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Interactions Between Hepatic Ascorbic Acid, Cytochrome P-450 and Lipids in Female Guinea Pigs with Different Ascorbic Acid Intake

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Summary

Changes in serum and liver lipids, hepatic ascorbic acid (AA) and cytochrome P-450 were investigated in female guinea pigs divided into three groups with different AA intake in drinking water (10, 100 and 1000 mg AA per liter) for 10 weeks. Serum and liver total cholesterol significantly decreased in guinea pigs receiving 100 and 1000 mg AA per liter of drinking water when compared with guinea pigs with suboptimal AA intake (10 mg/l). Similarly, serum triglycerides were decreased in the groups with higher AA intake. Liver AA concentration increased significantly in accordance with rising AA doses. High AA intake (1000 mg/l) for 10 weeks resulted in significant increase of both cytochromes P-450 and cytochrome b_5 and total haeme content in liver microsomes when compared to guinea pigs with suboptimal AA intake. A significant positive correlation between hepatic AA concentration and cytochrome P-450 content was observed. A close negative correlation between liver total cholesterol and cytochrome P-450 content in hepatic microsomes was also seen. Long-term suboptimal AA intake may unfavourably alter the blood and liver lipid profile as well as the capacity of hepatic drug metabolizing enzymes in both male and female guinea pigs.

Key words

Ascorbic acid – Cytochrome P-450 – Cholesterol – Guinea pigs

Introduction

The effects of ascorbic acid on lipid metabolism have been extensively studied in experimental animals (Ginter 1975, Kothari and Sharma 1988), as well as in human subjects (Ginter *et al.* 1977, Heine and Norden 1979). The relationships between vitamin C and cholesterol metabolism, which is believed to be one of the major risk factors for the development of cardiovascular diseases, are well documented. In guinea pigs which, like humans, do not synthetize ascorbic acid, AA-deficiency increased serum and tissue cholesterol and triglyceride levels (Ginter 1975, Jenkins 1980). Vitamin C stimulates the degradation of cholesterol by the activation of hepatic microsomal cytochrome P-450-dependent enzyme, cholesterol-7-α-hydroxylase, the rate limiting enzyme in the catabolism of cholesterol to bile acids (Ginter 1975, Björkhem and Kallner 1976). Ascorbic acid is also known to stimulate the activity of other hepatic cytochrome P-450-dependent monooxygenases, which catalyze a broad spectrum of oxidation reactions of xenobiotics and endogenous substrates (Sato and Zannoni 1974). On the other hand, AA deficiency decreases in liver microsomes of guinea pigs some specific forms of cytochrome P-450, which is the most important haemoprotein involved in the biotransformation of xenobiotics. A concomitant decrease in the activities of some monooxygenases is also observed (Zannoni *et al.* 1972, Rikans 1982, Kanazawa *et al.* 1991). AA can moderate in both experimental animals and man the toxic effects of many environmental pollutants such as cadmium, chromium, polychlorinated biphenyls etc. (Kato *et al.* 1977, Nagyová *et al.* 1994).

The aim of this study was to assess how different doses of AA can affect the interactions between serum and liver lipids and hepatic cytochrome

P-450 in guinea pigs. A special respect was paid to females, in which those relations may be influenced by sex-dependent factors.

Materials and Methods

Tricoloured female guinea pigs (Velaz, Prague) with an initial weight of 470–520 g were housed under standard laboratory conditions at 25 °C. During a two-week adaptation period, the animals were fed a standard laboratory diet for guinea pigs (Mok Velaz, Prague) with the addition of vegetables. Two weeks later, the animals were fed an ascorbic acid-free diet: sugar 100 g/kg, oat flakes 490 g/kg, milk powder 300 g/kg, butter 100 g/kg and salt 10 g/kg. The guinea pigs were then divided into three groups (12 animals in each group) according to the intake of ascorbic acid (Farmacon, Olomouc) in the drinking water. The first group, receiving 10 mg of AA per liter, was considered as the group with suboptimal AA intake. The second group, receiving 100 mg of AA per liter of drinking water, was considered as the group with adequate AA intake. The third group, receiving 1000 mg AA per liter, was considered as AA-saturated group. In all groups AA was administered for 10 weeks. Access to water and diet was *ad libitum* and the animals were weighed weekly. After overnight fasting, the guinea pigs were killed by decapitation and the livers were quickly removed and weighed. One part of the liver was extracted with chloroform:methanol 2:1 (v/v), and cholesterol was measured following the method of Zlatkis *et al.* (1953). Serum cholesterol and triglycerides were assayed by clinical commercial kits (Bio-Lachema, Brno). Ascorbic acid in the liver was determined by the dinitrophenylhydrazine method (Roe and Kuether 1943). Another part of the liver was chilled and homogenized in ice-cold 0.15 M KCl using a Potter-Elvehjem glass homogenizer with a teflon pestle. The 20 % (w/v) homogenate was centrifuged at 12 000 x *g* for 15 min and the liver microsomes were obtained by centrifugation of the supernatant fraction at 100 000 x *g* for 60 min. The microsomal pelet was resuspended in 100 mM Tris buffer pH 7.4, containing 1 mM EDTA and 30 % glycerol, and stored at −80 °C before use. In liver microsomes, protein and haeme concentrations (Lowry *et al.* 1951, Paul *et al.* 1953) as well as total cytochrome P-450 and cytochrome b_5 content (Omura and Sato 1964) were determined. For assessing cytochromes P-450 and b_5 a dual-wavelength spectrophotometer (UV/VIS Pye Unicam SP 8-100) was used.

The results, presented as means ± S.E.M, were statistically analyzed by analysis of variance (ANOVA) and regression analysis (Statgraphic). The minimal acceptable level of significance was $P < 0.05$.

Results

The body weight of guinea pigs in all groups increased during the experiment, but there was no significant influence of the different AA intake on the body weight (Fig. 1).

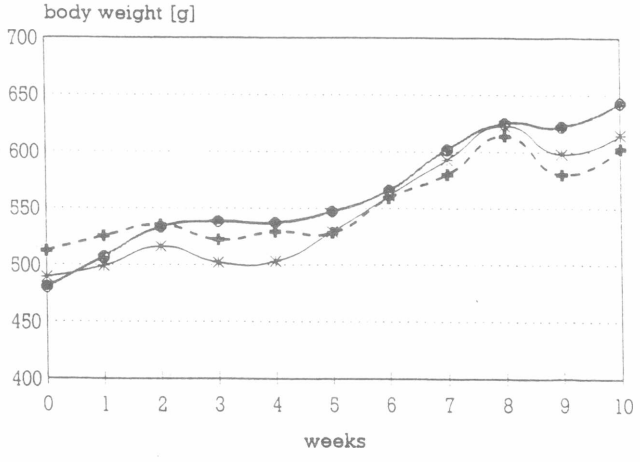

Fig 1
Body weight of female guinea pigs with different ascorbic acid intake: 10, 100 and 1000 mg/l drinking water.

Table 1 summarizes the effects of increasing doses of AA on serum and liver lipids. Serum and liver cholesterol levels significantly decreased in the groups receiving 100 and 1000 mg of AA per liter of drinking water for 10 weeks. Serum triglycerides also decreased in these groups (by 23% and 19%), but the effect was not significant. Ascorbic acid levels in the liver significantly increased in accordance with rising AA doses.

Table 2 summarizes the effects of increasing doses of AA on hepatic haeme and haemoprotein content. Microsomal haeme content was significantly higher in the groups with increased AA intake. The content of hepatic total cytochrome P-450 increased significantly (by 58 % and 76 %) with rising AA doses. The intake of 1000 mg of AA per liter of drinking water also significantly increased the content of cytochrome b_5 in liver microsomes (by 21 %). A close positive correlation between cytochrome P-450 and AA concentration in the liver (Fig. 2) as well as a negative correlation between cytochrome P-450 and total cholesterol in the liver (Fig. 3) were found.

Table 1
Serum and liver lipids and ascorbic acid levels in female guinea pigs with different ascorbic acid intake

	Ascorbic acid intake (mg per liter of drinking water)		
	10 mg	100 mg	1000 mg
Cholesterol			
Serum (mmol/l)	10.08 ± 1.11^{a}	7.51 ± 0.74^{b}	8.44 ± 0.61^{b}
Liver (mmol/kg)	22.30 ± 2.80^{a}	14.90 ± 1.30^{b}	13.40 ± 1.20^{b}
Triglycerides			
Serum (mmol/l)	2.01 ± 0.23^{a}	1.54 ± 0.11^{a}	1.62 ± 0.17^{a}
Ascorbic acid liver (mol/kg)	0.074 ± 0.015^{a}	0.294 ± 0.033^{b}	0.991 ± 0.067^{c}

Values are means ± S.E.M. for 12 animals per group. ^{a,b,c} *Different superscripts indicate significantly different means ($P < 0.05$) in the respective line*

Table 2
Haeme and haemoprotein concentrations in hepatic microsomes of female guinea pigs with different AA intake

	Ascorbic acid intake (mg AA per liter of drinking water)		
	10 mg	100 mg	1000 mg
Proteins (mg/g liver)	20.30 ± 1.30^{a} (12)	22.50 ± 0.90^{a} (12)	20.50 ± 1.00^{a} (12)
Haeme (nmol/mg protein)	1.18 ± 0.10^{a} (11)	$1.37 \pm 0.07^{a,b}$ (10)	1.50 ± 0.06^{b} (11)
Cytochrome P-450 (nmol/mg protein)	0.38 ± 0.04^{a} (11)	0.60 ± 0.05^{b} (10)	0.67 ± 0.04^{b} (12)
Cytochrome b_5 (nmol/mg protein)	0.28 ± 0.02^{a} (12)	0.26 ± 0.02^{a} (12)	0.34 ± 0.01^{b} (12)

Values are means ± S.E.M. The numbers in parenthesis represent the number of animals. ^{a,b} *Different superscripts indicate significantly different means ($P < 0.05$) in the respective line*

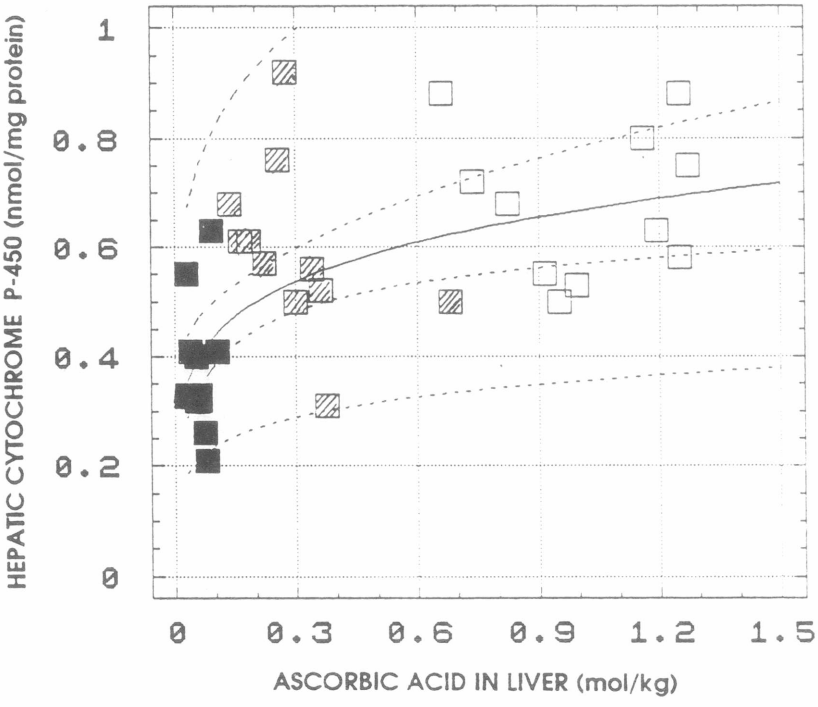

Fig 2
Correlation between hepatic cytochrome P-450 and ascorbic acid levels in the liver of guinea pigs with different AA intake. 10, 100 and 1000 mg/l drinking water. Multiplicative model: r = 0.610, P = 0.0002).

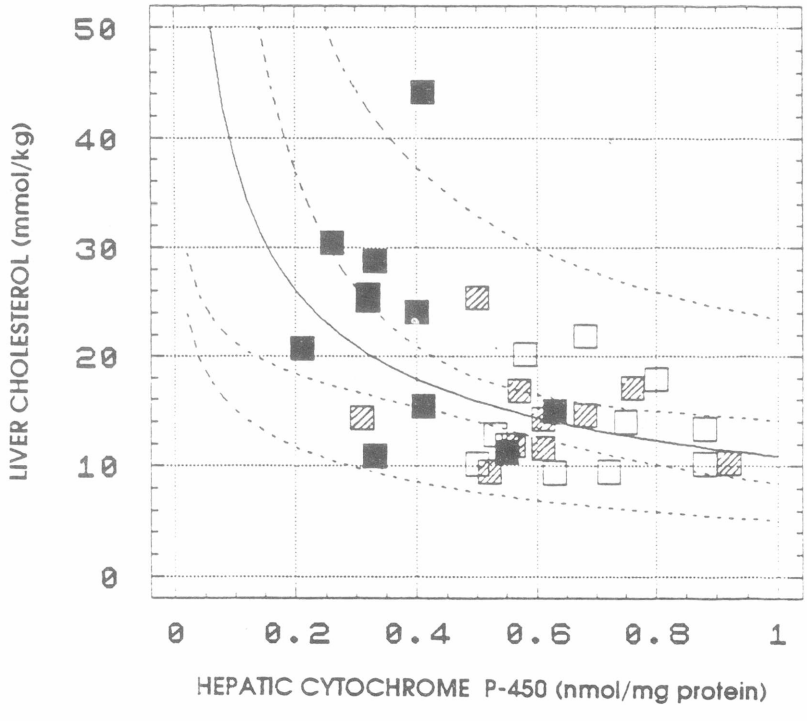

Fig 3
Correlation between hepatic cytochrome P-450 and liver cholesterol levels of guinea pigs with different AA intake. 10, 100 and 1000 mg/l drinking water. Multiplicative model: r = −0.500, P = 0.003).

Discussion

The principal organ for the synthesis and elimination of lipoproteins circulating in the plasma is the liver. The liver is also the main site in the body where the detoxication of xenobiotics, catalyzed by microsomal cytochrome P-450 dependent enzymes, occurs. Experimental studies have shown that higher hepatic microsomal enzyme activity may favourably affect the lipoprotein fractions which are characteristic for a low coronary risk (HDL cholesterol : total cholesterol, apolipoprotein A-I : apolipoprotein B ratios). Phenobarbital, a typical inducer of microsomal enzyme activities, retards cholesterol accumulation in the arterial wall and the formation of atherosclerotic plaque. It was concluded that the activation of liver

microsomal function can prevent atherogenesis in man (Luoma 1988). The same can be true for other naturally occuring compounds, which influence hepatic microsomal function and in this way may improve the serum lipoprotein profile. One the these compounds is ascorbic acid.

In guinea pigs, the liver AA levels affect the cholesterol catabolism. AA deficiency causes accumulation of cholesterol in the liver, hypercholesterolemia and the decrease of hepatic microsomal cholesterol-7-α-hydroxylase activity, the rate-limiting enzyme in the catabolism of cholesterol to bile acid (Ginter 1975, Björkhem and Kallner 1976). Multiple forms of cytochrome P-450 are involved in the activity of this enzyme. AA-supplementation decreases atherogenic low density lipoprotein (LDL)-cholesterol and favourably influences high density lipoproteins (HDL). The effect of vitamin C on HDL is probably also mediated by the cytochrome P-450 system.

Hypercholesterolemia and hypertriglyceridemia as well as cholesterol accumulation in the liver was also observed in female guinea pigs with suboptimal AA intake in this study. The cholesterol and triglyceride levels evidently decreased in AA-supplemented guinea pigs. Similar results have been reported for AA-deficient guinea pigs by other authors (Ginter 1975, Kothari and Sharma 1988).

Many experiments have been carried out to determine the mechanism by which limited intake of vitamin C decreases hepatic cytochrome P-450 content. Both, haeme synthesis and haeme degradation enzymes found to be were changed in AA-deficiency (Omaye and Turnbull 1980, Walsch and Degkwitz 1980). Since AA is an effective antioxidant *in vivo*, the possible involvement of lipid peroxidation in this phenomenon was also investigated (Tatara and Ginter 1994). Nevertheless, the results of Mori *et al.* (1992) suggested that there is mechanism(s) other than lipid peroxidation involved in the reduction of cytochrome P-450 content by AA-deficiency. In this study, total haeme content in liver microsomes of guinea pigs with suboptimal AA intake was significantly lower than in AA-saturated animals. The decreases correlate well with lowered cytochrome P-450 content ($r=0.558$, $P=0.0014$) and support the view about the stimulatory effect of AA on haeme and cytochrome P-450 synthesis.

It has been repeatedly proven that vitamin C deficiency markedly decreases the activity of cytochrome P-450 dependent monooxygenases and the ability to metabolize many xenobiotics in liver microsomes. In the case of a mild or marginal vitamin C deficiency, the level of total cytochrome P-450 does not change in some animals, but at the same time, the activity of several hydroxylation enzymes decreases. It is possible that a moderate vitamin C deficiency selectively influences only a small part of isoenzymes of cytochrome P-450. As a result, the activity of some monooxygenases decreases without more significant change in the concentration of total cytochrome P-450. It should also be noted that excessive doses of AA diminished the content of cytochrome P-450 and the activities of monooxygenases (Sutton *et al.* 1982, Ginter *et al.* 1984). Our results show that total hepatic cytochrome P-450 and cytochrome b_5 content gradually increased with rising of AA intake and its concentration in the liver. The increase of cytochrome P-450 in hepatic microsomes from AA saturated guinea pigs represents almost 80% of the values observed in the group with suboptimal AA intake.

The capacity of the liver to metabolize xenobiotics and endogenous substrates is sex-dependent and hence may change the serum and liver lipoprotein profile. The physiological significance of hepatic cytochrome P-450 in cholesterol metabolism was also confirmed in this study using female guinea pigs with different AA intake. When compared to our previous studies on male guinea pigs (Ginter *et al.* 1984), we concluded that the serum and liver lipid profile correlated with cytochrome P-450 content and was not sex-dependent, neither in guinea pigs with limited AA intake nor in AA-saturated guinea pigs.

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