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CERTAIN EXPERIMENTAL MODELS IN BIOMEDICAL RESEARCH OF HYPERTENSION

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Summary. *Experimental research in medicine can be performed in vitro on cell cultures, bacteria or insects, as well as on laboratory animals. Many animal diseases, functions or genes resemble those of people and this provides experimental models. According to bibliographic data, experimental research is conducted on rodents (mice, rats, guinea pigs, rabbits and other rodents) in 85% of all research. The selection of rodents implies determining the type of rodent, infestation and breed. Rats used in medical research originate from the gray Norwegian rat, i.e. Ratus norvegicus. There is a small number of inbred breeds, the most famous of which is the Lewis rat. The most frequent outbreed infestations in use are Wistar and Sprague-Dawely varieties. Hypertension is a multifactorial disease and experimental models of hypertension have provided valuable information on etiology, pathophysiology, complications and treatment. The choice of animal model will be determined by the research aim, financial and technical support. This article reviews certain experimental models of hypertension.*

Key words: *Research, model, hypertension*

Experimental research in medicine can be performed in vitro on cell cultures, bacteria or insects, as well as on laboratory animals. Many animal diseases, functions or genes resemble those of people and this provides experimental models. According to bibliographic data, experimental research is conducted on rodents (mice, rats, guinea pigs, rabbits and other rodents) in 85% of all research. The selection of rodents implies determining the type of rodent, infestation and breed. Rats used in medical research originate from the gray Norwegian rat, i.e. *Ratus norvegicus*. There is a small number of inbred breeds, the most famous of which is the *Lewis rat*. The most frequent outbreed infestations in use are Wistar and Sprague-Dawely varieties.

Type and Role of the Mediator in Hypertension

The term "endothelial dysfunction" was coined in the mid-eighties by Furgot and Zavazaki who noticed that acetylcholine influences existing cells to relax the underlying smooth musculature of the blood vessels. This single-cell layer in contact with different substances (circulating hormones, cytokines, medication, autacoids, physical and chemical stimuli) synthesizes and releases various factors which modulate angiogenesis, inflammatory response, chemostasis, as well as vascular tonus and permeability. Vasoactive substances execute relaxation (adenosine, prostacyclin PGI2, nitrogen monoxide NO, hydrogen peroxide H2O2, epoxyeicosatrienoic acid EET and C nitrogen uretic peptide CNP) and contraction (thromboxane A2, isoprostanes, 20-hydroxieicosatrienic acid, superoxide anion O2, hydrogen peroxide endothelin-1, angiotensin II and uridine adenosine tetraphosphate) (1).

Endothelial cells also have a direct effect on smooth-muscle cells through the myoendothelial gap which not only enables the establishment of the electronic tonus (such as endothelially caused hyperpolarization) with the aid of the endothelium-releasing hyperpolarization factor, but also enables the release of transfer ions or small molecules, such as calcium, and cyclic nucleotides. The great regulatory factor of local homeostasis is mostly balanced by the endothelium between vasodilatation and vasoconstriction. It also influences the proliferation and migration of smooth muscle cells, prevents and stimulates aggregation and adhesion of thrombocytes, as well as thrombogenesis and fibrinolysis. The lack of this fine balance emphasizes endothelial dysfunction, which could be an early marker of atherosclerosis according to certain authors.

Endothelial dysfunction also exists in many different diseases, in patients with kidney insufficiency, it cannot be explained only by reduced renal function, traditional (hypertension, diabetes, hypercholesterolemia) and non-traditional risk factors. Regardless of the consequences, increase in endothelial dysfunction causes vascular resistance, although it is unclear whether endothelial dysfunction causes hypertension or whether hypertension damages the endothelium (2,3,4,5).

In hypertension, there is damage of the main vasodilatation path dependent on cyclooxygenase, through two mechanisms dependent on nitrogen oxide (NO) and dependent on various factors belonging to the heterogeneous group of substances called (EDHF). It was noticed in potassium-sensitive Dahl rats, that there is no gene alteration when there is a reduction in the basal production of NO and expression of endothelium synthesized NO SHR. When caused by the reduction of EDHF, endothelial dysfunction is in relation to the reduction of NO activity. These changes are reversible during the application of antihypertensive medication and endothelia receptor antagonist. Hypertension is mostly the basis for endothelial dysfunction and damage of the blood vessels; it is a promoter of inflammatory activity of endothelial cells and the activator of vascular resistance (6).

Experimental models

The various types of animal models and methods of inducing hypertension being used are represented in Table 1.

Table 1. The various types of animal models and methods of inducing hypertension

1. Renovascular hypertension: Goldblatt method, Hypertension induced by external compression of renal parenchyma, Reduced renal mass, Glomerular sclerosis
2. Dietary hypertension: Increased salt intake
3. Endocrine hypertension: Mineralocorticoid induced hypertension, Adrenal regeneration hypertension
4. Neurogenic hypertension: Denervation of sinoaortic baroreceptors, Electrical or chemical stimulation of different areas of the brain
5. Psychogenic hypertension: Stress-induced hypertension (borderline hypertensive rats)
6. Genetic hypertension: model for essential hypertension as well as complication of hypertension (spontaneous hypertensive rat)
7. Other models: Obesity-related hypertension, Hypertension induced by cholinomimetic agents, Angiotensin II induced hypertension, hypertension induced by cadmium, Transgenic rat models, chronic nitric oxide inhibition-induced hypertension, uterine ischemia

The potassium-sensitive genetic model of hypertension

F1 rats with borderline hypertension are found between spontaneously hypertensive rats and normotensive rats Wistar-Kyoto, which is the potassium-sensitive genetic model of hypertension. Such rats, fed with 8% of potassium content in their food, develop characteristic alterations in sympathetic renal regulation and nerve regulation of the renal function similar to that of spontaneously hypertensive rats, while those fed with the normal salt content in their food are mostly normotensive and do not display sympathetic renal mechanisms. This renal sympathetic mechanism was formed as a quantitative complex which represents an intermediary phenotype. Testing was performed in such a way that rats with borderline hypertension, four to sixteen weeks old, were fed for 12 weeks with food containing 8% salt. The response to intravenous addition of a salt solution of 10% of their bodily weight appeared in 30 minutes in 81 subjects in a chronic experiment which was performed in such a way that instruments measured middle arterial pressure, sympathetic renal activity and potassium uresis. The result of the experiment was that middle arterial pressure did not correlate with the reduction of sympathetic renal activity and the increase of potassium uresis during volume overload (7).

The spontaneous hypertension

Spontaneously hypertensive rats, as animal models of essential hypertension, are used for testing cardiovascular diseases. It was devised by Okamoto et al. in 1960 and is the most frequently used model for hypertension. Hypertension develops from week 5 to week 6 with systolic hypertension which reaches 180-220 mm Hg in older subjects, and around week 40 - 50, there is a development of cardiovascular disease signs, such as vascular and coronary hypertrophy. The assumption that the kidney participates in the development of hypertension has been proven on this model. This was done by transplanting a kidney from a spontaneously hypertensive rat to a normotensive rat which became hypertensive and vice versa, i.e. after the kidney transplant from the normotensive to the spontaneously hypertensive rats, the pressure normalized in the recipient which proved the primary role of the kidney in the development of hypertension in spontaneously hypertensive rats. Although this model was used as a clear pathologic model, it shows interesting compensation capabilities, such as the ability of the recipient to have better renal morphology after the transplantation from the spontaneously hypertensive rat to the hypertensive rat. The animal model for ischemic stroke was developed by breeding the type of rats from spontaneously hypertensive to such that have a tendency to die from a stroke (8).

The genetic hypertension

The Milanese breed of hypertensive rats and their control group, the Milanese normotensive rat breed, illustrate the role of cations in the disorder of membrane transports in this genetic type of essential hypertension (9).

The relationship between the number of glomeruli and blood pressure was analyzed on one of the genetic models of hypertension in the Prague hypertensive rats and their normotensive line. On subjects between 7 and 53 weeks of age, research has shown that the absolute number of

glomeruli does not directly influence the value of blood pressure in both breeds of the rat older than 7 weeks. Gender or rats younger than 7 weeks also do not bear any influence. It is assumed that other possible causes have an influence. Therefore, humeral mechanisms, vascular abnormalities or transport changes, as well as protein receptors, have to be taken into consideration (10).

By administering recombined human relaxin in the chronic test, the glomerular filtration and effective renal plasma flow are increased, and effective renal resistance is decreased with insignificant alterations to the middle arterial pressure. The Munich albino Wistar rat displays progressive chronic nephritis with age and is used for proving functional and histological consequences of the effect of the recombined human relaxin on the remodeled renal matrix of older rats. Double blind tests have shown a significant accumulation of collagen in the tubules and glomeruli on the histological renal medication, and hyperfiltration and renal vasodilatation are noticed during the short administering of gelatinase for 24 hours. After 20 days, renal function is repaired at the expense of activity inhibition of gelatinase which shows the degradation of collagen in these rats, as well as permanent changes of the vasculature matrix (11).

The hemodynamic model

This breed of rat is used in the hemodynamic model for testing the transport of macromolecules through the glomerular capillaries, as well as for the determination of size of effective pores and their number. During the testing of this mechanism, polypeptide H-dextran D was given to seven Munich-Wistar rats prior to and during the intravenous infusion of angiotensin II 0.35 μg/kg/min. During the angiotensin II infusion, the total excretion of proteins through urine is increased on the different transcapillary hydraulic pressure of 34-43 mm Hg. Hemodynamic changes significantly influence proteinuria induced by angiotensin II. Testing was performed on the mechanism of increased filtration of serum proteins through the damaged glomerular membrane with polydisperse H-dextran D or anion sulphates DS, given in the infusion of 14 control and 16 puromycin aminonucleoside PAN-treated Munich-Wistar rats, after which the quantity of glomerular water filtration was measured and it was lower by 40% in PAN-treated rats (12).

The Angiotensin II induced hypertension

One of the hypertensive models used for defining the mechanisms based on angiotensine II with transgenic models harboring the mouse Ren/2 gene in genom Fiser's 344 rats. This new animal model is genetically defined for investigating the mechanisms of the development of glomerulosclerosis and pathogenesis of hypertensive damages. This model maintains the level of extrarenal expression of the genes and consequential induction of hypertension up to the fixed level of increased plasma renin which does not participate in the normal regulatory mechanism of the system rennin-angiotensin. As a contrast to the model with the infusion of angiotensin II, rats whose kidney was exposed to the raised level of rennin and angiotensin II consequently induced extrarenal transgenes. Therefore, this model on transgenic mice displayed the pathophysiological mechanism of hypertension which is the result of the elevated activity of circulating angiotensin II or from extranrenal tissue. The experiment was conceived in such a way that rats were split into 6 groups, i.e. with normal feeding, with food which had a low content of indole-3-carbinol (13C), which in 14 days induces a slow development of hypertension, then group fed with high doses of 13C for the induction of malign hypertension, group of rats which is transgenically negative, i.e. Fischer rats which were fed with 13C of high dosage, then the group chronically treated with 13C and angiotensine blocker - sartan. The final group of transgenic rats was treated by angiontensin receptor blocker and normal feeding (13).

The result of the experiment is that the level of angionetsin II in the plasma was not different in rats with malign hypertension and in those with slowly degrading hypertension. Therefore, it can be concluded that the levels in the plasma and intrarenal concentration are not controlled in the early period after induction with a high dosage of 13C (14).

The blood pressure (BP) in rats is measured directly (intravascular) or indirectly (bloodless) (15).

Conclusion

Experimental models were created with the goal of determining the role of different molecules and mediators in the complex mosaic between inflammation, vascular cells, structural changes on blood vessels and hypertension. Experimental models also showed the infiltration of renal tissue by macrophages, caused by inflammation as a consequence of hypertension. Whether this phenomenon is combined with inflammation, infiltration and oxidative stress in the renal interstitium plays a big role in the future development of hypertension. These findings have to be verified on the human population.

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NEKI EKSPERIMENTALNI MODELI U BIOMEDICINSKIM ISTRAŽIVANJIMA HIPERTENZIJE

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Kratak sadržaj: *Eksperimentalna istraživanja u medicini se mogu izvoditi in vitro na ćelijskim kulturama, bakterijama ili insektima, ali i na laboratorijskim životinjama. Mnoge bolesti, funkcije ili geni životinja podsećaju na ljudske i to obezbeđuje modele za eksperimente. Prema literaturnim podacima eksperimentalna istraživanja su u 85% svih istraživanja sprovedena su na laboratorijskim glodarima (miševi, pacovi, zamorci, kunići i dr glodari). Izbor glodara podrazumeva da je potrebno da se odredi vrsta glodara, zapat i soj. Pacovi koji se koriste u medicinskim istraživanjima vode poreklo od sivog norveškog pacova Ratus norvegicus. Postoji manji broj inbred sojeva od kojih su najpoznatiji Lewis rat. Od outbred zapata se najčešće koriste Wistar i Sprague-Dawely varijeteti.*

Ključne reči: *Istraživanja, modeli, hipertenzija*