Plasma concentration of vitamin C in dogs with a portosystemic shunt

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Abstract

Most mammals, including dogs, synthesize vitamin C in the liver. We measured the plasma concentration of vitamin C to assess the body vitamin C status in 15 dogs with a portosystemic shunt (PSS). The plasma biochemical parameters indicated liver abnormalities in all the dogs. In contrast, the plasma concentration of vitamin C ranged from 2.21 to 9.03 mg/L in the 15 dogs and was below the reference range (3.2 to 8.9 mg/L) in only 2 dogs. These findings suggest that vitamin C status is not impaired in dogs with PSS.

Résumé

La plupart des mammifères, incluant les chiens, synthétisent de la vitamine C dans leur foie. La concentration plasmatique de vitamine C a été mesurée afin d'évaluer le statut en vitamine C de l'organisme chez 15 chiens avec un shunt porto-systémique (PSS). Les paramètres biochimiques plasmatiques indiquaient des anomalies hépatiques chez tous les chiens. À l'opposé, la concentration plasmatique de vitamine C variait de 2,21 à 9,03 mg/L chez les 15 chiens et se situait sous l'écart de référence (3,2 à 8,9 mg/L) pour seulement 2 chiens. Ces résultats suggèrent que le statut en vitamine C n'est pas altéré chez les chiens avec PSS.

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Vitamin C (ascorbic acid + dehydroascorbic acid) is an essential dietary component for primates, guinea pigs, and flying mammals that lack L-gulono-γ-lactone oxidase, the final enzyme in the biosynthesis of ascorbic acid from glucose (1). Most mammals, including dogs, can synthesize enough ascorbic acid for their requirements, and ascorbic acid may be reversibly oxidized to dehydroascorbic acid in the body (2), indicating that it is a dispensable nutrient for these species. Ascorbic acid functions as a cofactor for enzymes involved in the biosynthesis of collagen, carnitine, and norepinephrine (3,4). In addition, it acts as a powerful antioxidant, quenching reactive oxygen species and reactive nitrogen species (5), as well as being the first line of defence against oxygen radicals in the water-soluble component (6). Furthermore, ingestion of a large quantity of dietary vitamin C has resulted in the improvement of neutrophil functions such as chemotaxis, phagocytosis, and superoxide production in patients with recurrent furunculosis and impaired neutrophil functions (7). Thus, vitamin C is crucial to preserving health and preventing disease in all mammals.

Generally, the plasma concentration of vitamin C is an indicator of the total body status of vitamin C. In fact, a negative relationship between vitamin C and 8-epiprostaglandin $F_{2\alpha}$ — one of the isoprostanes whose production is elevated by oxidative stress (8) — was detected in human plasma (9). In addition, responses to oxidative stress (DNA damage and accelerated production of carbonylated protein and malonaldehyde) were significantly smaller in humans with higher concentrations of plasma vitamin C (10). Furthermore, consumption of a diet high in hydrogenated fat resulted in increased oxidative stress and decreased plasma vitamin C in hamsters (11). Therefore, it is important to measure the plasma concentration of vitamin C to know the total vitamin C status of the animal.

Portosystemic shunts (PSSs) are abnormal anastomotic connections between the portal vein and other systemic veins. Congenital PSSs commonly occur in dogs, and the reduced functional hepatic mass may cause numerous clinical problems, including hepatic encephalopathy and abnormalities in erythrocyte volume and blood hemoglobin concentration (12,13). Because ascorbic acid is synthesized in the liver, impairment of body vitamin C status was considered to be likely in dogs with PSS. In fact, dogs have much lower rates of ascorbic acid production than other animals, such as cows, goats, rats, and rabbits (14). We surveyed the plasma concentration of vitamin C in dogs with a diagnosis of PSS.

Of all dogs referred from animal clinics to the Azabu University Veterinary Teaching Hospital from June 2003 to May 2005 because of clinical suspicion of an underlying PSS, 15 were included in this study. Clinical signs at the animal clinics are shown in Table I. Abdominal radiography, ultrasonography, and jejunal portography indicated that all the dogs had a single extrahepatic shunt. Four blood samples were collected from each animal by jugular vein puncture: 2 samples into heparinized tubes to measure the plasma concentration of vitamin C and biochemical parameters, including

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†Professor Hiroshi Fujise died October 10, 2004. We dedicate this paper to his memory.

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Table I. Clinical signs and concentrations of vitamin C and biochemical parameters in dogs with a portosystemic shunt (PSS)

Breed	Sex and age	Clinical signs	Body weight, kg	Concentration (and reference range[a]) Vitamin C[b] (3.2–8.9), mg/L	Total protein[b] (5.5–8.8), g/dL	Albumin[b] (2.5–3.5), g/dL	ALT[b] (0–100), U/L	ALP[b] (0–200), U/L	Total bile acid[c] (0–5), μmol/L	Ammonia[d] (0–68), μg/dL
Dachshund	M, 5 mo	Vomiting, sialorrhea	1.8	2.21	4.3	2.3	69	266	22.5	296
Dachshund	M, 5 mo	Malaise	3.0	5.96	4.2	2.7	373	1198	ND	627
Dachshund	M, 1 y	Inactivity	2.5	5.32	4.8	2.3	574	1391	25.6	543
Dachshund	F, 2 y	Vomiting	2.7	4.83	4.9	2.5	1166	1631	59.8	377
Maltese	M, 1 y	None	2.2	2.94	5.3	3.4	1678	158	79.4	74
Maltese	M, 3 y	Tremor	2.2	6.75	5.2	3.0	36	25	75.9	73
Maltese	F, 1 y	Sialorrhea, fugue	2.1	5.01	5.3	1.8	153	349	257.5	195
Maltese	F, 5 y	Dysbasia	2.8	6.19	5.5	3.0	79	83	ND	89
Papillon	M, 3 y	Staggering	2.9	4.39	4.9	2.5	449	261	84.3	356
Saluki	F, 1 y	Anorexia	12.6	6.42	4.8	2.1	51	60	32.5	508
Shih tzu	M, 4 mo	Unknown	2.2	6.58	4.9	2.3	652	348	80.1	118
Shih tzu	M, 7 y	None	4.8	5.04	5.4	2.5	106	636	89.9	321
Shih tzu	F, 2 y (spayed)	Sialorrhea, tremor	5.5	6.42	5.6	3.4	40	76	32.1	36
Yorkshire terrier	M, 1 y	None[e]	1.9	4.51	6.3	3.1	64	43	41.3	25
Yorkshire terrier	M, 3 y	Vomiting	2.7	9.03	5.8	3.1	54	31	10.8	40

ALT — alanine aminotransferase; ALP — alkaline phosphatase; M — male; F — female; ND — not determined

[a] On the equipment, except for vitamin C, for which the reference was the plasma ascorbic acid concentration as reported by Wang and colleagues (16) for various breeds of clinically normal dogs

[b] Plasma

[c] Serum

[d] Blood

[e] The dam had a PSS

total protein, albumin, alanine aminotransferase (ALT), and alkaline phosphatase (ALP); 1 sample into a tube without anticoagulant to measure the serum concentration of total bile acid; and 1 sample into a tube with ethylene diamine tetraacetic acid as an anticoagulant to measure the blood concentration of ammonia. The last 2 samples were obtained after the dogs had fasted.

For measurement of the plasma concentration of vitamin C, the plasma sample was mixed with dithioerythritol solution (final concentration 1.5 g/L) after centrifugation at 625 × *g* for 5 min at 4°C, followed by immediate freezing at −80°C until analysis within 48 h. The reduced plasma was thawed at room temperature and centrifuged at 2500 × *g* for 2 min at 4°C. The supernatant was passed through a 0.45-μm filter and the vitamin C content analyzed by high-performance liquid chromatography as described previously (15). The plasma levels of the biochemical parameters were analyzed by an automatic blood analyzer (COBAS INTEGRA 700; Roche Diagnostics, Tokyo, Japan) according to the manufacturer's protocol. The serum concentration of total bile acid was measured by the enzyme method with the use of 3α-hydroxysteroid dehydrogenase (Monolis, Tokyo). The blood concentration of ammonia was analyzed by an automatic blood analyzer (FDC100N; Fuji Film, Tokyo) according to the manufacturer's protocol.

Table I shows the plasma, serum, and blood concentrations of vitamin C and the biochemical parameters in the 15 dogs with PSS. The values for the parameters known to be altered in dogs with PSS were greater than the reference range in many of the dogs: the concentrations of ALT, a hepatocyte membrane leakage enzyme, and ALP, an inducible enzyme, were elevated in 8 of the 15 dogs; the concentration of total bile acid in serum was elevated in all 13 of the dogs in which it was measured; and the concentration of ammonia in blood was elevated in 12 of the 15 dogs. In fact, all the dogs exhibited elevation in the concentration of at least 1 parameter. These results are consistent with the diagnosis of a single extrahepatic shunt by abdominal radiography, ultrasonography, and jejunal portography (data not shown). In contrast, the plasma concentrations of total protein and albumin were below the reference range in 11 and 5 of the

15 dogs, respectively, which suggested reduced hepatic functional mass and reduced hepatic protein synthesis, respectively.

The plasma concentrations of vitamin C were within the reference range in 13 of the dogs and lower in only 2 dogs. The reference range had been determined by measuring the plasma ascorbic acid concentration in various breeds of clinically normal dogs (16). There are several methods for measuring the plasma concentration of vitamin C, which are based not only on colorimetric analysis but also on chromatographic analysis (2). Because the measured level is known to vary with the measurement method (16), caution is needed in comparing the data from different studies. Wang and colleagues (16) measured the plasma concentration of ascorbic acid using the HPLC-based method, whereas in this study we measured the plasma concentrations of both ascorbic acid and dehydroascorbic acid as vitamin C by the HPLC method after treating the plasma samples with a reducing reagent. Considering that most of the vitamin C in plasma exists as ascorbic acid (17), comparison of the plasma levels of vitamin C determined in the present study with the reference range suggested by Wang and colleagues (16) is reasonable. In fact, the plasma levels of vitamin C in apparently healthy Shiba dogs measured by the same method as we used were 4.8 to 9.2 mg/L (18).

There are 3 possible explanations for the normal vitamin C status in most of the dogs with PSS. First, vitamin C synthesis in the liver may not be decreased in dogs with liver dysfunction. High metabolic priority for the synthesis of vitamin C is suggested in cows: the measured plasma concentration of vitamin C did not differ between control cows and ketotic cows, in which glucose utilization is limited (19). It is likely that the remaining functional hepatocytes compensate in synthesizing vitamin C in dogs with PSS. Another possibility is that dogs with PSS may ingest adequate amounts of vitamin C in their diets. Although the content of commercial dog foods is not clearly defined, both commercial diets and prescription diets for dogs are often supplemented with vitamin C (M. Kaneko and Y. Asami, Nosan Corporation, Tsukuba, Japan: personal communication, 2005). A final possibility is that the hepatic dysfunction of these dogs was of insufficient magnitude to affect the production of vitamin C. In dogs with severe hepatic necrosis and chronic hepatic insufficiency induced by oral administration of dimethylnitrosamine, the plasma concentration of ascorbic acid was decreased (20).

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