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Paradoxical effects of atorvastatin in isoproterenol-induced cardiotoxicity in rats: Role of oxidative stress and inflammation

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ABSTRACT

Atorvastatin (ATV) was previously shown to improve oxidative stress, inflammation and endothelial dysfunction in several experimental and clinical studies yet other studies have reported a pro-oxidant and damaging effect upon ATV administration. The present study was directed to investigate the effect of ATV pre- and post-treatment in isoproterenol (ISO)-induced cardiotoxicity in rats. Myocardial damage was induced by ISO (5 mg/kg/day, s.c.) for 1 week. ATV (10 mg/kg/day, p.o.) was given for 2 weeks starting 1 week before or after ISO administration. ISO-treated rats showed significant alterations in electrocardiographic recordings, serum creatine kinase-MB (CK-MB) level as well as oxidative stress and inflammatory biomarkers. Moreover, ISO administration resulted in endothelial dysfunction and significant histopathological damage. Pre-treatment with ATV aggravated ISO-induced cardiotoxicity. On the other hand, ATV post-treatment succeeded to significantly improve oxidative stress and inflammatory biomarkers, endothelial dysfunction and myocardial degeneration. These results suggest that ATV might produce a synergistic pro-oxidant effect if given before or along with another pro-oxidant (ISO). Thus, caution should be applied upon the use of statin as a prophylactic therapy for primary cardiovascular disease prevention.

1. Introduction

Isoproterenol (ISO)-induced myocardial damage is a well-standardized model to study the protective effects of many drugs on cardiac function. Persistent β-adrenergic stimulation following ISO administration results in formation of reactive oxygen species (ROS) [1], cardiomyocytes injury [2], ventricular hypertrophy [3] and increased fibrosis and collagen deposition [4].

Production of low levels of ROS acts as signaling molecules stimulating the endogenous protective antioxidants in the body [5]. On the other hand, excessive high concentrations of ROS are associated with stimulation of detrimental inflammatory and apoptotic pathways [6]. Clinical studies have reported increased plasma levels of lipid peroxidation products and decreased activity of anti-oxidant enzymes in patients with compensated heart failure [7]. ROS produced in failing hearts causes mitochondrial dysfunction, inhibition of certain enzymes affecting vital metabolic pathways, release of inflammatory cytokines, endothelial dysfunction and cardiac muscle damage [8].

Oxidative stress induces inflammation via stimulation of transcription factors including nuclear factor-kappa B (NF-κB). The elevated levels of inflammatory cytokines enhance the expression of inducible nitric oxide synthase (iNOS) contributing to the generation of the highly reactive oxidant peroxynitrite in the presence of oxidative stress [9]. Moreover, the released pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) are involved in the depletion of endothelial nitric oxide synthase (eNOS) expression [10] which mediates the beneficial effects of nitric oxide (NO) through reduction of the vascular resistance, increase in blood flow, and regulation of leukocyte-endothelial adhesion [11]. Oxidative stress, cytokines elevation, and downregulation of eNOS expression are all associated with endothelial dysfunction that is directly correlated to impaired NO bioavailability and myocardial dysfunction [12].

Experimental and clinical studies have shown that statins therapy can prevent or ameliorate cardiac dysfunction through inhibition of oxidative stress [13,14,15], inflammation [16] and improvement of endothelial function [17]. Moreover, statins have shown to stabilize NO bioavailability through enhancement of eNOS activity and reduction of iNOS activity [18].

Although the use of statin could be regarded as a promising avenue for therapeutic intervention in various cardiovascular disorders including patients suffering from myocardial damage and heart failure [19,20], yet it has been reported that statin treatment reduces the

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NADPH-CoQ reductase activity resulting in the reduction of endogenous antioxidant coenzyme Q (CoQ) and subsequently decreases the body's resistance to oxidative stress [21,22]. This could explain the failure of ATV treatment to reduce oxidative stress in a previous experimental model of ISO-induced myocardial infarction [23].

Therefore, the aim of the present study was directed to assess the paradoxical effects of ATV when used as pre-and post-treatment in ISO-induced cardiotoxicity in rats and the possible involvement of oxidative stress in these interventions.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 180–210 g were obtained from the animal facility of Faculty of Pharmacy, Cairo University. Rats were housed under controlled temperature (25 ± 2oC) and constant light cycle (12 h light/dark) and allowed free access to standard rodent chow diet and water. The investigation 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 2011) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (Permit Number: PT 433).

2.2. Chemical reagents

ATV was obtained from Riva Pharma Pharmaceutical Inc (Cairo, Egypt) and ISO was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other used chemicals and reagents, unless otherwise specified, were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Experimental protocol

Rats were randomly divided into five groups, 9 animals each. Group I served as normal animals. Rats in groups II–V were given ISO (5 mg/kg/day, s.c.) for 7 days [24]. Rats in group II were sacrificed after the last dose of ISO and served as ISO pre-treatment group. Rats in group III were pretreated with ATV (10 mg/kg/day, p.o.) for 14 days starting one week before ISO injection. Rats in group IV were sacrificed two weeks after last dose of ISO and served as ISO post-treatment group. Rats in groups V were post-treated with ATV (10 mg/kg/day, p.o.) for 14 days starting after last dose of ISO. ATV was freshly prepared daily in saline where the used dose was selected from previous experimental studies [25,26]. Notably, normal rats treated with ATV for 2 weeks showed similar results to that observed in normal rats (data not shown). For each group, two sets of experiments were conducted; one (n = 5) for biochemical measurements and the other (n = 3) for histological examination.

2.4. Hemodynamic measurements

At the end of experiment, rats were anesthetized with thiopental (50 mg/kg, i.p.) and kept warmed with a heating lamp to prevent the incidence of hypothermia. Subcutaneous peripheral limb electrodes were inserted for electrocardiographic (ECG) recording (HPM 7100, Fukuda Denshi, Tokyo, Japan) to determine heart rate (HR), ST elevation, QT interval and QRS duration.

2.5. Biochemical measurements

After ECG recordings, blood sampling was collected from the retro-orbital sinus for estimation of serum creatine kinase-MB (CK-MB) level. Animals were then sacrificed by cervical dislocation under anesthesia and the whole ventricle was rapidly excised, washed with ice-cold saline, dried and weighed. Part of the ventricle was homogenized in ice-cold saline using a homogenizer (Heidolph Diax 900, Germany) to prepare 10% homogenate for determination of myocardial reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), tumor necrosis factor-alpha (TNF-α) and nitrotyrosine contents as well as catalase, superoxide dismutase and caspase-3 activities.

2.5.1. Serum creatine kinase-MB

Serum CK-MB was assessed using a commercially available kit (Stanbio, USA). Results were estimated kinetically at 340 nm using spectrophotometer (Thermo electron corporation, England) and expressed as U/l.

2.5.2. Lipid peroxidation products

Lipid peroxidation products were estimated by determination of the levels of thiobarbituric acid reactive substances (TBARS) that were measured according to the assay of Buege and Aust [27] and expressed as nmol/mg protein.

2.5.3. Reduced glutathione

Reduced glutathione(GSH) contents were determined using 5,50-dithiobis 2-nitrobenzoicacid (DTNB) (Sigma-Aldrich Chemical Co., USA), which produces a stable yellow color that can be measured spectrophotometrically at 412 nm [28] and expressed as μmol/g wet tissue.

2.5.4. Superoxide dismutase activity

Superoxide dismutase(SOD) activities were assessed according to the method of Marklund [29]. It simply resides on computing the difference between autooxidation of pyrogallol (Sigma–Aldrich Chemical Co., USA) alone and in presence of the fraction that contains the enzyme. Changes in the absorbance at 420 nm were recorded at 1-mininterval for 5 min. Results were expressed as U/mg protein. One unit is defined as the amount of enzyme that produces 50% inhibition of pyrogallol autooxidation.

2.5.5. Catalase activity

Catalase activities were assessed according to the method of Abei [30]. Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one min with sodium azide (Sigma–Aldrich Chemical Co., USA). The remaining H_2O_2 reacts with 3, 5- dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone (Sigma-Aldrich Chemical Co., USA) to form a chromophore with color intensity inversely proportional to the amount of catalase in the original sample. The color intensity was measured at 510 nm and the results were expressed as U/mg protein. One unit of catalase activity is defined as the amount of enzyme that degrades 1 μmol H_2O_2 per min at 25 °C.

2.5.6. Tumor necrosis factor-alpha

Myocardial TNF-α content was assessed using rat TNF-α ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/mg protein.

2.5.7. Nitrotyrosine

Myocardial nitrotyrosine content was assessed using rat nitrotyrosine ELISA kit (MyBioSource, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pmol/mg protein.

2.5.8. Caspase-3 activity

Myocardial caspase-3 activity was estimated using caspase-3 colorimetric assay kit (R&D Systems Inc, USA). The absorbance was read at 405 nm using a microplate reader (BioTek instruments, USA). The results were expressed as nmol pNA/h/mg protein.