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EFFECT OF INCREASED DEPLETION OF COPPER, SUPPLEMENTARY CHOLESTEROL DIET AND STRESS ON THE CHOLESTEROL CONCENTRATION IN WALL OF RAT THORACIC AORTA

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The effects of experimental copper deficiency by itself, and in combination with other factor resulting in ischaemic heart diseases (IHD), were investigated on the lipid composition and copper status of serum and aortic wall in rat. The depletion of absorbed copper was raised by complex formation (D-penicillamine-treatment). This provoked secondary copper deficiency was combined with a dietary cholesterol- and stress-loading. After treatment the levels of triglyceride (Tg), total cholesterol (Chol), HDL-cholesterol (HDL) (HDL = high density lipoprotein) and HDL_2-cholesterol (HDL_2) as well as copper (Cu) and zinc (Zn) in the serum and in aortic wall were measured by chemical analysis. It was pointed out, that increase in triglyceride and total cholesterol levels were promoted by the provoked copper deficiency in the serum and in the wall of the thoracic aorta. In combination with other risk factors it caused an increase in the elevation of Tg and Chol concentrations and has reduced the level of HDL_2, significantly.

Keywords: risk of ischaemic heart disease, cholesterol, secondary copper deficiency, analysis of IHD risk factors

Copper is an essential trace metal with an important role in the cardiovascular system. The tendency of copper status and zinc/copper ratio has proved to be an useful index in the aetiology of IHD [6]. The elasticity of blood-vessels is determinated by copper status, since copper-dependent collagenase and lysil-oxydase catalyse the synthesis of collagen and elastin [5]. Concentrations of zinc and copper in blood and heart tissue of patients with coronary artery disease are subnormal [9]. Copper depletion in animals is known to alter tissue fatty acid and lipid composition [2] as well as energy metabolism. Since fatty acids are the preferred energy substrate

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in the heart, experimental copper deficiency affects heart performance in part by interfering with cardiac lipid metabolism [3]. Experimental copper deficiency is associated with cardiac rhythm disturbances and a greater risk of myocardial infarction in both humans [6], and animals [14].

The problems of prevention of IHD heve been examined in our institute for several years [12, 10]. A weighed model of risk factor analysis has been developed, by which we can evaluate and prognose the risk of IHD in the regularly investigated human populations. In our opinion, beside examining the traditional risk factors, the prognostic value of this model could be enhanced by considering such parameters – like copper and zinc status – that may have an important role in the manifestation of IHD.

The effects of experimental copper deficiency were investigated by itself, and combined with other factors resulting in ischaemic heart diseases, on the copper status and lipid composition of aortic wall in rat.

Materials and methods

Adult male Sprague Dawley rats, weighing 210±25 g were divided into 5 group of 12 animals each. Every group was fed *ad libitum* with a standard diet sufficient in Cu (3 μg/g). Group I was the untreated control. Group II received 1 mg of D-penicillamine sc. (Byanodine, Biogal, Hungary) per day dissolved in 1 ml of normal saline [1] for 4 weeks. The animals of Group III were fed with standard diet supplemented with 2% of synthesized cholesterol for 8 weeks. Group IV was pretreated with D-penicillamine and fed with standard diet for 4 weeks, and an additional diet supplemented with 2% of cholesterol for the next 4 weeks. Group V was pretreated with D-penicillamine and fed with standard diet for 4 week, and an additional diet supplemented with 2% of cholesterol for the next 4 weeks. During the last 4 weeks, these animals were housed in special plastic cages for 4 hours per day (it resulted in stress-situation because of immobilization/hypokinesis). The time course of procedures is outlined in Fig. 1.

After 8 weeks, all animal were sacrificed after an overnight fasting, blood was collected and serum was used for subsequent enzymatic analysis of triglycerides and cholesterols (ELITECH DIAGNOSTICS, USA), and serum samples precipitated with $MnCl_2$ were used to the enzymatic analysis of HDL and the samples precipitated with $MnCl_2$ and dextran sulphate to the HDL_2 determination [13]. Arch of the aorta was dissected and after shaking with chloroform-metanol it was analysed for triglyceride and cholesterols by the method of Rappaport [11]. Zn and Cu content of serum and aortic wall were determined with atomic absorption spectrophotometry [4].

The statistical comparisons were performed with the Student's t-test.

Table I

Triglyceride and cholesterol concentrations in rat serum after 8 weeks of treatment

Group		I	II	III	IV	V
Triglyceride*	$\bar{x}$	1.2	1.6	3.4	4.5	4.8
	±SD	0.4	0.6	0.8	0.9	1.1
Cholesterol*	$\bar{x}$	2.3	2.6	3.8	4.3	7.6
	±SD	1.3	1.4	1.6	2.5	3.6
HDL*	$\bar{x}$	1.1	1.1	1.1	0.9	1.0
	±SD	0.3	0.3	0.3	0.4	0.5
HDL_2	$\bar{x}$	0.5	0.4	0.4	0.3	0.3
	±SD	0.1	0.1	0.1	0.1	0.1

* mmol/l

Table II

Cu content and the Zn/Cu rate in serum and in the wall of thoracic aorta in rat

Group		I	II	III	IV	V
Cu						
Serum	$\bar{x}$	1.57	1.50	1.55	1.53	1.43
(μmol/l)	±SD	0.17	0.19	0.22	0.07	0.18
Aorta wall	$\bar{x}$	0.79	0.67	0.74	0.40	0.48
(μg/g wet weight)	±SD	0.17	0.13	0.12	0.12	0.08
Zn/Cu						
Serum	$\bar{x}$	0.95	1.10	1.02	1.11	1.24
	±SD	0.17	0.19	0.22	0.07	0.15
Aorta wall	$\bar{x}$	0.89	0.91	0.90	1.00	0.94
	±SD	0.11	0.14	0.10	0.16	0.10

The disadvantageous changes occurred in the aortic wall are summarized by Fig. 2. As a function of time, the levels of cholesterol deposited in the aortic wall elevated ($p < 0.1$) proportional by the decrease of aortic Cu content ($p < 0.02$) in the pretreated groups that received additional cholesterol, but the process went on at an accelerated pace in the animals that received a combined cholesterol- and stress-loading.

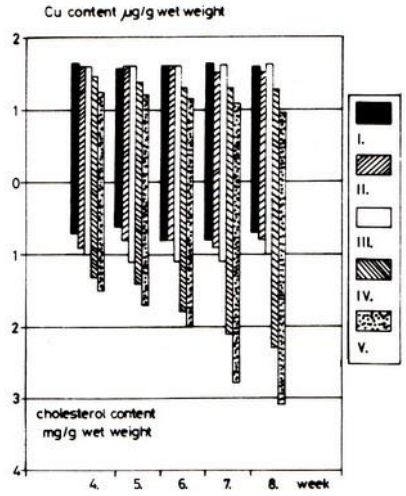

Fig. 2. Cu content and cholesterol deposition in the rat serum and wall of thoracic aorta as a function of time

To sum up, it can be established, that by itself, the provoked secondary Cu deficiency or dietary cholesterol result in hardly measurable changes in the parameters examined. The risk of disadvantageous changes in aorta, however, can be multiplied by simultaneous existence of several risk factors (elevated cholesterol intake, stress) in the presence of Cu deficiency.

Discussion

It is proved by Meissner [8] that in animals the Cu deficient diet increases the synthesis of cholesterol, reduces the decomposition of lipoproteins and disturbes the depletion of cholesterols.

Increased depletion of Cu, namely secondary Cu deficiency resulted in similar changes in our experimental animals – especially accompanied by the effect of other simultaneously existing well-known risk factors – which has been proved by the elevated cholesterol content of thoracic aortic wall and disadvantageous reduction of cardiovascular protecting factor HDL_2 in serum.

Relying upon these findings, we suppose that the prognostic value of our risk factor analysis for ischaemic heart disease could be improved by taking the Cu status into consideration, with special attention to the tendency of change in the calculated rate of Zn/Cu in serum.

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