunk was composed of several atrophied nerve fibers, and some muscle fibers appeared with irregular surfaces. Other sections showed degenerated myofibrils, severe atrophied muscle fibers, damaged pale areas, and deeply stained nuclei. From the recorded data, it is revealed that some of these myonuclei are detached from their normal peripheral location, and others apparently showed some degrees of karyolysis or vacuolation.

Furthermore, it is obviously detectable that the sarcolemma was thick and dark with a zigzag shape. Although the dense collagenous fibers were extended as septa among the muscle fibers, most muscle fibers had taper endings, and others looked as filaments.

The aggregation of the inflammatory cells and myonecrosis fibers were noticed among other intact fibers and very crowded myonuclei. Areas of extensive necrosis, haemorrhage, and rich blood capillaries with bundles of collagen fibers with inflammatory cells invade the connective tissue septa (Figures 4-9).

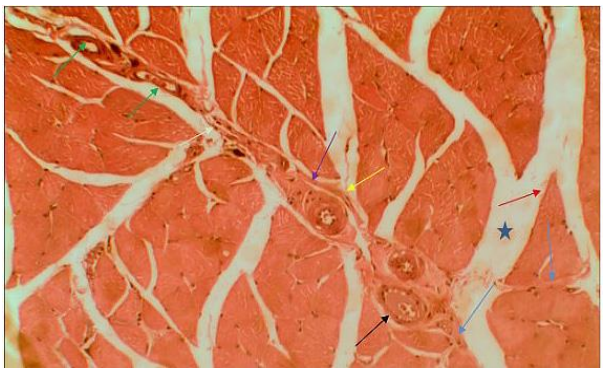

Figure 4. A section of the control rat's skeletal muscle tissue of the treatment group showing irregular degeneration in some muscle fibers ( ), strand of collagen fibers ( ), number of blood vessels ( ), extension from the epimysium ( ). Nerve trunk is also noticed ( ) with a small accumulation of the inflammatory cell ( ), taper endings ( ), and dilated intercellular spaces ( ) (H&E stain; 40×)

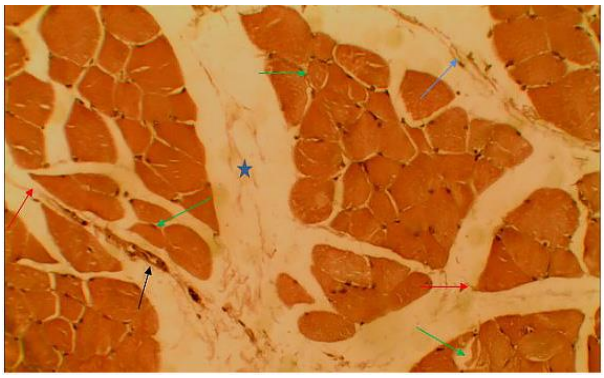

Figure 5. A transverse section on control rat skeletal muscle tissue of the treatment group showing dilated intercellular spaces ( ), infiltrate inflammatory cell ( ), and strand of collagen fibers ( ). Some muscle fibers atrophied and degenerated ( ) and others have taper endings ( ) (H&E stain; 40×)

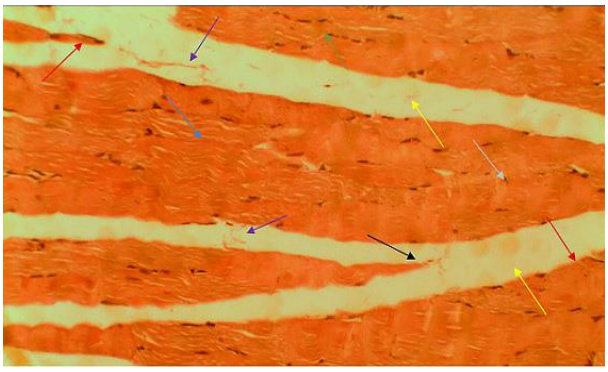

Figure 6. A section on the rat skeletal muscle of the treatment group showing muscle fibers with taper endings ( ). Other wave fibers ( ) also reveal some degenerated muscle fibers ( ) with irregular myonuclei ( ), dilated intercellular spaces ( ), sparse collagenous fibers ( ), and the surface of muscle fiber bundle showing irregular sarcolemma ( ) (H&E stain; 40×)

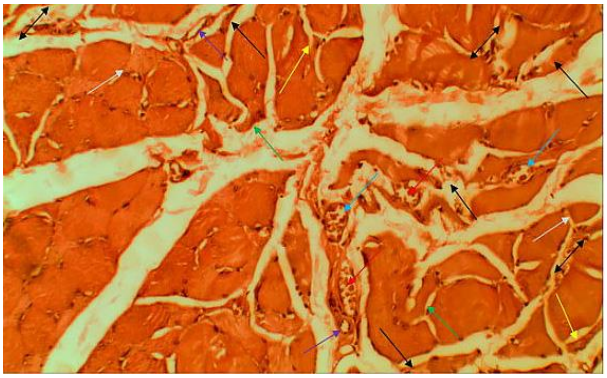

Figure 7. A section on the rat skeletal muscle tissue of the treatment group showing an increase in the damage area to include the superficial and deep layers ( ), muscle spindle ( ) with degenerated intrafusal muscle fibers ( ), foci inflammatory zone ( ), fragments of muscle fibers ( ), blood vessels ( ), atrophied muscle fibers ( ), and bifurcate muscle fibers ( ) (H&E stain; 40×).

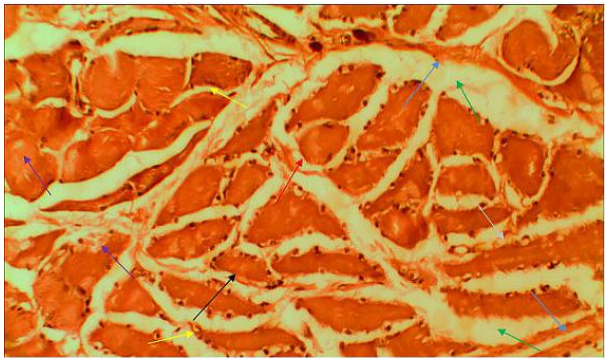

Figure 8. A section on the rat skeletal muscle tissue of the treatment group showing atrophied skeletal muscle fibers ( ), heavy deposition of collagenous fibers ( ), some oval muscle fibers ( ), splitting fibers ( ), dilated intercellular spaces ( ), pale region of degeneration ( ), as well as the degeneration of muscle fibers and barely surface ( ) (H&E stain; 40×)

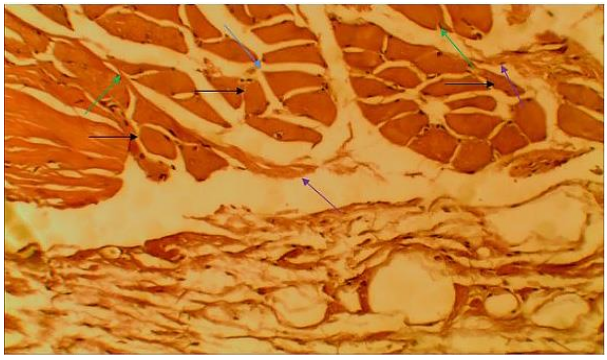

Figure 9. A section on the rat skeletal muscle tissue of the treatment group showing variable muscle fibers ( ) with taper endings ( ), dense epimysium ( ), rounded muscle fibers ( ), other splitting fibers ( ), strands of collagen fibers ( ), and disappearance of the myonuclei from some muscle fibers ( ) (H&E stain; 40×)

4. Discussion

Histologically observations on trapezius and vastus medialis of the rats' skeletal muscle tissue sections in the control group showed muscle fibers with normal structure. These fibbers were parallel and aligned with peripheral myonuclei. The perimysium connective tissues furnish the intercellular area that separated muscle fibbers. Small capillaries are extended in these spaces, and these results clarified that all rats' administration with neutral solution did not show any changes. These findings are similar to the recorded data from a study conducted by Anto Michel, Colberg (10) who mentioned the normal structure of the skeletal muscles.

Skeletal muscle tissues from the treatment group showed obvious alterations, such as distortion, mild atrophy, infarction, and irregularly arranged myonuclei. Most muscle fibers were wavy and had barely surfaces. The striation was less clear, and all these changes were due to the effect of atorvastatin (80 mg/kg/day), which was confirmed by the grading of degeneration of muscle fibers. These findings are in agreement with the results of a previously published study by Schaefer, Lawrence (12) who reported that in clinical practice, 55%-10% of patients receiving statins developed myopathy, and this myopathy was dose-dependent. Furthermore, this was in line with the results of a study by Silva, Matthews (13) who reported a 10-fold increase in myopathy in patients taking a high dose of atorvastatin or simvastatin, compared to patients on a lower dose.

Furthermore, sections on skeletal muscle tissue from the treatment group showed degenerated myofibrils and hypercontracted fibers with internal myonuclei more than peripheral location. Dense collagenous fibers were extended within dilated intercellular spaces in addition to the inflammatory cells and atrophied with fewer muscle spindles. In addition, the nerve trunk showed fewer branches and demyelinated with degeneration. These results were reported by some investigators using a variety of statins (12, 14, 15).

It could be concluded that the inflammatory reaction is a consequence of detrimental damages of the muscle fibers. This phenomenon was stated by Vaughan and Gotto (16) who mentioned that the deteriorated muscle fibers secret numerous inflammatory mediators which lead to mononuclear cellular infiltration. Several markers were mentioned as the signs of muscle fiber degeneration, such as the

centrally located nuclei observed in this study (17). Furthermore, the inadequate oxygen supply along with metabolites exchange to the enlarged and hypertrophied fibers may lead to the breaking of the muscle fibers (14).

Moreover, the histological findings of the current study was in good agreement with the results of a study performed by Pulipati and Davidson (15) who found that the consumption of statin lead to the muscles necrosis in type II fibers. It is approved that muscle fibers degeneration initiated in type II fibers. While the type I fibers were least sensitive to statins (12). In a study conducted by Smallwood et al. (14) they have stated that vastus medialis muscle is formed of type II white fibers.

5. Conclusion

From the recorded data in the current study, it is clearly evident that the atorvastatin administration for less than 2 months resulted in some sorts of myotoxic structural changes and apoptosis as evident by deformity and lack of striation, as well as the degeneration of nuclei and splitting of muscle fibers in the adult male rats' skeletal muscle.

Authors' Contribution

Study concept and design: K. M. H.
Acquisition of data: K. M. H.
Analysis and interpretation of data: K. M. H.
Drafting of the manuscript: K. M. H.
Critical revision of the manuscript for important intellectual content: K. M. H.
Statistical analysis: K. M. H.
Administrative, technical, and material support: K. M. H.

Ethics

All procedures were approved by the ethics committee of the University of Misan, Maysan, Iraq under project number 2020-4789-5478.

Conflict of Interest

The authors declare that they have no conflict of interest.

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