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Blood serum ascorbic acid evaluated in patients suffering from liver and pancreas diseases and in the liver, heart, kidneys and lungs of rats intoxicated with CCl_4 and galactosamine

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Summary

The aim of the study was to evaluate blood serum ascorbic acid levels in patients with hepatic cirrhosis, acute and chronic pancreatitis and in the liver, heart, kidneys and lungs of rats intoxicated with CCl_4 and galactosamine. The results revealed statistically significant increases in the blood plasma ascorbic acid levels in patients with acute and chronic pancreatitis and hepatic cirrhosis. In the group of patients with chronic pancreatitis, however, the blood plasma ascorbic acid levels were not different from the controls. In the group of control rats the highest ascorbic acid levels were observed in the liver and the lowest in the heart. In the intoxicated rats with CCl_4 and galactosamine the kidneys' ascorbic acid contents increased significantly after administration of both toxins in single doses but decreased after 3-days of administration of CCl_4. In the liver, decreased ascorbic acid contents were observed after single doses of CCl_4 and galactosamine, but after the 3-day administration its contents were increased. The content of ascorbic acid in the lung increased after each of the toxins used. In the heart, ascorbic acid contents decreased considerably after single and three-day CCl_4 and galactosamine administration as well.

Keywords: ascorbic acid, acute pancreatitis, chronic pancreatitis, CCl_4, galactosamine, rats

In western populations, blood plasma ascorbic acid concentration is 54.4 μmol/l for males and 64.0 μmol/l for females, in the USA the levels are 67.9 and 68.6 μmol/l respectively. Ascorbic acid does not bind with proteins, undergoes filtration in the renal glomerules, and is actively reabsorbed in renal tubules. In a healthy body the elimination threshold is 60-100 mg/24 hs when ascorbic acid is administered orally. When administered in doses > 100 mg or given IV, 25% of the vitamin is eliminated. A plasma saturation of 70 μmol/l is achieved at oral doses 200-400 mg/24 hrs. Ascorbic acid cellular availability depends on the efficiency of sodium dependent carriers. Half-life in the plasma is 10 hours. Ascorbic acid circulates in the blood in a reduced form (16).

Numerous experimental and clinical studies concerning diseases affecting the liver or pancreas carried out in animals or humans have found increased levels of lipid peroxidation products. Inflammatory cells, xantine oxidase, P450 cytochrome and nitric oxide synthetase are possible sources of free radicals. To eliminate the excess of free radicals, the body activates its defensive mechanisms such as enzymatic systems, e.g. glutathione peroxidase, superoxide dismutase and catalase. Other non-enzymatic mechanisms are also used to protect it from oxidative stress, e.g. antioxidants (vitamins C and E) and free radical removers, transient metal ions and metalothioneins.

Carbon tetrachloride is a strong toxin, especially toxic for the liver. This substance has been used to induce damage to the liver in experimental models for over 70 years. Single doses cause liver steatosis and cirrhosis in rats and repeated multiple injections lead to liver fibrosis. Both effects of CCl_4 on the liver are achieved in a different way and result from independent toxic activity (18). Liver steatosis develops as

a consequence of lipoprotein synthesis inhibition by CCl_4 or synthesis of their defective forms. Quite important are CCl_4 metabolites, i.e. CCl_3 and Cl free radicals formed by the hemolytic split of CCl_4 molecule in the P450 cytochrome system. Researchers stress the fact that free radicals are responsible for unsaturated lipid peroxidation contained in the membranes of the endoplasmic reticulum. That leads to their destruction and formation of secondary free radicals originating from membranous lipids (4).

Galactosamine is likewise a well known hepatotoxin. The injection of a single dose induces acute hepatitis. Its toxic effect is due to UDP-glucose and UDP-galactose deficiency and deficit of intercellular calcium. The deficiency causes damage to the cell membranes and organelles and impairs the synthesis of proteins and nucleic acids. Galactosamine administration alters the location of proteoglicanes in the liver and inhibits hepatocyte energetic metabolism. The toxin also damages the enzymes that facilitate the transport of substrates to the mitochondria and modifies the structure of cellular membranes (6).

The aim of the study was to evaluate blood serum ascorbic acid levels in patients with hepatic cirrhosis, acute pancreatitis (AP) and chronic pancreatitis (CP). In the experimental part of the study the contents of ascorbic acid in selected tissues (liver, heart, kidneys and lungs) of the rats intoxicated with CCl_4 and galactosamine were determined.

Material and methods

Blood plasma levels of ascorbic acid were determined in patients with hepatic cirrhosis (42 patients), AP (38 patients) and CP (39 patients). The blood specimens were collected on the day the patients were admitted to hospital. Ascorbic acid levels were determined according to Aye Kyaw method (1) and the results compared to the control group of healthy persons (40 persons). None of the examined persons received ascorbic acid supplementation.

Animal studies were carried out on Wistar rats, weighing 250-350 g. Group I (control group) received 2 ml olive oil; group II received CCl_4 administered i.p. in a single dose 0.5 ml/kg b.m. dissolved in 2 ml olive oil. Group III received CCl_4 during three consecutive days administered i.p. at the dose 0.5 mg/kg b.m. and group IV received galactosamine administered i.p. in a single dose of 800 mg/kg b.m. After 24 hrs from the administration the rats were decapitated and the organs, i.e. liver, heart, kidneys and lungs, harvested for further examinations. Ascorbic acid contents in the rat tissues were determined by Aye Kyaw method. The tissues homogenate were centrifuged at 4000 rpm for 10 minutes at 3°C and the supernatant was used for ascorbic acid analysis. The authors measured plasma and tissues ascorbic acid level using specific and sensitive to vitamin acid phosphoungstate. Absorbance of the blue-colored supernatant at 700 nm was measured against a blank. For every set a standard and a blank were run through the procedure.

The studies were approved by The Local Bioethical Board at The Medical University of Lublin (nr 40/AM/2004).

Results and discussion

The results revealed significantly increased blood serum ascorbic acid levels in patients with AP and hepatic cirrhosis. The results were similar in both groups of these patients. In the group of patients with CP, however, the blood serum ascorbic acid levels were not significantly different from the controls (fig. 1).

In the group of control rats the highest ascorbic acid contents were observed in the liver (fig. 2) and the lowest in the heart (fig. 5). In the kidneys and lungs of the control the results were similar (fig. 3 and 4).

The kidney demonstrated a statistically significant increase of the contents of ascorbic acid after the administration of both toxins given in single doses. Three-days of administration of CCl_4 decreased the contents of ascorbic acid (fig. 3). Decreased ascorbic

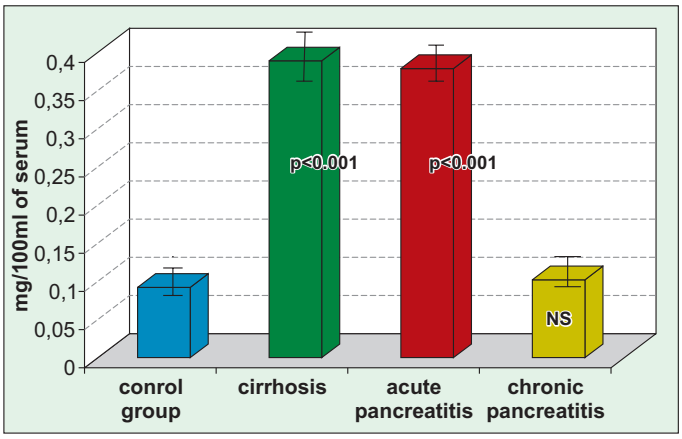

Fig. 1. Level of ascorbic acid in blood of patients

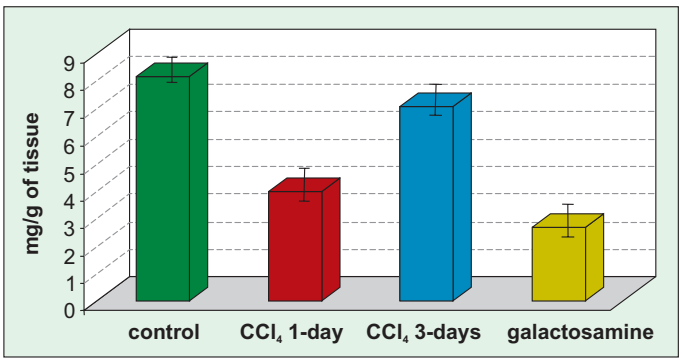

Fig. 2. Level of ascorbic acid in the liver of rats

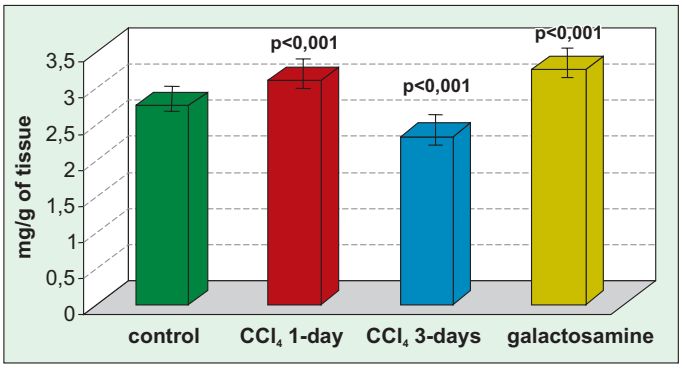

Fig. 3. Level of ascorbic acid in the kidneys of rats

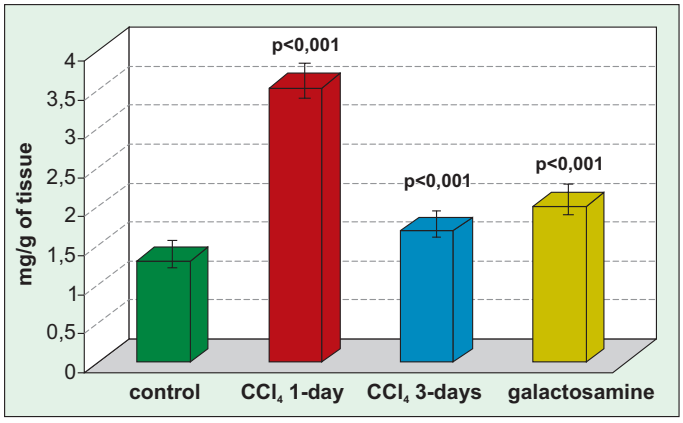

Fig. 4. Level of ascorbic acid in lungs

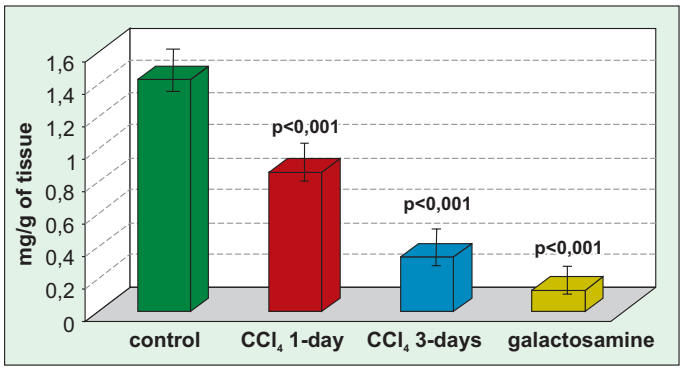

Fig. 5. Level of ascorbic acid in the heart of rats

acid contents were observed in the liver after single doses of CCl_4 and galactosamine; however, after 3-days of administration ascorbic acid contents were higher than after a single dose of these substances but lower in comparison with controls (fig. 2). The content of ascorbic acid in the lungs increased after each of the toxins used and the highest increase was observed after single doses of CCl_4 (fig. 4). In the heart ascorbic acid contents decreased considerably after single and 3-day CCl_4 administration and after galactosamine as well (fig. 5).

Despite numerous studies investigating the role of ascorbic acid in oxidative stress due to various diseases its involvement nevertheless remains unclear. High doses of ascorbic acid are thought not only to eliminate free radicals but also to decrease lipid peroxidation and affect cellular defensive functions (14, 20).

The studies found significantly increased blood serum ascorbic acid in patients with liver cirrhosis and AP. These results are similar to those obtained by Nalini et al. (13). They compared the level of antioxidants in the blood serum of the patients with non-alcohol and alcohol cirrhosis and healthy volunteers. They found increased blood serum lipid peroxidation products and increased ascorbic acid concentration, considerably decreased levels of reduced glutathione and peroxide dismutase activity. Likewise Lu et al. (12), investigating the levels of vitamins C, E and lipoperoxidase in the blood plasma of the patients with liver cirrhosis, observed considerably decreased levels of those parameters. It is difficult to explain the differences in these results, which may indicate the complex ascorbic acid involvement in the regulation of oxidative-antioxidative states and disorders. Similarly George (7) observed decreased ascorbic acid contents in the liver and in the blood serum after the induction of fibrosis due to administration of dimethylnitrozamine on 7^{th}, 14^{th} and 21^{st}. Increased use of ascorbic acid during the elimination of free radicals to decrease oxidative stress seems to explain this phenomenon.

Another benefit for patients from the use of ascorbic acid is decreased fibrosis observed in chronic ailments of the liver, both of metabolic type, e.g. non-alcoholic steatose hepatitis (NASH) (9) and viral in origin (2). High doses of ascorbic acid, i.e. 1000 mg were tolerated very well by patients (9). Ascorbic acid supplementation and its high blood plasma levels correlated significantly with slight fibrosis; however, they did not affect the intensity of inflammatory changes and transaminases activity in the patients with NASH (9). Nevertheless in patients with hepatitis C a decreased intensity of necrotic-inflammatory changes was observed (2).

The role of ascorbic acid in the course of AP is nonetheless controversial. Virlos et al. (19) did not observe positive effects of its administration aside from other antioxidants in severe AP. Ascorbic acid may produce beneficial effects in patients administered high doses of ascorbic acid (10 g/24 hrs for 5 days) from the onset of the disease (5). Similar results were observed by Sajewicz (15). Very high doses allow not only the elimination of free radicals and the reduction of damage due to lipid peroxidation, but also improve cellular immune response which often contributes to decreased mortality caused by septicemia (5). The researchers observed significantly decreased serum ascorbic acid that would increase during the first 5 days and correlate with the severity of the inflammatory condition. Likewise Scott et al. (17) observed decreased blood serum ascorbic acid in patients with AP compared to the controls. In the authors' investigation to determine the levels of ascorbic acid we drew blood from patients on admission to hospital. That likely accounts for higher ascorbic acid contents noted in our study in comparison to the control group. That may prove ascorbic acid got mobilized from the tissues (high values) to decrease in turn and be activated to prevent oxidative stress.

Least researched was blood serum ascorbic acid in the patients with CP and ascorbic acid supplementation in the course of the disease (3). The biggest benefit from antioxidant therapy is the reduction of pain and improved quality of life (8, 10).

Some mammals, including rats, are able to synthesize ascorbic acid on their own which the human body cannot produce (11, 18). This is the main reason why the results of the animal studies cannot be referred

directly to humans. Loo van der et al. (11) investigating rats of various age found that ascorbic acid content decreases with aging in the majority of tissues, e.g. the liver, heart, kidney, lung and skeletal muscles. However, he did not observe its decrease in the walls of the aorta or brain.

Ascorbic acid is undoubtedly an important contributor to antioxidative processes, but its full involvement needs further research to explain the mechanisms of activity.

Conclusions

1. Acute and chronic pancreatitis and liver cirrhosis in patients induce mobilization of ascorbic acid from the tissues, which is expressed as its higher blood serum contents.

2. In the control group of rats ascorbic acid contents in the tissues examined differed considerably.

3. CCl_4 and galactosamine decreased ascorbic acid contents in some of the tissues, in others they caused its increase, which may prove uneven exposure of the tissues to the toxins, thus to oxidative stress.

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