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Department and Institute of Histology and Embryology, Medical University of Lublin

TERESA KRYŁOWICZ, JADWIGA ROMANOWSKA-SARLEJ,
MIROSŁAW ŁAŃCUT, WŁODZIMIERZ MATYSIAK,
KRYSTYNA CZERNY

Ultrastructure of myocardium after application of angiotensin II receptor antagonist (losartan)

Angiotensin II receptor AT1 antagonists are drugs applied in the therapy of arterial hypertension and its organ complications (2, 3, 4, 14). The mechanism of its action is based on a specific blocking of type 1 receptors for angiotensin II (8, 10). In the pathologic states, which are accompanied by more intensive stimulation of renin-angiotensin system, act favourably in cardiovascular system, ebolishing unfavourable effects of stimulation AT1 receptors for angiotensin II (2, 8).

The aim of this study is the ultra-structural evaluation of rat cardiomyocytes after application of various doses of angiotensin II receptor antagonist (losartan).

MATERIAL AND METHODS

The studies were conducted on 50 (200 g) white Wistar rats from a laboratory animal breeding farm. The animals were divided into four experimental groups and one control group. The experimen--tal animals received losartan manufactured by Adamed Ltd, containing potassium losartane as an active substance. The drug was administered for the period of four weeks, in two doses: daily human therapeutic dose (50 mg) and in a ten times larger dose. Losartan was applied in the form of the water suspension, with the use of the stomach tube, according to the rat's body mass: experimental group I – 0.14 mg/day for the period of 4 weeks; experimental group II – the drug was administered in the same dose as in group I, then a 2-month currency period was applied, during which the rats were given, like in the control group, standard fodder and water to drink; experimental group III – losartan was administerd in the dose of 0.14 mg/day for the period of 4 weeks; experimental group IV – the drug was applied like in group III, then there was a 2-month currency period.

After the experiment was finished, the rats were decapitated and their myocardia of the left ventricle of the heart were collected for examination in an electron microscope. The ultrathin sections were watched and photographed in the transmission electron microscope Tesla BS 500.

RESULTS

The ultrastructure of the cardiomyocytes of the rats in the experimental groups was compared to the structure of the cardiomyocytes in control rats, which showed correct ultrstructural images.

In the group of animals receiving therapeutic dose of losartan (experimental group I) changes were observed in the structure of basic cellular organelle in cardiomyocytes. There was noticed a

significant accumulation of spherical-like mitochondria with abundance of crests. Near Z membranes broadened cross-sections through sarcoplasmic reticulum cisterns were visible, often situated in the vicinity of mitochondria. Some cardiomyocytes revealed distinct invagination of nuclear membrane (Fig. 1).

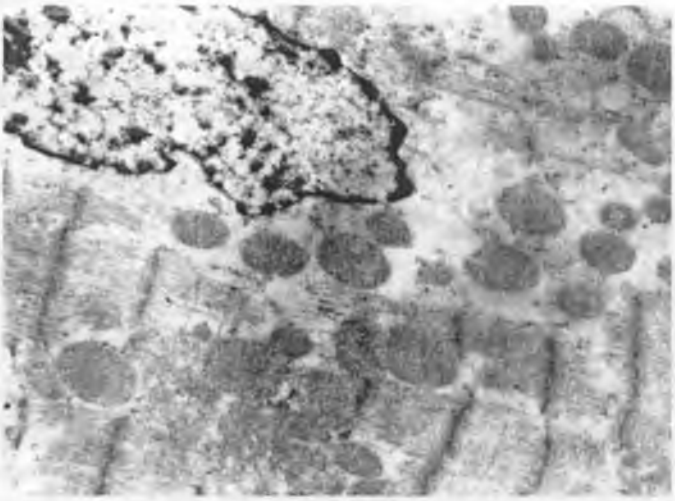

Fig. 1. Experimental group I. Some of cardiomyocytes with invagination of nuclear membrane. Magn. 8000x.

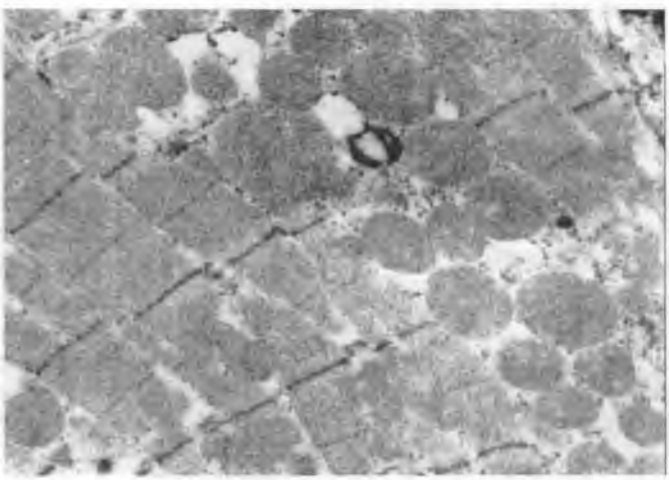

Fig. 2. Experimental group I. Secondary lysosomes and mitochondria with abundance of crests near SER. Magn. 10000x

Glycogen granules were distributed in irregular clusters in the area of the whole sarcoplasm, in a much larger amount compared to the control group. In the region of cellular nucleus and among the accumulation of mitochondria singular secondary lysosomes were visible (Fig. 2). Electronomicrophotograps of cardiomyocytes in experimental group II did not principally diverge from the control group image. In this group there were noticed features of retardation of the lesions

visible in experimental group I (Fig. 3). The ultrastructural image of left ventricle cardiomyocytes in experimental group III in principle did not diverge from the image of experimental group I; the changes visible in experimental group were expressed more intensely (Fig. 4). Experimental group IV. The ultrastructural image of left ventricle cardiomyocytes resembled the image of myocytes in experimental group II.

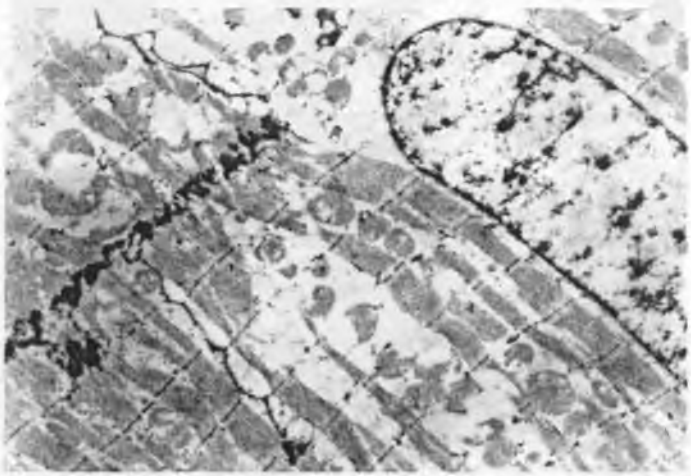

Fig. 3. Experimental group II. Cardiomyocytes and intercalated disc. Near the pole of nucleus – secondary lysosome. Magn. 4000x

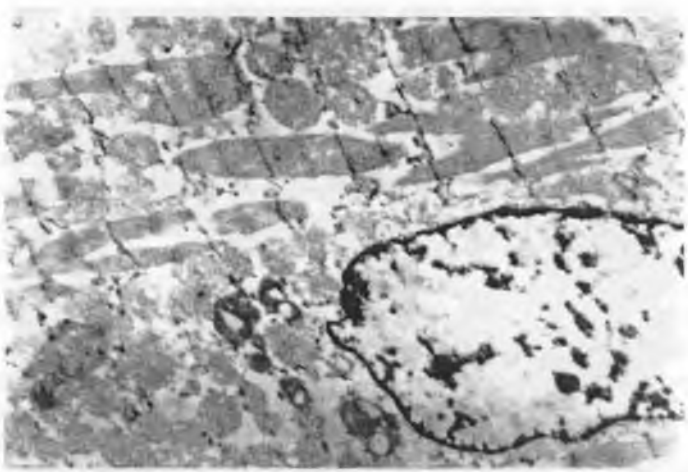

Fig. 4. Experimental group III. Fragment of nucleus, near secondary lysosomes. Near mitochondria – broadened sarcoplasmic reticulum cisterns. Magn. 6000x

DISCUSSION

AT1 receptors for angiotensin II are localized in cell membranes of cardiomyocytes and fibroblasts in the heart (6, 10). Angiotensin II produced in the heart, acting by AT1 receptors, takes part in regulation of function and growth of heart tissues. In experimental condition it was observed that angiotensin II AT1 receptor antagonists influence cardiomyocyte metabolism (10).

In our investigation after the application of losartan in a therapeutic dose (experimental group I) changes were observed in the structure of basic cellular organelle in cardiomyocytes: mitochondria, sarcoplasmic reticulum, secondary lysosomes, nucleus, glycogen granules. Danniels (1), Louch (7), Thomas (12) prove that losartan modulates contractile action of cardiomyocytes in condition of ischaemia, suggesting that losartan exerts an influence on calcium homeostasis in cell. Sarcoplasmic reticulum is a store of calcium ions in cells. Mitochondria and sarcoplasmic reticulum create a functional network that takes part in calcium signal transmission and maintaining calcium homeostasis in cell. Cell excitation, that is its answer to some stimuli, brings about the changes in concentration of calcium ions in cytoplasm (13). In our experience a close adjacency of broadened sarcoplasmic reticulum cisterns and mitochondria with an abundance of crests that certify to the concentrated form and low-energetic state of mitochondria, inform about an intensive response of these structures to stimulation of the cell. Shrinking of the nucleus certify to the intensity of metabolic processes that take place in it. Glycogen granules that are present in the cytoplasm are a sensitive marker of the oxygenation/ischaemia level of myocardium. Cardiomyocytes that include a larger amount of glycogen are more resistant to ischaemia (11), and in states like infarct of myocardium, the amount of glycogen decreases (9). Cardiomyocytes may absorb angioteninogen, renin, angiotensin I from plasm which explains the way in which angiotensin peptides may originate in myocardium (6). According to Jimenez (5), intracellular accumulation of angiotensin II near the nucleus supposedly takes place with participation of Golgi's apparatus. In case of our experiment secondary lysosomes, forming a functional unit together with Golgi's apparatus may contain accumulated intracellular angiotensin peptides. In experimental group III (a dose ten times larger than the therapeutic one) the observed changes were expressed more intensely. The changes subside after giving up the medicine, with the exception of mitochondria, that keep their own concentrated form of matrix, which proves the adaptative character of the observed changes. The occurring changes belong to features that favourably influence cardiomyocyte metabolism.

CONCLUSIONS

1. Losartan in the doses of 0.14 mg/day and 1.4 mg/day causes slight changes within myocardium that manifest themselves through: enlargement of mitochondria with abundance of crests, broadening of sarcoplasmic reticulum cisterns, presence of secondary lysosomes, presence of more numerous glycogen granules.
2. The applied doses of losartan did not significantly influence the intensity of the observed changes.
3. The observed slight changes are probably of adaptative nature that subside after giving up the medicine.
4. The occurring changes belong to features that favourably influence cardiomyocyte metabolism.

REFERENCES

1. Danniels M. C. G. et al.: Losartan prevents contractile dysfunction in rat myocardium after left ventricular myocardial infarction. Am. J. Physiol. Heart Circ. Physiol., 281, H2150, 2001.
2. Diez J. et al.: Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chaber stiffness in hypertensive patients. Circulation, 105, 2512, 2002.
3. Gaciong A., Leśniewska E.: Antagoniści receptora dla angiotensyny II. Med. po Dypl., 9, 8 (supl.): 1, 2000.

4. Goch A. et al.: Losartan and enalapril in the treatment of essential hypertension – effect on left ventricular mass and function. Arterial Hypertension, 5, 1, 21, 2001.
5. Jimenez E. et al.: Angiotensin II (AII) binding sites in nuclei from rat liver: partial characterization of the mechanism of AII accumulation in nuclei. J. Endocrinol., 143 (3), 449, 1994.
6. de Lannoy L. M. et al.: Localization and production of angiotensin II in the isolated perfused rat heart. Hypertension, 31, 1111, 1998.
7. Louch W. E. et al.: Losartan improves recovery of contraction and inhibits transient inward current in a cellular model of cardiac ischemia and reperfusion. The Journal of Pharmacology and Experimental Therapeutics, 295, 2, 697, 2000.
8. Ramahi T. M.: Antagoniści receptora angiotensyny II w leczeniu chorób układu krążenia i nerek. Medycyna po Dypl., 10, 8, 131, 2001.
9. de Sanchez V. C. et al.: Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long-term and integrative study. Can. J. Physiol. Pharmacol., 75, 1300, 1997.
10. Schmieder R. E.: Mechanisms for the clinical benefits of angiotensin II receptor blockers. American Journal of Hypertension, 18, 720, 2005.
11. Szczeklik E.: Enzymologia kliniczna. PZWL, Warszawa 1974.
12. Thomas G. P. et al.: Losartan exerts antiarrhythmic activity independent of angiotensin II receptor blockade in simulated ventricular ischemia and reperfusion. The Journal of Pharmacology and Experimental Therapeutics, 278, 3, 1090, 1996.
13. Waśniewska M.: Współzależność funkcjonalna mitochondriów oraz siateczki śródplazmatycznej a sygnał wapniowy. Postępy Biochemii, 46 (3), 247, 2000.
14. Zalewska-Kaszubska J., Górska D.: Inhibitory konwertazy angiotensyny oraz antagoniści receptorów AT1 – angiotensynowych w leczeniu nadciśnienia. Farmacja Polska, 58, 4, 165, 2002.

SUMMARY

The influence of losartan, angiotensin II type 1 receptor antagonist, on the ultrastructural image of myocardium of white rats was revealed. The drug was administered in the therapeutic and a ten times higher dose. It was manifested by the enlargement of mitochondria with abundance of crests, broadening of sarcoplasmic reticulum cisterns, presence of secondary lysosomes, presence of more numerous glycogen granules. After a 2-month break in the drug administration, features of retardation of the lesions were noticed after a period of 4 weeks of the drug application, which could indicate that the observed changes are of a transitory character.

Ultrastruktura miokardium po zastosowaniu antagonisty receptora angiotensyny II (losartan)

Wykazano wpływ losartanu – antagonisty receptora AT1 dla angiotensyny II, podawanego w dawce terapeutycznej i dziesięciokrotnie większej przez okres 4 tygodni, na obraz ultrastrukturalny miokardium szczurów białych. Wyrażało się to wzmożoną gęstością elektronową macierzy mitochondrialnej, poszerzeniem zbiorników siateczki sarkoplazmatycznej, zwiększeniem zawartości lizosomów wtórnych i ziarnistości glikogenu w sarkoplazmie kardiomiocytów. Po dwumiesięcznej przerwie w podawaniu leku zauważono cechy stopniowego wycofywania się obserwowanych zmian, co wskazywałoby na to, że obserwowane zmiany mają charakter przejściowy.