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THE EFFECT OF AMLODIPINE ON THE MYOCARDIAL ULTRASTRUCTURE OF THE HYPERTENSIVE RATS

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Abstract

As a group the calcium channel blockers are effective antihypertensive agents, but their cell protective properties are not investigated well. Rats were separated in three groups: Normotensive control group (normotensive Wistar-Kyotorats), Hypertensive control group (spontaneously hypertensive rats - SHR) and Amlodipine group (SHR that received a 10 mg/kg /day amlodipine dose during three months). We performed examination of the myocardium's fragments under a scanning electron microscope. Morphometric investigation of mitochondrions we performed using special program's named "ORGANELLE". Amlodipine significantly normalizes the ultrastructure of the myocardium. This antihypertensive drug prevents strengthening contractility of the myofibrils. Amlodipine causes the fusion mitochondrions which has compensatory character on theback ground of good safety of mitochondrial ultrastructures. Another feature of amlodipine is a significant increasing number of the blood capillaries. There are distributed ribosomes, polysomes, endoplasmic reticulums in the cytoplasm of the endothelial cells of these blood capillaries, which denotes biosynthetic activity of these cells. This cell-protective effect of the amlodipine in spontaneously hypertensive rats could be a significant additional factor in the treatment of hypertension, especially one that complicated with pathological changes in heart.

Key words: *hypertension, amlodipine, ultrastructure of cardiomyocytes*

1. INTRODUCTION

Anatomic and functional relationships between various body systems determine development of interrelated metabolic, functional and structural disorders in the organs during arterial hypertension (AH). In this case not only drugs with antihypertensive activity are very important for treatment of AH but also ones with cell protective activity or both effects. That's why momentous question of modern cardiology is to find antihypertensive drugs with cell protective properties.

In the recent years calcium channel blocker amlodipine has occupied superior position and that is caused by its effective therapeutic activity as antihypertensive agent [1,7,9]. However, till the present days there haven't been any detailed researches of amlodipine effects to the heart ultrastructure in the literature [4,5,11-13]. So, our study evaluate influence of amlodipine to the myocardium ultrastructures of hypertensive rats, furthermore we investigated mitochondrial morphology of the cardiomyocytes.

2. MATERIALS AND METHODS

Amlodipine was purchased from Glochem Industries Limited (India). Normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) of either sex were provided by the Animal Center of our University (National O.O. Bohomolets Medical University, Ukraine). They were housed with controlled temperature (23–25°C) and lighting, and with free access to standard food and drinking water. All of the animals used in this experiment received humane care; and the study was in compliance with the institutional guidelines for the health and care of experimental animals and in accordance with Directive 86/609/EEC (Ethic Committee permit No. 62 from 05.IV.2012).

The rats were separated into three groups of 21 animals each. The groups were composed as follows:

1) Normotensive control group – WKY received standard rat chow and fresh water ad libitum;

2) Hypertensive control group – SHR received standard rat chow and fresh water ad libitum, without treatment;

3) Amlodipine group – SHR received standard rat chow and fresh water ad libitum as well as a 10 mg/kg/day amlodipine dose via an orogastric tube during three months.

Under ketamine hydrochloride anesthesia animals were perfused through the left ventricle with physiological saline at 37°C, followed by 2,5% glutaraldehyde in 0,1 M phosphate buffer (pH 7,4). The excised fragments of the myocardium were further immersed in the same fixative for one hour, washed in 0,1 M phosphate buffer (pH 7,4) 3 times and postfixed in 1% OsO4 in the same buffer solution for one hour. After dehydration in a graded concentration of ethanol, the specimens were passed through isoamyl acetate, dried in a critical-point dryer. After that the fragments of the myocardium coated with uranyl acetate and lead citrate, and examined under a Hitachi scanning electron microscope.

Morphometric investigation of mitochondrions we performed using special program's named "ORGANELLE". In each experimental group we studied 30 areas of myocardium under the magnification of 20000-27000 times.

Data are expressed as means ±s.e.m. Statistical analyses were performed using the parametric Student's test. Differences were considered significant when the P value was < 0,05.

3. RESULTS AND DISCUSSION

Scanning electron microscopic studies showed that ultrastructure of myocardium from left ventricle of the rats line WKY have no deviation. Cardiomyocytes contain normal myofibrils that are organized in common sarcomere with A and I disks. Mitochondrions have oval or elongated form, also they have well-structured outer membrane, matrix moderate electron density and parallel cristas.

According to the morphometric analysis mitochondrions of normotensive rats occupy 30,3±2,9% volume of cardiomyocytes. Their quantitative density is (51,1±5,20)x10-2/μm3 with an average area of a cut (41,3±2,04)x10-2 μm2 (Table 1, Fig. 1).

Blood microvessels have open lumen. Also nuclear zone of the endothelial cells evaginates in this lumens. Peripheral areas of these cells of the moderate thickness contain mainly micropinocytic vesicles.

Table 1. Morphometric indexes that characterize changes of mitochondrions of the cardiomyocytes.

Indexes / Groups of animals	Volume density of mitochondrion (%)	Quantitative density of mitochondrion ($10^{-2}/\mu m^3$)	Average area of mitochondrion's cut ($10^{-2}\ \mu m^2$)
Normotensive rats	30,3±2,9	51,1±5,2	41,3±2,04
Hypertensive rats	21,16±2,47*	59,21±10,63	31,96±1,53*
Hypertensive rats + Amlodipine	27,46±1,76**	21,38±4,36*/**	81,16±7,69*/**

Values are means ±s.e.m. *P < 0,05 vs. normotensive rats, **P < 0,05 vs. hypertensive rats

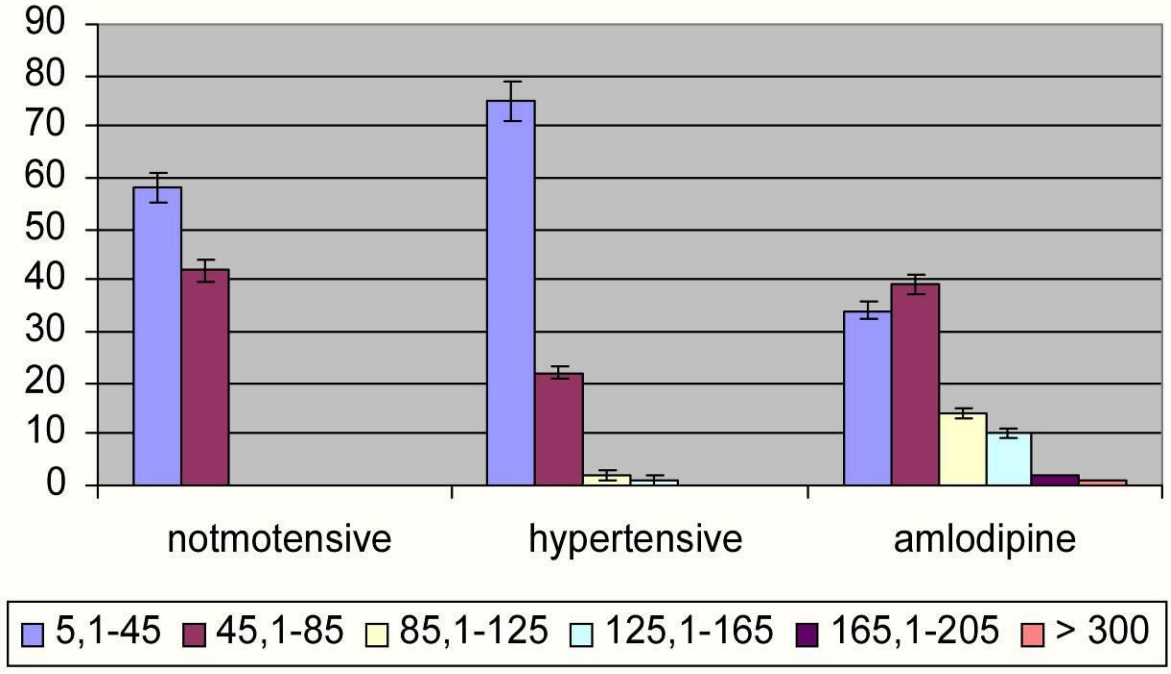


Figure 1. The distribution of mitochondrions by area.

On the **x-axis** – average area of mitochondrions' cut ($10^{-2}\ \mu m^2$); on the **y-axis** – volume density of mitochondrions (%).

The myocardium from left ventricular of SHR is characterized by mosaic ultrastructure. This is due to different depth and prevalence of injuries which primarily relate to contractile and the energy apparatuses of cardiomyocytes. In general there is lysis of the myofilaments in cardiomyocytes which sometimes extends to the entire sarcomere (Fig. 2a).

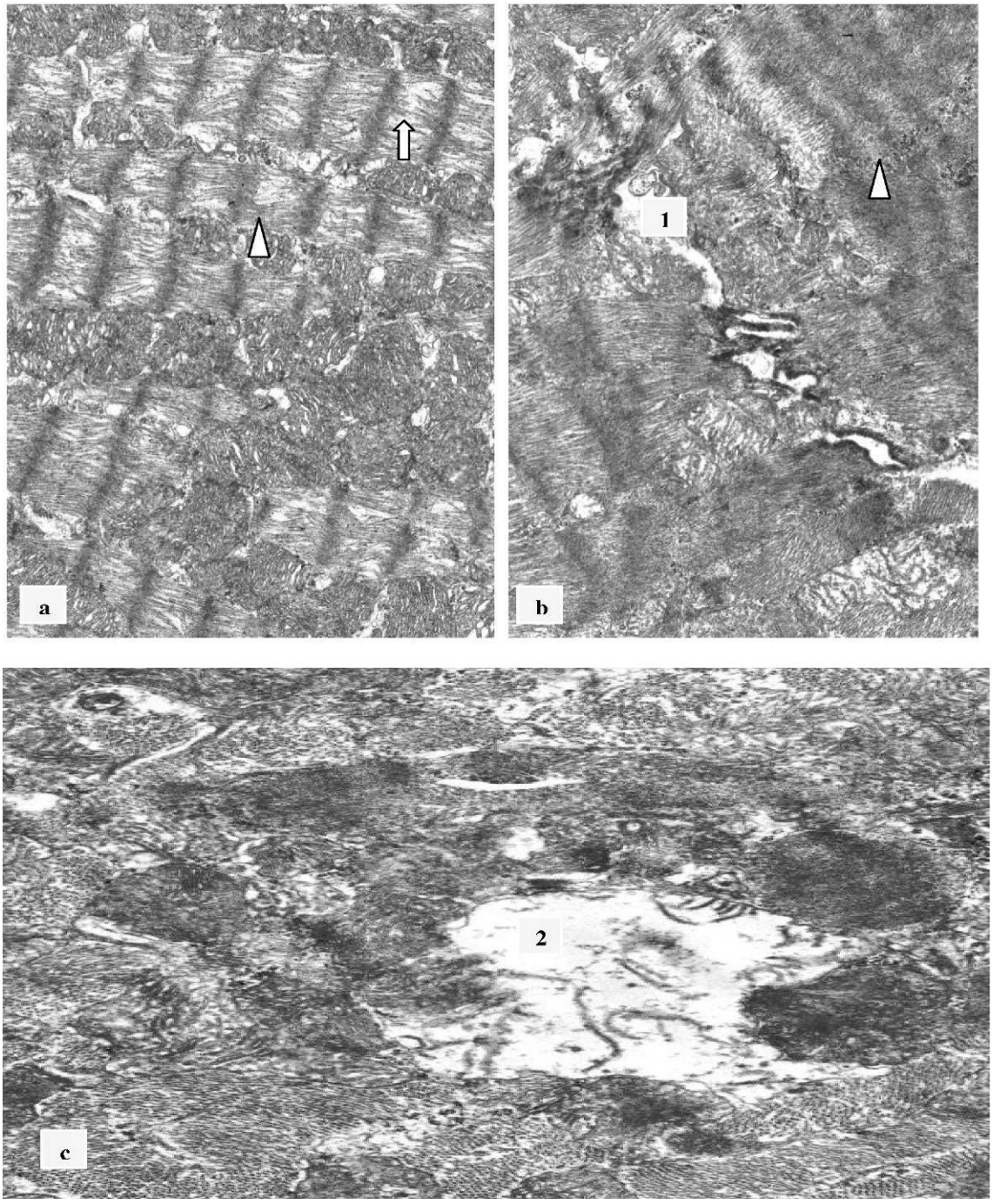


Figure 2. Electron micrographs of the left ventricular myocytes of the spontaneously hypertensive rats heart.

a, b – largely coarctate myofibrils (△); myofibrils with destructive features (⇧); intercalated disk (**1**) of the cardiomyocytes. **c** – swollen and injured mitochondrions (**2**).

Only rare cardiomyocytes contain myofibrils without destructive features. Myofibrils are largely coarctate, as a result sarcomeres contain indistinguishable A and I disks, and Z-lines are sharply close each other (Fig. 2b).

Violations of the myocardium contractile structures correlate with changes of intercalated disk to which they are attached. Between some cardiomyocytes slots there is expansion due to separation of plasmatic membranes of the neighboring cardiomyocytes (Fig. 2b). In another sites plasmalemma of the cells twists, and this changes the order between bridge contact and gap junction.

Also we've discovered changes that touch the system of T-tubules and sarcoplasmic reticulum, and which associate with the impaired contractility of the cardiomyocytes. In the SHR the tubules of these systems are expanded throughout, T-tubules penetrate deep into cardiomyocytes (Fig. 2a), which probably increase the release of calcium from these structures and increases the contractility of the myofibrils.

During arterial hypertension mitochondrions of the cardiomyocytes from left ventricular often become wavy due to invaginations of outer membranes. Sometimes there is a local lysis of outer membranes, which also may extend to all surface of the mitochondrion and as a result there remain only internal membranes – cristas (Fig. 2c).

Morphometric analysis showed that the number of mitochondrions is (59,21±10,63)x10-2/μm3, which is no different from normotensive control ($p > 0.05$) (Table 1). Also their volume density is significantly lower than in normotensive controls; and average area of mitochondrion's cut decreases. However the analysis of a mitochondrial distribution by the areas showed that in the cardiomyocytes of the rats with hypertension besides increasing quantity of small mitochondrions also appear large organelles that were not inherent in the control animals (Fig. 1). But the fact that the largest mitochondrions are swollen gives us grounds to consider that increasing of the size of these organelles not to be as a compensative hypertrophy but to be as the manifestation of dystrophic processes.

Although amlodipine significantly normalizes the ultrastructure of the myocardium, it doesn't completely restore one. This antihypertensive drug prevents strengthening contractility of the myofibrils, which displays a lack of reduced-size sarcomeres, parallel Z- and M-lines in the neighboring sarcomeres (Fig. 3a,b).

Also the structures that involve in calcium metabolism (T-tubules and sarcoplasmic reticulum) have no signs of damage (Fig. 3a). In general the number of the damaged myofibrils is lower than in untreated rats.

Mitochondrions have mostly elongated shape with well-preserved outer membrane. In general, the safety of outer and inner membranes of mitochondrions after using of amlodipine is much better than it is in the rats with hypertension (Fig. 3a,b).

Morphometric analysis showed that amlodipine causes significant increasing the size of mitochondrions. The average area of the cut is equal to (81,16±7,69)x10-2 μm2, which is almost twice higher than in normotensive and untreated rats with hypertension (Table 1). The analysis of the distribution of mitochondrions per areas showed that their average size increases due to decreasing number of small mitochondrions against the increasing number of medium-sized organelles and the appearance of giant mitochondrions (Fig. 1). On the other hand the simultaneous reduction of quantitative density of mitochondrions more than in two times leads to the fact that the volume they occupy in the cytoplasm remains statistically unchanged compare with normotensive rats. This gives grounds to suppose that amlodipine causes the fusion of mitochondrions which has compensatory character on the background of good safety of mitochondrial ultrastructures, coronary artery vasodilatation and improved myocardial perfusion. This mitochondrial fusion may be explained by activation mitofusins of their outer and inner membranes under the influence of the therapeutic effects of amlodipine [2,3,10].

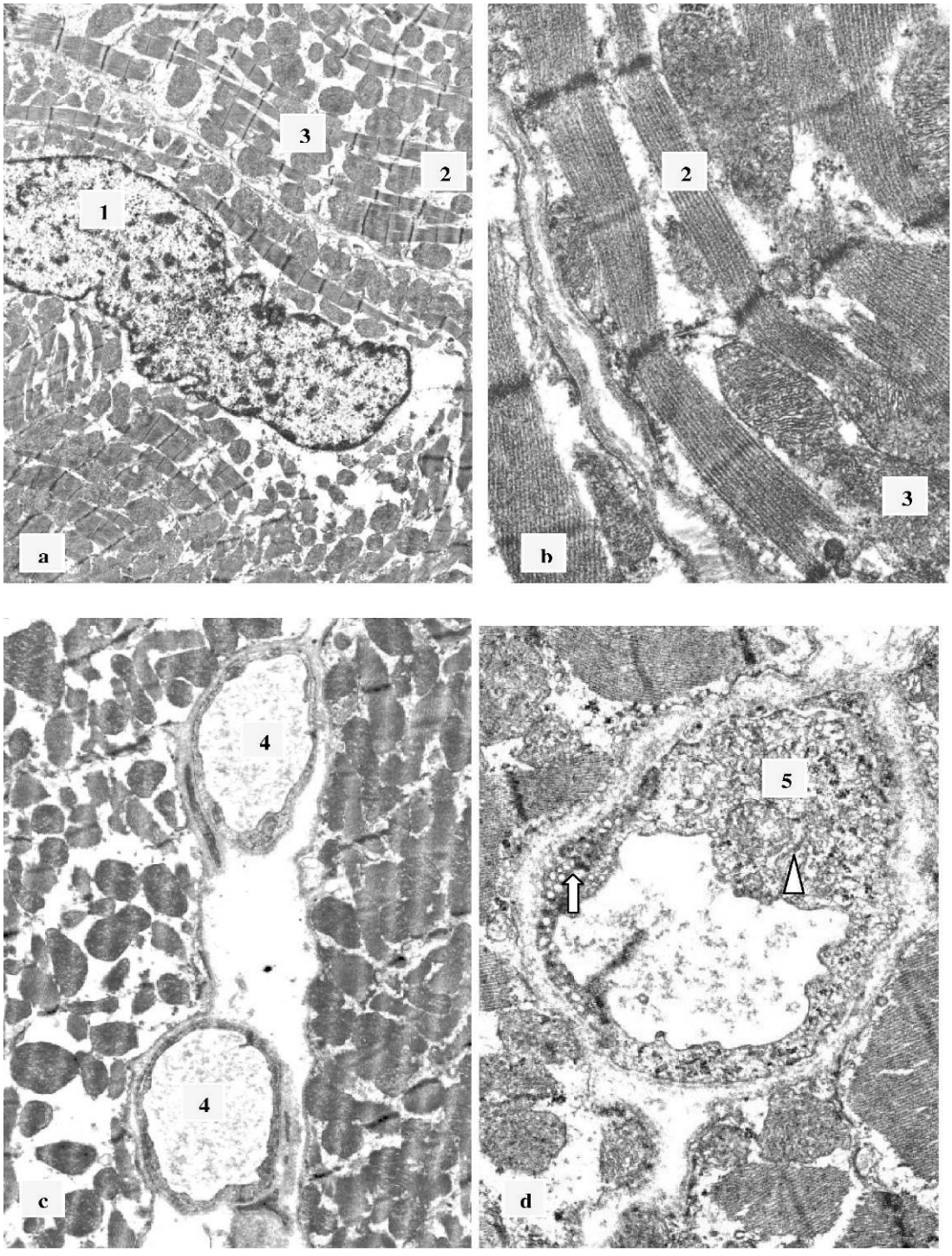


Figure 3. Electron micrographs of the left ventricular myocytes of the spontaneously hypertensive rats heart treated with amlodipine.

a, b – nucleus (**1**), myofibrils coarctate (**2**), mitochondrions (**3**) of the cardiomyocytes. **c** – blood capillaries with opened gaps (**4**). **d** – mitochondrions (**5**), endoplasmic reticulums ( ), micropinocytic vesicles ( ) of the endotheliocytes.

Also, the other feature of amlodipine is a significant increasing number of the blood capillaries. There are distributed ribosomes, polysomes, endoplasmic reticulums in the cytoplasm of the endothelial cells of these blood capillaries, which denotes biosynthetic activity of this cells (Fig. 3d, 4).



Figure 4. Electron micrographs of the left ventricular myocytes of the spontaneously hypertensive rats heart treated with amlodipine.

Nucleus (**1**), clusters of the micropinocytic vesicles (△) of the endotheliocytes.

Also there are placed micropinocytic vesicles in the peripheral areas of endotheliocytes that sometimes form clusters (Fig. 4). Small diameter and opened gaps of the capillaries, the proximity of capillaries to each other (Fig. 3c) indicates their active growth, which restores again reducing by arterial hypertension the number of microvessels. This angiogenesis probably caused by a normalizing effect of amlodipine on nitric oxide synthase level and an increasing functional activity of the endotheliocytes. It is known that the increasing functional activity of the endotheliocytes is the key to initiate migration and division of the endotheliocytes [6,8,14,15]. These newly formed vessels can serve as bypass for arteries affected by atherosclerosis and reduce the damage of the tissues that is caused by the lack of blood supply.

4. CONCLUSION

We conclude that newly identified protective effect of amlodipine on the myocardium cells could be a significant additional factor in the treatment of hypertension, especially one that complicated with pathological changes in heart.

REFERENCES

1. Chan D. 2006, 'Mitochondrial fusion and fission in mammals', Annu Rev Cell Dev Biol, vol. 22, pp. 79-99.
2. Chen H., Vermulst M., Wang Y.E., Chomyn A., Prolla T.A., McCaffery J.M., Chan D.C. 2010, 'Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations', Cell, vol. 14, no. 2, pp. 280-289.
3. Choi S.M., Kim J.E., Ahn B.O., Kwon J.W. 2006, 'Effects of amlodipine orotate on hypertension-related complications in spontaneously hypertensive rats', Arzneimittelforschung, vol. 56, no.1, pp. 12-17.
4. Hazama F., Ozaki T., Amano S. 1979, 'Scanning electron microscopic study of endothelial cells of cerebral arteries from spontaneously hypertensive rats', Stroke, vol. 10, no.3, pp. 245-252.
5. Khan N.A, Chattopadhyay P., Abid M., Pawdey A., Kishore K., Wahi A.K. 2012, 'Protective effects of amlodipine on mitochondrial injury in ischemic reperfused rat heart', J Environ Biol, vol. 33, no. 3, pp. 591-595.
6. Kizhakekuttu T.J., Wang J., Dharmashankar K., Ying R., Gutterman D.D., Vita J.A., Widlansky M.E., 2012, 'Adverse alterations in mitochondrial function contribute to type 2 diabetes mellitus-related endothelial dysfunction in humans', Arterioscler Thromb Vasc Biol, vol. 32, no.10, pp. 2531-2539.
7. Lee Y.J., Choi D.H., Cho G.H., Kim J.S., Kang D.G., Lee H.S. 2012, 'Arctium lappa ameliorates endothelial dysfunction in rats fed with high fat/cholesterol diets', BMC Complement Altern Med, vol. 12, no. 1, pp. 116.
8. Lu J.C., Cui W., Zhang H.L., Liu F., Han M., Liu D.M., Yin H.N. 2009, 'Zhang K., Du J., Additivebeneficialeffectsofamlodipineandatorvastatininreversingadvancedcardiachypertrophyinelderlyspontaneouslyhypertensiverats', Clin Exp Pharmacol Physiol, vol. 36, no. 11, pp. 1110-1119.
9. Makani H., Bangalore S., Romero J., Wever-Pinzon O., Messerli F.H. 2011, 'Effect of rennin-angiotensine system blockade on calcium channel blocker-associated peripheral edema', Am J Med, vol. 124, no. 2, pp. 128-135.
10. Mozdy A., Shaw J. 2003, 'A fuzzy mitochondrial fusion apparatus comes into focus', Nat Rev Mol Cell Biol, vol. 4, no. 6, pp. 468-478.
11. Sag C., Yokusoglu M., Cincik M., Ozkan M., Kayir H., Uzun M., Baykal B., Ozogul C., Baysan O., Uzbay I.T. 2006, 'The prevention of myocardial ultrastructural changes by perindopril, atenolol and amlodipine in chronic alcohol administered rats', Pharmacol Res, vol. 53, no. 2, pp. 142-148.
12. Singh A., Bangalore S. 2012, 'Do angiotensin receptor blockers prevent myocardial infarctions as well as other initial therapies?' CurrOpinCadiol, vol. 27, no. 4, pp. 381-385.
13. Sorop O., Bakker E.N., Pistea A., Spaan J.A., VanBavel E. 2006, 'Calcium channel blockade prevents pressure-dependent inward remodeling in isolated subendocardial resistance vessels'. Am J Physiol Heart CircPhysiol, vol. 29, no. 3, pp. 1236-1245.
14. Sun L., Bai Y., Du G. 2009, 'Endothelial dysfunction – an obstacle of therapeutic angiogenesis', Aqeing Res Rev, vol. 8, no. 4, pp. 306-313.
15. Vesterdal L.K., Mikkelsen L., Folkmann J.K., Sheykhzade M., Cao Y., Roursgaard M., Loft S., Møller P. 2012, 'Carbon black nanoparticles and vascular dysfunction in cultured endothelial cells and artery segments', ToxicolLett, vol. 214, no. 1, pp. 19-26.