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EFFECTS OF ZINC ON THE CONTRACTILITY OF THORACIC AORTA SMOOTH MUSCLES OF RABBITS IN CONDITIONS OF FORCED PHYSICAL RESTRAINT (HYPO-DYNAMIC STRESS)

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ABSTRACT: The effect of zinc was investigated on the contractility of smooth muscles of thoracic aorta of chinchilla male rabbits in a situation of prolonged hypo-dynamic stress (i.e. strict movement restriction) similar to that of pronounced sedentary life, which in humans is a major risk factor for cardiovascular disease. The sedentary stress was induced in the rabbits by keeping them for 48 days in small metal cages where movements were severely restricted. Half of the rabbits received daily oral zinc supplements as zinc acetate at the dose of 0.3 mg/kg body wt. The other half received no supplements. Additional rabbits were kept as no-intervention controls. The contraction force of smooth muscles as mediated by adrenaline (10^{-5} mol/l) was determined using a mechanotron and expressed in percentages. The levels of zinc, copper and manganese in blood plasma were determined by atomic absorption spectrophotometry. The data show that hypo-dynamic stress increases the tone of smooth muscles in aorta walls, while Zn and Mn levels in plasma are decreased and Cu levels are increased. However, dietary Zn supplementation to the rabbits under hypo-dynamic stress reduced smooth muscle tone, thus exerting a beneficial effect by releasing tension in aorta walls. Whether these finding could be extrapolated to human vascular function is still hypothetical.

Introduction

It is well known that the onset, course and complications of cardiovascular disease are all related to disorders in coronary blood circulation. This in turn depends on the contractility of smooth muscles, which is controlled by vasoactive agents such as nitric oxide (NO), prostacyclin, thromboxane, endothelin, etc. These are synthesized and released by the vascular endothelium (Palmer et al., 1987; Waldron et al., 1996). Vegetative nervous system disorders, which also manifest themselves in stressogenic situations (Gustein et al., 1984; Cohen, 1986), may affect blood vessel contractility. Moreover, stressogenic situations are associated with oxidative stress. Zinc, an anti-atherogenic element and a cofactor of numerous enzymes, protects cells from oxidative stress (Aiuto, Powell, 1995; Hennig et al., 1996). It is a structural element in transcription factors (e.g., p21, A20) involved in the mechanism of apoptosis (Geng, 1997; Favier, 1998). This element, being a part of the structure of "fingering" protein, plays a role in NF-kB (nuclear transcription factor) binding to DNA (Hennig et al., 1996; Favier, 1998). Zinc also inhibits Ca^{2+}/Mg^{2+}- endonucleases and cysteine protease (caspase-3), thus in turn damaging the poly(ADP-ribose) polymerase (a DNA-repairing enzyme) (Favier, 1998). Zinc may thus be significant for maintenance of integrity of cellular ultrastructure of endothelial cells and at the same time can have a positive effect on ATP synthesis and on vasodilation. Because zinc has a synergistic effect on manganese metabolism and a non-synergistic effect on copper metabolism, zinc supplements may have an effect on the Mn and Cu concentration in tissues.

Changed levels of manganese and copper may be influenced by smooth muscle contractility because, according to literature data (Donnelly, 1978; Kasten et al., 1994; Yan et al., 1998), these metals affect blood-vessel tone. This may be one of the indirect effects of zinc on the contractility of smooth muscles.

In addition, functional analysis using segments from aorta or coronary artery revealed that similarly to the human situation these conductance vessels develop a NO dependent endothelial dysfunction (Gödecke et al., 2002). Risk factors of ischemic heart disease such as arterial hypertension, hypercholesterolemia, smoking and other factors are related to endothelium-dependent relaxation as much in coronary arteries as in peripheral arteries (Stroes et al., 1995; Plotnick et al., 1997; Neunteufl et al., 1998).

Since sedentary life is a risk factor in cardiovascular disease, we wanted to study the effects of zinc on the contractility of thoracic aorta smooth muscles in conditions of forced sedentarism (hypo-dynamic stress). This type of investigation may help to better understand the mechanisms of vasoconstriction and the possibility to correct adverse vascular effects through zinc administration.

Materials and methods

Induction of hypo-dynamic stress

The animals (n=27) were bred, studied and sacrificed according to the well-defined code adopted by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (license no.0006).

Hypo-dynamic stress of 48-day duration was produced according to B.M. Fiodorov (1991) in chinchilla rabbits (weight 2.5–3.0 kg; n=18) by placing them in small metal cages, which closely shrouded their bodies. The rabbits, however, could freely eat and drink. Every day during these 48 days, nine of the rabbits received oral zinc doses of 0.3 mg/kg body wt as zinc acetate. Other nine animals were also placed in metal cages for 48 days but did not receive zinc supplementation. The control rabbits (n=9), which had not been submitted to intervention and received no zinc supplementation, were kept in normal vivarium conditions.

Procedures in zinc supplementation

All the rabbits used in the study received the same food, which was not purified with respect to trace elements. To produce the zinc supplement a solution of zinc acetate was used, containing 1mg of zinc per ml of solution (i.e. 15.3mmol/l of zinc). The solution was administered in relation to the body weights: a dose of zinc 0.3mg/kg was added with an automatic pipette to one of the food components, namely, barley meal pellets (8g in weight). The pellets thus prepared were administered to one half (n=9) of the rabbits under hypo-dynamic stress every day starting from the second experimental day.

Vascular studies

After the 48-day hypo-dynamic regimen the experimental rabbits were anesthetized using thiopental-sodium (35mg/kg) and sacrificed. On opening the chest, samples of thoracic aorta were taken to test smooth muscle contractility. Isolated preparations of thoracic aorta in experimental and control rabbits were excised to test smooth muscle contractility. Isolated samples were tested for adrenaline effect by perfusing them with an oxygenated Ringer solution containing (in mmol/l): NaCl (139); KCl (3.5); $CaCl_2$ (2.5); $NaHPO_4$ (2.4); $MgCl_2$ (1.7); $NaH2PO_4$ (0.67); $NaHCO_3$ (1.5); and glucose (5) at pH 7.2–7.4 at 37.5°C and by dividing them into rings of 2.0–2.5 mm wide. In order to determine the endothelium-dependent tone of smooth muscles of thoracic aorta, their contraction force was measured both in the presence of endothelium and without it in all groups of rabbits: in the controls, in the rabbits kept under hypo-dynamic stress, and in the rabbits that had received zinc during hypo-dynamic stress. Potassium solution (80 mmol/l KCl) was used as a functional test of contractility of thoracic aorta preparations. The contraction force in an isometric regimen was determined using a mechanotron 6MXIC, a device which transforms the mechanical contraction force of smooth muscles into an electric signal. The initial tension was 10 mN for all vessels. An adrenaline solution (10^{-5}mol/l) was prepared at the time of use. The effect of adrenaline was recorded every 3 min (3, 6, 12, 15 min). The contraction force of the smooth muscle preparation was expressed in percentages. One hundred percent was the maximum contraction force that developed after administration of 80 mmol/l KCl solution to the smooth muscle preparation.

Endothelium denudation

Endothelium was eliminated mechanically with a fine brush and the quality of endothelium elimination was afterwards evaluated histologically. For this purpose after endothelial denudation the preparations were immersed, fixed, dehydrated and embedded in a mixture in the same way as preparations for electron microscopic investigations. The preparations were then cut transversely on a sledge microtome in 1–1.5μm tick sections and stained with methylene blue and basal fuchsine. Sections were observed under light microscopy. Ten replications were done per animal.

Investigations on plasma zinc, copper and manganese concentrations

The concentrations of zinc, copper and manganese were investigated in plasma and in the heart left ventricle. To determine plasma concentrations, blood samples were taken from the ear vein of the rabbits before and after stress exposure. Flame atomic absorption spectrophotometry (Perkin-Elmer 503) was used to determine concentrations of trace elements.

Reagents and laboratory preparation

For the preparation of standard solutions necessary to determine concentration of trace elements the following reagents were used (Aldrich, 1999–2000):

1) Copper atomic absorption standard solution (1 g/l of Cu in 1 % HNO_3), catalogue No 20.707-1;

2) Zinc atomic absorption standard solution (1 g/l of Zn in 1% HCl), catalogue No 20.766-7;

3) Manganese atomic absorption standard solution (1g/l of Mn in 1% HNO_3), catalogue No 20.728-4.

For preparation of the mixture of acids the following acids were used:

1) Perchloric acid, 70%, double distilled, PPB/Teflon grade [7601-90-3], catalogue No 38.008-3;

2) Nitric acid, 70%, double distilled, PPP/Teflon grade [7697-37-2], catalogue No 38.009-1.

An original method (Ryselis, 1996) was used for preparing labware free of metal contamination. According of this method the labware used for all media was immersed for 24 hours in 1:5 diluted extra-pure nitric acid (Aldrich, 2000; cat. No 22.571-1) and rinsed five times in extra-pure deionized water.

Analyses

Flame atomic absorption spectrophotometry (Perkin-Elmer 503) was used to determine concentrations of zinc, copper and manganese in the plasma. Blood from the ear vein of the rabbits before and after stress was collected in heparinized test tubes and centrifuged at 1500 rpm for 30 min. After specimen collection, 3 ml of plasma were placed in individual 50 ml Erlenmeyer flasks.

Each sample was collected in duplicate. A 15 ml mixture of two parts of nitric acid and one part of perchloric acid, as mentioned above, was added to the plasma specimens. The flasks were placed on asbestos-covered plates and heated to 200°C to dryness. The sediments were diluted in distilled deionized water to 3 ml to yield mother solutions. Two ml of the plasma mother solutions were used to determine manganese concentrations. The remaining 1 ml of each solution was diluted in distilled deionized water to 4 ml. Of these solutions, 4 ml were used to determine zinc and copper concentrations. To determine the reliability of the atomic absorption spectrophotometry analyses, standard solutions were prepared as described in the operating instructions of the equipment and the reagent mentioned above were used.

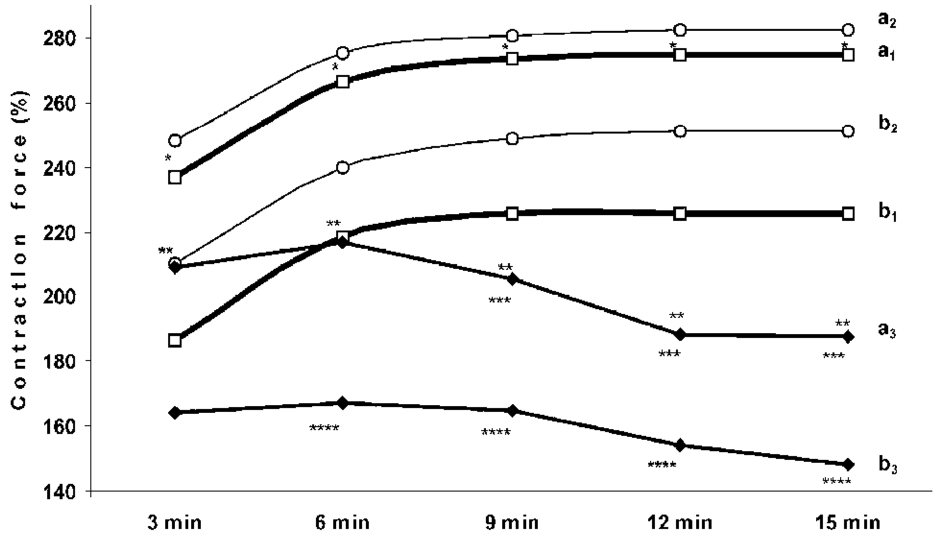

Fig. 1. Contractility changes of smooth muscle in thoracic aorta walls elicited by 10^{-5} mol/l adrenaline in chinchilla rabbits.

a_1 — contraction force of aortic smooth muscle preparations without endothelium in control rabbits;

b_1 — contraction force of aortic smooth muscle preparations with endothelium in control rabbits;

a_2 — contraction force of aortic smooth muscle preparations without endothelium in rabbits exposed to 48-day hypo-dynamic stress;

b_2 — contraction force of aortic smooth muscle preparations with endothelium in rabbits exposed to 48-day hypo-dynamic stress;

a_3 — contraction force of aortic smooth muscle preparations without endothelium in rabbits exposed to 48-day hypo-dynamic stress supplemented with zinc;

b_3 — contraction force of aortic smooth muscle preparations with endothelium in rabbits exposed to 48-day hypo-dynamic stress supplemented with zinc;

** P <0.05-0.01, a_1 compared with b_1;*

*** P <0.05-0.01, a_3 compared with b_3;*

**** P < 0.05-0.01, a_3 compared with a_1 and a_2;*

***** P <0.05-0.01, b_3 compared with b_1 and b_2.*

Analysis was controlled by including an internal and an external quality control. For internal quality control we used control plasma of known concentration of trace elements. External quality control was realized in collaboration with the Institute for Biomedical Research, Kaunas University of Medicine, Lithuania and with the Agrochemical Research Center of Lithuanian Institute of Agriculture. Concentration of zinc, copper, manganese, magnesium, calcium in the same samples of blood plasma, but also of heart, hair, saliva, and water were separately determined and data were compared between the collaborating institutes.

Statistical analysis

Values are presented as mean±SEM. Data derived from repeated measures were analyzed by a two-way ANOVA. Otherwise data were compared with Student t-test. Differences were considered to be significant at $P < 0.05$.

Results

Smooth muscle contractility as affected by adrenalin in rabbits receiving or not Zn supplementation under hypo-dynamic stress:

Results show that in rabbits given zinc supplement and in the control group the reaction force of smooth muscles with endothelium (Fig.1: b_3, b_1, respectively) under the influence of adrenaline was significantly ($p < 0.05$) weaker than that without endothelium (Fig.1: a_3, a_1, respectively). On the other hand, under the influence of adrenaline (10^{-5} mol/l) the contractility of the thoracic aorta smooth muscles with (Fig.1: b_2) and without endothelium (Fig.1: a_2) did not differ significantly ($p > 0.05$) in rabbits given no zinc supplements during the 48-day hypo-dynamic stress period. Thus, this effect of hypo-dynamic stress shows an increase in the tone of blood-vessels.

Besides, in rabbits given zinc supplement during hypo-dynamic stress, the contraction force of smooth muscles with endothelium (Fig.1: b_3) at 6, 9, 12, 15 minutes and without endothelium (Fig.1: a_3) at 9, 12, 15 minutes was significantly ($p < 0.05$) weaker than that in those who received no zinc (Fig.1: b_2, a_2, respectively) and in control rabbits (Fig.1: b_1, a_1, respectively).

Concentration of zinc, copper and manganese in plasma of rabbits treated with and without zinc supplement during hypo-dynamic stress:

After the hypo-dynamic stress, rabbits receiving no zinc supplement showed statistically lower concentrations of plasma zinc and manganese ($p < 0.05$) as compared to those before stress, but copper level was increased.

In situation when rabbits were given zinc during hypo-dynamic stress, the concentration of zinc, copper and manganese in blood plasma before and after inter-

Table 1. Concentration of trace elements in blood plasma (μmol/l) under the influence on hypo-dynamic stress with or without zinc supplementation.

	Zinc M ± m (n = 9)	Copper M ± m (n = 9)	Manganese M ± m (n = 9)
Hypo-dynamics: Blood plasma			
Before intervention	28.0 ± 1.4	9.9 ± 0.36	3.75 ± 0.14
After intervention	22.0 ± 1.7*	16.0 ± 1.6*	3.2 ± 0.29*
Hypo-dynamics + zinc:			
Before intervention	27.1 ± 3.08	10.2 ± 1.3	3.44 ± 0.36
After intervention	26.5 ± 1.85	12.5 ± 1.3	3.26 ± 0.4

Note: n — number of rabbits. Values are mean ± standard error of the mean. *P <0.05–0.01 after vs. before hypo-dynamic stress.

vention did not change statistically. This may indicate normalization of the Cu and Mn concentrations due to the effect of zinc.

Discussion

The results demonstrate that in control rabbits the contractility force of smooth muscles with endothelium is statistically different from the contractility force of smooth muscles without endothelium (Fig.1: a_1, b_1), but in rabbits under the effect of hypo-dynamic stress the difference of contractility force of smooth muscles with endothelium and without it is not significant (Fig.1: a_2, b_2). Thus, an effect of hypo-dynamic stress is the increase in the tone of blood-vessel walls. This is associated with a lowered plasma concentration of zinc and manganese (Table 1). On the other hand, dietary zinc supplementation to rabbits under hypo-dynamic stress reduces the smooth muscle tone in thoracic aorta (Fig.1: a_3, b_3). The favorable effects of zinc on the contractility of smooth muscles may manifest themselves directly or indirectly via many mechanisms. One of the indirect mechanisms may be related to the synthesis and release in the endothelium of nitrous oxide (NO), a vasodilating agent. NO is synthesized by the NO-synthetase, the catalyzing function of which requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Soriano et al., 2001). NADPH synthesis occurs also through Zn-activation of glucose-6-phosphate dehydrogenase (Heinitz, 1978). Zinc is also involved in the first antioxidation protection system against reactive oxygen species as a cofactor of superoxide dismutase. This zinc-enzyme is of major importance in protecting all aerobic cells from the potentially damaging effects of oxygen: $O_2^{\cdot-}$ + biomolecule——>bioradical (damage); $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$ (protection) (Willson, 1989). Zinc may thus prolong the half-life of NO by inhibiting the production of peroxynitrite (produced by NO interaction with $O_2^{\cdot-}$) (Landino et al., 1996). Manganese also may prolong the half-life of NO (Kasten et al., 1994) as manganese-induced relaxation of aortic segments is both endothelium-dependent and concentration-dependent. Cyclic GMP concentrations in the aortic segments were increased 2-and 4-fold with 5 and 300 micromole Mn, respectively. N-monomethyl-l-arginine pretreatment of aortic rings abolished the relaxation and the cyclic Mn-mediated GMP accumulation. Manganese also inhibits postsynaptically the contractile response of norepinephrine in isolated porcine right coronary artery (Yan et al., 1998). Therefore, normal concentration of manganese in plasma of rabbits that received zinc during hypo-dynamic stress may also positively affect the contractility of smooth muscles in thoracic aorta. On the other hand, decreased concentration of zinc and manganese in tissues and plasma of rabbits receiving no zinc supplement in hypo-dynamic stress can suppress the activity of zinc proteins while reactive oxygen species may at the same time impair the biomolecules and their functioning, and stimulate the synthesis of biologically active substances. For example, lipid peroxides and/or peroxynitrite activate prostaglandin H synthetase, thus leading to increased amounts of prostaglandin endoperoxide and thromboxane, which bind to the same receptor and cause vasoconstriction. Prostacyclin is reduced as a result of extensive lipid peroxidation and/or peroxynitrite (which preferentially inhibits prostacyclin synthetase activity), resulting in decreased vasodilation (Davidge, 2001). The increased smooth muscle tone in rabbits that showed decreased plasma concentrations of zinc and manganese and did not receive Zn supplement during the 48-day hypo-dynamic stress, can be associated with these mechanisms. High plasma copper levels observed in rabbits given no Zn supplements may affect the maintenance of increased smooth muscle tone. This may be related to the activation of adrenoreceptors as copper is involved in catecholamine metabolism. For example, dopamine-β-hydroxylase, a copper-containing enzyme, facilitates the conversion of dopamine to epinephrine — a precursor of adrenaline (Eliasson, 1984).

Conclusions

The data show that hypo-dynamic stress increases smooth muscle tone, but dietary zinc supplementation in these conditions reduces the tone.

Zinc appears therefore to favorably influence smooth muscle contractility by reducing it and releasing tension.

Whether administration of zinc can exert beneficial effects also in human blood circulatory function as affected by sedentarism remains a conjecture.

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