Original Article

Effect of Carnitine

Therapeutic Effect of Carnitine on Atorvastatin induced Mechanical Myotoxicity of Gastrocnemius Muscles of Rats

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ABSTRACT

Objective: To Study Therapeutic Effect of Carnitine on Atorvastatin-induced Mechanical Myotoxicity of Gastrocnemius Muscles of Rats.

Study Design: Observational study

Place and Duration of Study: This study was conducted at Department of Physiology, University of Karachi, from 21st January 2012 to 30th December 2013.

Materials and Methods: Present study showed that effect of statin on mechanical properties of gastrocnemius muscle of rats and use of carnitine as prevention of statin induced myopathies. Animals were injected statin for 6 weeks in one group and carnitine to another group along with statin to study the possible therapeutic effect. After treatment period, animals were decapitated and gastrocnemius muscles were isolated. Twitch and tetanus of muscles were recorded in each group.

Results: Our results showed that treatment of statin reduced the body weight of animals and increased the resting length (106%) of isolated gastrocnemius muscles. We also observed that force of contraction of both twitch and tetanus in statin treated group were significantly reduced (P>0.0001). This negative effect of statin on twitch and tetanus parameters of muscle was partially decreased by an additive treatment of carnitine.

Conclusion: Thus, carnitine plays a vital role in improving muscle contractile ability caused by statins. Our study demonstrated the potential preventive measure of atorvastatin-induced myopathy using carnitine and its impacts on mechanical function of muscles.

Key Words: Hypercholesterolemia, Skeletal muscles, Statin, Carnitine, Muscle twitch, and Muscle tetanus

INTRODUCTION

Statins are the most preferred and widely used drugs for the treatment of hypercholesterolemia and prevention of various cardiovascular diseases due to their efficacy and tolerance[1]. Despite their life saving advantages, these drugs are reported to cause serious side effects in skeletal muscles. The chronic use of atorvastatin leads to adverse effects ranging from myalgias, myopathies and muscle cramps to fatal condition like rhabdomyolysis[2,3,4]. A number of mechanisms of damage have been proposed including deleterious effects on mitochondrial respiratory chain, energy production by skeletal muscle, membrane cholesterol[5] and degenerative changes in skeletal muscle[6]. Insufficient cholesterol in the muscle membrane due to statin treatment may alter membrane fluidity[7] and makes it vulnerable to acidosis, because leakage of ions and mitochondrial dysfunction respectively. Both these negative effects ultimately damage the contractile properties of skeletal muscles.

Statin induced side effects could be prevented as well as treated by giving a combination therapy of statins with L-carnitine[8]. Carnitine is a quaternary ammonium compound essential for the breakdown of fats into energy in the body[9]. However, possible protective effect of carnitine on mechanical properties of skeletal muscles is yet not well known. Therefore, the purpose of the present study was to determine the therapeutic effect of carnitine on contractile properties of gastrocnemius muscle for statin-induced myotoxicity. Physiological profile of statin induced muscle damage can be measured by mechanical parameters that are valid indicators of myopathy.

MATERIALS AND METHODS

Chemicals and Drugs: Commercially available statin (Lescol, Novartis) in the vehicle methylcellulose (CMC) suspension were used for the chronic dosing of rats in vivo. All chemicals used in the study were purchased from Sigma Aldrich until otherwise stated.

Grouping and Treatment of Animals: Albino rats of 250 to 300 grams were housed in different cages with 5 rats in each cage and the cages were labeled. The animals were randomly divided into three groups as follows:

Group I: the control group consisting of normal untreated animals

Group II: statin treated group to see the adverse effects of statins

Group III: statin + carnitine treated group, to see the prevention from adverse effects of statin.

After habituation process, statin was given at a dose of 10 mg/kg/day by rat feeding needle for 6 weeks. In parallel, control group was given 1 ml 0.5% of CMC (10) and 1 ml of carnitine 300mg/kg[11] with statin was administered to group III. After 6 weeks, animals were weighted again and dissection of animals followed by recording of mechanical properties of gastrocnemius muscle.

Muscles Dissection and Fixation: Gastrocnemius muscle from both the limbs was dissected out and isolated muscle was kept in oxygenated Krebs solution at 37^0 C in the recording chamber. Composition of Kreb's solution (in mM): NaCl 119, KCl 4.8, $CaCl_2$ 3.2, $MgSO_4$ 1.2, $NaHCO_3$ 24, NaH_2PO_4 1.2, EDTA 0.02 and glucose 11[12]. Solutions were continuously gassed with 95% O_2 and 5% CO_2. 1mM tetrodotoxin was added to the solutions to avoid spontaneous contraction of muscle. The pH of solutions was maintained between 7.2 and 7.3 during each experiment.

The gastrocnemius muscle was fixed in the chamber and connected to the force transducer that was finally connected to the power lab. The stimulating electrodes were connected via lead with the stimulator and were placed under the muscle belly.

Mechanical Parameters Recording: Before recording of mechanical parameters, resting length was determined. Muscle was fixed in the organ bath and was kept flaccid. This flaccidity was tightened by mm and the muscle was stimulated at Frequency 1 Hz. This procedure was continued until maximum tension was developed in the muscle. At this point, length of the muscle was measured and was denoted as resting length.

In order to record the simple muscle twitch, the power lab was kept at 50V strength and 0.5m.Sec duration with frequency of 1 Hz. Tetanus was recorded by giving continuous stimulation for a short period of time. Low frequency tetanus was recorded at a frequency of 1 Hz while high frequency tetanus was recorded at frequency 80 Hz.

Statistics: SPSS software version 17 was used for the analysis of data. The results were expressed as mean ± SE. Student independent t test were performed for the determination of difference between group means. The significance level were set as P<0.05.

RESULTS

1) Effect on Body Weight: Body weights of animals of each group are shown in the table 1 on first day and last day of treatment. Values clearly indicated that chronic statin treatment was the main cause of profound decrease in body weight of the animals.

2) Resting Length: In the present experiments using gastrocnemius muscles of rat, the contraction properties were studied in both control and treated muscles. In the first step, the resting length of muscles was determined to obtain maximum muscle contractions. The **resting length** of the muscles was significantly increased (106.6%) in statin treated muscles (control 3.0±0.006, statin 3.2±0.03; P<0.0001). However, use of statin along with the carnitine significantly reduced the resting length back to the range of control muscles (control 3.0±0.006, Statin + carnitine3.03±0.01; P=0.037, Figure 1)

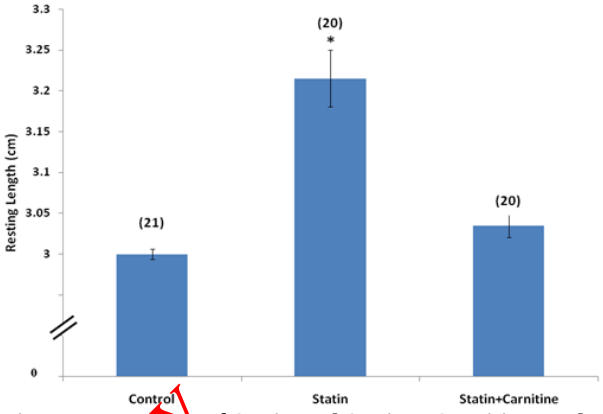

Figure No.1: Effect of Statin and Statin + Carnitine on the Resting Length of Gastrocnemius Muscles of Rats

Figure 1 Resting length of gastronomies muscle of rats chronically treated with atorvastatin (5 mg kg/day) and from control rats. Each bar is represent Mean ± SE * represents a significant change (P < 0.01) when compared with control. ϕ represents a significant change when a comparison was made between statin treated and statin + carnitine treated groups. Values in parentheses indicate number of experiments.

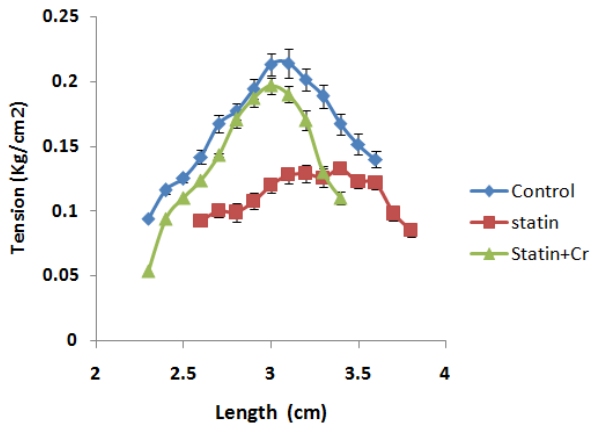

Figure No.2: Statin and Statin + Carnitine induced Length tension relationship of gastrocnemius muscles

Figure 2: Length-tension relationship of rat gastrocnemius muscles from statin and statin + carnitine group. Tension was plotted vs. muscles length during the twitch recording. The data were obtained from 20 muscles. Each data point indicated values in mean ± SEM.

3) Twitch parameters: Force of contraction (FOC) and **contraction time** (CT) were significantly reduced

by statin treatment. (FOC control 0.24±0.005, statin 0.14±0.004; P<0.0001) and (CT control 19.0±0.17, statin 17.3±0.48; P<0.002) .This was regained near to normal when given along with carnitine. Similarly **rates of rise and relaxation** of twitch tension were significantly reduced (P<0.001) with chronic statin treatment but carnitine could not reverse this effect significantly (Table 2).

Table No.1: Effect of Statin on body weight

	Before treatment	**After Treatment**
Control (21)	275±5.1	265±6.5
Statin (20)	270±4.2	240±5.5*
Statin + Carnitine (20)	283±5.5	290±5.2

The values represent Mean ±SE. * shows a significant change (P value < 0.01) when compare with control. Values in parentheses indicate number of experiments.
The values represent Mean ± SE * shows a significant change (P value < 0.01) when compare with control. ϕ shows a significant change when a comparison was made between statin treatment and statin + carnitine treated groups. Values in parentheses indicate number of experiments.

Peak Duration and **Total Twitch Duration** were significantly increased by statin treatment (P<0.0001 and P<0.005 respectively), however only Peak Duration could be brought back to normal by carnitine while no improvement was seen in Total Twitch Duration. In contrast, no effect of statin treatments was observed on **Half Relaxation Time** of the skeletal muscle. (Table 2)

Table No.2: Effect of Statin and Stain + Carnitine on Twitch Parameters of Rat Gastrocnemius Muscles

Parameters	Control (21)	Statin (20)	Statin + Carnitine (20)
Force of Contraction (Kg/cm^2)	0.24±0.005	0.14±0.004*	0.25±0.012 ϕ
Rate of Rise of Tension (g/sec)	26.1±0.7	16.0±0.6*	14.7±0.96*
Rate of Relaxation (g/sec)	17.2±1.1	11.8±0.8*	13.5±0.8
Contraction Time (mS)	19.0±0.17	17.3±0.48*	19.0±0.34 ϕ
Half Relaxation Time (mS)	9.0±0.55	10.0±0.55	13.5±1.1* ϕ
Peak Duration (mS)	4.1±0.14	7.15±0.29*	3.5±0.13*ϕ
Total Twitch Duration (mS)	32.0±0.54	35.3±0.91*	36.4±0.75*

Table No.3: Effect of Statin and Statin + Carnitine on Tetanus of Rat Gastrocnemius Muscle

Parameters	Control	Statin	Statin + Carnitine	Control	Statin	Statin + Carnitine
	At Low Frequency			At High Frequency		
Force of Contraction (Kg/cm^2)	0.22±0.006 (21)	0.13±0.004* (20)	0.21±0.009 ϕ (20)	0.91±0.038 (21)	0.55±0.02* (20)	1.0±0.06 ϕ (20)
Half Relaxation Time (mS)	9.7±0.35 (18)	10.8±0.56 (18)	12.7±0.76* (20)	71.0±2.8 (18)	52.0±1.76* (18)	51.0±1.5* (20)
Rate of Rise of Tetanus (g/sec)	21.7±0.84 (21)	15.5±0.54* (20)	18.5±0.5* ϕ (20)	10.7±0.52 (21)	7.8±0.35* (20)	11.7±0.39 ϕ (20)
Rate of Relaxation (g/sec)	16.7±1 (18)	8.6±0.56* (20)	10.69±0.77* (15)	2.0±0.16 (18)	2.4±0.18 (20)	4.3±0.255* ϕ (15)

The values represent Mean ±SE. * shows a significant change (P value < 0.01) when compare with control. ϕ shows a significant change when a comparison was made between statin treatment and statin + carnitine treated groups. Values in parentheses indicate number of experiments

3) Length Tension Relationship: We plotted muscle tension against its length to see the relationship between these parameters. Graph shows that with increase in length of muscle, force of contraction was also increased up to a certain point and then started to decline with further increase in the length of muscle (Figure 2). Stain significantly reduced the force of contraction by each cm increase in length of muscle and reached to a maximum strength on 3.2±0.03 cm, in contrast to 3.0±0.006 cm in control. Force of contraction in the carnitine group reversed this effect back to the control group in the initial lengths of muscle

and also showed resting length very close to control. While the force of contractions of carnitine group after resting length was declined more rapidly than control.

4) Tetanus Parameters: Force of Contraction of tetanus at low frequency was decreased by statin treatment upto 59% (control 0.22+0.006, statin 0.13+0.004; P<0.0001) while treatment of carnitine along with statin reversed this effect. Rates of rise and relaxation of Tetanus were also markedly reduced by Statin treatment up to level of significance (P<0.0001). Improvement by carnitine was brought only in rate of rise of tetanus (Table 3)

At high frequency, force of contraction, half relaxation time and rate of rise of tetanus were significantly decreased by statin treatment (P<0.0001). Carnitine also showed similar effect as was observed in low frequency. However, carnitine treatment had no effect on half relaxation time (Table3).

We also calculated twitch-tetanus ratio of each group at both low and high frequency. We observed no significant change in the ratio of force of contraction of single twitch to tetanus at both low and high frequency. (Table 4)

Table No.4: Effect of Statin and Statin + Carnitine on Twitch-Tetanus Ratio of Rat Gastrocnemius Muscle

Parameters	Control	Statin	Statin + Carnitine
Ratio with low frequency	1.089±0.013 (21)	1.10±0.042 (20)	1.16±0.016 (20)
Ratio with high frequency	0.272±0.009 (21)	0.0277±0.012 (20)	0.240±0.0093 (20)

The values represent Mean ± SE. * shows a significant change (P value < 0.01) when compare with control. ϕ shows a significant change when a comparison was made between statin treatment and statin + carnitine treated groups. Values in parentheses indicate number of experiments

DISCUSSION

We assessed the effects of chronic statin treatment on the mechanical properties of rat gastrocnemius muscles and use of carnitine along with statin to judge its preventive effect. For this purpose, we chose lipophilic atorvastatin because it is the most widely used drug all over the world. Other lipophilic statin simvastatin is already withdrawn from the market due to its deleterious effects like rhabdomyolysis[13].

Atorvastatin treatment resulted in a marked reduction in the amplitude of force of contraction during twitch and tetanus stimulation. Possible mechanism of muscle damage including mitochondrial dysfunction associated with the generation of oxidative stress, calcium imbalance[14] and enhanced energy metabolism[15] have been reported previously. Mechanical properties of skeletal muscle are directly related to electrical properties. In our study electrical properties were also found to be altered (unpublished results).

Structural and functional modifications in skeletal muscle by statins are more prominent in fast twitch muscle[16]. We used gastrocnemius muscle which is a combination of fast and slow twitch fibers.

In our study, we observed that carnitine prevented this adverse effect of atorvastatin and increased the amplitude of force of contraction. Similar protective results were seen by carnitine in which tetanic contraction of soleus muscles was increased after ischemic reperfusion[17]. In another study, contractile functions of muscles were decreased at high frequency stimulation in the type 2 diabetic animal models and detected reduced level of carnitine[18]. This suggests that statin might have decreased carnitine level in the muscle and serum that led to altered contractile properties of skeletal muscle. Sufficient level of carnitine in the plasma prevented the generation of free radicals[19]. It has also been established that availability of carnitine limits the rate of fat oxidation. In addition, carnitine also prevents cytotoxicity by reducing mitochondrial permeability[20].

CONCLUSION

In conclusion, adverse effects of atorvastatin on contractile properties of skeletal muscle can be reduced with the use of carnitine.

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REFERENCES

1. Assmann G, Carmena R, Cullen P, Fruchart JC, Jossa F, Lewis B. Coronary heart disease; reducing the risk: a worldwide view. Circulation 1999; 100:1930-1938.
2. Thompson PD, Clarksonm P, Karas RH. Statin associated myopathy. JAMA 2003;138:1681-1690.
3. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 2004;350: 1495-1504.
4. Schaefer WH, Lawrence JW, Loughlin AF, Stoffregen DA. Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal muscle myopathy in rats. Toxicol Appl Pharmacol 2004;194(1):10-23
5. Christopher Kirsch, Gunter P Eckert. statin effects on cholesterol micro-domains in brain plasma membranes. Biochemical Pharmacol 2003;65(5): 843–856.
6. Lancut M, Jedrych B, Lis-Sochoka M, Czerny K. Histological and ultrastructural changes in cross

striation muscle cells, under the influence of atorvastatin reductase HMG-CoA inhibitor. Ann. Univ. Mariae Curie Sklodowska Med 2004;59(2): 32-37.
7. Richard A. Cooper. Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells. J Supramolecular Structure 1978;8(4): 413–430, 1978
8. Arduini A, Peschechera A, Carminati P. Method of preventing or treating Statin-induced toxic effects using L- carnitine or an alkanoyl L-carnitine 2001 @ {patent: 6245800}
9. Wutzke KD, Lorenz H. The effect of L-carnitine on fat oxidation, protein turnover, and body composition in slightly overweight subjects. Metabolism 2004;53(8):1002–1006
10. Pierno S, Didonna MP, Cippone V, Lucca AD, Pisoni M, Frigeri A. Effect of chronic treatment with statins and fenofibrate on rat skeletal muscle: A biochemical, histological and electrophysio-pysiological study. Bri J Pharmacol 2006;149: 909-919.
11. Li J, Peng LN. Effects of carnitine on respiratory chain and metabolism of oxygen radical in mitochondria of skeletal muscle after exhaustive running in training rat. Acta Physiologica Sinica 2013;65(6): 631–636
12. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of Epoxyeicosatrienoic Acids as Endothelium-Derived Hyperpolarizing Factors. Circulation Research 1996;78: 415-423.
13. Pierno S, De Luca A, Tricarico D, Roselli A, Natuzzi F, Ferrannini E, et al. Potential risk of myopathy by HMG-CoA reductase inhibitors: a comparison of pravastatin and simvastatin effects on membrane electrical properties of rat skeletal muscle fibers. J Pharmacol Exp Ther 1995; 275(3): 1490-6.
14. Liantonio A, Giannuzzi V, Cippone V, Camerino GM, Pierno S, Camerino DC. Fluvastatin and atorvastatin affect calcium homeostasis of rat skeletal muscle fibers in Vivo and in Vitro by impairing the sarcoplasmic reticulum/mitochondria Ca+2 release system. J Pharmacol and Exp Therapeutics 2007;321.
15. Wang W, Chi-Wai Wong. Statins enhance peroxisome proliferator-act ivated receptor γ coactivator-1a activity to regulate energy metabolism. J Mol Med 2010;88:309 –.317
16. Ozek NS, Bal B, Sara Y, Onur R, Severcan F. Structural and functional characterization of simvastatin-induced myotoxicity in different skeletal muscles. Biochimica et Biophysica Acta 2014;1840:406–415.
17. Demirel M, Kaya B, Cerkez C, Ertunc M, Sara Y. L-Carnitine Pretreatment Protects Slow-Twitch Skeletal Muscles in a Rat Model of Ischemia–Reperfusion Injury. Vasc Endovascular Surg 2013.
18. Bin Aleem S, Hussain MM, Farooq Y. Serum levo-carnitine levels and skeletal muscle functions in type 2 diabetes mellitus in rodents. J Coll Physicians Surg Pak 2013; 23(2):132-6.
19. Guardia PG, Alberici LC, Ravagnani FG, Catharino RR, Vercesi AE. Protection of rat skeletal muscle fibers by either L-carnitine or coenzyme Q10 against statins toxicity mediated by mitochondrial reactive oxygen generation. Front Physiol 2013; 15(4):103.
20. Costa RAP, Fernandes MP, de Souza-Pinto NC, Vercesi AE. Protective effects of L-carnitine and piracetam against mitochondrial permeability transit ion and PC3 cell necrosis induced by simvastatin. Europ J Pharmacol 2013;701:82 –86.

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