European Journal of Pharmacology 762 (2015) 270–274

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Coenzyme Q10 remarkably improves the bio-energetic function of rat liver mitochondria treated with statins

Afshin Mohammadi-Bardbori [a,*], Asma Najibi [a], Najmeh Amirzadegan [a], Raziyeh Gharibi [a], Ayat Dashti [a], Mahmoud Omidi [a], Arastoo Saeedi [a], Ali Ghafarian-Bahreman [a], Hossein Niknahad [a,b]

[a] Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Fars 71345-1583, Shiraz, Iran
[b] Pharmaceutical Science Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article history:
Received 6 March 2015
Received in revised form
18 May 2015
Accepted 20 May 2015
Available online 22 May 2015

Keywords:
Coenzyme Q10
Atorvastatin
Simvastatin
Mitotoxicity
HFD (high fat diet)
HMG-CoA reductase inhibitors

ABSTRACT

CoQ10 shares a biosynthetic pathway with cholesterol therefore it can be a potential target of the widely available lipid-lowering agents such as statins. Statins are the most widely prescribed cholesterol-lowering drugs with the ability to inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase. Preclinical and clinical safety data have shown that statins do not cause serious adverse effects in humans. However, their long-term administration is associated with a variety of myopatic complaints. The aim of this study was to investigate whether CoQ10 supplementation of animals under high fat diet (HFD) treated with statins is able to bypass the mitochondrial metabolic defects or not?

Animals were divided into 7 groups and fed with either regular (RD) or HFD during experiments. The first group considered as regular control and fed with a RD. Groups 2–7 including HFD control, CoQ10 (10 mg/kg), simvastatin (30 mg/kg), atorvastatin (30 mg/kg), simvastatin+CoQ10 or atorvastatin+CoQ10 treated orally for 30 days and fed with HFD. At the end of treatments, the animals were killed and blood samples were collected for biochemical examinations. The rat liver mitochondria were isolated and several mitochondrial indices including succinate dehydrogenase activity (SDA), ATP levels, mitochondrial membrane potential (MMP) and mitochondrial permeability transition pore (MPP) were determined.

We found that triglyceride (Tg), cholesterol (Chol) and low-density lipoprotein (LDL) were augmented with HFD compared to RD and treatment with statins remarkably lowered the Tg, Chol and LDL levels. Mitochondrial parameters including, SDA, ATP levels, MMP and MPP were reduced with statin treatment and improved by co-administration with CoQ10.

1. Introduction

Statins including atorvastatin, pravastatin, fluvastatin, lovastatin, pitavastatin and simvastatin are the most widely prescribed lipid-lowering drugs with the ability to inhibit HMG-CoA reductase, and lower serum cholesterol (Farmer, 1998; Grundy, 1988; Marks et al., 2003). On the other hand, statins have HMG-CoA reductase inhibitor properties (Desager and Horsmans, 1996; Lea and McTavish, 1997; Plosker et al., 2000) thereby they are also able to reduce coenzyme Q10 production (Folkers et al., 1990; Ghirlanda et al., 1993; Laaksonen et al., 1994; Nawarskas, 2005). Therefore, statins administration is associated with CoQ10 diminution in plasma and muscles of experimental animals and human (Ghirlanda et al., 1993; Laaksonen et al., 1994; Mortensen et al., 1997).

Coenzyme Q10 (CoQ10), a naturally occurring quinine and a lipid soluble compound is present in blood and all cellular membranes of human (Crane, 2001; Villalba et al., 1995) with highest concentrations in heart, kidney and liver (Aberg et al., 1992). The major regulatory step of CoQ10 biosynthesis is HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase, an enzyme common to the cholesterol synthetic pathway (Ranganathan et al., 1981). HMG-CoA reductase is a transmembrane protein and the rate-controlling enzyme of the metabolic pathway that produces cholesterol and the other compounds, such as CoQ10. In humans this enzyme is thus the target of the widely available cholesterol-lowering drugs such as statins. CoQ10 is predominately found within the inner mitochondrial membrane (Turunen et al., 2004). It accepts electrons from mitochondrial complexes and also from

* Corresponding author.
E-mail addresses: toxicology@sums.ac.ir, Afshin.mohammadibardbori@ki.se (A. Mohammadi-Bardbori).

http://dx.doi.org/10.1016/j.ejphar.2015.05.041
0014-2999/

β-oxidation of fatty acids (Ernster and Dallner, 1995; Forsmark-Andree et al., 1997). At the same time it transfers protons to the outside of the mitochondrial membrane (Mitchell, 1975).

In this study effects of CoQ10 supplementation during statin therapy to recover the mitochondrial metabolic defects in rats were examined.

2. Materials and methods

2.1. Chemicals

Compounds were obtained from following suppliers: atorvastatin and simvastatin were kindly provided by Kharazmi Pharmaceutical Co., Iran. All the other reagents including CoQ10, MTT, Rhodamine 123 were purchased from Sigma-Aldrich, Germany.

2.2. Animals

Male Sprague-Dawley rats (200–250 g) were obtained from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. The rats were housed environmentally ($t=25$ °C) and air humidity controlled room (60%) and kept on a standard laboratory diet and were maintained on a 12-h light–dark cycle for one week before the start of the experiments. They were allowed to feed standard laboratory chow and tap water ad libitum. The animals were treated according to the guideline of the Ethics Committee of Shiraz University of Medical Sciences.

2.3. Experimental design

Animals were divided into 7 groups ($n=6$) and fed with either regular (RD) or high fat diet (HFD; 90% RD+10% fat) during experiments. The first group considered as regular control and fed with a RD. Groups 2–7 including HFD control, CoQ10 (10 mg/kg), simvastatin (30 mg/kg), atorvastatin (30 mg/kg), simvastatin (30 mg/kg)+CoQ10 (10 mg/kg) or atorvastatin (30 mg/kg)+CoQ10 (10 mg/kg) treated orally for 30 days and fed with HFD during experiments. At the end of the treatments, the animals were killed and blood samples were collected for biochemical examinations. The rat liver mitochondria were isolated and several mitochondrial indices were measured.

2.4. Biochemical parameters determination

Animal blood samples were collected in the glass tube containing an anticoagulant substance (heparin). The biochemical parameters including triglyceride (Tg), cholesterol (Chol), low-density lipoprotein (LDL) and high density lipoprotein (HDL) were measured by automated hematology analyzer (KX-21N™).

2.5. Preparation of mitochondria

Rats were anaesthetized by injection of 60 mg/kg thiopental and the liver was removed with small scissor and minced in a cold manitol solution containing 0.225 M D-manitol, 75 mM sucrose and 0.2 mM ethylenediaminetetraacetic acid (EDTA). Approximately 30 g of the minced liver was gently homogenized in a glass homogenizer with a Teflon pestle and then centrifuged at 700*g* for 10 min at 4 °C to remove nuclei, unbroken cells and other non-subcellular tissues. The supernatant was centrifuged at 7000*g* for 20 min. The dark packed lower layer (heavy mitochondrial fraction) was resuspended in the manitol solution and recentrifuged twice at 7000*g* for 20 min. The heavy mitochondrial sediments were suspended in Tris solution containing 0.05 M Tris–HCl buffer (pH 7.4) 0.25 M sucrose, 20 mM KCl, 2.0 mM $MgCl_2$ and 1.0 mM $Na_2H\ PO_4$ at 4 °C before assay.

2.6. MTT assay

In our study the quantitative colorimetric method for determination of cell viability by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was modified for rat liver mitochondria suspension in tubes (succinate dehydrogenase activity; SDA) (Ghazi-Khansari et al., 2006; Mohammadi-Bardbori and Ghazi-Khansari, 2007).

2.7. Mitochondrial membrane potential (MMP) assay

Rhodamine 123 was used to assess perturbations in MMP. The mitochondrial fractions were added into the reaction mixture containing 150 mM sucrose, 4 mM $MgCl_2$, 5 mM potassium phosphate, 30 mM KOH–HEPES (pH 7.4) in a total volume of 1 ml at 37 °C for 5 min. The reaction was initiated by adding 10 μl of 26 μM Rhodamine 123 and fluorescence was measured with excitation at 507 nm and emission at 527 nm (Zhang et al., 2008).

2.8. The mitochondrial permeability transition pore (MPTP) assay

The activation of the MPTP was determined and confirmed by adding 250 μM Ca^{2+} to isolated rat mitochondria. Opening of the MPTP causes mitochondrial swelling, which is measured spectrophotometrically as a decrease in the absorbance at 540 nm as described previously (Haworth and Hunter, 1979). Isolated rat liver mitochondria were re-suspended in the swelling buffer consisting of 125 mM sucrose, 50 mM KCl, 5 mM HEPES, 2 mM KH_2PO_4 and 1 mM $MgCl_2$. Absorbance was monitored at 540 nm for 1 h. After 25 min, Ca^{2+} at concentration of 250 μM was added.

2.9. ATP determination by HPLC

Samples (0.5 ml) of aliquot isolated mitochondria were added to .025 ml of 3 M perchloric acid kept on ice, and stored at −70 °C until further analysis by means of HPLC. Before measurement of ATP, perchloric acid was neutralized with KOH and samples were centrifuged before injection to the HPLC. The ATP levels were analyzed using an in-line solid-phase extraction column coupled to a reverse-phase LC-18 column, as previously described (Jones, 1981). In brief, the samples were injected onto the extraction column and separated on the analytical LC-18 column. The separation was performed using a mobile phase consisting of KH_2PO_4 0.1 M (pH 6). ATP was detected using wavelengths of 254 nm. Finally, the amount of ATP was normalized to cellular protein content determined as below.

2.10. Protein concentration

Mitochondrial protein concentrations were determined using the method developed by Bradford (1976). Briefly, 100 μl of mitochondrial suspensions were added to a 96 well plate and gently mixed with Bradford reagent. After 5 min the absorbance was measured at 595 nm.

2.11. Statistical analysis

In this study the sample size was calculated based on a simple method of sample size calculation so called resource equation method (Charan and Kantharia, 2013). All values were expressed as Mean ± S.E.M of 6 animals. For comparison between more than two experimental groups, one-way ANOVA followed by Tukey multiple comparison tests were used. For comparison between