

EVALUATION OF NUTRITIONAL AND BIOCHEMICAL PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS FOLLOWING ANTIHYPERTENSIVE TREATMENT*

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ABSTRACT

Introduction. One side effect of antihypertensive drugs is their impact on nutritional status and metabolism. The purpose of this study was to assess the nutritional and biochemical parameters in spontaneously hypertensive rats following treatment with antihypertensive drugs.

Material and methods. The experiment was performed on 50 male spontaneously hypertensive rats (SHR), which were assigned to five groups: control (C), with perindopril (PR), with metoprolol (MT), with indapamide (ID), and with amlodipine (AM). All rats were provided ad libitum standard diet (with or without drugs) and distilled water. After 45 days, the animals were weighed and killed. Liver, kidney, heart, spleen, pancreas, and blood samples were collected. Concentrations of glucose, cholesterol, triglycerides, and albumin were assayed in serum. Morphology parameters, such as white blood cell, red blood cell, hematocrit, and lymphocyte counts were measured in the blood. Blood pressure was measured using a tail-cuff plethysmograph.

Results. The results obtained indicate that the hypotensive drugs under investigation had no effect on the selected nutritional parameters. Perindopril significantly decreased the relative mass of the heart and amlodipine markedly decreased the relative mass of the pancreas. A markedly higher concentration of glucose in the group with indapamid, and a significantly lower concentration of triglycerides in the group with metoprolol, were observed. Indapamide and amlodipine markedly increased the value of red blood cells and hematocrit in the blood of SHR.

Conclusions. Long-term therapy with antihypertension drugs may influence tissue mass and biochemical and morphological status in the body.

Key words: antihypertensive drugs, nutritional status, glucose, lipids, hematocrit, spontaneously hypertensive rats

INTRODUCTION

A large percentage of the human population worldwide suffer from chronic diseases. Patients diagnosed with these diseases must be treated with large

quantities of prescription drugs. There is a direct correlation between the number of medications taken by a patient and the incidence of side effects. Drug

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treatment can have a detrimental effect on nutritional status, especially by impairing the intake of food or the absorption, metabolism, and excretion of nutrients. Drugs are also associated with weight changes. Long-term therapy may influence carbohydrate, lipid, and mineral metabolism [Van Zyl 2011, Anderson and Fox 2012].

Hypertension is a common disease in Western societies, and is usually treated with hypotensive drugs that are prescribed long-term. The antihypertensive drugs are angiotensin-converting enzyme inhibitors (ACEi), diuretics, calcium-channel blockers, beta blockers, alpha blockers, and angiotensin receptor blockers (ARB). Some studies have indicated that hypotensive drugs may affect body weight and disturb glucose and lipid metabolism [Mathai et al. 2008, Sharma et al. 2001, Boschmann et al. 2006].

In our previous studies, we found that the inclusion of ACEi in combined therapy significantly reduces the sensitivity of taste in hypertensive patients [Suliburska et al. 2012 a]. Moreover, we observed in *in vitro* studies that selected hypotensive drugs impair pepsin activity and were suggested to affect the process of digestion [Suliburska et al. 2012 b]. It was also found that antihypertensive drugs influence the potential bioavailability of minerals from food [Suliburska et al. 2011].

Because the influence of hypotensive drugs on nutritional and metabolic status is poorly understood, we have examined the effects of perindopril, metoprolol, indapamide, and amlodipine on selected nutritional, biochemical, and morphological parameters in spontaneously hypertensive rats.

MATERIAL AND METHODS

Animals

This study was approved by the local bioethics committee for animal studies (approval no. 49/2009). Eight-week-old male spontaneously hypertensive rats were, derived from Wistar Kyoto rats with elevated blood pressure at the Kyoto School of Medicine, were purchased from Charles River Laboratories, Germany. The animals adapted to laboratory conditions during the first five days. The mean weight of the rats was 195 ± 21 g. The animals were housed individually in stainless steel cages coated with metal-free enamel, and kept under cycles of 12 hours light to 12 hours

dark. Room temperature was maintained at $21 \pm 1^\circ\text{C}$ with 55-65% humidity.

Experimental design

The experiment was performed using 50 rats. Animals were randomly assigned to five groups of 10 rats each: the control group (C), a group with perindopril (PR), a group with metoprolol (MT), a group with indapamide (ID), and a group with amlodipine (AM). All rats were fed a standard diet (maintenance diet for rats 1320, Altromin), whose full composition is presented in Table 1. In the diet of the noncontrol groups, perindopril, metoprolol, indapamide, and amlodipine were added at a rate of 0.2, 3.0, 0.03, and 0.2 mg/kg body mass of the rat, respectively. The drug was administered in the diet, and fresh solutions were prepared every day. The drug concentrations were adjusted so that the doses (calculated as milligrams per kilogram per day) were kept constant, regardless of dietary intake and body weight. The intake of diet was monitored daily. All rats were provided *ad libitum* diet and distilled water for 45 days. Body weight was recorded each week before food distribution.

Blood pressure measurements

Blood pressure was measured with a tail-cuff plethysmograph using a blood pressure measuring system (MODEL MK-1030, Muromachi Kikai). Blood pressure was measured following 15 min warming at 37°C in an animal holder made of dark brown acrylic, allowing blood pressure measurement under relatively stress-free conditions. An average of five readings was recorded for each animal.

Tissue and serum collection

At the end of the experimental period, the animals were weighed and their lengths measured. After 12 h of fasting, the rats were anesthetized with a sodium thiopental injection (40 mg/kg body weight) and killed by cardiac puncture. The liver, kidney, heart, spleen, and pancreas were dissected and weighed. The blood samples were collected in serum-separated tubes to obtain serum, and another with heparin sodium to obtain whole blood. The coagulated blood was left to clot at room temperature for 30 min, and then centrifuged for 15 min at 2,000 r.p.m. at 4°C . The supernatant fluid was then separated.

Table 1. Composition of the diet

Ingredient	Amount	Ingredient	Amount
Total energy, kcal/kg	2 844		
Total protein, % of energy	24	Biotin, µg/kg	60
Total fat, % of energy	11	Nicotinic acid, mg/kg	36
Total carbohydrate, % of energy	65	Pantothenic acid, mg/kg	21
Protein, g/100 g	19	Choline chloride, mg/kg	600
Fat, g/100 g	4	Calcium, g/kg	9
Fiber, g/100 g	6	Phosphor, g/kg	7
Vitamin A, IU	1 500	Magnesium, g/kg	3
Vitamin D3, IU	600	Sodium, g/kg	2
Vitamin B1, mg/kg	18	Potassium, g/kg	1
Vitamin B2, mg/kg	12	Iron, mg/kg	165
Vitamin B6, mg/kg	9	Manganium, mg/kg	75
Vitamin B12, µg/kg	24	Zinc, mg/kg	70
Vitamin C, mg/kg	36	Copper, mg/kg	13
Vitamin K3, mg/kg	3	Iodium, mg/kg	1.5
Vitamin E, mg/kg	75	Selenium, mg/kg	0.6
Folic acid, mg/kg	2	Cobalt, mg/kg	0.3

Biochemical measurements

Total cholesterol and triglyceride levels in serum were measured using commercial kits (Randox Laboratory Ltd., UK). The concentration of glucose in the blood serum was estimated using the glucose oxidase method. Albumin was measured by the immunoassay method using a rat kit. Morphological parameters were assayed in the whole blood in a diagnostic laboratory using routine methods.

Statistical analysis

Detailed statistical analysis was performed using Statistica for Windows 10.0 (StatSoft, Poland). The results were expressed as arithmetic means with standard errors. One-way analysis of variance (ANOVA) and a post hoc Tukey's test were used to compare data between groups. The significance was set at the $P < 0.05$ level.

RESULTS

Average dietary intake, weight gain, feed efficiency, and obesity indices were found to be comparable across all groups (Table 2). It was found that hypotensive treatment led to a decrease in both systolic (SBP) and diastolic blood pressure (DBP), but a significant decrease was observed only in those groups with perindopril (−23.3% for SBP, −27.9% for DBP) and metoprolol (−19.7% for SBP, −30.9% for DBP) compared with the control group (Table 3).

The relative heart mass in the group with perindopril was significantly lower than in the control group (0.32% vs. 0.38%). Treatment with amlodipine led to a marked decrease in the relative mass of the pancreas, as compared to the control group (0.24% vs. 0.30%; Table 2).

Table 2. Blood pressure and puls

Groups	Parameters		
	Puls	SBP mm Hg	DBP mm Hg
C (<i>n</i> = 10)	390.4 ±33.8	197.2 ±27.7	123.9 ±25.7
PR (<i>n</i> = 10)	367.5 ±19.8	151.3 ±16.4*	49.3 ±15.7*
MT (<i>n</i> = 10)	378.8 ±21.5	158.3 ±31.3*	85.8 ±19.1*
ID (<i>n</i> = 10)	369.6 ±37.1	180.2 ±20.7	101.1 ±29.4
AM (<i>n</i> = 10)	388.8 ±36.6	170.2 ±30.2	103.6 ±26.9

C – control group, PR – group with perindopril, MT – group with metoprolol, ID – group with indapamid, AM – group with amlodipine, *n* – number of rats in the group.

*Significantly different vs C group.

Table 3. Nutritional parameters

Groups	Parameters								
	dietary intake g	weight gain g	feed efficiency	obesity index	liver %	spleen %	heart %	pancreas %	kidney %
C (<i>n</i> = 10)	23.1 ±1.1	92.9 ±12.2	10.9 ±0.4	291.6 ±11.2	2.75 ±0.11	0.19 ±0.02	0.38 ±0.02	0.30 ±0.04	0.70 ±0.03
PR (<i>n</i> = 10)	24.1 ±1.0	92.5 ±11.8	10.5 ±0.5	291.8 ±12.0	2.71 ±0.09	0.18 ±0.02	0.32 ±0.02*	0.31 ±0.03	0.71 ±0.03
MT (<i>n</i> = 10)	23.3 ±0.9	95.0 ±10.8	11.0 ±0.8	287.5 ±11.7	2.77 ±0.08	0.17 ±0.03	0.37 ±0.01	0.28 ±0.04	0.72 ±0.02
ID (<i>n</i> = 10)	24.0 ±0.8	93.6 ±11.3	10.9 ±0.5	291.9 ±12.1	2.76 ±0.07	0.19 ±0.01	0.36 ±0.01	0.26 ±0.04	0.72 ±0.02
AM (<i>n</i> = 10)	23.9 ±1.1	92.2 ±11.7	10.5 ±0.6	287.1 ±11.6	2.72 ±0.07	0.18 ±0.02	0.36 ±0.01	0.24 ±0.03*	0.72 ±0.03

C – control group, PR – group with perindopril, MT – group with metoprolol, ID – group with indapamide, AM – group with amlodipine, feed efficiency was calculated as feed intake divided by weight gain, obesity index was calculated by dividing the cubic root of the body weight (grams) by the nasoanal length (millimeters) × 10⁴, *n* – number of rats in the group.

*Significantly different vs C group.

Markedly higher concentrations of glucose in the group with indapamid (+11.2%), as well as markedly lower concentrations of triglycerides in the group with metoprolol (–18.6%), were observed. The amount of red blood cells and the hematocrit value were significantly higher in the groups with indapamid (+11.3%

and +12.1%) and amlodipine (+11.6% and +10.6%) than in the control group (Table 2). The concentrations of cholesterol, albumin, and hemoglobin, and amount of white blood cells and lymphocytes, were comparable across groups.

Table 4. Biochemical and morphological parameters

Groups	Biochemical parameters				Morphological parameters				
	glucose mg/dl	choles- terol mg/dl	triglycer- ides mg/dl	albumin g/dl	WBC 10 ³ /μl	RBC 10 ⁶ /μl	HGB g/dl	HCT %	Lym 10 ³ /μl
C (<i>n</i> = 10)	109.2 ±10.8	44.0 ±7.8	31.7 ±4.8	4.0 ±0.3	2.3 ±0.7	8.6 ±0.9	15.0 ±0.9	40.6 ±3.5	90.6 ±3.4
PR (<i>n</i> = 10)	116.4 ±16.2	45.6 ±7.5	34.8 ±2.5	3.9 ±0.2	2.0 ±0.6	9.2 ±1.1	15.6 ±0.5	42.5 ±4.9	88.3 ±4.4
MT (<i>n</i> = 10)	104.8 ±17.7	39.6 ±5.1	25.8 ±4.3*	4.1 ±0.3	2.8 ±0.4	9.2 ±0.4	15.5 ±0.6	43.1 ±2.3	89.2 ±5.2
ID (<i>n</i> = 10)	121.4 ±10.5*	41.1 ±5.4	31.7 ±6.3	3.9 ±0.3	2.9 ±0.6	9.7 ±0.4*	16.0 ±0.3	45.5 ±1.6*	90.5 ±7.8
AM (<i>n</i> = 10)	110.0 ±10.3	40.8 ±5.1	30.4 ±6.0	3.9 ±0.2	3.0 ±0.7	9.6 ±0.4*	14.6 ±4.8	44.9 ±1.8*	91.8 ±4.0

C – control group, PR – group with perindopril, MT – group with metoprolol, ID – group with indapamide, AM – group with amlodipine, WBC – white blood cells, RBC – red blood cells, HGB – hemoglobin, HCT – hematocrit, Lym – lymphocytes, *n* – number of rats in the group.

*Significantly differences vs C group.

DISCUSSION

In this study, it was found that antihypertensive treatment can influence the relative mass of the heart and pancreas and the biochemical and morphological parameters in spontaneously hypertensive rats. Moreover, the results show that the investigated drugs had no effect on food intake or body weight in rats. Clinical studies have also indicated a lack of change in the intake of food and in weight gain in patients with selected hypotensive therapy [Zanella et al. 2008]. However, there is some evidence that antihypertensive treatment with β -blockers is associated with an increase in body weight, probably via an influence on energy metabolism in the body [Sharma et al. 2001]. It has also been found that oral treatment of Sprague-Dawley rats with perindopril resulted in reduced body fat mass but did not influence daily food intake or lean mass [Mathai et al. 2008]. Velkoska et al. [2010] found in turn that low doses of perindopril reduced food intake in Sprague-Dawley rats. The positive effect of perindopril was also observed in clinical studies, with Nedogoda et al. [2011] showing that 10 mg of perindopril diminished the fat mass in hypertensive patients

with obesity during six weeks of treatment. In this study, comparable levels of albumin in serum were observed across the groups. This may be the result of other, similar nutritional parameters, such as food intake and weight gain, in all groups after treatment. Hostmark et al. [2005] found a positive association between serum albumin and blood pressure in a large population of men and women. In this study, the level of albumin did not correspond with a change of blood pressure in rats.

Left-ventricular hypertrophy (LVH) is a frequent manifestation of organ damage in hypertension, which is associated with increased cardiovascular risk. Antihypertensive treatment generally results in a regression of cardiac hypertrophy, and reduces the risk of cardiovascular complications [Cuspidi et al. 2012]. The results of several experimental and clinical studies indicate that angiotensin-converting-enzyme inhibitor effectively reduces left ventricular hypertrophy [Black et al. 1996, 2001, Kobayashi et al. 1990]. In comparison with other antihypertensive drugs, perindopril significantly prevents the development of left ventricular hypertrophy in hypertensive patients and laboratory rats [Black et al. 1996, Kuperstein and Sasson 2000,

Malmqvist et al. 2001]. In this study, we also found that only perindopril significantly decreased the mass of the heart in SHR. The lower relative mass of the heart in the PR group, as compared with the control group, was probably the result of the diminished cardiac hypertrophy due to perindopril treatment.

In the present study, we also found that amlodipine treatment markedly decreases pancreas relative mass. This change in pancreatic mass may lead to exocrine and endocrine pancreatic insufficiency, and can impair the digestive and absorptive processes [Meier and Beglinger 2006]. However, it seems that the low mass of the pancreas was not associated with impaired beta cell function or insulin secretion, because the glucose level in serum was not affected in the AM group.

Disturbances in glucose status were observed in the group with indapamide. Some studies showed that use of thiazide-type diuretics was associated with glucose intolerance and increases glucose levels [Mathai et al. 2008, Sica et al. 2011]. Zhang et al. [2010] observed that indapamid, when combined with angiotensin-converting enzyme inhibitors, impairs glucose tolerance in hypertensive patients. Piecha et al. [2007] found that treatment with indapamide was followed by a significant decrease of plasma adiponectin concentration, and an increase in insulin resistance in patients with hypertension. They suggested that the change in adiponectin level may contribute to the pathogenesis of carbohydrate metabolism disturbances often found in patients treated with thiazide-type diuretics.

Several meta-analyses have indicated that treatment with thiazide and beta-blockers significantly increases the risk of development of diabetes [Standl et al. 2012]. In our study, this thesis proved to be the case only for thiazides (indapamide), and not for beta-blockers (metoprolol).

One frequent side effect of antihypertension drugs, especially the diuretics and beta-blockers, is lipid metabolism disorder. Some experimental evidence has demonstrated that beta-blockers adversely affect plasma triglyceride levels in serum [Maitland-van der Zee et al. 2001]. Numerous clinical studies have established that nonvasodilating beta-blockers (e.g., metoprolol) are associated with more negative effects on lipid profiles than vasodilating beta-blockers [Fonseca 2010]. Malmqvist et al. [2001] and Jacob et al. [2004] observed an increase in the serum triglyceride

concentration in hypertensive patients both with and without diabetes. In this experimental study, we observed an inverse relationship, as metoprolol significantly reduced the level of triglycerides in rats.

In this study, it was also found that treatment with indapamide and amlodipine was associated with increased red blood cell count and hematocrit values in serum SHR. High hematocrit in hypertension in the group with thiazide-type diuretics could reflect an increase in red blood cell mass caused by a reduction in plasma volume. Some studies have indicated that hematocrit is positively correlated with systolic and diastolic blood pressure [Cirillo et al. 1992], and have suggested that high hematocrit is a risk factor for the development of hypertension [Cirillo et al. 1992, Nakanishi et al. 2001]. In our study, we observed a connection between higher blood pressure and higher RBC and hematocrit values. Only slightly decreased blood pressure was found in the rat groups with significantly increased RBC and hematocrit in the blood (ID and AM groups) after treatment.

In conclusion, the results obtained in this study indicate that antihypertension treatment influences both tissue mass and the biochemical and morphological status of the body. The increase in red blood cells and hematocrit values following treatment with indapamide and amlodipine is connected with an ineffective decrease in blood pressure in SHR.

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OCENA PARAMETRÓW ŻYWIENIOWYCH I BIOCHEMICZNYCH U SZCZURÓW Z NADCIŚNIENIEM TĘTNICZYM (SHR) PO ZASTOSOWANIU LEKÓW HIPOTENSYJNYCH

STRESZCZENIE

Wstęp. Jednym z czynników ubocznych stosowania leków hipotensyjnych jest ich wpływ na stan odżywienia i metabolizm. Celem pracy jest ocena parametrów żywieniowych i biochemicznych u szczurów z nadciśnieniem tętniczym (SHR) po zastosowaniu leków hipotensyjnych.

Materiał i metody. Doświadczenie przeprowadzono na 50 samcach szczurów z nadciśnieniem tętniczym (SHR), które podzielono na pięć grup: kontrolną (C), z perindoprilem (PR), z metoprololem (MT), z indapamidem (ID) oraz z amlodypiną (AM). Wszystkie szczury miały stały dostęp do diety standardowej (z lekiem lub bez leku) oraz do wody destylowanej. Po 45 dniach zwierzęta zważono i uśpiono. Pobrano próbki krwi, wątrobę, nerki, serce, śledzionę i trzustkę. W surowicy oznaczono stężenie glukozy, cholesterolu, triglicerydów i albumin. W pełnej krwi oznaczono takie parametry, jak: białe krwinki, erytrocyty, hematokryt i limfocyty. Ciśnienie krwi zmierzono na ogonie za pomocą pletysmografu.

Wyniki. Otrzymane wyniki wskazują na brak wpływu analizowanych leków na parametry żywieniowe. Perindopril istotnie obniżył masę serca, a amlodypina spowodowała wyraźne zmniejszenie masy trzustki. Zaobserwowano istotnie większe stężenie glukozy we krwi u grupy z indapamidem oraz istotnie mniejsze stężenie triglicerydów w surowicy krwi szczurów z metoprololem. Indapamid i amlodypina wpłynęły na wyraźny wzrost ilości erytrocytów oraz wartości hematokrytu w krwi szczurów SHR.

Wnioski. Długoterminowa terapia lekami hipotensyjnymi może mieć wpływ na masy narządów wewnętrznych oraz parametry biochemiczne i morfologiczne w organizmie pacjentów.

Słowa kluczowe: leki hipotensyjne, stan odżywienia, glukoza, lipidy, hematokryt, szczury SHR

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