INFLUENCE OF SELENOMETHIONINE ON THE MORPHOLOGY OF RABBITS’ ORGANS IN EXPERIMENTAL ATHEROSCLEROSIS

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Abstract

The study was performed on male rabbits. The animals were divided into control and 2 experimental groups. Animals from the experimental groups were fed a cholesterol diet consisting of 0.5 g of cholesterol/100 g of fodder/rabbit/d. Additionally, animals from the second experimental group were supplemented with selenomethionine in the amount of 12.5 µg/kg of body mass/24 h. After 3 months of the experiment blood was collected for biochemical analyses and during the autopsy the heart, kidneys, liver and aorta were collected for histopathological examination. The concentration of LDL cholesterol, HDL cholesterol and triglycerides (TG) in plasma was determined. The conducted study showed the preventive influence of selenomethionine on atherogenesis. Selenomethionine reduced the development of atheromatosis changes in aorta walls as well as in heart arteries and steatosis in the liver and kidneys.

Key words: rabbit, atherosclerosis, selenomethionine.

Intensive studies bring new data on atherosclerosis pathogenesis. The latest theory on atherosclerosis formation suggests, that it is a specific inflammatory response to stimulating or damaging factors (25). Numerous endogenous and exogenous factors participate in the formation and development of atherosclerosis. A high level of lipids in the serum plays a major role in histopathological examination. The concentration of LDL cholesterol, HDL cholesterol and triglycerides (TG) in plasma was determined. The conducted study showed the preventive influence of selenomethionine on atherogenesis. Selenomethionine reduced the development of atheromatosis changes in aorta walls as well as in heart arteries and steatosis in the liver and kidneys.

Macrophages and platelets. SMC proliferate and synthesize extracellular matrix in the intima (25).

Studies performed in recent years indicate, that oxidative processes play an important role in atherosclerosis pathogenesis (15, 26). Atherosclerosis oxidative theory states that free oxygen radicals (O₂ and OH) are responsible for the damage of the vessel wall structure, modifications of serum lipoproteins, modification of cell impulse transduction, cellular interaction, genes expression, and immune response. They are formed in the body upon oxidation processes in mitochondria, in reactions catalyzed by oxidases (NADPH, xanthic, aldehyde, lipoxygenase) and in phagocytosis processes. Free radicals concentration is controlled by antioxidative systems on the enzymatic path (superoxidative dismutase, catalase, selenium-independent and selenium-dependent glutathione peroxidases and, glutathione reductase) (1, 3, 8, 28). A significant role is also played by antioxidative non-enzymatic system, which protects against the effects of free oxygen radicals (27). In this group of antioxidants there are vitamins A, E, and C, β-carotene (1, 8, 14, 18, 19, 22, 24, 28) garlic extract (35, 36). Preventive use of antioxidants is linked with the lowering of the risk of cardiovascular disease development (8, 24). A similar action is also displayed by selenium (7, 15, 17, 21, 23). In the nature it occurs in the form of inorganic compounds (selenians and selenins) and organic compounds (selenomethionine and selenocysteine). It participates in metabolic changes through selenocysteine (31). Selenocysteine is a component of selenoproteins, among which the most important are: glutathione peroxidase (GSHPx), iodothyronine deiodinase, selenoprotein P and selenoprotein W. Biologic activity of selenium is seen through these protein compounds. Selenium affects the uptake, degradation and inhibition of free oxygen radicals, thus protecting the body cells against premature ageing, cell damage, and metabolism disturbances which
lead to atherosclerosis and tumors. There are four known types of GSHPx: cellular, plasmatic, intestinal, and glutathione peroxidase of phospholipid superoxides (PHGSHPx). The best known so far, is a cellular peroxidase which catalyses H₂O₂ and lipid peroxide inactivation reaction. Bound with cell membranes, PHGSHPx inhibits membraneous lipid peroxidation and protects the cells against autooxidation. Selenium deficiency leads to a decrease in antioxidative activity of GSHPx in the body. It may also disturb the balance between prostacycline and tromboxane and result in an intense thrombocyte aggregation. In case of atherosclerosis, a major role may also be played by iodothyronine deiodinase which conditions triiodothyronine formation – the hormone lowering the cholesterol level. The latest studies prove that selenium may be of great significance in disease prevention, in the pathogenesis of which oxidative processes play an important part (20).

Material and Methods

The experiment was carried out on 18 male rabbits of the New Zealand breed with the initial body weight of 3 000 g ± 50 g. The animals came from the Central Experimental Animal Quarters of the Medical University of Silesia in Katowice. Before the experiment, the animals underwent a 2 weeks adaptation to the experimental environment. Only animals with normal lipid profiles were accepted for the experiment. The animals were divided into 3 groups, each consisting of 6 animals:

- control group (K) - receiving regular standard fodder;
- experimental group CH - receiving an atheromatosis diet consisting of 0.5 g of cholesterol/100 g of fodder/rabbit/24 h;
- experimental group CH + SM - receiving an atheromatosis diet (as above) supplemented with selenomethionine in the amount of 12.5 µg/kg of body mass/24 h.

The experiment lasted for three months. Animals were given water ad libitum. Every month blood was collected from their auricular marginal vein for biochemical analysis. The concentration of LDL cholesterol in plasma was determined with the enzymatic method using the BioMerieux kit (France) and the concentration of HDL cholesterol and triglycerides (TG) was determined with the Alpha Diagnostics kit (Germany). The results were statistically analysed using the Statistica PL software. The U’Manna Whitney test was used to compare differences between particular groups. Statistical significance was restricted by P≤0.05.

During the autopsy, the aorta, liver, heart, and kidneys were collected for histopathological examination. The organs were fixed in aqueous solution of formaldehyde. The pathomorphological changes were assessed on the basis of paraffin preparations, stained with haematoxylin and eosin (H-E). The slides used for histochemical studies were obtained on a freezing microtome and stained with Sudan III for neutral fats (34). Colour microphotographs were taken with the Docuväl microscope equipped with the photo device (Carl Zeiss Jena).

Results

Biochemical examination. Table 1 shows changes in the lipid concentrations in plasma of rabbits belonging to both groups fed a cholesterol diet. Statistically significant increase in the concentration of TG is shown after the 3-month experiment when compared with the control group (P=0.004). Also, there was a significant increase – when compared to the control group – in the concentration of LDL cholesterol in both the CH and CH+SM groups after every month of the experiment (P=0.004). Statistically significant change in the HDL cholesterol concentration was noticed after the 3 months in group CH+SM.

Macroscopic assessment. The presence of creamy atheromatous plaques was noticed in the area of arch and abdominal part of the aorta in the CH and CH+SM groups. In the CH group, the plaques were large and covered the entire circumference of the vessel, while in the CH+SM group they were of much lesser intensification.

There was a colour change – xanthochromia – noticed in the liver of the CH rabbits. There was no such a change in the liver of the CH+SM rabbits; also, there were no macroscopic changes in the kidneys and heart in rabbits of either group.

Microscopic assessment.

Aorta. The histopathological change observed in both CH and CH+SM groups was a focal hyperplasia of the intima, completely covered with laminas (CH group) or single, non-complete small atheromatous plaques (CH+SM group). There were numerous foam cells in the atheromatous plaques, and fat was also noticed in intercellular spaces. Figs 1A and 1B show aorta microscopic pictures.

Liver. There was a distinct steatosis of hepatocytes in CH rabbits. The steatosis was increased around the central veins of hepatic lobule (Fig. 2A). In the hepatocytes located on the periphery of hepatic lobule, there was either no steatosis or it was minimal. In the CH+SM group, the steatosis of hepatocytes was of lesser intensity (Fig. 2B).

Kidneys. There was little steatosis in the tubule epithelium cells noticed only in kidneys of the CH group (Fig. 3A). There were also atheromatous changes in the arterial vessels of the kidneys of the CH group, evidenced by hyperplasia of artery intima in the form of atheromatous plaques (Fig. 3B). They were observed in lobar arteries of 4 rabbits. In the CH+SM group, no steatosis in the tubule epithelium cells was noticed (Fig. 3C). Slight atheromatous changes in the lobar arteries were noticed in 2 rabbits of the CH+SM group (Fig. 3D).
Fig. 1 A. Group CH. Aorta. Atheromatous plaque. Foam cells in the intima. H-E staining. 130x.

Fig. 1 B. Group CH+SM. Aorta. Atheromatous plaque and foam cells in the intima. H-E staining. 180x.

Fig. 2 A. Group CH. Liver. Steatosis of hepatocytes around the central vein. Lixiviated fat. H-E staining. 280x.

Fig. 2 B. Group CH+SM. Liver. Steatosis of hepatocytes around the central vein. Lixiviated fat. H-E staining. 140x.
Fig. 3 A. Group CH. Kidney. Steatosis of canaliculus epithelium (lixiviated fat). H-E staining. 160x.

Fig. 3 B. Group CH. Kidney. Atheromatous plaque in the artery. H-E staining. 130x.

Fig. 3 C. Group CH+SM. Kidney. Normal pattern. H-E staining. 150x.

Fig. 3 D. Group CH+SM. Kidney. Atheromatous plaque in the artery. H-E staining. 130x.
**Table 1**

Schematic presentation of different concentrations of lipids in plasma of rabbits being on the 0.5 g% cholesterol diet of the CH+ SM group when compared to the K and CH groups

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Compared to Group</th>
<th>K</th>
<th>K</th>
<th>CH</th>
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<tr>
<td></td>
<td>after 3 months</td>
<td>↑</td>
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TG: Lipid concentration increased after 1 month and after 3 months.

LDL: Lipid concentration increased after 1 month, after 2 months, and after 3 months.

HDL: Lipid concentration increased after 1 month, after 2 months, and after 3 months.

LDL/HDL: Lipid concentration increased after 1 month, after 2 months, and after 3 months.

↑: Statistically significant increase when compared to the K or CH group.
↓: Statistically significant decrease when compared to the K or CH group.
–: No statistically significant change.

**Fig. 4 A.** Group CH. Heart. Atheromatous plaque in arteriole. Lixiviated fat. H-E staining. 150x.

**Fig. 4 B.** Group CH+SM. Heart. Atheromatous plaque in arteriole. H-E staining. 200x.
Heart. The changes noticed in the heart were localized in arterial vessels and consisted in focal proliferation and steatosis of the intima (atheromatous plaques). There were foam cells loaded with lipids present in the atheromatosis laminas. In the CH group the above-described changes were intense and were observed in arteries of different size (Fig. 4A), while in the CH+SM group atheromatous plaques were found only in 3 rabbits (Fig. 4B). The presence of fats in the atheromatosis laminas as well as in the renal tubule cells and hepatocytes was confirmed during histochemical studies. Fats were stained with Sudan III into orange colour.

Discussion

The obtained results of microscopic examination showed histopathological changes in all the examined organs of rabbits being on cholesterol diet. Proliferation of the internal layer in the form of atheromatous plaques was observed in the aorta, coronary vessels of the heart and renal arteries. Inside the plaques there were numerous foam cells. Lipids were also present in intercellular spaces. The obtained results showed that cholesterol in the applied dose exhibited atherogenic action, and the result correlated with the observation of Bacon et al. (2) and Jakinen et al. (12). As it was shown in experimental studies, the intensity level of atherogenesis in the aorta depends on cholesterol contents in a diet (2).

Rabbit cholesterol overload triggered, apart from atheromatous changes in vessels, regressive alterations in the liver and kidneys, manifested by hepatocyte steatosis and focal steatosis of renal tubule epithelium cells. In the liver, steatosis was found mainly around central veins, which is in accordance with metabolic processes (central part of the lobule synthesizes fats) (6). According to Brzozowski and Krus (5, 16) liver steatosis is a sign of disturbances in lipid and lipoprotein metabolism, leading to their excessive accumulation.

Steatosis of collective tubule epithelial cells, observed in the group CH, overloaded with cholesterol, probably results from fat metabolism disturbances in the liver. This is usually observed in liver steatosis.

Similar retrogressive alterations were observed in the liver, kidneys, heart, muscle, and other organs during clinical studies in people fed a diet containing a lot of fats and cholesterol.

In the experimental group CH+SM, receiving cholesterol with selenomethionine, similar pathomorphological changes were shown in arterial vessels, however, their intensity was a little lower. Fatty alterations in hepatocytes were more weakly expressed, and there was no steatosis in tubule epithelial cells. This proves a protective action of selenium, administered as an organic combination with methionine. The obtained results of pathomorphological evaluation in CH and CH+SM groups show correlation with the results of biochemical examinations (a significant increase in cholesterol fraction in relation to the control group). The observed decrease in cholesterol level in CH+SM group did not show any significance in relation to CH cholesterol group. In studies on rabbits, Birkner (4) observed an elevated malonic dialdehyde (MDA) level - a product of lipid peroxidation, following a cholesterol diet administration. The author also showed that the MDA increase was accompanied by an increase in antioxidative enzyme superoxide dismutase and glutathione peroxidase activity. Addition of selenomethionine to the fodder had a favourable effect, resulting in a decrease in MDA concentration, with simultaneous increase in antioxidative enzymes activity in the blood plasma (4). Reports from previous studies indicate significant changes of antioxidative system following the administration of selenium compounds (9-11, 13, 32, 33).

It was shown that selenium in the form of selenine (32), yeast preparation (9) and sodium selenide (13) administered to rabbits or rats with experimental hypercholesterolemia leads to a decrease in MDA concentration in their blood serum. It should be emphasized that selenium administered in the form of organic combinations more easily builds itself into selenodependent proteins, hence its action is more effective. Less intensive retrogressive alterations in the liver and kidneys show correlations with the index values of oxidation-reduction system in the organs of rabbits (4).

The conducted study showed the preventive influence of selenomethionine on atherogenesis. Selenomethionine reduced the development of atheromatosis changes (small plaques in aorta walls as well as in the heart and kidney arteries) and steatosis in the liver and kidneys.

References


