

Effect of the addition of L-ascorbic acid in megadoses to the diet on the morphology of liver cells in rats*

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ABSTRACT

The aim of the study was to determine the effect of megadoses of L-ascorbic acid, as a dietary supplement for rats, on the morphological status of the liver. Forty male rats were divided into 4 groups and fed mixtures that were supplemented with ascorbic acid in the following quantities in the particular groups (in % of the mixtures): I, 0.0; II, 0.3; III, 0.6; and IV, 0.9. The results showed that L-ascorbic acid supplemented to the mixtures at a level of 0.9% caused necrotic damage of hepatocytes.

KEY WORDS: rats, L-ascorbic acid, hepatocyte, pathomorphology

INTRODUCTION

Ascorbic acid, the most important antioxidant of the aqueous phase of the body (Banhegyi et al., 1997) counteracts the generation of reactive oxygen species (ROS), consequently preventing the production of toxic compounds causing degradation of cellular structures. Although the majority of animals synthesize L-ascorbic acid, due to environmental stress, production intensity or age, demand for this vitamin may considerably exceed its endogenous synthesis. Megadoses of vitamin C are employed in human and animal nutrition for prophylactic and therapeutic purposes. However, the pro-oxidative effects of vitamin C often observed in *in vitro* studies (Paolini et al., 1999) raise constant controversies concerning its influence on homeostasis of the organism (Podmore et al., 1998).

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The aim of the conducted studies was to determine the effect of megadoses of L-ascorbic acid added to diets for rats on the morphological status of the liver.

MATERIAL AND METHODS

Forty growing male Wistar rats (average initial body weight 235.0 g) were divided into 4 groups \times 10 animals and were kept in individual cages for 41 days under standard conditions: temperature 22°C, air humidity, 50-70%, light/dark 12/12 h. The rats received semi-purified mixtures containing (in %): maize starch I, 62.22; II, 61.92; III, 61.62; IV, 61.32; casein, 14.0; sucrose, 10.0; cellulose, 5.0; rapeseed oil, 5.0; mineral mix, 3.5; vitamin mix, 1.0; L-cystine, 0.18; and choline chloride, 0.1. The mixtures were supplemented with ascorbic acid in groups (in % of the mixtures): I, 0.0; II, 0.3; III, 0.6; and IV, 0.9. L-ascorbic acid, mineral and vitamin mixtures were obtained from ICN Biomedicals. After the experiment, the rats were euthanised by an overdose of ketamine and immediately after sacrifice, samples of liver were collected from the right lobe, 1 cm³ on average. They were fixed in 10-% neutral buffered formalin, embedded in paraffin, sectioned at 6 μ m and stained with haematoxylin-eosin and periodic acid-Schiff (PAS). The samples for determination of fat were frozen, sectioned and stained using Sudan III. The cells in one field were counted, using 50 \times magnification. Detailed examination of the cells was performed under 400 \times magnification.

The results were analysed by one-way analysis of variance ANOVA, using the Statgraphics 4.1 computer program.

RESULTS AND DISCUSSION

The histopathological picture of the liver of rats from groups I and III shows the normal structure of liver lobes and hepatocytes with a typical morphology (Figure 1). Single necrotic hepatocytes are visible within the particular lobes. In the liver of rats from groups II and IV, the histomorphological structure is preserved, but focal necrosis of hepatocytes is seen (about 25%) (Figure 2). In group IV, the smallest

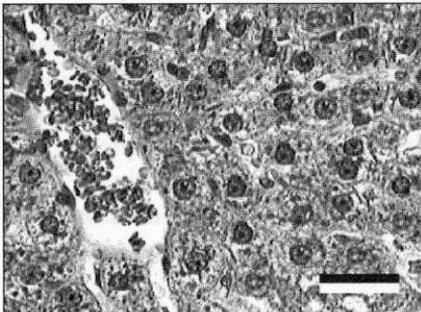


Figure 1. Normal liver of rats (group 1) (H-E, Bar 5 μ m)

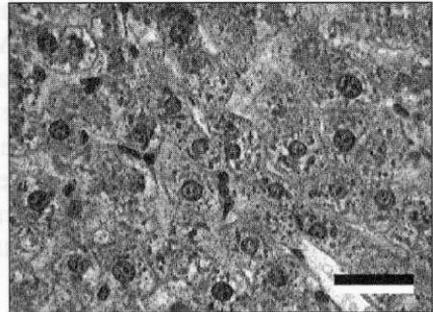


Figure 2. Focal liver necrosis of rats (group 4) (H-E, Bar 5 μ m)

number of two-nuclei hepatocytes was observed (Table 1). The lowest number of cells was found in group IV and the largest hepatocytes were in group II.

TABLE 1

Morphometry of cells and percentage of cells with different number of nuclei in the liver of examined rats

Items	Dietary ascorbate			SEM	P	
	0.3	0.6	0.9			
	%					
Morphometry of cells						
area, μ^2	2704 ^a	3783 ^{bc}	2391 ^{hd}	2680 ^d	127.24	0.000
diameter, μ	6.26 ^a	6.74 ^{bc}	5.95 ^{bdc}	7.25 ^{df}	5.948	0.000
Number of cells %						
without nuclei or with degenerated nuclei	1.5	26.6	8.7	25.5		
with 1 nucleus	91.9	68.6	84.9	73.4		
with 2 nuclei	6.6	4.8	6.4	1.1		

ab,cd,ef P<0,001

The anti-oxidative properties of ascorbic acid lie in its strong reducing capacities. An excessive level of reduction may be, however, the reason for generation of excessive quantities of reduced metal ions in the organism, which may stimulate the Fenton reaction, and by this, synthesis of the most aggressive hydroxyl radical (Levine et al., 1998). Generation of the hydroxyl radical leads to degradation of most cellular structures, including DNA (Halliwell, 1996). A considerable quantity of necrotic hepatocytes in those rats receiving 0.3 and 0.9% ascorbic acid in the mixtures, as observed in the present experiment, is undoubtedly evidence of cell degradation caused by the administered vitamin C. The pro-oxidative effect of vitamin C has been demonstrated *in vitro* (Carr and Frei, 1999). However, according to some authors, megadoses of vitamin C have been used in the treatment of cancer. The investigations of Shamaan et al. (1998) showed that numerous neoplastic nodules, stimulated in the liver, seemed to decrease after vitamin C supplementation. The cited studies focused on the decrease in the number of two-nuclei cells and increase of necrotic cells in the liver after supplementation of 0.9% ascorbic acid. It seems that the pro-oxidative properties of vitamin C observed in healthy rats might be beneficial in cancer. This type of action of ascorbate may explain the anticancer properties of vitamin C. The obtained results concerned, however, healthy rats; the effect of vitamin C may probably cause neoplastic cell damage.

Vitamin C at a 0.6% level did not result in degradation of hepatocytes, which supports previous results obtained by authors who found that mixtures supplemented with 0.6% vitamin C did not increase the serum level of L-ascorbic

acid or total antioxidant status in comparison with 0.3 and 0.9% vitamin C in the mixtures (Niemiec and Sawosz, 2004).

CONCLUSIONS

It may be concluded that the results obtained in our experiments show that L-ascorbic acid supplemented to mixtures at a level of 0.9% caused necrotic damage to liver cells.

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STRESZCZENIE

Wpływ dodatku megadawek kwasu L-askorbinowego do diety na morfologię komórek wątroby szczurów

Celem podjętych badań była ocena wpływu diety zawierającej megadawkę kwasu L-askorbinowego na morfologię wątroby szczurów. Doświadczenie przeprowadzono na 40 samcach szczurach rasy Wistar. Zwierzęta podzielono na 4 grupy i żywiono mieszankami, które różniły się zawartością kwasu L-askorbinowego w diecie (w %): I - 0,0; II - 0,3; III - 0,6 i IV-0,9. Na podstawie uzyskanych wyników stwierdzono, że dodatek kwasu L-askorbinowego do mieszanki (0,9%) wywołał uszkodzenia nekrotyczne komórek wątroby.