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Pathobiodynamics: Reduced Ascorbate Excretion by Rats with Severe Inflammation (39807)

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Drug oxidation and conjugation with glucuronic acid by liver microsomal enzymes may be profoundly subnormal in rats with adjuvant-induced arthritis or severe acute local inflammation (1-3).[3]

Ascorbic acid (vitamin C) is a major metabolite of D-glucuronic acid in many animals, including the rat. Its synthesis involves at least one hepatic microsomal enzyme, L-gulonolactone oxidase (4). Urinary ascorbate excretion is enhanced considerably by administration of drugs and chemicals that induce hepatic microsomal enzymes (5, 6). Guinea pigs (which cannot synthesize ascorbic acid) may require this vitamin to maintain normal levels of hepatic microsomal drug metabolism (7-9).

We now find that urinary ascorbate excretion by inflamed rats is reduced in parallel with the severity of inflammation. However, daily administration of ascorbic acid did not affect the development of the inflammatory disease, adjuvant arthritis, when given either prophylactically or therapeutically.

Experimental. Male inbred Dark Agouti rats (200-250 g) were used throughout. Groups of four to six animals were routinely housed in plastic boxes with bedding of wood shavings and free access to food and water. For urine collection, animals were housed individually in metabolism cages. Chronic inflammation was induced by subcutaneous injection of an adjuvant (=50 μl of liquid paraffin containing 0.5 mg of heat-killed *Mycobacterium tuberculosis*) in the tail. Acute inflammation was evoked by a subcutaneous injection of either 50 μl of oleyl alcohol or 0.1 ml of 1% carrageenan-saline in both rear paws.

Twenty four-hour urine collections were made before and after injection of these inflammagens. Urine was collected into bottles containing either 2 ml of 8% oxalic acid (for ascorbate analysis) or no preservative (for D-glucarate analysis), made up to 10 ml with water and stored at $-20°$ until analyzed. Ascorbate levels in urine, blood, and liver were determined colorimetrically using 2,4-dinitrophenylhydrazine (10). D-Glucarate in urine was measured enzymatically after conversion to glucaro-1:4-lactone (11).

To study the effects of ascorbic acid administration on adjuvant arthritis, an aqueous solution of ascorbic acid was prepared daily and given orally in a dose of 100 mg/rat from Day 0 to Day 24 after the injection of adjuvant. Pair-fed controls received 1 ml of water daily by oral intubation. Local inflammation (paw swelling) was measured with a screw-gauge micrometer. Sleeping was induced by an ip injection of 25 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, Sydney).

Results. Ascorbate and glucarate excretion. Table I records data from two experiments determining urinary ascorbic excretion. The absolute rates of ascorbate excretion by different groups of control animals varied considerably over a number of weeks. Within this limitation the decrease in urinary ascorbate excretion over 24 hr was highly significant 48 hr after injecting either of the two oily irritants (i.e., adjuvant, oleyl alcohol). By contrast, local inflammation induced by carrageenan did not affect the urinary ascorbate excretion within 24 hr after the injection. (The inflammatory response caused by carrageenan had largely subsided within this period.)

Animals injected with the mycobacterial

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[3] G. J. Barritt and M. W. Whitehouse, unpublished observations.

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adjuvant developed polyarthritis after 10 to 12 days and showed a long-lasting decrease in their urinary ascorbate excretion. Oleyl alcohol caused maximum paw swelling 72 hr after its injection; thereafter the local inflammation subsided. After 30 days no significant difference was observed in urinary ascorbate excretion between oleyl-alcohol-injected animals and the pair-fed controls.

Table II shows that the liver ascorbate was reduced significantly in oily-irritant-injected rats at the same times as significant decreases in urinary ascorbate excretion were noted. However, there were no significant differences in apparent blood ascorbate levels between inflamed animals and the controls, except in the 21-day levels of arthritic rats (=20% higher than the controls).

Urinary D-glucarate excretion by this strain of rats was low compared with their ascorbate excretion, which was approximately 10-fold higher. It did not alter following induction of acute inflammation. However, the arthritic rats excreted rather less glucarate in their urine 21 days after receiving adjuvant (Table III).

Effects of ascorbate administration. L-Ascorbic acid was given daily to rats from the day of adjuvant injection. In arthritic rats, body weight decreased after development of polyarthritis and the pentobarbital sleeping time was prolonged markedly. Considerable mortality occurred following injection of sodium pentobarbital (25 mg/kg).

Daily administrations of a relatively high dose of ascorbic acid (ca. 500 mg/kg) had no effect on the following: body weight loss, development of polyarthritis, severity of inflammation, (prolonged) sleeping times, pentobarbital toxicity (Table IV).

Discussion. Ascorbate levels in urine and liver decreased markedly after inducing severe local inflammation in rats. The enhanced urinary excretion of L-ascorbate, following administration of xenobiotics such as

TABLE I. URINARY ASCORBATE EXCRETION BY MALE RATS BEFORE AND AFTER EXPERIMENTAL INFLAMMATION.[a]

Experiment	Inflammagen[b]	Before	After 48 hr	14 Days	21 Days	60 Days
I	Adjuvant	10.18 ± 0.75	3.14 ± 0.19*		1.00 ± 0.12*	1.63 ± 0.04*
	Oleyl alcohol	3.10 ± 0.11	1.75 ± 0.16*		1.54 ± 0.14*	2.44 ± 0.06
	None (control)				3.10 ± 0.11	2.54 ± 0.04
II	Adjuvant	1.56 ± 0.08	0.90 ± 0.03**	0.94 ± 0.17*	0.56 ± 0.06*	
	None (control)			2.88 ± 0.29	2.27 ± 0.31	
	Carrageenan	2.27 ± 0.31	1.65 ± 0.14***			

[a] Values are for 24-hr ascorbate excretion (μmole ± SEM), four rats per group.

[b] Relative edemagenic activities measured by average percentage increase in paw thickness (n = 7) at 4 hr, 24 hr, and 4 days after local injection (see *Experimental*) were 85, 40, and 20% for carrageenan, 60, 80, and 100% for oleyl alcohol, and 20, 70, and 110% for adjuvant, respectively.

* $P < 0.01$.

** $P < 0.05$.

*** After 24 hr only, N.S.

TABLE II. BLOOD AND LIVER LEVELS OF ASCORBIC ACID IN RATS WITH EXPERIMENTAL INFLAMMATION.

Experiment	Inflammagen	(Time of sacrifice)	No. of animals	Blood levels (μmole/100 ml)[a]	Liver levels (μmole/g wet wt)[a]
I	Carrageenan	(48 hr)	5	3.62 ± 0.18	1.80 ± 0.11
	Adjuvant	(14 days)	5	3.16 ± 0.22	0.87 ± 0.08*
	Untreated		5	3.74 ± 0.41	1.91 ± 0.07
II	Oleyl alcohol	(72 hr)	5	4.60 ± 0.14	1.53 ± 0.05**
	Adjuvant	(21 days)	5	6.18 ± 0.19**	1.28 ± 0.04*
	Untreated		5	5.17 ± 0.33	1.74 ± 0.07
III	Oleyl alcohol	(30 days)	4	5.17 ± 0.26	2.04 ± 0.10
	Adjuvant	(60 days)	4	4.77 ± 0.57	1.38 ± 0.04*
	Untreated		6	4.88 ± 0.95	1.92 ± 0.05

[a] ± SEM.

* $P < 0.01$.

** $P < 0.05$.

TABLE III. URINARY EXCRETION OF D-GLUCARATE BY MALE RATS BEFORE AND AFTER EXPERIMENTAL INFLAMMATION.[a]

Inflammagen	Before	After 48 hr	14 Days	21 Days
Adjuvant	0.29 ± 0.02	0.22 ± 0.02	0.31 ± 0.02	0.12 ± 0.01*
None (control)		0.19 ± 0.03	0.27 ± 0.01	0.21 ± 0.04
Oleyl alcohol	0.21 ± 0.04	0.29 ± 0.02[b]		
Carrageenan	0.27 ± 0.01			

[a] Values are for 24-hr glucarate excretion (μmole ± SEM), four rats per group.
[b] Determined at 24 hr.
* $P < 0.05$.

TABLE IV. EFFECT OF ADMINISTERING ASCORBIC ACID (VITAMIN C) ON BODY WEIGHT GAIN, PAW SWELLING, AND PENTOBARBITAL-INDUCED SLEEP IN RATS WITH ADJUVANT-INDUCED ARTHRITIS (AIA).[a]

Day of observation	Group	Vitamin C	Weight gain[b] (g)	Increased paw thickness[b] (%)	Sleep time (min)	Mortality[c]
14	AIA	−[d]	−35 ± 4	65 ± 8	82 ± 4	0/7
	AIA	+	−38 ± 4	60 ± 8	68 ± 6	0/7
	Contr.	−	7 ± 1	5 ± 2	16 ± 4	0/5
24	AIA	−[d]	−35 ± 5	106 ± 20	>180	3/15
	AIA	+	−35 ± 3	89 ± 14	>180	3/15
	Contr.		24 ± 1	15 ± 2	29 ± 7	0/5
31	AIA	−[d]	−31 ± 4	99 ± 6	>180	5/12
	AIA	+	−31 ± 4	88 ± 11	>180	6/13
	Contr.		21 ± 3	16 ± 4	47 ± 8	0/5
49	AIA		28 ± 4	49 ± 4	39 ± 4	0/10
	Contr.		31 ± 5	12 ± 4	14 ± 2	0/5

[a] Ascorbic acid (100 mg/rat/day, po) was administered in 1 ml of H_2O from the day of adjuvant injection (Day 0) to Day 23. Contr. = control animals without adjuvant. Values = mean ± SE.
[b] Compared to weight and paw size on Day 0.
[c] In response to single dose of 25 mg/kg of Na pentobarbital (used to determine sleeping times).
[d] Control group of AIA rats receiving only H_2O (no vitamin C).

phenobarbital or carcinogens to rats, is believed to reflect stimulation of L-ascorbate synthesis from D-glucose via D-glucuronic acid (5, 6, 12).

Urinary excretion of another D-glucuronic acid metabolite, D-glucarate, also decreases with chronic inflammation (arthritis). D-Glucarate can be formed either wholly by liver cytosol enzymes, or with the participation of one microsomal enzyme, lactonase II (13). Marked decreases in both L-ascorbate and D-glucarate levels in rat urine have been noted after 3-day fasting (14). Although rats with established arthritis certainly lose weight compared with controls, their food consumption is actually as much as that of their weight-matched controls and often greater.

Prolongation of pentobarbital-induced sleep in rats affords an index of the severity and chronicity of a systemic response to peripheral inflammation (1, 2, 15). Prolonged sleeping times and decreased urinary ascorbate excretion were still evident in adjuvant-injected rats as late as 50 days after the adjuvant injection. At this time, almost all the peripheral inflammation had subsided and ankylosis occurred in many joints.

Preliminary studies have indicated that L-gulonolactone oxidase activity (4) may be very low in unwashed microsomes prepared from livers of arthritic animals compared with enzyme activity in normal liver microsomes (G. E. Turner and M. W. Whitehouse, unpublished).

A differential effect of ascorbic acid on rat arthritis has been reported: Paw swelling in animals with a peritonitis-polyarthritis was inhibited by 20 mg/kg/day of ascorbate, but doses of 50 mg/kg/day produced much less inhibition of the conventional adjuvant arthritis (16). We have been unable to confirm this, since none of our rats with adjuvant-induced peritonitis ever developed overt arthritis. In this present study, ascorbic acid loading (>400 mg/kg/day) neither prevented the development and progress of the arthritis nor affected some other param-

eters of the systemic disease (e.g., aberrant pentobarbital metabolism). It therefore seems unlikely that the various pathologies associated with the adjuvant disease in rats merely reflect impaired hepatic ascorbate biosynthesis and consequent insufficiency of this vitamin.

Patients with rheumatoid arthritis were found to have low blood ascorbate levels (as measured with dichlorophenolindophenol) despite a sufficient diet, believed to be due to a low renal threshold for this vitamin (17).

Summary. (1) Severe acute inflammation in rats (induced by oleyl alcohol) depresses urinary excretion and hepatic levels of ascorbate. (2) Rats with a chronic inflammation (polyarthritis) excrete reduced quantities of both L-ascorbate and D-glucarate in their urine. (3) In arthritic animals, blood ascorbate was increased but liver ascorbate levels were subnormal. (4) High daily doses of ascorbic acid failed to moderate the onset and progress of the adjuvant arthritis.

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