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The influence of selected antihypertensive drugs on zinc, copper, and iron status in spontaneously hypertensive rats

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Perindopril (PubChem CID: 107807)
Amlodipine (PubChem CID: 2162)
Indapamide (PubChem CID: 3702)
Metoprolol (PubChem CID: 4171)
Zinc gluconate (PubChem CID: 158040)
Copper gluconate (PubChem CID: 10692)

ABSTRACT

Mineral homeostasis in hypertensive patients may be affected by hypotensive drugs. The aim of this study was to assess the influence of selected antihypertensive drugs on mineral homeostasis in a rat model of hypertension. Eight-week-old male spontaneously hypertensive rats (SHRs) were treated with perindopril, metoprolol, indapamide, amlodipine, or no drug for 45 days. In another experiment, the SHRs were treated with indapamide or amlodipine in the presence of zinc and copper gluconate supplement. Lipids, glucose, and insulin levels along with superoxide dismutase and catalase activities were assayed in serum. Iron, zinc, and copper concentrations in serum, erythrocytes, and tissues were determined using the flame atomic absorption spectrometry. Blood pressure was measured using a tail-cuff plethysmograph. Treatment with indapamide and amlodipine was found to significantly lower zinc levels in serum, erythrocytes, livers, and spleens of the SHRs, as well as copper levels in the kidneys, compared with the control no-drug group. A markedly higher concentration of glucose was found in the indapamide-treated rats. Supplementing the indapamide-treated SHRs with zinc and copper gluconate resulted in a significant decrease in both systolic and diastolic blood pressure, and also lowered serum glucose and triglyceride concentrations and HOMA (homeostasis model assessment-insulin resistance) values. The results show that indapamide and amlodipine disturb zinc and copper homeostasis in SHRs. Supplementation with zinc and copper restores mineral homeostasis in SHRs treated with indapamide and amlodipine, and also corrects metabolic imbalances while improving the antihypertensive efficiency of indapamide.

1. Introduction

Hypertension is a common disease in Western societies, and is usually treated with hypotensive drugs that are prescribed long-term. Antihypertensive drugs include angiotensin-converting enzyme inhibitors (ACEi), diuretics, calcium-channel blockers, beta blockers, and angiotensin receptor blockers (ARB); alpha blockers are also frequently used. These drugs are taken alone (monotherapy) or in combination (polytherapy). The long-term use of hypotensive drugs may cause side effects, such as inverse disturbances in electrolyte homeostasis, which is relatively common with the use of diuretics and ACEi (Braun and Rosenfeldt, 2003).

In clinical and experimental studies, including our own, an association is observed between disordered sodium, potassium, magnesium, and calcium homeostasis and essential hypertension (Joosten et al., 2013; Pikilidou et al., 2007). It has been suggested that long-term antihypertensive therapy affects zinc pharmacokinetics, particularly in patients with conditions associated with disturbances of zinc homeostasis (Braun and Rosenfeldt, 2003). Antihypertensive therapy may significantly affect mineral status, and may require systematic monitoring (Pikilidou et al., 2007; Sulibursk a et al., 2011). Moreover, disorders in mineral status may impact lipid and glucose metabolism, and also mineral-dependent enzyme activity, such as that of superoxide dismutase (SOD) and catalase (CAT), in the body (Sulibursk a et al., 2011; Zuo et al., 2006). Drugs generally are capable of influencing the metabolism of trace elements in many ways from intestinal absorption to bioavailability and elimination. It is not known whether mineral supplementation or dietary modification might be an optimal

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treatment for hypertensive patients. However, in studies of mineral homeostasis in patients, the drugs used are rarely taken into consideration.

Because the interactions between hypotensive drugs and nutrients are poorly understood, we have examined the effect of perindopril, metoprolol, indapamide, and amlodipine on iron, zinc, and copper homeostasis in the SHR model. We also studied the effect of the combined mineral supplements and drugs on mineral homeostasis in SHRs.

2. Materials and methods

2.1. Animals

Eight-week-old male spontaneously hypertensive rats (SHRs) (Charles River Laboratories, Germany), derived from Wistar Kyoto rats with elevated blood pressure at the Kyoto School of Medicine, were used. 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 h light and 12 h dark. The room temperature was maintained at 21 ± 1 °C with 55–65% humidity. The animal procedures were approved by the local bioethics committee (approval no. 49/2009).

2.2. Experimental design

Two experiments were performed. In the first (without supplementation), SHRs were randomly assigned to five groups of 10 animals each: the control, diet without drugs (C1), diet with perindopril (PR1), diet with metoprolol (MT1), diet with indapamide (ID1), and diet with amlodipine (AM1). The rats were fed a standard diet (maintenance diet for rats 1320, Altromin). The full composition of the diet is presented in Table 1. All rats were provided ad libitum diet and distilled water for 45 days. Dietary intake was recorded daily. Body weight was recorded each week prior to food distribution. 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 rat, respectively. The drug was administered in the diet, combined with chow, to which the rats had free access. 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 diet intake and body weight. In this study, relatively low doses of these drugs were used.

Table 1
The composition of the base diet.

Ingredient	Amount	Ingredient	Amount
Total energy (kcal/kg)	2844		
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)	1500	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

Table 2
The amount of supplements in the diet in the experiment with supplementation.

Group	Supplement (g/kg diet)		Total zinc (mg/kg)	Total copper (mg/kg)
	Zinc gluconiate	Copper gluconiate		
C2 ($n=10$)	–	–	70	13
ID2 ($n=10$)	0.627	0.129	160	31
AM2 ($n=10$)	0.627	0.129	160	31

C-control group, ID2-group with indapamide and zinc and copper supplements. AM2-group with amlodipine and zinc and copper supplements.

The second experiment (with supplementation) was designed on the basis of the first experiment. In this experiment drugs were used with potential influence on mineral status. Thirty animals were randomly assigned to three groups of 10 animals each: control diet (C2); diet with indapamide, zinc, and copper gluconate (ID2); and diet with amlodipine, zinc, and copper gluconate (AM2). The supplemented diets were prepared by mixing an appropriate proportion of the diet with the supplements (USP Merck). The proportions of the diet and supplements in each mix are shown in Table 2. The procedure of the second experiment followed that of the first.

The experiments were begun following a 5-day period to allow the rats to adapt to laboratory conditions.

2.3. Blood-pressure measurements

Blood pressure was measured with a tail-cuff plethysmograph using a blood-pressure measuring system (MODEL MK-1030, Muromachi Kikai). The systolic and diastolic blood pressure was measured following 15 min warming at 37 °C in an animal holder made of dark brown acryl, allowing blood-pressure measurement under relatively stress-free conditions. An average of five readings was recorded for each animal. The rats were first habituated to the measurement device and remained unperturbed in the chamber throughout the inflation–deflation cycles.

2.4. Tissue and serum collection

The animals were fasted for 12 h prior to termination of the experiment. At the end of the experimental period, the animals were weighed and killed. The rats were anesthetized with a sodium thiopental injection (40 mg/kg body weight) and killed by cardiac puncture. The tissues (liver, spleen, kidneys, heart, pancreas, gonads) were dissected, weighed, and stored frozen (at −70 °C) for mineral content analysis. Each blood sample obtained by cardiac puncture was divided between a serum separator tube and a tube containing heparin sodium. The samples in the serum separator tubes were left undisturbed at room temperature for 30 min, and then centrifuged for 15 min at $1048 \times g$ at 4 °C; the supernatant was removed and stored at −70 °C. To isolate erythrocytes, whole blood in heparin sodium-containing tubes was centrifuged for 15 min at $1048 \times g$ at 4 °C, and the plasma was removed. Blood cells were washed thrice with 5 ml of 0.9% saline solution and centrifuged at $2493 \times g$ for 10 min at 4 °C. Following each centrifuging, saline solution was separated and the erythrocyte mass was placed in demineralized Eppendorf tubes and stored at −70 °C for minerals analysis.

2.5. Biochemical measurements

Total cholesterol (TC), triglyceride (TG), and fasting glucose levels in serum were measured using commercial kits (Randox Laboratory Ltd, UK) at a diagnostic laboratory in Poznań, Poland. The concentration of cholesterol and triglycerides in serum was