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Elucidation of the mechanism of atorvastatin-induced myopathy in a rat model

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ABSTRACT

Myopathy is among the well documented and the most disturbing adverse effects of statins. The underlying mechanism is still unknown. Mitochondrial dysfunction related to coenzyme Q10 decline is one of the proposed theories. The present study aimed to investigate the mechanism of atorvastatin-induced myopathy in rats. In addition, the mechanism of the coenzyme Q10 protection was investigated with special focus of mitochondrial alterations. Sprague-Dawely rats were treated orally either with atorvastatin (100 mg/kg) or atorvastatin and coenzyme Q10 (100 mg/kg). Myopathy was assessed by measuring serum creatine kinase (CK) and myoglobin levels together with examination of necrosis in type IIB fiber muscles. Mitochondrial dysfunction was evaluated by measuring muscle lactate/pyruvate ratio, ATP level, pAkt as well as mitochondrial ultrastructure examination. Atorvastatin treatment resulted in a rise in both CK (2X) and myoglobin (6X) level with graded degrees of muscle necrosis. Biochemical determinations showed prominent increase in lactate/pyruvate ratio and a decline in both ATP (>80%) and pAkt (>50%) levels. Ultrastructure examination showed mitochondrial swelling with disrupted organelle membrane. Co-treatment with coenzyme Q10 induced reduction in muscle necrosis as well as in CK and myoglobin levels. In addition, coenzyme Q10 improved all mitochondrial dysfunction parameters including mitochondrial swelling and disruption. These results presented a model for atorvastatin-induced myopathy in rats and proved that mitochondrial dysfunction is the main contributor in statin-myopathy pathophysiology.

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1. Introduction

Lowering plasma cholesterol level especially low-density lipoprotein (LDL) has widely been related to reduction in cardiovascular-related mortality and morbidity. Statins represent the first line treatment for atherosclerotic disorders related to hypercholesterolemia. They competitively inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate limiting step in cholesterol biosynthesis (Sirtori, 2014). Despite their different physicochemical properties, all statins are capable to decrease LDL-C with different potencies (Grundy et al., 2004) The use of high dose of statins is sometimes needed especially in high risk patients to achieve therapeutic goals on all atherogenic parameters. Doubling of statins dose had shown a 5% to 7% decrease in LDL-C levels (Nicholls et al., 2010), however with increased myopathic incidence and drug discontinuation (Silva et al., 2007).

Generally, statins have a good safety profiles except for muscle toxicity and increase in liver enzymes. Myopathy is the most significant and well documented side effect described with statin use (Hu et al., 2012). While clinical studies reported myopathy incidence as 0.1% (Rallidis et al., 2012), latest estimates indicate that up to 29% of statin users complain from musculoskeletal side effects (Stroes et al., 2015). Myopathy may comprise myositis, myalgia with or without CK increase. The most severe form of myopathy is rhabdomyolysis characterized by muscle destruction and myoglobin release (Armitage, 2007).

The exact mechanism of statin-induced myopathy has not been clearly elucidated. Different theories have been suggested including altered membrane fluidity and excitability (Pierno et al., 1995), impaired calcium homeostasis (Liantonio et al., 2007), induction of

Abbreviations: LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; CoQ10, coenzyme Q10; LDH, lactate dehydrogenase enzyme.

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apoptosis (Dirks and Jones, 2006), activation of ubiquitin-proteasome pathway (Hanai et al., 2007) and mitochondrial dysfunction (Kaufmann et al., 2006). The main suggested theories relay on inhibiting mevalonate downstream products particularly; cholesterol, prenylated proteins and ubiquinone. It was suggested that reduced serum cholesterol may lead to altered muscle membrane fluidity and excitability. Prenylated proteins are thought to be essential in many signaling pathway. Dysprenylation of small GTPases causes vacuolation of myofibers, degeneration and swelling of organelles and eventually apoptosis (Abd and Jacobson, 2011).

Mitochondrial dysfunction is one of the most compelling theories to elucidate statin myopathy. Mitochondrial dysfunction could be related to decrease in coenzyme Q10 (CoQ10) or ubiquinone. Coenzyme Q10 is a powerful antioxidant and an essential cofactor in the electron transport chain. Through inhibiting the mevalonate pathway, statins could reduce CoQ10 level which in turn disrupts cellular respiration, producing muscle related effects (Tomaszewski et al., 2011). Studies have shown that statins decrease CoQ10 levels in serum (Folkers et al., 1990; Willis et al., 1990) and skeletal muscle (Nakahara et al., 1998; Päivä et al., 2005). A recent study had shown a decrease in mitochondrial oxidative phosphorylation capacity accompanied by a decrease in muscle CoQ10 in simvastatin-treated patients (Larsen et al., 2013). Nevertheless, some studies failed to show any decrease in muscle CoQ10 following statins treatment (Fukami et al., 1993; Laaksonen et al., 1996) or to correlate that decrease with mitochondrial dysfunction (Nakahara et al., 1998).

This study aimed to elucidate the mechanism underlying atorvastatin-induced myopathy in a rat model. Myopathic markers including biochemical, functional and histopathological alterations were assessed. Mitochondrial dysfunction parameters and ultrastructural changes were examined with special focus on type IIB fiber muscle. In addition, the elucidated changes in myopathic indices and mitochondrial parameters determinations were further assessed after co-administration of coenzyme Q10.

2. Materials and methods

2.1. Chemicals

Atorvastatin calcium was a gift form Borg pharmaceuticals, Alexandria, Egypt. Coenzyme Q10 was purchased from Selleckchem, Houston, USA. Both drugs were suspended in 0.5% carboxymethyl cellulose (CMC). All other chemicals or reagents are used with high grade and purity.

2.2. Animals and experimental design

Male albino Sprague Dawely rats weighing 200–230 gm. were obtained from the animal house of Pharos University, Alexandria, Egypt. Rats were housed under controlled temperature (25 ± 2 °C) and constant light cycle (12 h light/dark) and allowed free access to water and controlled diet (approximately 20 g standard rodent chow diet/200 g rat). The procedures used for the care and euthanasia of animals complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

Rats were divided into three groups. Group I (ATV) received atorvastatin (100 mg/kg). Group II received both atorvastatin (100 mg/kg) and coenzyme Q10 (100 mg/kg) (CoQ10 + ATV). Groups III was the control group (C) receiving the vehicle (0.5% CMC). Group IV (CoQ10) received coenzyme Q10 (100 mg/kg) alone. All drugs were administrated by oral gavage for 21 days. The dose of atorvastatin was used based on pilot study examining 10, 30 and 50 mg/kg. Only 50 mg/kg showed some myopathic signs in some rats, so the dose was escalated to 100 mg/kg.

At the end of the experiment (day 22), biceps femoris muscle was removed, weighed and frozen at −80 °C. Frozen muscles were grinded and homogenized (homogenizer1600 MiniG, New Jersey, USA) in ice-cold phosphate buffer (pH = 7.4). The supernatant was then separated and used for further biochemical determinations.

2.3. Myopathy indices

2.3.1. Body and muscle weight determination

Rats' body weights were determined on day zero, 7, 14 and 21 and percentage weight change was calculated compared to pretreatment weight. On the day of sacrifice, biceps femoris from the other hindlimb was isolated, washed in iced saline and weighed. The muscle was dried at 60 °C till constant weight. Muscle weight was calculated as mg/g body weight. Percentage muscle weight change was calculated in both wet and dry conditions and compared among groups.

2.3.2. Motor activity assessment

An accelerating rotarod device designed for rats (Orchid scientific & Innovative, India) was used for motor function evaluation (Trapani et al., 2011). One week before the start of the experiment, rats were subjected to training for three days. At the start of the experiment, rats were allowed to accommodate then start running on a rotating rod at 24 rpm until falling down. Performance of rats was expressed as latency to fall, measured in seconds. Three different measurements were recorded and the average was calculated. Rats were allowed to rest and taken back to the cage for at least 10 min between each measurement. Percentage change in the performance was calculated for day 14 and day 21 with reference to day zero values.

2.3.3. Serum creatine kinase determination

Serum samples were separated on day zero, 14, and 21 and examined for creatine kinase activity using a Cobas autoanalyser (Roche diagnostics, USA) .The X-fold increase of creatine kinase activity was calculated by comparing day 14 and day 21 to day zero values.

2.3.4. Serum myoglobin determination

Serum myoglobin content was measured on day 21 samples using ELISA kit (Cusabio, China) and expressed as ng/ml.

2.3.5. Muscle lactate dehydrogenase enzyme content

Lactate dehydrogenase enzyme (LDH) was analyzed in muscle homogenate as a marker of cell damage using enzyme-linked immunosorbent assay ELISA kit (Cusabio, China).

2.3.6. Histopathological examination

Semimembranosus and extensor digitorum longus were isolated for histopathological examination. Muscles were fixed in buffered 10% formalin, processed to wax blocks. Paraffin embedded samples were sectioned transversely and longitudinally and stained with haematoxylin and eosin for examination by light microscopy. Necrosis was graded blindly into; grade 0 = no necrosis; grade 1 = mild (up to 20% of fibers in section affected); grade 2 = moderate (20–50% of fibers in section affected); grade 3 = severe (more than 50% of fibers in section affected) (Westwood et al., 2005).

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