

Effect of high doses of L-ascorbic acid on the antioxidative/oxidative state in the rats

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ABSTRACT

The objective of this study was to determine the effects of mega-doses of vitamin C (0.3, 0.6 and 0.9% of diet) as a dietary supplement for rats on selected indices of the antioxidative/oxidative state in 40 growing Wistar rats (4×10). It was found that L-ascorbic acid and Total Antioxidative State (TAS) in plasma did not increase with increasing vitamin C supply. The results indicate that high doses of L-ascorbic acid (0.3 and 0.9 but not 0.6%) increased the concentration of this antioxidant in plasma. Supplementation of vitamin C above 0.3% to the diets had pro-oxidative effects on lipid structures, while application of 0.9% promoted oxidative degradation of rat livers.

KEY WORDS: ascorbic acid, antioxidative/oxidative state, rats

INTRODUCTION

Ascorbic acid is an important micromolecule for animals and humans, as it is involved in many biological mechanisms. It is a powerful antioxidant acting both directly *via* scavenging of reactive oxygen species and indirectly through regeneration of other antioxidant systems. Paradoxically, under certain conditions (low concentration, presence of metal ions) it can exert a pro-oxidant effect, increasing oxidative damage of lipids, proteins and DNA (Auroma et al., 1999). The objective of this study was to determine effects of high doses of vitamin C on antioxidative/oxidative indicators of redox homeostasis in growing rats.

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MATERIAL AND METHODS

Forty growing male Wistar rats (235 g body weight) were divided into 4 groups \times 10 animals and were kept in individual cages for 41 days under standard conditions: temperature 22°C, humidity 50-70%, light/dark 12/12 h. The rats received a semi-purified mixture, containing (in %): maize starch, 62, casein, 14, saccharose, 10, cellulose, 5, rapeseed oil, 5, mineral and vitamin mix., 3.7, L-cystine, 0.2 and chloride choline, 0.1. The mixture was supplemented with 0 (control group), 0.3, 0.6 and 0.9% of L-ascorbic acid.

Vitamin C, mineral and vitamin mixtures were obtained from ICN Biomedicals. At the end of the experiment the rats were fasted for 12 h, sedated by intramuscular ketamine at a dose of 50 mg/kg body weight. Blood was sampled from the heart into heparinized tubes, cooled to 4°C and analysed. The animals were euthanized by ketamine overdose and then the livers were removed. Determination of ascorbate in plasma was performed by HPLC-UV detection. Total Antioxidative State (TAS) in plasma, activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the red blood cells were analysed by Randox Laboratories Ltd. Kits in a COBAS FARA II analyser. 8-oxo-2'-deoxyguanosine (8-oxo-dG) in livers was determined using HPLC with an electrochemical and UV detector. Thiobarbituric acid reactive substances (TBARS) were measured by a colorimetric method using 1.2.3.3.-tetraethoxypropane (TEP) as the standard.

The results were analysed by monofactorial ANOVA and Duncan's multiple range test, using the Statgraphic 4.1 Plus software package.

RESULTS AND DISCUSSION

The concentration of C vitamin in blood serum of rats receiving diets with 0.3 and 0.9% of C vitamin was higher than in the control and 0.6% groups (Table 1). It has been demonstrated that C vitamin stimulates antioxidant potential (El-Missiry, 1999) and decreases the concentration of the final products of the peroxidation of cell structures (Bano and Parihar, 1997; Chen et al., 2000). The addition to a diet of 0.3% of C vitamin increased the concentration of TAS in plasma, which is in agreement with measurements on growing pigs (Sawosz, 1999). It may be considered that the addition of 0.3% of C vitamin to a diet can influence a reductive potential of plasma. Furthermore, it can be expected that the addition of 0.6% of C vitamin can activate a feedback mechanism of ascorbic acid biosynthesis in rat hepatocytes (Banhegyi et al., 1997) causing decreasing concentration of C vitamin and a lower level of TAS in serum. However, the elevation of C vitamin up to 0.9 % did not increase the TAS level.

Table 1. Concentration of ascorbic acid, TAS, TBARS in the plasma; SOD, GPx activity in red blood cells and concentration of 8-oxo-2'-deoxyguanosine in the liver of rats

Parameter	Dietary ascorbate, %			SEM	P	
	0.3	0.6	0.9			
Ascorbic acid, $\mu\text{mol/L}$	26.223 ^a	35.952 ^b	30.429 ^a	53.530 ^c	2.905	<0.001
TAS ¹ , mmol/L	0.610 ^b	0.816 ^c	0.482 ^a	0.658 ^b	0.034	<0.001
SOD ² , U/gHb	1947.72	1619.74	2006.52	1833.32	312.2	NS
GPx ³ , U/gHb	20.96	18.93	20.09	22.70	1.337	NS
TBARS ⁴ , $\mu\text{mol/L}$	2.838 ^a	3.245 ^b	3.439 ^b	3.226 ^b	0.081	<0.001
8-oxo-2'-deoxy guanosine, 8-oxodG/10 ⁶ dG	4.386 ^a	4.898 ^{ab}	4.040 ^a	5.888 ^b	0.343	0.009

^{a,b,c} means with different superscripts are significantly different ($P < 0.05$)

¹ total antioxidant status, ²superoxide dismutase, ³glutathione peroxidase, ⁴tiobarbituric acid reactive substances

An increase in the products of lipid peroxidation, evaluated on the basis of TBARS level, may suggest that C vitamin added above 0.3% of a diet promotes oxidative lipid degradation (Table 1). Furthermore, Nyssonen et al. (1997) demonstrated that supplementing 3 g C vitamin/kg body weight for smokers increased the concentration of TBARS in plasma. In the present investigation, C vitamin was administrated to animals that were not being stressed and were able to synthesize it and, consequently, high doses of C vitamin could tip the redox balance to the side of oxidative reactions.

Lipid peroxidation may be activated by numerous reactive oxygen species in contrast to DNA oxidation, which is less prone to reactive oxygen species. The antioxidative defence system effectively protected DNA from oxidation when 0.3 and 0.6% C vitamin were added (Table 1). However, supplementation of the diet with 0.9% led to oxidative DNA damage in the liver of rats.

The pro-oxidative capabilities of ascorbic acid have been demonstrated by measuring the content of 8-oxo-2'-deoxyguanosine (Podmore et al., 1998). A DNA adduct, 8-oxo-2-deoxyguanosine, is formed as the result of guanine being attacked by hydroxyl radicals or singlet oxygen. Vitamin C not only scavenges free radicals, but also reduces redox-active transition metal ions (Fe, Cu) and can be involved in generation of hydroxyl radicals via the Fenton reaction *in vitro* (Halliwell, 1999).

Vitamin C is thus a very active and rapidly penetrating compound, and when administrated at high doses may promote synthesis and generation of carcinogenic substances -TBARS and DNA adducts.

CONCLUSIONS

High doses of L-ascorbic acid (0.3, 0.6 and 0.9%) in diets for rats increased the level of final products of lipid peroxidation. Although supplementation of 0.3%

of C vitamin stimulated the total antioxidant state in serum, the diet with 0.9% caused oxidative degradation of DNA in the liver of rats.

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STRESZCZENIE

Wpływ megadawek kwasu L-askorbinowego na stan antyoksydacyjno/oksydacyjny u szczurów

Celem pracy było określenie wpływu megadawek witaminy C, podawanych w ilościach 0,3; 0,6 i 0,9% mieszanki, na stan antyoksydacyjno/oksydacyjny szczurów. Badania przeprowadzono na 40 szczurach Wistar (4×10 sztuk). Stwierdzono, że poziom kwasu L-askorbinowego oraz całkowity potencjał antyoksydacyjny (TAS) w osoczu nie zwiększał się proporcjonalnie do ilości dodawanej do mieszanki witaminy C. Dodatek kwasu L-askorbinowego do diety (0,3 i 0,9% vs 0,0 i 0,6%) spowodował zwiększenie jego stężenia w osoczu szczurów. Dodatek do diety 0,3% witaminy C działał prooksydacyjnie w stosunku do struktur lipidowych organizmu, a zastosowanie witaminy C w ilości 0,9% wpłynęło na oksydacyjną degradację DNA wątroby szczurów.