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Combined atorvastatin and coenzyme Q10 improve the left ventricular function in isoproterenol-induced heart failure in rat

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ABSTRACT

The effect of atorvastatin on cardiac remodeling, function, and homodynamic parameters in isoproterenol-induced heart failure was evaluated in the present study. A subcutaneous injection of isoproterenol (5 mg/kg/day) for 10 days was used for the induction of heart failure. Isoproterenol administration produced intensive myocardial necrosis and fibrosis with a significant decrease in the arterial pressure indices, heart rate, contractility ($LVdP/dt_{max}$) and relaxation ($LVdP/dt_{min}$), but an increase in the left ventricular end-diastolic pressure. Rats were randomly assigned to control, treatment with only atorvastatin, and treatment with atorvastatin plus coenzyme Q10. Histopathological analysis showed a marked attenuation of myocyte necrosis and interstitial fibrosis in all atorvastatin treated groups ($P<0.001$). A low dose of atorvastatin (5 mg/kg/day) significantly improved the left ventricular systolic pressure, contractility and relaxation ($P<0.01$). On the contrary, a high dose of atorvastatin (20 mg/kg/day) worsened the isoproterenol-induced left ventricular dysfunction by a further reduction of $LVdP/dt_{max}$ from $+2780\pm94$ to $+1588\pm248$ (mm Hg/s; $P<0.01$) and $LVdP/dt_{min}$ from -2007 ± 190 to -2939 ± 291 (mm Hg/s; $P<0.05$). Co-administration of coenzyme Q10 with atorvastatin reversed the hemodynamic depression and the left ventricular dysfunction to a high level ($P<0.001$). There was a lower level of LVEDPs in the atorvastatin + coenzyme Q10 treated groups (3 ± 1 and 4 ± 1.4 versus 8 ± 3.5 and 14 ± 3.6 mm Hg, respectively), thereby suggesting improvement in the myocardial stiffness by the combined coenzyme Q10 and atorvastatin treatment. The atorvastatin therapy attenuated myocardial necrosis and fibrosis in isoproterenol-induced heart failure. However, a high dose of the drug considerably worsened the left ventricular dysfunction and hemodynamic depression, which was reversed by coenzyme Q10 co-administration.

1. Introduction

Statins possess pleiotropic beneficial effects which are independent of their cholesterol lowering actions. Some of these effects include inhibition of cellular proliferation and migration (Glynn et al., 2008) and improvement of the endothelial function (liu et al., 2009). A large number of clinical trials have established the benefits of statins on coronary heart disease events and strokes (Heart Protection Study Collaborative Group, 2003; Law et al., 2003; Shepherd et al., 1995). Atorvastatin has been shown to decrease mortality (Vrtovec et al., 2008) and improve the left ventricular ejection fraction and symptoms of heart failure (Sola et al., 2006; Yamada et al., 2007). In a recent study of randomized controlled trials of statin versus placebo in patients with heart failure, Lipinski et al. (2009) demonstrated that statins are safe and help to improve left ventricular ejection fraction as well as decrease hospitalization for deteriorating heart failure. However, some other trials have challenged the full beneficial effects of statins (Kjekshus et al., 2007; Silver et al., 2004; Tavazzi et al., 2008). Coenzyme Q10 participates in the electron transport during oxidative phosphorilation in mitochonderia and is involved in the production of ATP (James et al., 2004). Deficiencies of coenzyme Q10 were found in patients with heart failure and the observed level of coenzyme Q10 deficiency was correlated to the severity of the disease (Mortensen, 2003). The use of coenzyme Q10 as part of a maintenance therapy in patients with chronic heart failure was suggested. Statins are also known to reduce coenzyme Q10 levels in plasma and myocardium through inhibition of HMG-CoA reductase. Therefore, treatment with statins may further decrease the existing low levels of

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coenzyme Q10 in patients with heart failure (McMurray et al., 2010). The above mentioned fact, the role of coenzyme Q10 in ATP production in mitochondria and the importance of mitochondria in myocardial function has provoked the hypothesis that statin-induced CoQ10 deficiency may worsen the cardiac function in patients with heart failure. In addition to a reduction in CoQ10 synthesis, statins also influence other isoprenoid intermediates (farnesyl and geranylgeranyl diphosphates) of mevalonate pathway more potently. By inhibition of mevalonate synthesis, statins prevent geranylgeranylation and farnesylation of Ras, RhoA and Rac1 (Liao, 2004), which are the key mediators of hypertrophic responses (Proud, 2004; Sugden, 2003). In a rat model of cardiac hypertrophy, simvastatin prevented the left ventricular hypertrophy (Indolfi et al., 2002) when induced by a pressure overload. Further, another study reported that simvastatin was even more potent in its reduction of left ventricular dysfunction in comparison to captopril (Luo et al., 1999). Isoproterenol is a synthetic β- adernoceptor agonist that its subcutaneous injection induces heart failure and suppressed cardiac functions because of myocardial hypertrophy and fibrosis (Ojha et al., in press). The present study aims to investigate the effects of atorvastatin on cardiac function, remodeling, and progression to heart failure in isoproterenol induced heart failure in rats. It also aims to examine whether co-administration of atorvastatin with coenzyme Q10 has an impact on these effects.

2. Materials and methods

2.1. Animals

Male Wistar rats (260 ± 20 g) were used in this study. The animals were given food and water ad libitum. They were housed in the Animal House of Tabriz University of Medical Sciences at a controlled ambient temperature of 25 ± 2 °C with 50 ± 10% relative humidity and a 12-h light/12-h dark cycle. The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran (National Institutes of Health Publication No 85-23, revised 1985).

2.2. Chemical reagents

Atorvastatin was a generous gift from Sobhan Pharmaceutical Inc (Tehran-Iran). Isoproterenol was bought from Sigma Chemicals Co, while coenzyme Q10 (Ubiquinon) was purchased from Viva Pharmaceutical Inc (Canada). The other reagents were of a commercial analytical grade.

2.3. Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected subcutaneously to rats (5 mg/kg) daily for 10 consecutive days at an interval of 24 h to induce experimental heart failure (Benjamin et al., 1989).

2.4. Experimental protocol

The animals were randomized into 8 groups consisting of 6 rats each. Rats in group 1 (normal control) received a subcutaneous injection of physiological saline (0.5 ml) and were left untreated for the whole period of the experiment. Rats in group 2 received oral administration of normal saline for 25 days and at the 15th day were subcutaneously injected 5 mg/kg of isoproterenol once at an interval of 24 h for 10 consecutive days. Rats in groups 3 to 5 were pretreated orally, using gastric gavages, with atorvastatin (5, 10 and 20 mg/kg, respectively) for 25 days and at the 15th day were subcutaneously injected 5 mg/kg of isoproterenol once at an interval of 24 h for 10 consecutive days. Rats in group 6 were given coenzyme Q10 (Ubiquinon; 10 mg/kg) orally for 25 days and at the 15th day were subcutaneously injected 5 mg/kg of isoproterenol once at an interval of 24 h for 10 consecutive days. Rats in groups 7 to 8 were pretreated orally with atorvastatin (5 and 20 mg/kg; respectively) plus 10 mg/kg coenzyme Q10 for 25 days and at the 15th day were subcutaneously injected 5 mg/kg of isoproterenol once at an interval of 24 h for 10 consecutive days. All the rats were made to fast overnight. However, they had free access to water at the last administration of the drug.

2.5. Hemodynamic measurements

At the end of the experiment, the animals were anesthetized with sodium pentobarbital (60 mg/kg; i.p). The trachea was cannulated for artificial respiration when the rats no longer responded to external stimuli. Next, the systemic arterial blood pressure was recorded from a catheter inserted into the left carotid artery. A standard limb lead I ECG was monitored continuously throughout the experimental period. The mean arterial blood pressure was calculated from the systolic and diastolic blood pressure traces. The heart rate was calculated from the ECG. To evaluate the cardiac left ventricular function, a Mikro Tip catheter transducer (Millar Instruments, INC) was advanced to the lumen of the left ventricle. This helped to measure the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum and minimum rates of developed left ventricular pressure ($LVdP/dt_{max}$ and $LVdP/dt_{min}$) and

Table 1
General animal characteristics parameters and tissue weight ratios in the control group and in rats treated with isoproterenol (i.p; Heart failure) or with isoproterenol + atorvastatin or + coenzyme Q10 (orally).

Groups **N = 6**	Baseline BW (g)	Δ BW (g)	LV wet to dry weight ratio (g/g)	LV wet to HW ratio (g/g)	Wet HW to BW ratio (g/kg)
Control	262 ± 8	23 ± 1.1	4.24 ± 0.04	0.63 ± 0.03	3.10 ± 0.26
Isoproterenol	269 ± 9	11 ± 0.9[a]	4.62 ± 0.10	0.65 ± 0.02	4.82 ± 0.29
Atorvastatin (5 mg/kg) + isoproterenol	278 ± 12	15 ± 1.3	4.35 ± 0.05	0.69 ± 0.015	4.08 ± 0.09
Atorvastatin (10 mg/kg) + isoproterenol	274 ± 13	14 ± 1.5	4.22 ± 0.37	0.67 ± 0.02	4.02 ± 0.05
Atorvastatin (20 mg/kg) + isoproterenol	282 ± 15	14 ± 1.8	4.25 ± 0.70	0.70 ± 0.03	3.95 ± 0.15
Coenzyme Q10 (10 mg/kg) + isoproterenol	263 ± 7	13 ± 0.8	4.36 ± 0.06	0.69 ± 0.02	4.18 ± 0.20
Atorvastatin (5 mg/kg) + Q10 + isoproterenol	275 ± 9	28 ± 1.5[d]	4.08 ± 0.2[b]	0.66 ± 0.04	3.79 ± 0.09[c]
Atorvastatin (20 mg/kg) + Q10 + isoproterenol	258 ± 13	17 ± 1.1[b]	4.28 ± 0.05	0.65 ± 0.05	4.27 ± 0.18

Data are expressed as mean ± sem. Baseline BW, body weights immediately prior to the drug administration; Δ BW, increase in body weight over treatment period. LV, left ventricle; W, weight; and HW, heart weight.
[a] P<0.01 from respective control value.
[b] P<0.05 from isoproterenol treated group using one way ANOVA with Student–Newman–Keuls post hoc test.
[c] P<0.01 from isoproterenol treated group using one way ANOVA with Student–Newman–Keuls post hoc test.
[d] P<0.001 from isoproterenol treated group using one way ANOVA with Student–Newman–Keuls post hoc test.