INFLUENCE OF VITAMIN E ON THE DEVELOPMENT OF MORPHOLOGICAL CHANGES IN RABBITS’ ORGANS IN EXPERIMENTAL HYPERCHOLESTEROLAEMIA

BARTOKA STAWIARSKA-PIĘTA, EWAA SZAFLARSKA-STOJKO, EWA BIRKNER*, EWA GRUCKA-MAMCZAR*, MAGDALENA WYSZYŃSKA, RAFAŁ STOJKO, IRENA WRÓBLEWSKA-ADAMEK AND AGATA KABAŁA-DZIK

Department of Pathology, Faculty of Pharmacy in Sosnowiec, *Department of General Biochemistry, Faculty of Medicine in Zabrze, Silesian Medical University, 40-952 Katowice, Poland
e-mail: jkpcpm@poczta.onet.pl

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Abstract

The experiment was conducted on male rabbits of the New Zealand breed divided into three groups: a control group and two experimental groups. The experimental groups were on a cholesterol diet consisting of 0.5 g of cholesterol/100 g of fodder/rabbit/24 h. In addition, rabbits from the 2nd experimental group were receiving vitamin E in the amount of 10 mg/kg of body mass/24 h. After three months, blood was collected for biochemical analysis, and the heart, kidneys, liver and aorta were collected during postmortem examination for histopathological tests. The pathomorphological changes were assessed in paraffin sections, stained with haematoxylin and eosin, as well as in frozen sections, stained with Sudan III for neutral fats. The experiments showed that the addition of vitamin E to the cholesterol diet inhibits the development of atheromatosis in artery walls and regressive changes in examined organs.

Key words: rabbits, cholesterol, atheromatosis, vitamin E, histopathology.

Aggregation of thrombocytes, increased production of PDGF, as well as proliferation of smooth muscles cells and extracellular matrix of internal membrane (28).

Many studies in recent years have shown a significant role of free oxidation radicals and radicals secondarily generated by them in the development and proliferation of atheroma. Among the later ones, apart from radicals deriving from unsaturated fatty acids, there are the active compounds arising from the influence of free radicals on polyunsaturated acids - such as MDA and 4-HNE - that play an important role (22). The oxidation theory of atheromatosis development has led to a number of studies estimating the role of antioxidants in the prevention of atheromatosis. It has been assumed that the circulating LDL are to some extent protected from free radicals, thanks to the antioxidants diluted in their lipid fraction (11).

Some vitamins belong to active antioxidants. They determine the correct course of life processes and along with other endogenic antioxidant substances (such as glutathione, uric acid or cysteine) protect the organism from the toxic activity of reactive forms of oxygen (10, 11). Vitamin E (α-tocopherol) is a compound of special interest among the researchers of atheromatosis. This can be explained by the fact that vitamin E is the first defense line against the peroxidation of fatty acids (16, 20, 33). Vitamin E - due to its ability to sweep out free radicals - inhibits the production of cytokines, integrines, and adhesive particles ICAM-1 and VCAM. It also increases the resistance of LDL to oxidation, which as a result successfully prevents the adherence of cells to the endothelium. Through inhibiting the activity of C protein kinase in inflammatory cells, vitamin E also significantly decreases the production of free radicals in monocytes which in turn decrease the synthesis of...
inflammation mediators. It also inhibits the proliferation of smooth muscles of vessel walls (15, 24). So far the presented results are not only ambiguous, but also often controversial (3, 6, 9, 14, 17-19, 23, 25, 29, 30).

The aim of this study was the morphological assessment of rabbit’s organs: aorta, heart, kidney and liver in experimental atheromatosis caused by a cholesterol diet, as well as the defining of the influence of vitamin E on the atherogenesis process.

Material and Methods

The study was performed 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 Silesian Medical University in Katowice. Before the experiment, the animals were adapted for 2 weeks 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 GLK fodder;
- experimental group I (CH) - being on a atheromatosis diet consisting of 0.5 g of cholesterol/100 g of fodder/rabbit/24h;
- experimental group II (CH + vit. E) - being on a atheromatosis diet (as above) supplemented with vitamin E in the amount of 10mg/kg of body mass/24h.

The experiment lasted for three months. Animals were given water ad libitum. Every month blood was collected from their ear border 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 post-mortem examination the aorta, liver, heart and kidneys were collected for histopathological tests. 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 (37). Colour microphotographs were taken with the Docuval microscope equipped with the photo device (Carl Zeiss Jena).

This study was approved by the Bioethical Committee for Animal Testing of the Silesian Medical University.

Results

Biochemical examination. Table 1 shows changes in the lipids concentrations in plasma of rabbits belonging to both groups on a cholesterol diet. Statistically significant increase in the concentration of triglycerides (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+vit.E groups after every month of the experiment (P>0.004).

Table 1
Schematic presentation of different concentrations of lipids in plasma of rabbits being on the 0.5% cholesterol diet of the CH+vit. E group when compared to the K and CH groups

<table>
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<th>compared to group:</th>
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↑ Statistically significant increase when compared to the K or CH group.
No statistically significant change.
Administration of vitamin E along with cholesterol (CH+vit. E) caused a downward tendency in the LDL concentrations when compared to the CH group. No statistically significant change in the HDL cholesterol concentration was noticed in either of the groups.

**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+vit. E groups. In the CH group, the plaques were large and covered the entire circumference of the vessel while in the CH+vit. E 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+vit. E 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 of CH and CH+vit. E groups was a focal hyperplasia of the intima, completely covered with laminas (CH group) or single, non-complete small atheromatous plaques (CH+vit. E group). There were numerous foam cells in the atheromatous plaques, and fat was also noticed in intercellular spaces. Figs. 1 and 2 show aorta microscopic pictures. Changes in the tunica media were noticed in the CH group – its focal thickening was noticed with no signs of fibrosis as well as there was a focal proliferation of macrophages in the proximity of *membrana elastica interna*.

**Liver.** There was a distinct steatosis of hepatocytes in CH rabbits. The steatosis was increased around the central veins of hepatic lobule (Fig. 3). In the hepatocytes located on the periphery of hepatic lobule, there was either no steatosis or it was minimal. In the CH+vit. E group, no steatosis in the hepatic cells was noticed (Fig. 4).

**Kidneys.** There was little steatosis in the tubule epithelium cells noticed only in kidneys of the CH group (Fig. 5). There were also atheromatous changes in the arterial vessels of kidneys of the CH group, evidenced by hyperplasia of artery intima in the form of atheromatous plaques (Fig. 6). They were observed in lobar arteries of 4 rabbits. In the CH+vit. E group, no steatosis in the tubule epithelium cells was noticed (Fig. 7). Slight atheromatous changes in the lobar arteries were noticed in 2 rabbits of the CH+vit. E group.

**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. 8), while in the CH+vit. E group atheromatous plaques were found only in 2 rabbits (Fig. 9).

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 yellow-orange colour.

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**Fig. 1.** Group I (CH). Aorta. Atheromatous plaque. Foam cells in the intima. H-E.

**Fig. 2.** Group II (CH+vit.E). Aorta. Small atheromatous plaque. Foam cells in the intima. H-E. x 150.

**Fig. 3.** Group I (CH). Liver. Steatosis of hepatocytes around the central vein. H-E. x 280.
Fig. 4. Group II (CH+vit.E). Liver. Normal appearance. H-E. x 140.

Fig. 5. Group I (CH). Kidney. Steatosis of tubulus epithelium. H-E. x 160.

Fig. 6. Group I (CH). Kidney. Atheromatous plaque in the artery. H-E. x 130.

Fig. 7. Group II (CH+ vit.E). Kidney. Normal appearance. H-E. x 140.

Fig. 8. Group I (CH). Heart. Atheromatous plaque in arteriole. H-E. x 150.

Fig. 9. Group II (CH+ vit.E). Heart. Atheromatous plaque in arteriole. H-E. x 150.
Discussion

The conducted studies showed that cholesterol is the main perpetrator of artery atheromatosis. It also leads to retrogressive changes, such as fatty degeneration in the liver and kidneys. There is no doubt that cholesterol is the main atherogenic factor (2, 9, 14, 17, 18, 30, 31, 36). The lipid profile depends not only on the amount of fat in the diet, but also on its autosomal metabolism. The biochemical tests showed a statistically significant increase in the concentration of its most atherogenic fraction – the LDL cholesterol. Recent studies show that the oxidative modification of LDL facilitates the accumulation of lipids in tissues and causes atherogenesis (29).

The conducted study showed the preventive influence of the well-know antioxidant, i.e. vitamin E, on atherogenesis. Vitamin E reduced the development of atheromatosis changes (small plaques in aorta walls as well as in the heart and kidney arteries). The biochemical tests in vitamin E-treated group showed only the downward tendency in the concentration of LDL cholesterol in plasma when compared to the CH group. No change in the concentration of the HDL fraction was observed. An increase in TG was shown which along with the LDL cholesterol intensifies the oxidative stress and disturbs the regular functioning of the epithelium (8). Also, Birkner (2) noticed - in the biochemical studies of animals being on a 0.5% cholesterol diet with the supplement of vitamin E – a statistically significant increase in superoxide dismutase (SOD) activity in plasma when compared to the control group after 3 months of experiment. In the biochemical tests, as in the presented study, the concentration of MDA (the oxidation stress marker) was determined (21). There was an increase in its concentration in the group receiving the 0.5% cholesterol diet, while the addition of the antioxidant – vitamin E to the diet caused a decrease in the MDA concentration to that of the control group (2). Therefore, the preventive action of vitamin E resulted in this case most likely from the prevention of epithelium dysfunction (1). The results showing the positive influence of vitamin E on the rate of oxidation processes point to its anti-atherogenic activity and correlate with the presented results of pathomorphological assessment.

The results obtained correspond to the findings of many previous experimental and clinical studies showing the positive anti-atherogenic activity of vitamin E (9, 17, 18, 25, 28). Numerous studies point to the effectiveness of combined therapy (administration of a few antioxidants together). Usually, vitamin E is administered along with selenium, methionine and/or vitamin C (34, 35). Although some clinical studies did not show a significant benefit of using vitamin E in the development of atherogenesis changes (7, 19, 27). There have also been reports pointing to the prooxidative properties of vitamin E (3, 29, 30).

It is necessary to notice that the influence of vitamin E on atherogenesis changes depends on the degree of their progression. Early changes in the vessels are reversible and we may assume that even low doses of vitamin E can successfully reverse them. Advanced changes are practically irreversible and therefore safe doses of vitamin E can be ineffective. It is also necessary to remember that uncontrolled use of antioxidants in patients with heart ischemia receiving other medicaments may lower their effectiveness (19). Hepatocyte steatosis in rabbits’ liver showed in this experiment suggests dysfunction in lipid and lipoprotein metabolism in the liver of animals loaded with cholesterol. Since the synthesis processes in the hepatocytes are mostly intensified around the central veins of the lobules and metabolism and degradation are most active in the periphery, the early fatty degeneration changes take place in the central part of the lobule (4). Liver steatosis may be accompanied by the steatosis of renal loop epithelium cells and collective tubuleus, which is connected with the disordered metabolism of lipids of the liver (13). The presence of degenerative changes in hepatocytes as well as in the renal tubule epithelium cells may lead to necrosis of these cells and very serious consequences, such as hepatic cirrhosis or kidney fibrosis (12).

To sum up, in the accomplished research it was proved that vitamin E administered together with the atherogenic diet restrains atheromatosis changes in aorta, heart and kidney arteries and steatosis in the liver and kidneys.

References

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