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Research Article

Adenosine Triphosphate Exerts Cardioprotective Effect on High-Dose Atorvastatin-Induced Heart Damage in Rats

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Abstract

Atorvastatin is a statin derivated hypolipidemic drug used in the treatment of hyperlipidemia. High-dose atorvastatin has been shown to significantly reduce adenosine triphosphate (ATP) levels in the heart tissue. Reduction of ATP by atorvastatin causes increased production of reactive oxygen species (ROS), decreased antioxidants, subsequent cell membrane and mitochondrial damage. The present study aimed to biochemically investigate the protective effect of ATP against possible cardiac damage caused by high dose atorvastatin in rats. Male Wistar rats were divided into atorvastatin (ATR), atorvastatin+ATP (AAT) and healthy control (HG) groups. ATP at a 25 mg/kg dose was injected intraperitoneally (ip) to the AAT (n-6) group. 0.9% NaCl as solvent was applied to the ATR (n-6) and HG (n-6) groups by the same route. Afterward, atorvastatin was administered orally at a dose of 20 mg/kg to the AAT and ATR groups. This procedure was repeated once daily for four weeks. At the end of this period, blood samples were taken into tubes to analyze troponin-I (TP-I) by cardiac puncture before animals were sacrificed with high-dose anesthesia. In addition, heart tissues were removed and malondialdehyde (MDA), total glutathione (tGSH), total oxidant (TOS) and total antioxidant (TAS) levels were measured. Biochemical test results showed that in the heart tissues of the ATR group, the oxidative parameters MDA and TOS significantly increased, while the antioxidant parameters tGSH and TAS significantly decreased compared to AAT and HG. Atorvastatin alone administration significantly increased blood TP-I levels, a marker of cardiac tissue damage. However, ATP administration to AAT group animals brought oxidative parameter levels closer to HG, despite high-dose atorvastatin treatment. In addition, the significant decrease in antioxidant levels was prevented by ATP application. High doses of atorvastatin can cause heart damage. ATP treatment was able to prevent atorvastatin-induced oxidative heart damage.

Keywords: Atorvastatin, oxidative stress, ATP, rat.

Adenozin Trifosfatın Sıçanlarda Yüksek Doz Atorvastatinin Neden Olduğu Kalp Hasarı Üzerine Kardiyoprotektif Etkileri

Öz

Atorvastatin, hiperlipidemiyi tedavi etmek için kullanılan statin türevli bir hipolipidemik ilaçtır. Yüksek doz atorvastatinin kalp dokusundaki adenozin trifosfat (ATP) seviyelerini önemli ölçüde azalttığı gösterilmiştir. Atorvastatinin neden olduğu ATP eksikliği, reaktif oksijen türlerinin (ROS) üretiminin artmasına neden olur. Bu çalışmada, sıçanlarda yüksek doz atorvastatinin olası kardiyak hasarına karşı ATP'nin koruyucu etkisinin biyokimyasal olarak araştırılması amaçlandı. 18 adet erkek Wistar rat atorvastatin (ATR), atorvastatin+ATP (AAT) ve sağlıklı kontrol (HG) gruplarına eşit olarak ayrıldı. ATP, AAT grubuna 25 mg/kg dozda intraperitoneal (ip) olarak enjekte edildi. Daha sonra AAT ve ATR gruplarına atorvastatin oral olarak 20 mg/kg dozunda uygulandı. Bu prosedür, dört hafta boyunca günde bir kez tekrarlandı. Biyokimyasal test sonuçları, AAT ve HG'ye kıyasla ATR grubu MDA ve TOS'un önemli ölçüde arttığını, tGSH ve TAS'ın ise önemli ölçüde azaldığını gösterdi. Ek olarak, atorvastatinin tek başına uygulanması kan TP-I düzeylerini önemli ölçüde arttırdı. Bununla birlikte, ATP'nin AAT grubu hayvanlara uygulanması, yüksek doz atorvastatin tedavisine rağmen oksidatif parametre seviyelerini HG'ye yaklaştırdı. Yüksek dozlarda atorvastatin kalp hasarına neden olabilir. ATP tedavisi, atorvastatin kaynaklı oksidatif kalp hasarını önleyebildi.

Anahtar Kelimeler: Atorvastatin, oksidatif stres, ATP, sıçan.

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1. Introduction

Statins are widely used in first-line therapy by reducing the mortality and cardiovascular morbidity associated with atherosclerosis [1]. A statin derivated, atorvastatin, disrupts cholesterol synthesis in the liver by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [2]. The favorable effects of statins in reducing major cardiovascular events have mainly been attributed to lowering LDL cholesterol [3]. Additional favorable outcomes are decreasing total cholesterol and very low-density lipoproteins while increasing the amount of high-density lipoprotein (HDL) and its receptors [4]. However, in patients receiving atorvastatin, side effects such as creatine phosphokinase elevation, arthralgia, dyspepsia, diarrhea, nausea, nasopharyngitis, insomnia, urinary tract infection have been previously reported [5]. It has been shown that atorvastatin significantly reduces adenosine triphosphate (ATP) levels, which meets a large part of the heart's energy demand [6]. In some case reports, it has been suggested that there is a decrease in cardiac function after initiating statin therapy [7, 8]. Decreased coenzyme Q10 (CoQ10) has been held responsible for deteriorating diastolic parameters in statin therapy [9]. CoQ10 is an antioxidant molecule which plays a vital role in mitochondrial ATP synthesis [10]. Furthermore, some papers argue atorvastatin treatment may cause ATP to decrease, increase reactive oxygen species (ROS) production, and reduce tissue antioxidants and induce mitochondrial damage [6, 11].

Considering that the mitochondria themselves are an important source of ROS, the high dependence of cardiomyocytes on mitochondrial ATP means that these cells are susceptible to mitochondrial ROS [12]. This literature information indicates that atorvastatin's possible side effect on the heart is due to ATP reduction and subsequent oxidative stress.

ATP is an organic compound containing carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and phosphate (P) [13]. ATP is continuously produced in mitochondria via oxidative phosphorylation [14]. The tissue ATP amounts establish oxidant/antioxidant balance [15]. Similarly, a recent study reported that ATP protects the heart tissue from oxidative damage [16]. The present study aims to biochemically investigate the protective effect of ATP against possible heart damage induced by high-dose atorvastatin in rats.

2. Material and Methods

2.1. Animals

A total of 18 male Albino Wistar rats weighing 275-288 grams were used for the experiment. All animals were obtained from Ataturk University Medical Experimental Application and Research Center. During the experiment, animals were housed and fed under appropriate conditions in a laboratory environment at average room temperature (22 °C).

2.2. Chemicals

For the experiment, thiopental sodium was obtained from İ.E ULAGAY (Turkey), ATP from Zdorove Narodu (Ukraine) and atorvastatin from Pfizer İlaçları Ltd.Şti (Turkey).

2.3. Experimental groups

A total of 18 albino Wistar male rats were divided into three groups, six in each: atorvastatin alone (ATR), atorvastatin+ATP (AAT) and healthy control (HG) groups.

2.4. Experimental procedure

ATP was injected intraperitoneally (ip) at a 25 mg/kg dose to the AAT group of experimental animals. 0.9% NaCl saline was administered in the same volume by the same route to the ATR and HG groups. One hour after ATP and 0.9% NaCl administration, atorvastatin was administered orally at a dose of 20 mg/kg to the AAT and ATR groups. This procedure was repeated once daily for four weeks. At the end of this period, blood samples were taken into tubes to analyze troponin-I (TP-I) by cardiac puncture before animals were sacrificed with high-dose anesthesia (50mg/kg thiopental sodium). In addition, heart tissues were removed and malondialdehyde (MDA), total glutathione (tGSH), total oxidant (TOS) and total antioxidant (TAS) levels were measured. All groups were compared with each other.

2.5. Biochemical analyzes

2.5.1. Preparation of Samples

Before dissection, all tissues were washed with phosphate-buffered saline solution. Tissues were homogenized in cold phosphate buffers (50 mM, pH 7.4) appropriate for the variant to be measured. Tissue homogenates were centrifuged at 5,000 rpm for 20 minutes at 4°C and supernatants were extracted to analyze tGSH and MDA. All tissue results were expressed as grams/protein. All spectrophotometric measurements were made via a microplate reader (Bio-Tek, USA).

2.5.2. Tissue MDA and tGSH determination

MDA measurements were based on the method used by Ohkawa et al., which includes the spectrophotometric measurement of the absorbance of the pink-colored complex formed by thiobarbituric acid (TBA) and MDA [17].

tGSH measurement was made according to the method described by Sedlak J and Lindsay RH [18].

2.5.3. TOS and TAS determination

TOS and TAS tissue homogenates were determined using a new automated measurement method and commercially available kits (Rel Assay Diagnostics, Turkey), both developed by Erel [19, 20].

2.5.4. TP-I analysis

TP-I levels were measured with the VIDAS Troponin I Ultra kit using the ELFA (Enzyme-Linked Fluorescent Assay) technique.

2.6. Statistical Analysis

Experimental results were expressed as "mean value ± standard deviation" (x ± SEM). The significance of the difference between groups was determined using the one-way ANOVA test. In the follow-up, Fisher's post-hoc LSD (least significant differences) was performed. All statistical operations were performed in the "SPSS for Windows, 18.0" statistical program and $p<0.05$ value was accepted as significant.

3. Results and Discussion

3.1. MDA and tGSH analysis results

As seen in Figure 1, ATR group heart tissue MDA levels were significantly higher than those of the HG and AAT groups ($p<0.001$). However, the difference between HG and AAT groups in terms of MDA levels was statistically insignificant ($p>0.05$). Atorvastatin alone administration significantly decreased tGSH levels in rat heart tissue ($p< 0.05$). While the difference in tGSH levels between the ATR and AAT groups was significant, the difference in tGSH between the HG and AAT groups was calculated to be insignificant ($p>0.05$) (Figure 2).

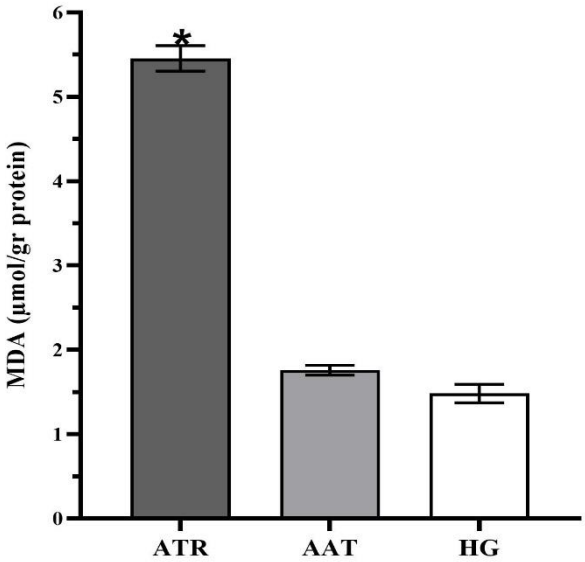

Figure 1. Heart tissue MDA levels of all study groups (n=6).
*=p<0.001 according to HG and AAT groups.

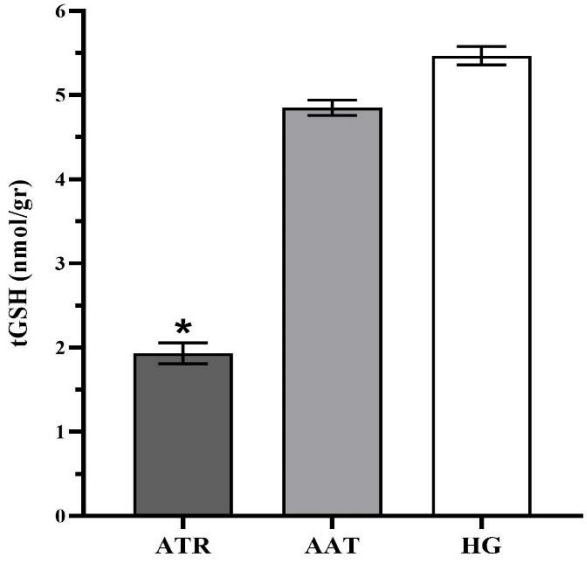

Figure 2. Heart tissue tGSH levels of all study groups (n=6).
*=p<0.05 according to HG and AAT groups.

3.2. TOS and TAS analysis

When the heart tissues of the ATR group were compared with the AAT, TOS levels were significantly increased and TAS levels were found to be decreased (p<0.01). However, AAT group TOS and TAS levels were close to HG, and their difference was calculated as insignificant (p>0.05) (Figure 3, 4).

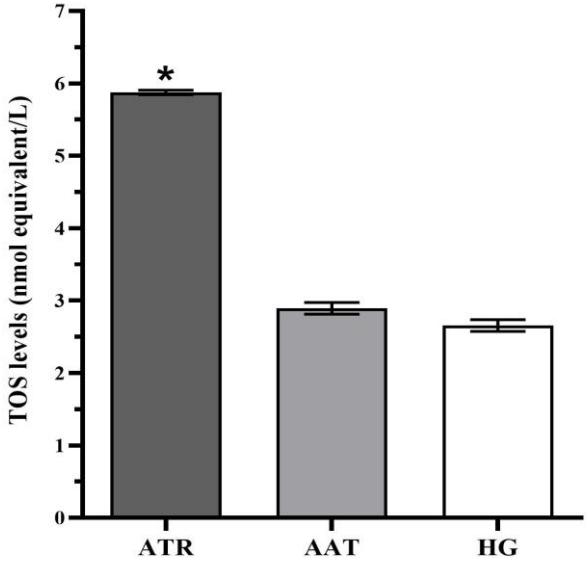

Figure 3. Heart tissue TOS levels of all study groups (n=6).
*=p<0.01 according to HG and AAT groups.

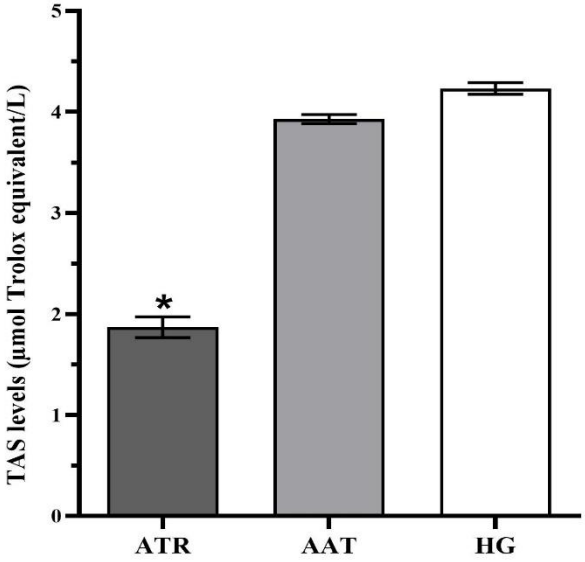

Figure 4. Heart tissue TAS levels of all study groups (n=6).
*=p<0.01 according to HG and AAT groups.

3.3. TP-I analysis in blood serum

When the ATR and AAT groups were compared, blood TP-I levels were significantly increased in favor of the ATR group ($p<0.05$). However, the difference in TP-I levels between AAT and HG groups was calculated to be insignificant ($p>0.05$) (Figure 5).

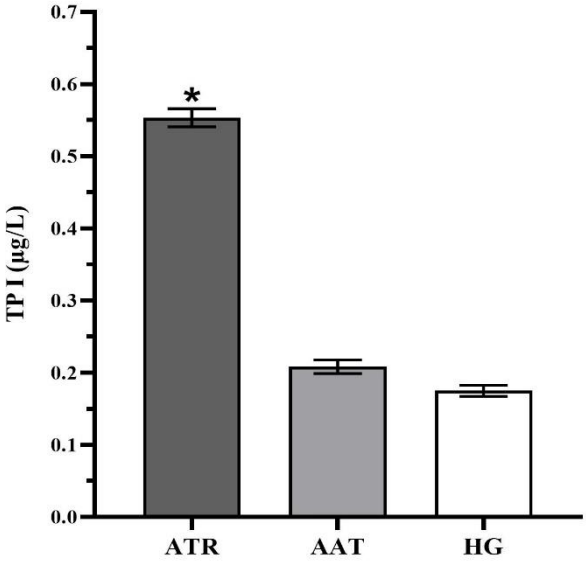

Figure 5. Heart tissue TP-I levels of all study groups (n=6).
*=p<0.05 according to HG and AAT groups.

3.4. Discussion

This study was conducted to investigate the protective effect of ATP against high-dose atorvastatin-induced heart damage biochemically. Evidence-based guidelines recommend using high-dose statins to reduce LDL below a certain level [21]. In this context, the present study methodology was designed particularly to investigate the side-effect profile of high-dose atorvastatin and the therapeutic potential of ATP. Depleted ATP stores and increased ROS production plays a key role in the pathogenesis of atorvastatin-induced mitochondrial dysfunction 11. The present study biochemical test results showed that the amount of malondialdehyde (MDA), known as lipid peroxidation (LPO) toxic product, increased in the rat heart tissues administered atorvastatin at a dose of 20 mg/kg. MDA is a widely used oxidant parameter in oxidative stress-induced LPO [22]. Actually, the studies have reported that atorvastatin inhibits MDA and proinflammatory cytokine production and protects heart tissue from ischemia-reperfusion injury [23]. Another experimental study designed with cadmium chloride, atorvastatin treatment prevented oxidative liver tissue damage [24]. However, an experimental study has demonstrated that the high dose (20 mg/kg) atorvastatin increases the MDA levels in animal myocardial cells [25]. In addition to the difference in the context of the dose, the degree of side effects may vary in different tissues. For instance, atorvastatin significantly lowered ATP levels, especially in the heart and kidney tissues, while slightly lowered in the liver, muscle and brain [6].

In the present study, atorvastatin alone treatment decreased tGSH heart tissue levels. GSH is an antioxidant molecule in a tripeptide structure consisting of L-glutamate, L-cysteine and glycine found in many tissues. Under glutathione peroxidase (GPO) catalysis, GSH reacts with toxic H2O2, protecting cells from ROS damage [26]. There is no definite information in the literature that atorvastatin reduces GSH in myocardial cells. However, it has been suggested that statins may cause cardiomyopathies by inhibiting GPO biosynthesis, which suppresses the LPO reaction [27]. Another study supporting the present research results has shown that the statin-related hepatocellular side effect attributed to a decrease in cellular GSH stores [28].

In the current study, total TOS and TAS levels were measured to investigate further atorvastatin's oxidant/antioxidant balance in heart tissue. TOS and TAS reflect the total tissue status of all oxidants and antioxidants [19, 20]. Therefore, TOS levels are used to practically measure ROS and TAS levels to evaluate the total antioxidant status. The change of this balance in favor of oxidants is considered as oxidative stress [29]. The present experimental results indicate that high-dose atorvastatin treatment changes the heart tissue oxidant/antioxidant balance in favor of oxidants.

The present study determined that ATP significantly prevented the increase in MDA and TOS levels and also prevented the decrease in tGSH and TAS levels in the rat heart tissue. In the literature review, no studies were found investigating the effect of ATP particularly on atorvastatin-induced oxidative heart damage. However, ATP treatment showed a favorable impact against sunitinib-induced oxidative heart damage by reducing the level of MDA and preventing excessive reduction of tGSH [16].

Another study showed that ATP has a protective effect by preventing the increase in MDA and TOS levels induced by methanol in the optic nerve tissue and also preventing a decrease in the tGSH and TAS levels [30]. Similarly, in a recent study, ATP improved propofol-induced myopathy and reduced oxidative damage [31].

TP-I is used as a sensitive marker for myocardial damage confirmation in coronary artery disease [32]. Studies have shown that oxidative stress-induced damage to the myocardial cell membrane causes TP-I release into the circulation, as in coronary artery disease [16, 33]. The current study results determined that TP-I levels were higher in ATR group animals' blood serum than in AAT. Parenteral ATP administration prevented atorvastatin-induced blood TP-I increase. The fact that ATP treatment limits TP-I leakage into the circulation by reducing oxidative stress, suggests that it preserves the integrity of the membrane structure. Similarly, consistent with our study, ATP treatment lowered blood TP-I levels against vandetanib-induced heart damage [34].

4. Conclusion

High-dose atorvastatin increased oxidant parameters and decreased antioxidant parameters in the rat heart tissues. ATP prevented oxidative stress induced by high dose atorvastatin, possibly reducing ROS overproduction. Present study experimental results indicate that ATP may be useful in preventing atorvastatin-related oxidative heart damage.

Ethics in Publishing

The local Animal Experiments Ethics Committee approved the protocol and procedures (Date: 30/4/2020. Meeting No: 4/59).

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