THE EFFECT OF ASCORBIC ACID ON LIVER AND PLASMA CHOLESTEROL LEVELS OF MALE RATS

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SUMMARY : The possible hypocholesterolemic effect of ascorbic acid feeding in mature and immature (up to one month old) male rats was investigated by the liver and the plasma cholesterol level determination together with the ascorbic acid concentration in plasma. The feeding period was six weeks. The mature and the immature rats were divided into three feeding groups. The first group of rats were exposed to regular feeding (the control group). The second group was given a diet consisting of 0.2 g ascorbic acid/100 ml of water whereas the third group was fed with 0.5 g ascorbic acid/100 ml of water. It was observed that, both ascorbic acid concentrations lowered the plasma and the liver cholesterol levels significantly ($p<0.005$) whereas in mature rats only the plasma cholesterol levels were significantly lowered ($p<0.0005$).

Key Words : Ascorbic acid, cholesterol, liver, plasma, lipid metabolism.

INTRODUCTION

The distribution of cholesterol between the various fractions of plasma lipoproteins is important from the aspect of atherogenesis (2). Cholesterol in low-density lipoproteins is considered to be atherogenic, while cholesterol in high density lipoproteins is credited with a protective action against the onset of atherosclerotic lesions. Even a short-term ascorbate deficiency provokes changes in the lipid and apoprotein composition of plasma lipoprotein in guinea pigs, which results in a rise of the atherogenic index of the blood serum (3,4). The cholesterol accumulation in the blood and the tissues may be caused by a change in the rate of a number of processes: a. Altered cholesterol distribution between plasma and the tissues, b. Enhanced absorption of exogenous cholesterol from the diet, c. Enhanced synthesis of endogenous cholesterol, d. Decreased cholesterol excretion in the form of neutral sterols, e. Decreased cholesterol transformation to bile acids.

The oxidation of cholesterol to bile acids is dependent on the ascorbic acid status, but it can not be further stimulated by ascorbic acid when the animals are already on an adequate intake of the vitamin (4,7). It was reported that vitamin C is necessary for cholesterol transformation to bile acids at the rate limiting steps of bile acid biosynthesis. When double hydroxylation at C7 and C26 takes place, cholesterol is changed into the principle bile, which is chenodeoxycholic acid. The ascorbic acid does not affect the hydroxylation of the C26 of the cholesterol side chain. Vitamin C is important for the hydroxylation of the cholesterol nucleus on C7. This reaction is catalyzed by the cholesterol 7-α-hydroxylase and requires oxygen, NADPH, cytochrome p-450 and vitamin C. This reaction is inhibited by latent ascorbic acid deficiency and this leads to high concentration of cholesterol in the liver and the plasma (6,7).

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The aim of the present experiment was to study the effects of two different vitamin C concentrations on the plasma and the liver cholesterol levels in rats capable of synthesizing ascorbate. The influence of age was also studied.

MATERIALS AND METHODS

The following experiments were carried out by 30 male albino rats (15 mature rats and 15 immature rats) weighing 300-350 g. There was an insignificant weight increase among the animals of vitamin C supplemented groups. The animals were fed on a regular solid diet. Supplemented vitamin C feeding was applied through their drinking water which was given ad libitum. 5 rats in each age group were taken as the control group, 5 rats were fed with 0.2 g ascorbic acid/100 ml of water and 5 rats were fed with 0.5 g ascorbic acid/100 ml for 6 weeks. The ascorbate solutions were prepared immediately before use each day. The animals were kept at the animal house in proper cages at RT. The animal room was maintained on a 12-hour light dark cycle (light on at 700). The animals were sacrificed at the end of each test period and the blood and the liver were removed immediately and used freshly. The determinations of ascorbic acid (5) and cholesterol in plasma (8), as well as the determination of cholesterol in liver (8) were carried out. The absorbances were read in a LKB Ultra-spectrophotometer M4050.

Table 1: The concentrations of Ascorbic Acid in plasma and the cholesterol levels in plasma and the liver of immature control rats.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.050	1.39	0.30
2	0.031	2.20	0.32
3	0.023	2.60	0.42
4	0.041	1.28	0.28
5	0.057	1.80	0.41
$\bar{x}$	0.0405	1.795	0.347
S.D.	0.01344	0.5727	0.06429

RESULTS AND DISCUSSION

The measured concentrations of ascorbic acid in plasma and the cholesterol levels in plasma and the liver of the three different groups of the immature rats (Tables 1-3) and the mature rats (Tables 4-6) are given. The statistical evaluations of the experimental data among all the groups is given in Table 7.

It was previously reported that the ascorbate levels in the tissues of animals synthesizing L-ascorbic acid are saturated, and they are only slightly influenced by exogenous vitamin C. Therefore the effect of ascorbate on cholesterol metabolism in such animals is small (2). During the present study, the liver cholesterol levels in mature rats were not affected with excess vitamin C feeding whereas the plasma cholesterol levels significantly decreased (Table 7).

Table 2: The three measured concentrations in the group of immature rats fed by 0.2 g ascorbic acid/100 ml water.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.039	0.84	0.16
2	0.036	0.82	0.22
3	0.052	1.20	0.24
4	0.053	1.05	0.23
5	0.048	1.15	0.22
$\bar{x}$	0.0456	1.012	0.214
S.D.	$7.7x10^{-3}$	0.17484	0.0313

Table 3: The three measured concentrations in the group of immature rats fed by 0.5 g ascorbic acid/100 ml water.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.050	1.05	0.20
2	0.055	0.65	0.30
3	0.067	0.25	0.15
4	0.050	0.83	0.29
5	0.104	1.00	0.17
$\bar{x}$	0.0652	0.756	0.222
S.D.	0.02037	0.32354	0.0691

These findings correlate with the results of other investigators (2). However, in immature rats both the plasma and the liver cholesterol levels decreased significantly with excess vitamin C feeding (Table 7). Therefore, it can be

Table 4: The concentrations of ascorbic acid in plasma and the liver of mature control rats.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.050	0.85	0.804
2	0.069	0.60	0.482
3	0.055	0.70	0.536
4	0.048	0.85	0.780
5	0.068	0.59	0.434
$\bar{x}$	0.058	0.72	0.607
S.D.	9.85×10^{-3}	0.126	0.172

Table 5: The three measured concentrations in the group of mature rats fed by 0.2 ascorbic acid/100 ml water.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.09	0.700	0.670
2	0.08	0.375	0.536
3	0.10	0.360	0.407
4	0.16	0.360	0.375
5	0.07	0.725	0.771
$\bar{x}$	0.10	0.504	0.552
S.D.	0.3536	0.191	0.169

Table 6: The three measured concentrations in the group of mature rats fed by 0.5 ascorbic acid/100 ml water.

Rat No.	Plasma Ascorbic Acid (mg/100 ml)	Plasma Cholesterol (mg/100 ml)	Liver Cholesterol (mg/1.5 g)
1	0.093	0.625	0.402
2	0.109	0.500	0.500
3	0.103	0.625	0.600
4	0.114	0.375	0.375
5	0.140	0.350	0.360
$\bar{x}$	0.112	0.495	0.474
S.D.	0.0176	0.132	0.112

concluded that the tissue vitamin C saturation in young animals is not attained before they mature. In this study, the urine excretion of vitamin C was not checked. However, a significant increase in excretion is not expected with increasing doses of vitamin C up to the tissue saturation levels (1).

According to the results presented in Tables 1, 2, 3, 4, 5 and 6, the following conclusions can be derived:

1. For all the study groups, the immature rats had less plasma ascorbic acid concentration compared to the mature rats. This difference was observed to be much more pronounced for the groups on ascorbic acid diet.

2. For all the study groups, the immature rats showed much higher concentration of cholesterol in plasma than those of the mature rats. Whereas their liver cholesterol levels were found to be much less than those of the mature rats.

3. For the mature rats, it was observed that as the ascorbic acid intake increased to 0.2 g/100 ml of water, than to 0.5 g/100 ml of water, the plasma ascorbic acid concentration increased, the plasma and the liver cholesterol concentrations decreased. The standard deviations in all these measured quantities declined in correlation to the increase in ascorbic acid intake level up to 0.5 g/100 ml. It can then be said that for the mature rats 0.5 g/100 ml ascorbic acid dose gives the lowest and the least varying cholesterol levels in both plasma and liver.

Table 7: The statistical data evaluation of the all experimental groups by Student's t-test.

		Plasma Ascorbic Acid	Plasma Cholesterol	Liver Cholesterol
Immature Rats	Control vs 0.2 g	N.S.	$P<0.01$	$P<0.005$
	Control vs 0.5 g	$P<0.05$	$P<0.005$	$P<0.01$
	0.2 g vs 0.5 g	N.S.	N.S.	N.S.
Mature Rats	Control vs 0.2 g	$p<0.02$	$p<0.0005$	N.S.
	Control vs 0.5 g	$p<0.0005$	$p<0.005$	N.S.
	0.2 g vs 0.5 g	N.S.	N.S.	N.S.

N.S. : Not significant

4. For the immature rats, it was observed that as the ascorbic acid intake increases to 0.2 g/100 ml, the cholesterol levels in plasma and in liver were substantially decreased and the concentration of ascorbic acid in plasma was increased whereas the standard deviations in

those quantities were all decreased. A further increase in the ascorbic acid intake to 0.5 g/100 ml caused further decrease in the cholesterol concentration in liver. The standard deviations in all these quantities were significantly increased, indicating that the optimum ascorbic acid intake level for the immature rats was 0.2 g/100 ml in contrast to the mature rats.

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