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The prevention of myocardial ultrastructural changes by perindopril, atenolol and amlodipine in chronic alcohol administered rats

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Abstract

The effects of perindopril, an angiotensin converting enzyme inhibitor, atenolol, a beta adrenergic receptor blocker and amlodipine, a calcium channel blocker were investigated in chronic alcohol administered rats. Adult male Wistar rats (240–320 g) were used in the present study. Alcohol was given to rats by a modified liquid diet for 21 days. Perindopril (2.5 and 5 mg kg^{-1}), atenolol (5 and 10 mg kg^{-1}) and amlodipine (5 and 10 mg kg^{-1}) were injected to rats in different groups intraperitoneally for 21 days. Control rats were pair fed by an isocaloric liquid diet containing sucrose as a caloric substitute for alcohol. Saline was injected to control rats for 21 days. Rats were anesthetized with ether. Their hearts were removed and 1 mm^3 samples from left ventricles were fixed. Five fields per heart were examined and photographed with transmission electron microscope. Blood alcohol levels were also measured spectrophotometrically. Daily alcohol consumption of the rats was in a range of 12.09–15.5 g kg^{-1}. Blood alcohol concentrations were found as 145.63 mg dl^{-1} at 21st day of alcohol consumption. Chronic alcohol consumption caused some marked myocardial injuries. Perindopril and atenolol but not amlodipine produced some significant beneficial effects on alcohol-induced myocardial damages. Our results imply that perindopril and atenolol but not amlodipine have protective effects on heavy chronic alcohol consumption-induced myocardial injury in rats.

Keywords: Amlodipine; Perindopril; Atenolol; Alcohol consumption; Myocardial ultrastructure

1. Introduction

Alcohol abuse and dependence remain among the greatest substance abuse problem worldwide [1]. There was evidence for a dose–response relationship between level of alcohol consumption and risk of harm for liver cirrhosis, cancers of the oropharynx, larynx, oesophagus, rectum, liver and breast, and stroke. Cardiovascular disease is one of the most important risk factors and the leading cause of death in most regions of the world [2]. Although there was evidence for a protective effect of low alcohol consumption against risk of coronary heart disease, an increased risk of cardiac arrhythmias, cardiomyopathy and sudden coronary death were associated with heavy drinking [3]. In addition, chronic alcohol consumption is closely linked to hypertension [4] and hypertension together with hyperlipidemia is one of the most significant risk factor for cardiovascular diseases [5,6].

It has been shown that chronic ethanol ingestion caused left ventricular dysfunction in rats [7]. Both chronic moderate and heavy alcohol consumption exacerbate myocardial ischemia-reperfusion injury [8]. Coronary death also contributed significantly to the excess mortality in alcohol-dependent men and an increased vulnerability for sudden coronary death to persist for a considerable time after discharge from detoxification [9]. In that point, drug selection and use in alcoholic patients with cardiovascular disease have been gained a significant importance.

Antihypertensive drugs such as angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers and beta adrenoceptor blockers have already been used for reducing cardiovascular risk factors in patients with cardiovascular diseases [10]. The various kind of antihypertensive drugs show marked

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differences in their ability to prevent or reverse cardiac problems such as myocardial hypertrophy, differences primarily related to their mechanism of action [11,12]. The effectiveness and safety of these drugs in alcoholic patients or chronic heavy alcohol users that have also cardiovascular risk factors or diseases have not been clearly known. Oral chronic alcohol treated rat model [13] could be useful for investigating the harmful effects of alcohol on cardiovascular system and evaluating the effects of drugs. Thus, this study was organized to investigate the effects of perindopril, an ACE inhibitor, amlodipine, a calcium channel blocker, and atenolol, a beta adrenoceptor receptor blocker, on chronic alcohol-induced myocardial injury in rats.

2. Materials and methods

2.1. Animals and laboratory

All procedures in the present study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). Forty-two adult male Wistar rats (240–320 g) were the subjects in the present study. They were housed in a quiet and temperature- and humidity-controlled room ($22 \pm 2\,^{\circ}C$ and $60 \pm 5\%$, respectively) in which a 12-h light/12-h dark cycle was maintained (07:00–19:00 h light).

2.2. Chronic ethanol administration to rats

The rats were housed individually in metal cages. Ethanol was given to rats by a liquid diet for 21 days as previously described [13]. The rats received a modified liquid diet with or without ethanol ad libitum. No extra chow or water was supplied. The composition of the modified liquid diet with ethanol is cow's milk 925 ml (Mis Süt, Turkey), 25–75 ml ethanol (96.5% ethyl alcohol; Tekel, Turkish State Monopoly), Vitamin A 5000 IU (Akpa İlaç Sanayi, Turkey) and sucrose 17 g [14]. This mixture supplies 1000.7 kcal L^{-1}.

At the beginning of the study, rats were given modified liquid diet without ethanol for a week. Then, liquid diet with 2.4% (v/v) ethanol was administered for three days. The ethanol concentration was increased to 4.8% (v/v) for the following 3 days and finally to 7.2% (v/v) for 14 days. An isocaloric liquid diet containing sucrose as a caloric substitute to ethanol was also given to control rats. Liquid diet was prepared daily and presented at the same time of the day (10:00 h). The weight of the rats was recorded every day and the daily ethanol intake was measured and expressed as grams per kilogram per day.

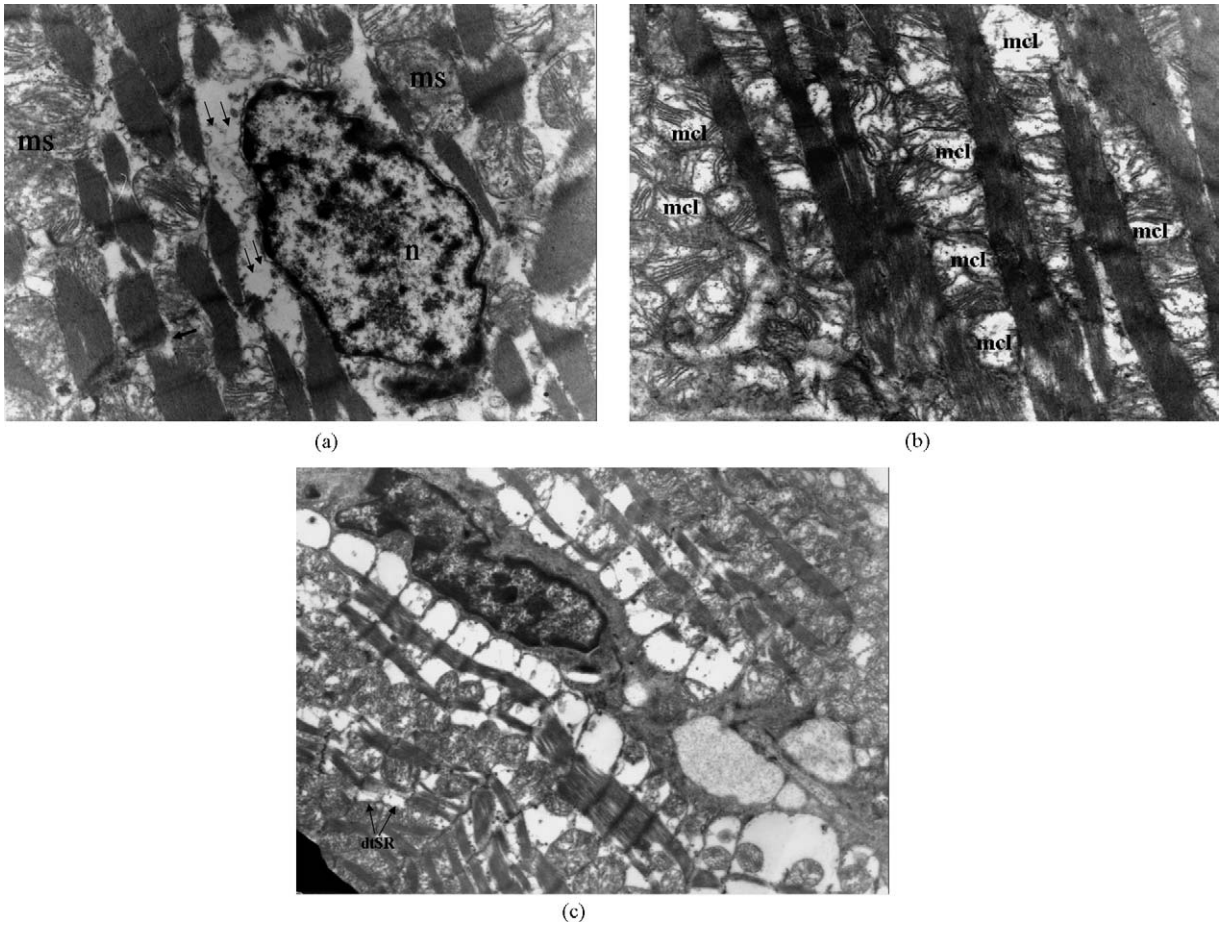

Fig. 1. Thin section from the ventricle of rat in control group. (a) Loss of myofibrils involving perinuclear area (double arrow), mitochondrial swelling (ms), breaks in myofibrils (thick arrow), nucleus (n). (b) Prevalent mitochondrial cristalysis (mcl). (c) dilated tubules of sarcoplasmic reticulum (dtSR) which are confined to perinuclear areas and areas of myofibrillar loss + SR proliferation.

2.3. Experimental procedure

The rats which were exposed to ethanol for 21 days were grouped ($n = 6$) as follows:

Group I (Control): The rats that were exposed to ethanol without drug.
Group II: Exposed to ethanol with 2.5 mg kg^{-1} day^{-1} perindopril.
Group III: Exposed to ethanol with 5 mg kg^{-1} day^{-1} perindopril.
Group IV: Exposed to ethanol with 5 mg kg^{-1} day^{-1} amlodipine.
Group V: Exposed to ethanol with 10 mg kg^{-1} day^{-1} amlodipine.
Group VI: Exposed to ethanol with 5 mg kg^{-1} day^{-1} atenolol.
Group VII: Exposed to ethanol with 10 mg kg^{-1} day^{-1} atenolol.

Rats were anesthetized with ether. Their hearts were removed and 1 mm^3 samples from left ventricles were fixed for 48 h in 2% gluteraldehyde solution in phosphate buffer (PBS). The samples were then washed in 1/15 molar PBS (pH 7.25) five times and post-fixed in 2% osmium tetroxyde (OSO_4) in distilled water at 4 °C for 1.5 h. Following several washes with PBS at room temperature, samples were dehydrated in alcohol and embedded in Araldite (Firm: SERVA, Name: Araldite CY 212) that was polymerized for 48 h at 60 °C.

One-micron semi-thin sections were used to select blocks with longitudinally oriented fibers and areas that demonstrated degeneration or not. Semi-thin sections were stained with toluidine blue and areas of interest were chosen. Then these blocks were cut into 60–90 nm ultra thin sections (OM 42, Reichert, Hamburg, Germany) mounted on copper grids and stained with uranylacetate and lead citrate. Five fields per heart were examined and photographed with transmission electron microscope (Carl Zeiss EM900-Germany).

2.4. Evaluation of ultrastructural changes

We evaluated the myocardial ultrastructure by using the following scoring system.

0	Normal
1	Dilatation of sarcoplazmic reticulum tubules
1.5	Decrease in number and the size of mitochondria
2	Mitochondrial crystallizes
2.5	Mitochondrial swelling
3	Loss of myofibrils

Each photograph was graded by an experienced histologist, who did not know the group of the photograph.

2.5. Evaluation of blood ethanol levels

Blood ethanol levels were measured spectrophotometrically at 340 nm (Schimadin UV, Model 2100S, Kyoto, Japan) using a commercial ethanol assay kit which employs alcohol dehydrogenase (EL.IIII) (Sigma chemical, St Louis, MO, USA) levels were expressed as milligrams per decilitre.

Blood samples were taken by intracardiac puncture. Under light ether anesthesia on the third day of 2.4% ethanol consumption and 14th day of 7.2% ethanol consumption. The samples were taken 6 h after daily renewal of the diet (i.e. at 16:00 h). The samples were collected into test tubes and centrifuged (4500 rpm—2268 × g for 5 min) at room temperature. The supernate were separated and divided into two portions for measuring drug quantity and blood ethanol levels. Samples were stored at −20 °C until analyzed.

Table 1
The electron microscopic scores of the groups.

Group	Median	Mean (minimum–maximum)
Control	2	2.16 (1.5–3)
Perindopril 2.5 mg	2	1.93 (1–3)
Perindopril 5 mg	1	0.81 (0–2)
Atenolol 50 mg	1.75	1.51 (0–2.5)
Atenolol 100 mg	1	1.03 (0–2.5)
Amlodipin 5 mg	2	2.05 (1–3)
Amlodipin 10 mg	2	1.88 (1–3)

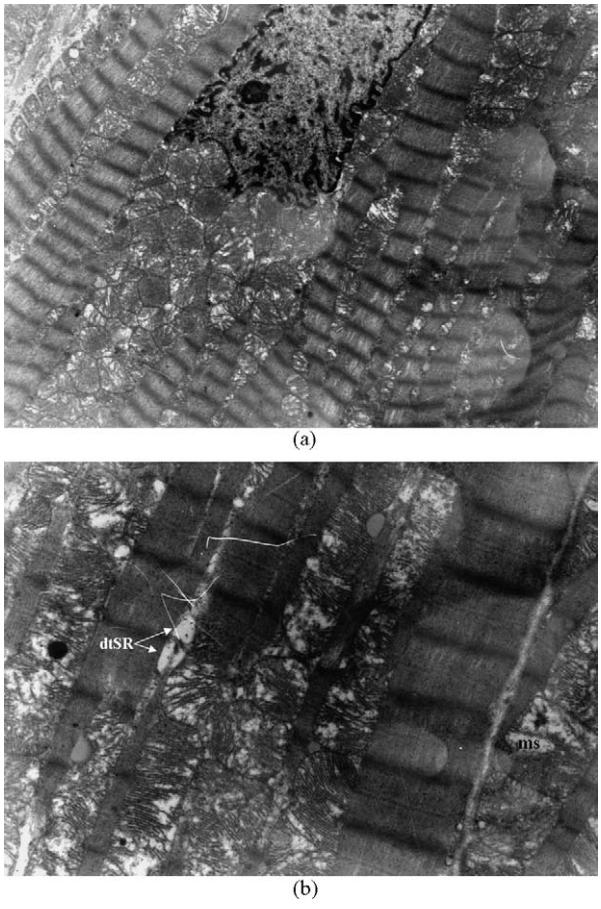

Fig. 2. Thin section from the ventricle of the rat which was administered 2.5 mg perindopril. (a) Nuclear chromatin and myofibrills are normal, no mitochondrial cristalysis. (b) Cristae are lightly sparse in some areas but there are no cristalysis. Rare mitochondrial swelling (ms) and dilated tubules of sarcoplasmic reticulum (dtSR) are inconspicuous.

2.6. Statistical analysis

Kruskal–Wallis test was used for the non-parametric analysis of the electron microscopic scores, and the level of significance was set at $p < 0.05$.

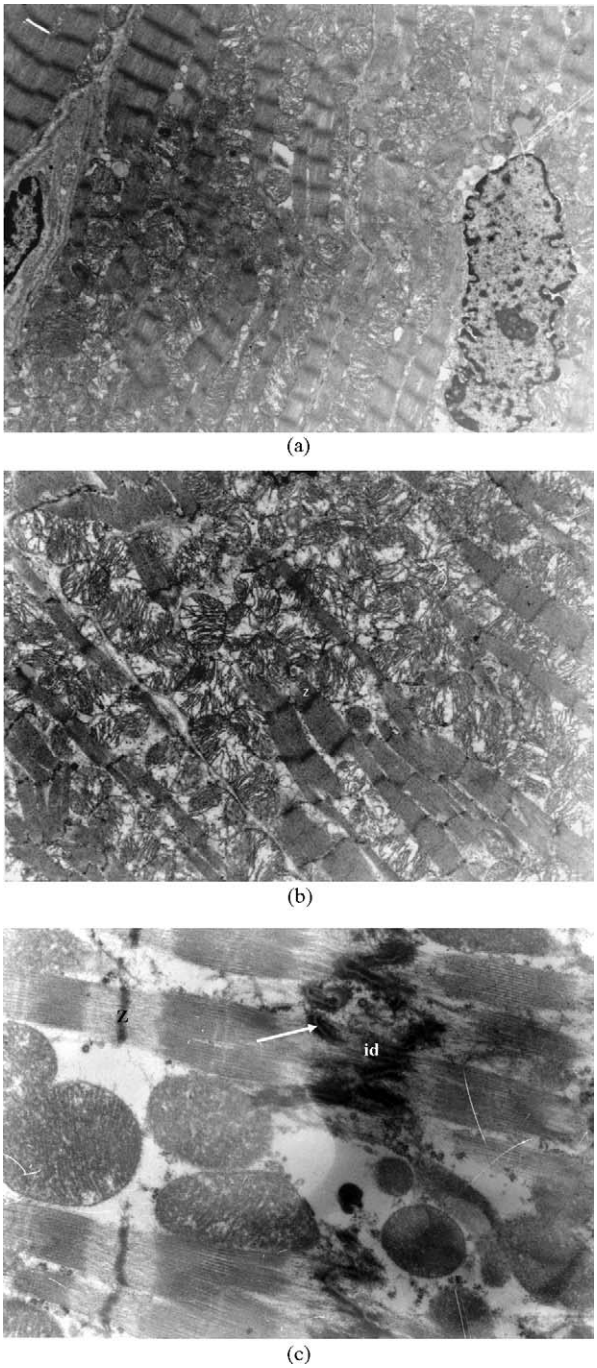

Fig. 3. Thin section from the ventricle of the rat which was administered 5 mg perindopril. (a) Normal myocardium: nuclear chromatin and myofibrills are normal. (b) Normal myocardium: the thin filaments insert at the electron dense Z bands (Z), the myofibrils are paralel to the long axis of the cell with lateral alignment of the Z bands. There is no loss of myofibrils; mitochondria and tubules of SR are normal. (c) Intercalate discs (id), desmosome (d) type connections in them and Z bands (Z) are normal and clearly visualized.

3. Results

3.1. Daily ethanol consumption and blood ethanol levels

Daily ethanol consumption of the rats was in a range of 12.09 ± 3.17 to $15.50 \pm 2.56\,g\,kg^{-1}$ during the exposure to ethanol (7.2%, v/v). No significant difference between the ethanol-ingesting groups was observed (data not shown).

Serum ethanol concentrations were found as 37.25 and $145.63\,mg\,dl^{-1}$ at third and 21st days of ethanol consumption, respectively.

3.2. Electron microscopic findings

Electron microscopic findings have been presented in Figs. 1–5. Ultrastructural changes in control group are seen in Fig. 1(A–C), perindopril $2.5\,mg\,kg^{-1}$ group in Fig. 2(A–B), perindopril $5\,mg\,kg^{-1}$ in Fig. 3(A–C), amlodipine $5\,mg\,kg^{-1}$ group in Fig. 4A, amlodipine $10\,mg\,kg^{-1}$ group in Fig. 4B, atenolol $5\,mg\,kg^{-1}$ in Fig. 5A and atenolol $10\,mg\,kg^{-1}$ in Fig. 5B.

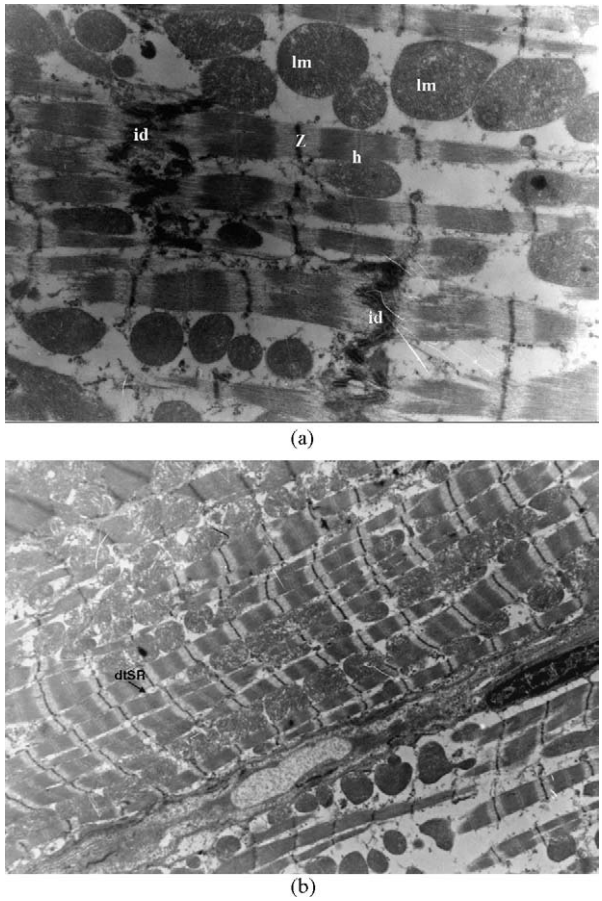

Fig. 4. Thin section from the ventricle of the rat which was administered 5 mg (a) and 10 mg (b) amlodipin. (a) Cardiomyocytes generally have normal structure, rare of them have myofibrillar loss. Some mitochondria are larger than normal (lm), intercalate discs (id), Z bands (Z) and H bands (h) are normal. (b) Rare dilated tubules of SR (dtSR) are present, other structures are normal.

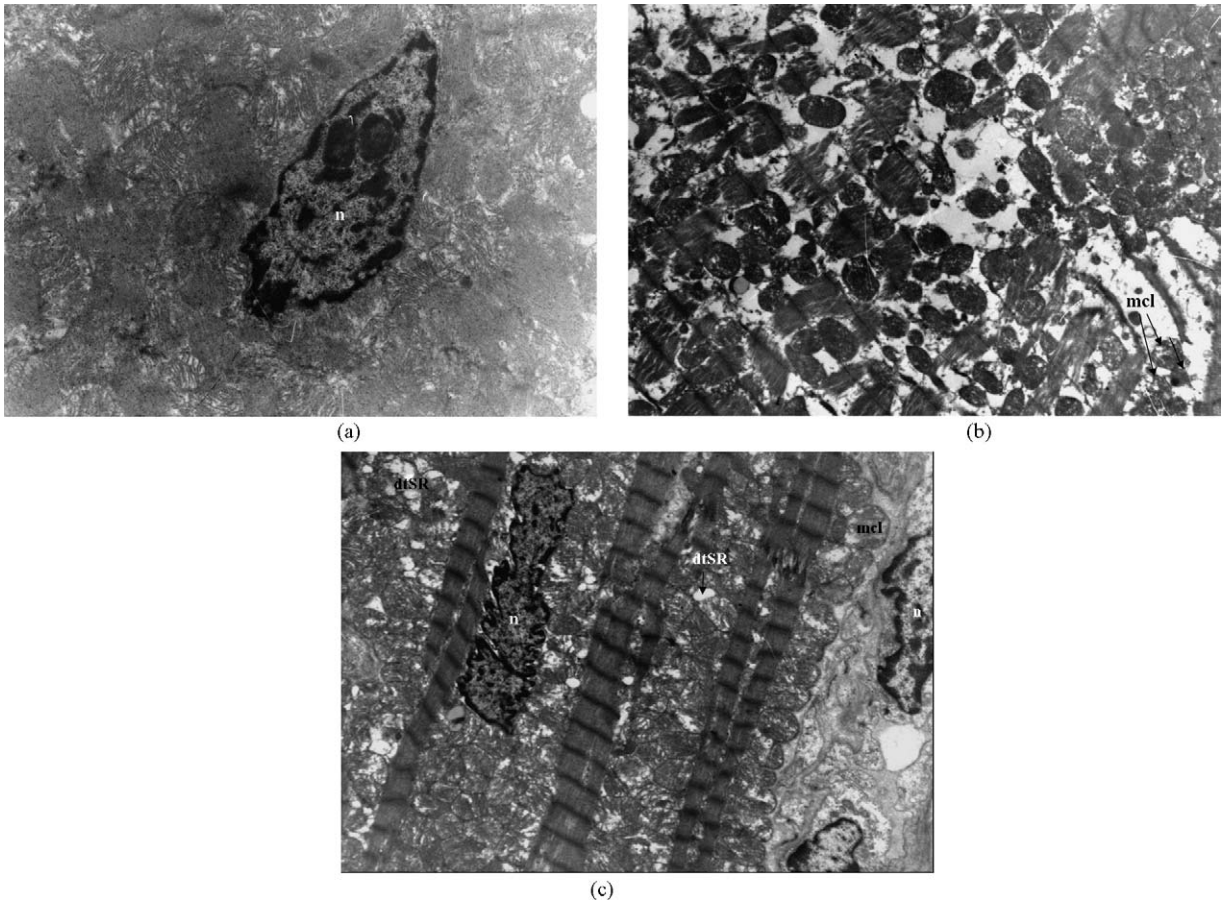

Fig. 5. Thin section from the ventricle of the rat which was administered 5 mg (a) and 10 mg (b) atenolol. (a) Nuclear (n) and myofibrillar formation are normal. (b) Myofibrillar loss and slight mitochondrial cristalysis (mcl) is present in some cells. (c) Mitochondrial cristalysis (mcl) is present in some areas, rare dilated sarcoplasmic reticulum tubules (dtSR) are prominent. Nucleus (n) is normal.

The mean electron microscopic scores in ascending order of the groups were perindopril 5 mg kg^{-1}, atenolol 10 mg kg^{-1}, atenolol 5 mg kg^{-1}, amlodipin 10 mg kg^{-1}, amlodipin 5 mg kg^{-1}, perindopril 2.5 mg kg^{-1}, and control group, respectively. In Table 1 the electron microscopic scores of the groups and in Fig. 6 the statistical comparison of the groups are illustrated.

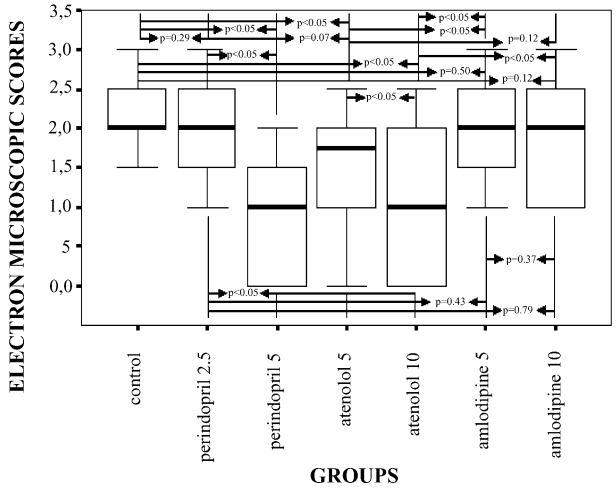

Fig. 6. The statistical comparison of the groups.

While there was no statistically significant difference between the control group and perindopril 2.5 mg kg^{-1}, amlodipin 5 mg kg^{-1} and amlodipin 10 mg kg^{-1} groups ($p > 0.05$), the difference between the control group and the groups of perindopril 5 mg kg^{-1}, atenolol 5 mg kg^{-1} and atenolol 10 mg kg^{-1} was statistically significant ($p < 0.05$).

4. Discussion

The results of the present study clearly show that perindopril and atenolol but not amlodipine produced some marked beneficial effects that can be detected by electron microscopy on alcohol-induced myocardial damages in rats. In our study, perindopril at high dose was also found to be more effective than atenolol.

Previous studies indicated that chronic ethanol consumption above 9 g kg^{-1} day^{-1} had some neurotoxic behavioural impairment such as locomotor activity and motor coordination in rats [14–16]. In addition, high blood alcohol concentration (143 mg dl^{-1}) was detected in rats at 21st day of chronic ethanol consumption (just before the experiments). According to the results of our previous studies [17–21] daily ethanol consumption ranged from 12 to 15 g kg^{-1} for 21 consecutive days has also been produced physical dependence in rats. Thus, as it was

expected, in the present study, the heavy ethanol consumption caused some marked injuries in myocardial tissues of the control rats. This observation implies that chronic heavy alcohol administration to rats by a liquid diet technique for almost three weeks might be used as a myocardial damage model in rats. This observation also indirectly suggests that there may be a relationship between heavy chronic alcohol consumption and myocardial injuries in rats. A recent study from our laboratory indicated that chronic alcohol administration by liquid diet to rats did not produce any significant change on myocardial ischemia, implying that the injury is mediated through other than ischemic mechanisms [22].

Normal human myocardial cells are arranged in parallel and have specialized end-to-end connections that form the intercalated discs. The discs occupy the entire length of the end-to-end cellular contacts. They are present at the level of the thermal 2 bands in the myofibrils and step from one Z band level to the next, forming an irregular line. The myofibrils are mainly composed of thin (actin) and thick (myosin) filaments in a highly organized arrangement. They have a characteristic-banding pattern seen by electron microscopy. The cytoplasm of myocardial cells contains many mitochondria distributed between myofibrils. Glycogen granules and few lipid droplets are present scattered between other cytoplasmic components. Small golgi bodies, rough endoplasmic reticulum, lysosomes, and lipofuscin pigment granules are found in the perinuclear areas [23]. In the present study, we ultrastructrally demonstrated the degenerative changes in myocardium of rats with chronic ethanol intake. We determined that these degenerative changes due to chronic alcohol intake are partially reversed in experimental groups, which were administered perindopril, an ACE inhibitor, and atenolol, a beta adrenoceptor receptor blocker. Both ACE inhibitors and beta adrenoceptor blockers have been found protective and beneficial on cardiovascular problems in human [24,25]. In experimental studies, both perindopril [26,27] and atenolol [28,29] exhibited some marked beneficial effects in several models of cardiovascular damages in rats. Our findings are in line with the previous studies. In addition to the models in previous studies, we showed that both perindopril and atenolol also had marked beneficial effects in heavy chronic alcohol-induced myocardial injuries.

Amlodipine, a calcium channel blocker, was not as effective as other two drugs which were used in our study. Our results suggested that ACE inhibitors and beta adrenergic receptor blockers might have more protective effect on chronic alcohol-induced injuries in the rat myocardium as compared to amlodipine, a calcium channel blocker. Some experimental studies performed in rats indicated that amlodipine had some beneficial effects on cardiovascular damages. For example, Nayler showed that amlodipine reduced cardiac hypertrophy and vascular damages in rats [30]. Yamazaki et al. also suggested that amlodipine treatment inhibited development of cardiac remodelling in rats [31]. In another recent study, it has been indicated that amlodipine provided a marked improvement on cardiac dysfunction induced by burn trauma in rats [32]. In our study, although amlodipine did not have any marked beneficial effects on alcoholic injuries in the rat myocardium, it had some minor constructive effects. Moreover, amlodipine treatment did not worse alcoholism-induced injuries. Incapable dose of amlodipine may be responsible for the ineffectiveness in the present study. We did not use higher doses because the chosen doses were previously shown to lower blood pressure in rats [33–36]. The higher doses would make hemodynamical deterioration, which could make the results difficult to interpret. In addition, heavy chronic alcohol-induced damages in rat myocardium may not be sensitive to amlodipine effects. On the other hand, differences between the action mechanisms of the drugs using in our study may be related to this situation. Further studies including more doses of amlodipine need clarification of the effects of this drug on chronic alcohol-induced myocardial damages.

We did not measure the arterial blood pressure of the rats during the study; therefore, it is not clear whether the protective effects are mediated through the decrease in blood pressure (indirect effect) or direct mechanism. Thus, the mechanism of the protective effect was beyond the scope of this study and requires further investigation. However, in our study, degeneration of mitochondria have been clearly seen and the beneficial effects of ACE inhibitor and beta blocker using in our study may be related to direct protective effects on the cardiomyocytes.

In conclusion, our results imply that perindopril and atenolol but not amlodipine have some significant beneficial effects on heavy chronic alcohol consumption-induced myocardial injury in rats. However, it must be taken into consideration that these results are obtained from an animal study. Adaptation of the results on human clinical practice must be implemented after long-term randomized clinical studies on humans.

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