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Histopathological Effects of Cholesterol and Protective Effects of Vitamin E and Selenium on the Morphology of Liver

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Abstract: In this study, effects of cholesterol and protective effects of vitamin E (vit E) and Selenium (Se) on the morphology of the liver in rats were observed. Cholesterol (Chol) supplemented diet, Chol + vit E, Chol + Se and Chol + vit E + Se were applied to the rats. Portal fibrosis with bile ductal proliferation and an increased inflammatory infiltration were seen in Chol group. In this group, ultrastructurally, granular endoplasmic reticulum (GER) arrangement was seen in irregular shape in hepatocyte cytoplasm. The membranes of GER and mitochondria were not seen clearly. A morphological improvement was seen in the other groups. It has been concluded that the mechanism of the injury may be involved in the accumulation of toxic bile acids or the generation of free radicals from cholesterol oxidation products. In addition it has been realized that the growth factors may be released into the hepatic sinusoids to activate stellate cells and to initiate fibrogenesis.

Key Words: Cholesterol, liver, vitamin E, Selenium

Introduction

The liver plays an important role at the cholesterol metabolism in S. Dawley rats (1). Hepatic fibrosis was reported to be induced by Chol supplemented diet (2, 3). It has been shown that it causes Chol accumulation in cells by high Chol diet (4, 5). Most forms of chronic liver disease are associated with the development of the fibrosis (6, 7). It has been stated that, the stimulation of non parenchyma cells, i.e., myofibroblasts, could participate in the mechanism of the hepatic fibrosis (7, 8). Therefore, it is known that myofibroblast like cells (MFB) have ability to synthesis a wide spectrum of collagenous and non collagenous matrix proteins and to express and to secrete growth factors, i.e., TGF-b (9, 10). It has been observed that vit E prevents development of atherosclerotic plaques in an experimental study (11). It is known that vit E and Se have anticancerogenic effect in experimentally developed pancreas cancer (12). In addition, these protective effects may be the result of antioxidant effects.The purpose of our study was to investigate whether vit E and Se had a protective effect on the liver morphology.

Materials and Methods

Animals and Laboratory Studies

Thirty-five adult male S. Dawley rats (250-300 g) were utilized in the present study. Control group was fed rat chow ad libitum. Treatment groups were divided into four groups: Chol group, Chol + vit E group, Chol + Se group, Chol + vit E + Se (combined) group. Chol was given chow supplemented with 1% cholesterol (0.5 g/kg/day, orally, in 0.5 ml of corn oil; Riel de Hain). Vit E (dl- a tocopherol; Roche) was given 600 mgr/kg, three times a week intraperitoneally (i.p). Se (Sodium selenite, Merck) was given 2.5 mgr/kg, three times a week, i.p in 0.5 ml of 0.9% NaCl. The rats in various experimental diets were sacrificed and their livers were removed at the

end of eight weeks. Tissues were fixed in 10% formaldehyde. Five mm thick paraffin sections were stained with Haematoxylin Eosin (H&E) and Masson's trichrome for light microscopical examination (13, 14). Tissues were fixed in 2.5% glutaraldehyde in phosphate buffer for 48 hours, postfixed in 1% osmium tetroxide, embedded in Epon 812, for ultrastructurally study (15). Thin sections were double stained with uranyl acetate and lead citrate, examined and photographed with Jeol-100 C TEM.

Statistics

Statistical significance of differences between groups was analysed with the Kruskal-Wallis variance analysis, followed by Duncan's test. A probability (P value) of 0.05 or less was taken to indicate statistical significance.

Results

Light microscopical findings

In the control group, hepatocyte plates were normal and fibrosis, ductal dilatation and proliferation or inflammatory infiltration was not observed. In addition, in the control group, hepatocellular vacuolization was not seen. Degenerative changes in various degrees were observed in microscopic examination of liver in Chol fed group. In this group, portal tract fibrosis with bile ductal proliferation and as clusters of inflammatory cells surrounding portal area or within the parenchyma was seen. Cytoplasmic vacuolization within hepatocytes and bile ductal dilatations were observed and was generally associated with periductal fibrosis in the Chol group (Fig. 1). A decreasing in degenerative changes was noticed in Chol + vit E group as compared to the Chol group. There was a moderate decreasing of portal tract fibrosis with bile ductal proliferation, inflammatory infiltration in Chol + vit E compared to Chol fed group (Fig 2). Hepatocellular damage and portal tract fibrosis with bile ductal proliferation and inflammatory infiltration were markedly decreased in Chol + Se group compared to Chol fed group (Fig 3). No hepatocellular damage, portal fibrosis and inflammatory infiltration were found in the combined group. It was noticed that this group has the same histological appearance as the control group (Fig.4).

Ultrastructural findings

In the control group, membranes of GER and mitochondria had normal ultrastructurally appearance.

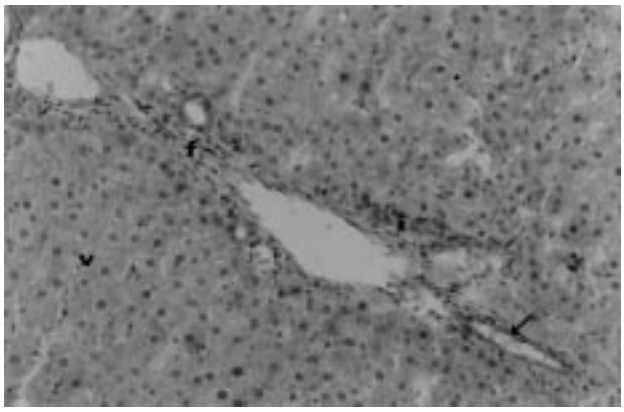

Figure 1. An area of fibrotic liver in Chol fed rat. Note to hepatocellular vacuolization (v), portal fibrosis (f) with ductal dilatation (arrow) and inflammatory infiltration (i). H &E. X 20.

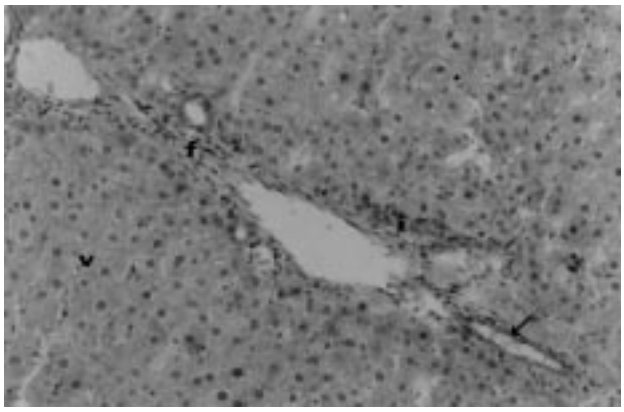

Figure 2. Portal area and hepatocyte plates. Note the decreased hepatocellular vacuolization, portal fibrosis with ductal dilatation (arrow) and inflammatory infiltration (i) in Chol+ vit E group. H &E. X 20

GER cisternae were arranged regularly. GER were seen to have abnormal appearance in hepatocyte cytoplasm in Chol fed group. The arrangement of GER membranes were irregular. Most of them were fragmented or scattered. The membranes of mitochondria and GER were not clearly observed. Most of the mitochondria showed poor electron-dense matrices with rounded profiles. The cristae of mitochondria was not seen clearly (Fig. 5). In contrast, hepatocyte damage was less in vit E group than Chol fed group. In this group, the scattering of GER membranes decreased and it was more regular and the cristae of mitochondria were seen clearly (Fig.6). Minimal hepatocellular damage was seen in treated Se group than Chol fed group and Chol + Vit E treated group. The appearance of GER was regular and mitochondrial membranes and cristae were seen to be

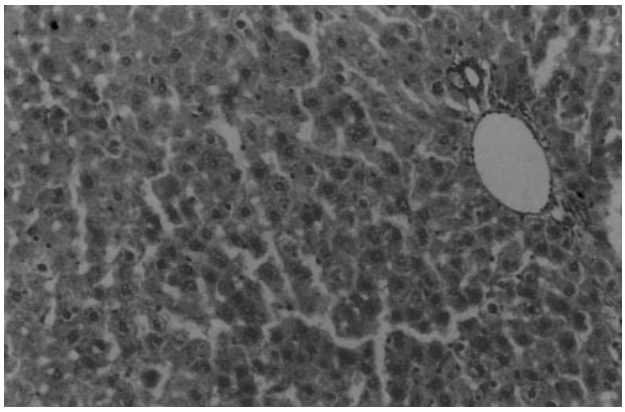

Figure 3. Portal area and hepatocyte plates. Note the portal fibrosis, ductal proliferation and inflammatory infiltration were minimal in Chol+ vit E group. H &E. X 20

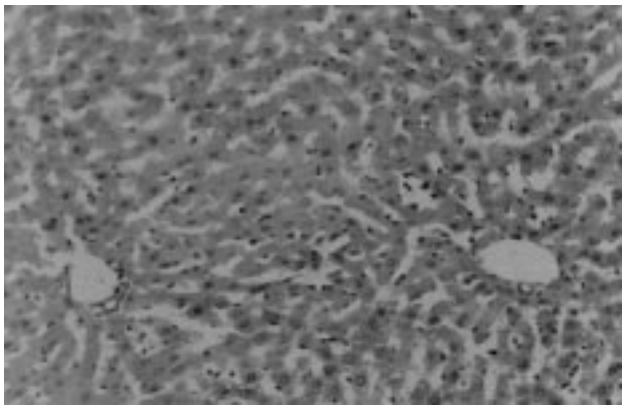

Figure 4. Note the portal area and hepatocyte plates in the combined group showing similar appearance with the control group. H &E. X 20

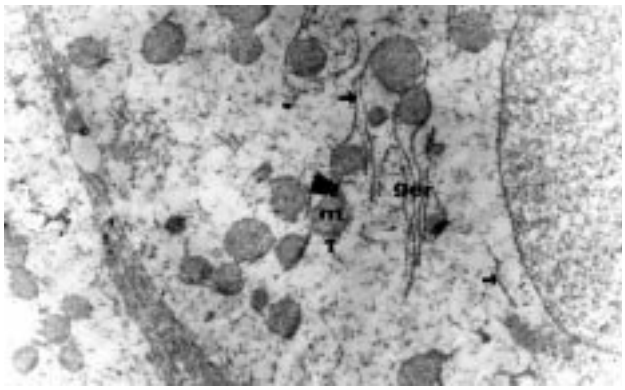

Figure 5. Note the abnormalities of GER (ger) and mitochondrial (m) membranes, breaking, fragmentation and scattering with in cytoplasm in Chol fed rat (arrows). X 10.000

normal within the cytoplasm in Se treated group (Fig. 7). Findings of the combined group were similar to the control group. The morphology of GER and mitochondria were in normal appearance (Fig. 8).

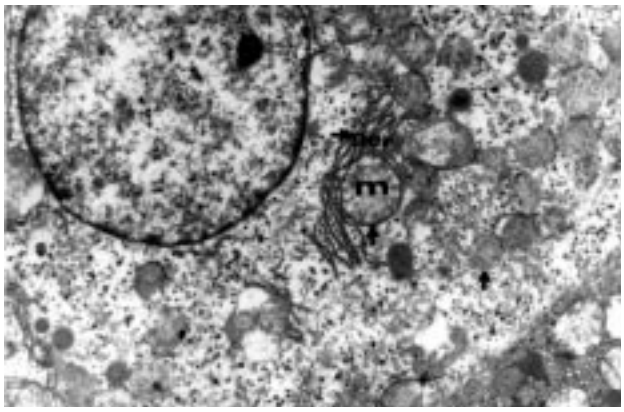

Figure 6. GER (ger) and mitochondrial (m) membranes abnormalities were decreased (arrows). Note the decrease of scaterring in GER in Chol + vit E group. X 10.000.

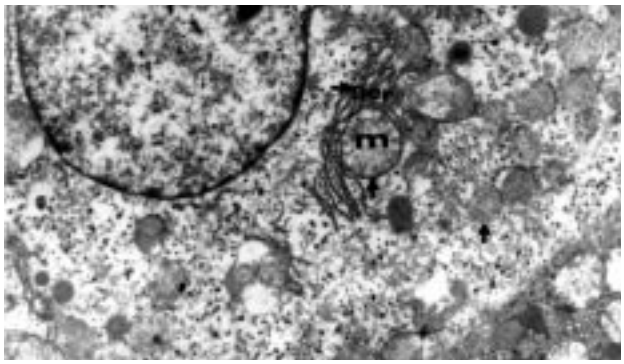

Figure 7. GER (ger) and mitochondrial (m) membranes were regular and improved in Chol + Se groups (arrows). X 5200.

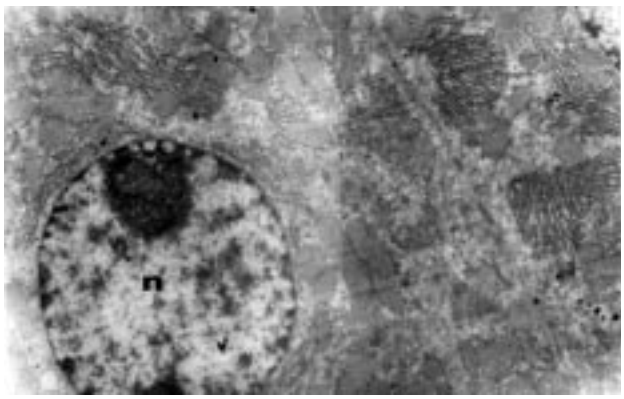

Figure 8. Nucleus (N) and organelles were in normal appearance and was preserved in the combined group (arrows). X 10.000.

Other findings

Plasma Chol levels were elevated after 8 weeks on the Chol diet. Chol + vit E treated group had a lower plasma

Chol level than the Chol fed group for eight weeks ($p< 0.05$). Chol + Se treated group had a lower plasma Chol level than the control group, Chol fed group and Chol + vit E treated group for eight weeks ($p< 0.05$). Chol + vit E + Se group had a lower plasma Chol level than the control group, Chol fed group, Chol + vit E treated group and Chol + Se treated group for eight weeks ($p< 0.05$) (Table 1).

Table 1. The means of plasma Chol levels ± SD in different groups.

Groups	Chol in Plasma (mg/dl)
Contol (n=7)	52 ± 18 ♦
Chol (n=7)	77 ± 32 ♦
Chol+Vit E (n=7)	47 ± 14 ♦
Chol+Se (n=7)	38 ± 9 ♦
Chol+ it E+Se (n=7)	27 ± 5 ♦

* $P < 0.0001$ (Kruskal-Wallis).

♦ $P < 0.05$ (Duncan test).

Discussion

Hypercholesterolemia is said to contribute to hepatic injury as a result of Chol accumulation. Lack of underlying basement membranes facilitates the flow of LDL Chol in circulating plasma between sinusoidal lumen and hepatocytes, because in normal liver sinusoids are limited by fenestrated endothelial cells (7, 8). The normal liver tissue has Chol oxidase. Chol generates Chol oxidation products and free radicals by Chol oxidase. Chol oxides include cytotoxic and steatotic effects (4, 5). Lipid accumulation leads to oxidative stress in hepatic tissue. The oxidative stress may contribute to peroxidation of LDL (6, 16). These peroxidative fatty acids and reactive oxygen species induce hepatic damage. Hausner et al. showed that cholesterol supplemented diet caused high plasma Chol level with hepatic and adrenal cell degeneration (2). GER and mitochondria swelling have been reported within the hepatocyte cytoplasm. In our study, intensely cell degeneration of hepatocyte was determined with abnormal organelles in Chol fed group. At some of the organelles, i.e., GER and mitochondrial membranes fragmentation, scattering and breaking was observed. These results indicated that the metabolism of Chol oxidation products may generate free radicals, resulting in peroxidation of membrane lipids and subsequent hepatocellular injury. High cholesterol diet enhanced Chol accumulation in hepatocyte and bile ducts (17,18). This accumulation may cause cholestasis, and therefore increasing of bile contraction and developing of bile ductal proliferation and dilatations are known. Some observers have showed the bile ductal proliferation and dilatation in portal area by conventional light microscopy in high Chol diet (2, 3). We also reached the same results. We observed bile ductal dilatation generally associated with periductal fibrosis. This morphologic evidence demonstrated that bile acids and Chol contributed to bile ductal contraction and dilatation. They have shown that hepatic fibrosis develops rapidly for several weeks in the rats of fed Chol. Growth factors have an important role in developing of fibrosis. Lypocytes which transforming MFB like cells by effect of growth factors, increase synthesis of extracellular proteins, especially collagen (10, 19). In our study, we observed marked inflammatory reaction and this reaction was an indication of active expression inflammatory mediators as TGF-b and PDGF. Morphologic evidence of LDL-Chol transport activity has been revealed by presence of cytoplasmic vacuolization (3, 20). In previous studies, it was suggested that vit E prevents occurring of atheroma plaques in atherosclerotic lesions. Vit E is a potent antioxidant and so it prevents oxidation of polyunsature fatty acids in cell membranes (11, 21). In the present study, decreasing of hepatic fibrosis, bile ductal proliferation and inflammatory infiltration were observed as protective effects of vit E. Ultrastructurally, cell morphology was protected well to Chol toxicity. Se is a main component of glutathione peroxidase (Gpx) (22). GPx causes reduction of H2O2 to nontoxic elements. As H2O2 is a first element of free radicals, it showed minimal hepatocellular damage in Se treated rats. Vit E and Se have a synergistic effect, so their antioxidant effects increase. In the present study, the results as the combined group seem to be the same as in the control group. As a result, we can say that, combined Vit E + Se therapy decreases hepatocellular damages and these antioxidant agents have protective effects on hepatocytes.

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