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RESEARCH ARTICLE

Blood Biochemical Parameters and Lysosomal Enzyme Activity in Experimental Models of Arterial Hypertension and The Effectiveness of Antihypertensive Therapy

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Abstract

Summary. The aim of the study was to establish an experimental model of hypertension in rabbits and to evaluate biochemical blood parameters, the activity of lysosomal enzymes, the microscopic structure of the heart and aorta, as well as the effectiveness of the administered fixed triple antihypertensive therapy

Research materials and methods. The experiment has included 23 rabbits of Chinchilla breed weighing 2.5-3.0 kg, which the AH model has been created in. Of these, 8 rabbits which have not received antihypertensive therapy (AHT) have made up the control group. After 4 weeks of model creation, AHT has been started in 15 rabbits of the main group within 1 month. Over the course of 8 weeks, maximal (SBP) and minimal arterial therapy (DBP) have been measured in rabbits using a veterinary electronic tanometer. Along with this, the level of creatinine, urea, residual nitrogen in the blood plasma, the activity of the enzymes lactate dehydrogenase (LDH) and the CK fraction of creatinine phosphokinase (CK- KFK) have been determined, ECG studies have been performed. All rabbits have been prescribed Exforge H (80/12.5/5 mg and 160/25/10 mg), choosing an individual dose depending on blood pressure once a day per os. The hearts of the decapitated rabbits were removed from the body and weighed using an electronic balance. After macroscopic examinations, the hearts were dissected into sections and fixed in 10% formalin solution. Tissue samples obtained from the aorta and various regions of the heart were processed using standard histological techniques in the Morphology and Histology Department of ETM. Subsequently, histological slides were prepared, stained with hematoxylin–eosin, and examined under a SKOPE (Netherlands) microscope

Results. During the study, along with an increase in blood pressure, an increase in the level of creatinine, residual nitrogen and urine in the blood plasma has been revealed when creating a model of hypertension, which has been accompanied by an increase during the month. A decrease in the activity of the enzyme LDH and MV-KFK has been also noted. After the therapy hold for 1 month, positive dynamics have been found both in the level of blood pressure and indicators of biochemical analysis. Since creatinine concentration has decreased by 28.5%, residual nitrogen – by 36%, urea – by 40%, LDH activity has increased by 30%, MV-KFK by 40%.

Conclusion. Thus, in the arterial hypertension (AH) model, a dynamic assessment of lysosomal enzyme activity in the blood demonstrates a decrease in the LDH and CK-MB fractions, while cardiac remodeling continues to develop. Under fixed triple antihypertensive therapy, an increase in lysosomal enzyme activity is observed, indicating that the components of this combination exert multidirectional effects on cellular metabolism.Additionally, the fixed antihypertensive combination exerts only a modest influence on the cardiac remodeling processes that have developed secondary to elevated arterial pressure—namely, cardiomyocyte hypertrophy and interstitial fibrosis.

Keywords: experimental models of arterial hypertension, lysosomal enzymes, fixed combination drug Exforge H.

Introduction

Despite the achievements of modern medicine, arterial hypertension (AH) remains an important and current issue. AH is a widespread non-infectious disease and plays a major role as the primary risk factor in the development of stroke, myocardial infarction, and cardiac death. To study the causes of impaired regulation of arterial pressure (AP) in greater depth, various AH models are used in animal experiments (3,8,9). In experiments on monkeys, dogs, rabbits, rats, and other animals, models of dysfunction of the central nervous system, endocrine glands, kidneys, and other systems are created to induce AH. Creating such models is a convenient

method that allows investigation of the mechanisms underlying the onset and stabilization of elevated AP. Results obtained from these models have played an exceptional role in forming modern views on the etiology and pathogenesis of primary and symptomatic AH (6,8,9). These models also serve as a basis for developing effective preventive and therapeutic measures for the disease.

Considering the important role of the kidneys in maintaining hemodynamic balance and in the development of changes in AP, studying nephrogenic models becomes essential to clarify the reasons for persistently elevated AP. In the experiment, the method proposed by H. Goldblatt in the last century (1934, 1939) is used for modeling hypertension (6). The experimental renal hypertension model developed by H. Goldblatt has played a special role not only in understanding symptomatic but also essential hypertension. According to H. Goldblatt, AH observed in experimental animals resulted from the development of renal ischemia due to constriction of the renal artery, leading to decreased renal blood flow and increased renin concentration.

Taking all of this into account, our aim was to create an AH model in rabbits, study biochemical indicators and the activity of lysosomal enzymes in the blood, and evaluate the effectiveness of a fixed triple antihypertensive therapy.

Materials and Methods

Twenty-three Chinchilla rabbits weighing 2.5–3.0 kg were used in the experiment to create an AH model. The experiment lasted 8 weeks. Eight rabbits that did not receive antihypertensive treatment formed the comparison group, while 15 rabbits formed the main group. Four weeks after the model was induced, the effectiveness of antihypertensive treatment over a period of one month was evaluated. To create the AH model, the method proposed by Goldblatt H. and Kahn J. (1938) was used, involving compression of the abdominal aorta at the point where the renal arteries branch off [6]. This method is minimally invasive and enables the induction of a higher and more sustained elevation in arterial pressure. The operation was performed under sterile conditions using calypsol (ketamin) anesthesia at a dose adjusted for body weight. When the anterior abdominal wall was opened, the abdominal aorta was completely ligated at one-third of its length—where the renal arteries originate—and the incision was sutured layer by layer. For the next five days, the wound was treated with betadine, and antibiotic therapy was administered.

For 8 weeks, systolic (SAP) and diastolic arterial pressure (DAP) were measured in the rabbits using the CONTEC08A-VET veterinary electronic tonometer on the forelimb, hind limb, or sometimes the tail. At the same time, biochemical blood analysis was performed (creatinine, urea, and residual nitrogen levels were determined in plasma), and the activity of lactate dehydrogenase (LDH) and creatine phosphokinase-MB fraction (CK-MB) was measured using traditional methods on an MS BioScreen-500 biochemical analyzer with reagents from the German company "HUMAN." Alongside laboratory analyses, electrocardiography (ECG) was performed before the model was created, and on day 10, month 1, and month 2 of the experiment using a 3-channel "HeartScreen 60G" ECG device (Innomed, Hungary).

Four weeks after the modeling procedure, antihypertensive therapy was initiated. All rabbits received the fixed triple-combination drug Exforge H (80/12.5/5–160/25/10 mg), with the dose selected according to AP level and administered once daily per os.

During statistical analysis, arithmetic mean (M), standard error (m), and minimum–maximum ranges were determined for homogeneous groups. In the analysis of qualitative indicators, the absolute number of the groups and their proportional distribution expressed as percentages were determined. To compare quantitative indicators between groups, the non-parametric Mann–Whitney U test was used. Differences were considered statistically significant when $p < 0.05$. Statistical processing was performed on a personal computer using modern software—Microsoft Excel and IBM SPSS Statistics.

Results and Discussion

At the beginning of the study, SAP in all rabbits was 120.2 ± 2.5 mmHg, and DAP was 90.0 ± 1.7 mmHg. In the rabbits subjected to ligation, an increase in arterial pressure was achieved both through the direct activation of the renin–angiotensin system and the enhanced formation of angiotensin II, and also indirectly, by elevating sympathetic nervous system activity and catecholamine levels, thereby increasing peripheral vascular resistance.

In the comparison group, biochemical analysis at the time of model induction showed mean creatinine of 88.4 ± 9.3 μmol/L, residual nitrogen 25.5 ± 1.7 mmol/L, and urea 8.6 ± 0.6 mmol/L. LDH activity was 422.1 ± 44.2 U/L, and CK-MB 563.2 ± 41.2 U/L. On day 15 after modeling, an increase in biochemical parameters and a decrease in lysosomal enzyme activity were observed: creatinine 93.9 ± 8.0 μmol/L, residual nitrogen 31.8 ± 3.7 mmol/L, urea 11.0 ± 1.8 mmol/L. LDH activity decreased to 372.4 ± 18.1 U/L, and CK-MB to 415.6 ± 36.2 U/L. Only CK-MB showed a statistically significant change ($p < 0.05$). SAP rose to 140.3 ± 4.3 mmHg and DAP to 100.7 ± 2.1 mmHg. At month 1 of the study, a significant increase in both SAT and DAT was observed in this group. At the same time, during this period, a statistically significant increase in all 3 biochemical indicators in the blood was noted. Although LDH activity decreased to 330.3±10.9 U/l, and CK-MB to 378.2±7.5 U/l, a statistically significant difference was determined in the level of CK-MB.

At month 1 after model induction, all indicators differed significantly: creatinine 130.9 ± 8.0 μmol/L ($p < 0.01$), residual nitrogen 50.6 ± 1.6 mmol/L ($p < 0.001$), urea 20.3 ± 0.8

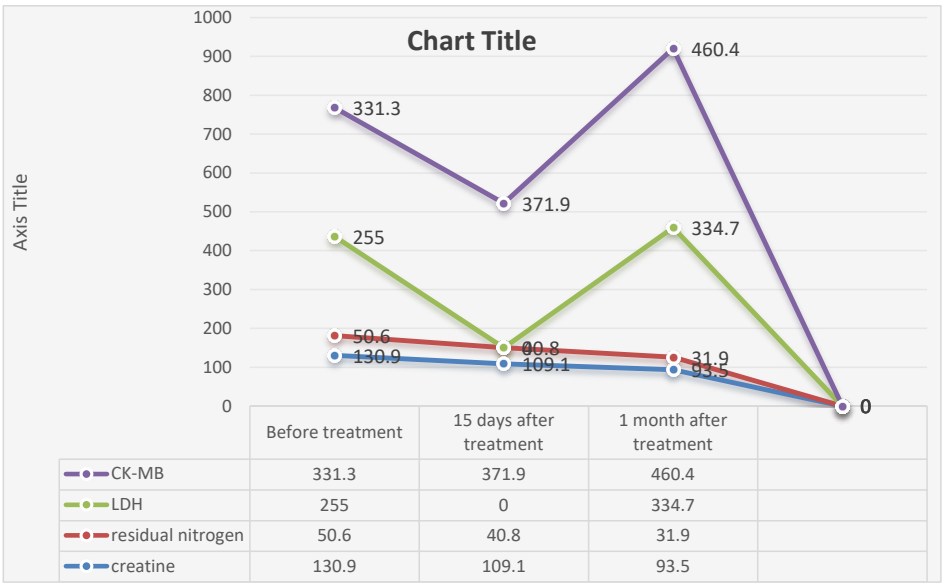

Figure 1: Effect of the fixed triple-combination drug Exforge H on blood creatinine, residual nitrogen, urea concentration, and the activity of LDH and CK-MB fraction in the model

mmol/L ($p < 0.001$). LDH decreased to 255.0 ± 14.1 U/L ($p < 0.05$) and CK-MB to 331.3 ± 17.9 U/L ($p < 0.01$).

As seen, LDH activity significantly decreased in the renovascular AH model. Reduced LDH activity leads to accumulation of pyruvic acid (pyruvate) in the organism due to disruption of lactic acid formation. A significant decrease in CK-MB fraction indicates the development of destructive processes and intensification of myocardial energy metabolism.

Evaluation of Treatment Effectiveness

The effectiveness of the fixed triple-combination therapy was also assessed during the study. During treatment with Exforge H (80/12.5/5 mg), AP levels and blood concentrations of creatinine, residual nitrogen, urea, LDH, and CK-MB fraction were studied over time (Figure 1). On day 15 of treatment, SAP decreased to 100.5 ± 7.8 mmHg and DAP to 70.3 ± 1.2 mmHg. Creatinine was 109.1 ± 2.7 µmol/L, residual nitrogen 40.8 ± 2.2 mmol/L, and urea 15.4 ± 2.7 mmol/L, all statistically significant ($p < 0.05$). Lysosomal enzyme activity increased with treatment: LDH reached 291.6 ± 9.7 U/L, and CK-MB 371.9 ± 3.1 U/L.

Although changes in the studied parameters were observed in the first month of treatment, statistically significant results were again recorded only in the biochemical indicators of blood. During treatment, cases of AP decreasing to 114/50 mmHg were noted. One month after treatment, blood creatinine concentration decreased to 93.5 ± 1.5 µmol/L ($p<0.01$), residual nitrogen to 31.9 ± 0.5 mmol/L ($p<0.01$), and urea to 11.0 ± 0.3 mmol/L ($p<0.01$). After one month of therapy, an increase in the activity of lysosomal enzymes was already observed. During this period, LDH activity reached 334.7 ± 9.7 U/L, and CK-MB fraction reached 460.4 ± 3.1 U/L, which differed statistically significantly from the pre-treatment levels ($p<0.01$).

The significant increase in LDH activity during therapy can be explained by the ability of ACE inhibitors to regulate carbohydrate metabolism. It is known that the CK-MB fraction plays a role in the "transport" of macroergic phosphate compounds: it plays a special role in the transfer of energy from the mitochondria to the cytoplasm of the cell (1,3,9). Although a statistically significant increase in CK-MB fraction was observed as a result of treatment, normal enzyme activity levels were not restored. The increase in the activity of the main enzyme responsible for the energy supply of metabolism indicates that ACE inhibitors, by blocking the renin–angiotensin system and reducing the formation of angiotensin II, promote the restoration of metabolic processes in the organism (5,8).

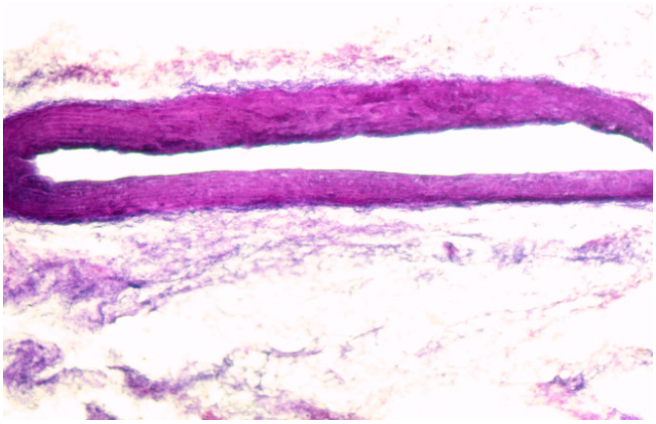

Figure 2: Histological appearance of the proximal aorta after one month of Exforge-H treatment in a rabbit with one-month arterial hypertension. Hematoxylin–eosin, 100×

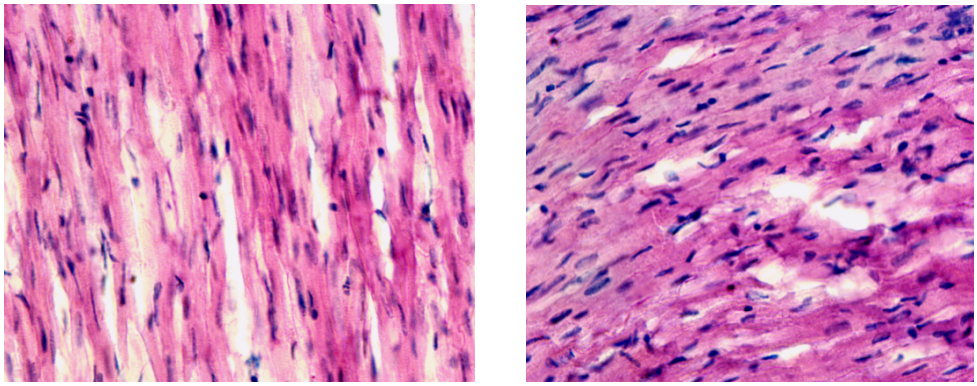

Figure 3: Histological appearance of the left ventricular wall following one month of Exforge-H treatment in a rabbit with one-month arterial hypertension. Hematoxylin–eosin, 100×.

The results of the histological analyses demonstrated that, following one month of Exforge-H treatment in rabbits with an experimentally induced renovascular stage III arterial hypertension model, no significant structural alterations occurred in the proximal segment of the aorta (10,11). The aortic lumen remained unobstructed, and the inner wall was intact. A slight adhesion of blood cells was observed on the endothelial surface. The subendothelial layer also retained a normal histological architecture. In some samples, a mild thickening of elastic fibers in the tunica media was noted (Figure 2).

In preparations obtained from the left ventricular wall of rabbits treated with Exforge-H for one month, the endothelial lining appeared normal. No notable thickening or calcification was detected in the subendothelial layer. The intramural blood vessels were preserved and exhibited normal morphology (Figure 3).

Signs of myocardial remodeling—such as hypertrophy of cardiomyocytes and interstitial fibrosis—were still present in many samples. In most preparations, the characteristic cross-striated pattern of cardiomyocytes was not clearly visible. Interstitial fibrosis remained evident (Figure 3).

Thus, in the arterial hypertension model, a decrease in the activity of lysosomal enzymes (LDH and CK-MB fractions) in the blood, as well as cardiac remodeling, is observed. In contrast, the fixed triple antihypertensive therapy leads to an increase in lysosomal enzyme activity and simultaneously exerts a beneficial effect on the cardiac remodeling processes—namely, cardiomyocyte hypertrophy and interstitial fibrosis.

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