Nutritional Significance of Ascorbic Acid for the Metabolism of Xenobiotics and Cholesterol in ODS- and Conventional Rats

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I. Introduction

Nearly all species of animals synthesize L-ascorbic acid (AsA, vitamin C) and do not require it as an essential nutrient. However, humans and other primates, guinea pigs, Indian fruit-bats, and a certain species of bird cannot synthesize the vitamin because they are deficient in L-gulono-γ-lactone oxidase (GLO, EC1.1.3.8). Guinea pigs are generally used as an experimental animal for examining the physiological significance of AsA, although they are not as convenient as rats in size, feeding habits and nutritional background.

Makino and Katagiri (1) established a colony of mutant Wistar rat, that is ODS rat, with a hereditary defect in AsA-synthesizing ability that is controlled by a single autosomal recessive gene. The genotype of ODS rat is designated by the gene symbol od, and represented as od/od. The genotype of a mother strain of ODS rat is designated as +/+ . ODS(od/od) rat is maintained as a coisogenic strain of +/+ rat. The inability to synthesize AsA in ODS rats was traced to a deficiency of GLO in the liver (2).

We believe that ODS rat is valuable and convenient for examining the physiological significance of AsA. In this study, we examined (I) the requirement of dietary AsA in ODS rats, (II) the physiological role of AsA in drug metabolism in ODS rats, and (III) the effect of dietary AsA in cholesterol metabolism in ODS rats.

II. Requirement of dietary AsA in ODS rats (3)

In this study, rats were fed purified diet containing 30% casein, whose composition was described previously (3). The metabolic pathway of AsA biosynthesis, that is D-glucuronic acid pathway, is shown in Fig. 1. Firstly, hepatic activities of several enzymes involved in this pathway were measured in ODS rats or +/+ rats. ODS rats maintained the normal activities of UDPglucose dehydrogenase, UDPglucuronosyl transferase and β-glucuronidase as compared with those in +/+ rats (Fig. 2). However, no activity of GLO was detected in ODS rats. Fig. 3 show Northern blot analysis of hepatic GLO in ODS rat, od/+ (heterozygous) rat or +/+ rat. RNA from ODS rat liver contained GLO mRNA having the same size as that of the mRNA of +/+ rat. The levels of the RNAs detected in both od/od and +/+ rat were comparable to each other. RNA from od/+ rat liver also contained a comparable level of GLO mRNA. Nishikimi and his coworkers (4) previously reported the same result as that observed in this study. They speculated that there might be a mutation in GLO gene and that the mutation might not affect the transcriptional efficiency, the processing of the primary transcript, and the stability of the mRNA. These results indicate that ODS rat is unable to synthesize AsA due to the lack of GLO activity.

Fig. 1. D-Glucuronic acid pathway.
(1) UDPglucose dehydrogenase
(2) UDPglucuronosyl transferase
(3) β-Glucuronidase
(4) L-gulono-γ-lactone oxidase
The requirement of dietary AsA was examined in male ODS rats. The level of dietary AsA was 0, 50, 150 or 300 mg/kg diet, and experimental period was 20 days. +/- rats, that is a control group, were fed a AsA-free diet. Body weight gains are shown in Fig. 4. In the group fed an AsA-free diet, body weight began to decrease on day 12. Liver, spleen and adrenal levels of AsA increased as dietary AsA level increased in ODS rats. Hemorrhage in muscle and leg joints, lower content of hepatic cytochrome P-450 and lower activities of hepatic drug-metabolizing enzymes, higher serum and adrenal levels of corticosterone and lower urinary hydroxyproline were observed in AsA-deficient ODS rats than those in ODS rats fed a diet supplemented with 300mg AsA/kg diet or +/- rats fed an AsA-free diet. From these results, it is concluded that dietary addition of about 300mg AsA/kg diet is enough to prevent signs of AsA deficiency and to achieve maximum growth.

Fig. 4. The activities of hepatic UDPglucose dehydrogenase, UDPglucuronosyl transferase, β-glucuronidase and L-gulono-γ-lactone oxidase in ODS rats and +/- rats.

Fig. 3. Northern blot analysis of L-gulono-γ-lactone oxidase mRNA from livers of od/od, od/+ and +/- rats.

III. AsA requirement for the induction of hepatic drug-metabolizing enzymes (5)

AsA deficiency causes a decrease in hepatic activity of drug-metabolizing enzyme and a decrease in hepatic content of cytochrome (cyt.) P-450. The mechanism of lowering effect of AsA deficiency on hepatic content of cyt.P-450 is not clear.

Firstly, we examined the effect of AsA deficiency on hepatic levels of xenobiotics-inducible cyt.P-450's mRNA. We used polychlorinated biphenyls (PCB) as an inducer of cyt. P-450s. PCB markedly induces both phenobarbital (PB)-inducible cyt.P-450s and methylcholanthrene (MC)-inducible cyt.P-450s. Fig. 5 shows the effect of AsA deficiency on hepatic level of PB-inducible cyt.P-450s (cyt.P-450b and e) mRNA and on hepatic activity of PB-inducible drug-metabolizing enzyme (aminopyrine-N-demethylation). Hepatic level of cyt.P-450b+e mRNA and hepatic activity of aminopyrine-N-demethylase were lower in AsA deficiency than those in ODS rats fed a diet supplemented with 300mg AsA/kg diet. Fig. 6 shows the effect of AsA deficiency on hepatic level of MC-inducible cyt.P-450s (cyt.P-450c and d) mRNA and on hepatic activity of MC-inducible drug-metabolizing enzyme (benzo(a)pyrene hydroxylase). AsA deficiency affected neither the level of cyt.P-450c+d mRNA nor the activity of benzo(a)pyrene hydroxylase. From these results, it is concluded that AsA deficiency might decrease the content of PB-inducible cyt.P-450s due to the reduction of their mRNA in ODS rats.

As a next experiment, we examined the possibility that the dietary requirement of AsA might be increased by the administration of xenobiotics, such as PCB, for the maximum induction of hepatic drug metabolism. Dietary levels of AsA tested were 0, 300, and 3,000mg/kg diet in ODS rats.
Fig. 5. Effect of AsA deficiency on hepatic activity of aminopyrine-N-demethylase and hepatic level of Cyt.P-450b+e mRNA in ODS rats. Asterisk denotes a significant difference from the value of control group (p<0.05).

Fig. 6. Effect of AsA deficiency on hepatic activity of benzo(a)pyrene hydroxylase and hepatic level of cyt.P-450c+d mRNA in ODS rats.

Fig. 7. Effect of dietary levels of MA on hepatic activities of aniline hydroxylase and aminopyrine-N-demethylase, and hepatic content of Cyt.P-450 in ODS rats or +/+ rats. Asterisk denotes a significant difference from the value of control group (p<0.05). Shaded bars indicate the groups fed PCB. Bars not followed by the same superscript letter are significantly different (p<0.05).

+/- rats were fed a AsA-free diet as a control group. When ODS rats were fed diets without PCB, the supplementation of 300mg AsA/kg diet was sufficient to maintain a normal activity of hepatic aminopyrine-N-demethylase or aniline hydroxylase, and a normal level of hepatic Cyt.P-450 (Fig. 7). On the other hand, when ODS rats were fed diets with PCB (200mg/kg diet), significantly higher activity of aniline hydroxylase or aminopyrine-N-demethylase, and significantly higher level of Cyt.P-450 were observed in ODS rats fed a diet supplemented with 3,000mg/kg diet than those in rats fed a diet supplemented with 300mg/kg diet. It is concluded that the requirement of AsA is increased severalfold by the administration of xenobiotics for the maximum induction of hepatic drug metabolism.

IV. AsA and cholesterol metabolism in ODS rats (6,7)

Ginter and his coworkers (8) reported that AsA deficiency in guinea pigs caused a marked elevation of serum cholesterol, an accumulation of cholesterol in liver, and a decrease in conversion of cholesterol into bile acids. The rate-limiting enzyme in bile acid synthesis from cholesterol is cholesterol 7α-hydroxylase. This enzyme is one of the Cyt.P-450-family proteins.
Firstly, we examined the effect of AsA deficiency or excessive intake of AsA on cholesterol and bile acid metabolism in ODS rats fed a diet containing 2% cholesterol. AsA deficiency caused a higher level of serum cholesterol, a lower activity of hepatic cholesterol 7a-hydroxylase, and a lower excretion of fecal bile acids as compared with those in ODS rats fed a diet 300mg AsA/kg diet (Fig. 8). However, the intake of excessive amount of AsA (30,000mg AsA/kg diet) did not affect serum level of cholesterol, the activity of hepatic cholesterol 7a-hydroxylase or fecal excretion of bile acids. From these results, AsA deficiency might cause a hypercholesterolemia due to the depression of bile acid synthesis in ODS rats fed a cholesterol-containing diet.

In addition, we examined the effect of AsA deficiency on serum levels of HDL-, LDL-, VLDL- and chylomicron-cholesterol (9) in ODS rats fed a cholesterol-free diet. As shown in Fig. 9, higher level of cholesterol and higher level of LDL-cholesterol were observed in AsA-deficient rats as compared with those in control rats. AsA deficiency did not affect serum level of HDL-, VLDL- or chylomicron-cholesterol as compared those in control rats. In ODS rats fed a cholesterol-free diet, AsA deficiency might cause a hypercholesterolemia due to the elevation of the level of LDL-cholesterol.

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