Some Anti-Inflammatory Properties of Ascorbic Acid (36181)

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Vascular and cell membrane permeability increases, the immune (allergic) response, and lysosomal hydrolase activity are interrelated in the inflammatory process. Ascorbic acid has been shown to play a stabilizing role in several of these processes indirectly in scorbutic animals. Ascorbic acid deficiency leads to separation of the cells in the vascular endothelium (1). A similar effect has been observed after histamine injection (2). The evidence for ascorbate stabilization of cellular membranes is not well documented (3). The anti-allergic effect of ascorbic acid has been demonstrated through its ability to reduce anaphylaxis in the guinea pig (4), mouse (5), and rat (6), but not in the dog (7). We have shown previously that ascorbic acid can inhibit lysosomal β-glucuronidase (8). These results led us to investigate the effect of ascorbic acid in several animal models of inflammation. This report compares the inhibitory actions of ascorbic acid, aspirin, and phenylbutazone against rat liver lysosomal β-glucuronidase (*in vitro*), UV erythema in guinea pigs, carrageenan edema in rats, adjuvant arthritis and peritonitis–polyarthritis in rats.

Materials and Methods. Animals used in these studies were (1) male, Cox/Holtzman rats for lysosomal β-glucuronidase and PPA, (2) female, Harlan/Holtzman rats for carrageenan edema and adjuvant arthritis, and (3) female albino guinea pigs for UV erythema. L-Ascorbic acid (Eastman), aspirin, and phenylbutazone (Geigy) were made up in stock solutions 20× final concentration in ethanol/isotonic saline (1/1) for *in vitro* studies and five-fold concentration in carboxymethylcellulose for animal studies. Lysosomes were prepared by the method of deDuve *et al.* (9). The lysosomal pellets from five grams of rat liver were suspended in 200 ml of Mg-free Krebs–Ringer-Phosphate buffer, pH 7.4. Fifty μl of stock solution of drug was added to 1 ml of lysosomal suspension. Control tubes contained 1 ml of lysosomal suspension and 50 μl of ethanol/saline. The tubes were incubated for 60 min at 37°. Then, 250 μl of 5 m*M* phenolphthalein-β-glucuronide (sodium salt) (Sigma) in 0.5 *M* acetate buffer, pH 5.0, was added to each tube. Incubation was continued for an additional 10 min. The enzyme reaction was stopped by the addition of 2.0 ml of 0.4 *M* glycine buffer, pH 10.7, containing 0.25% sodium dodecyl sulfate to clear the suspension. Phenolphthalein was determined with a Zeiss PMQ II spectrophotometer at 560 nm.

UV erythema was induced on guinea pigs by a modification of the method of Winder *et al.* (10). A Hanovia lamp (Kromayer model 10) was placed in contact with the skin on the back of a guinea pig for seven seconds. The animals were dosed *po* 15 min before UV exposure. The erythemas were examined at 1 hr then half hour intervals until two and one-half hours after irradiation. The severity of the burn was rated 0–3. The rating was multiplied by 4 at 1 hr, 3, 2, 1 for each of the successive intervals. Control irradiated animals rated 120 by this technique.

Edema was produced in rat paws with carrageenan by the method of Winter *et al.* (11). Drugs were given *po* two and one-half and one-half hour before the carrageenan injection. Paw volumes were determined after three hours by mercury displacement using a pressure transducer. The injected right hind paw was compared to the left, noninjected paw.

Adjuvant arthritis was produced according

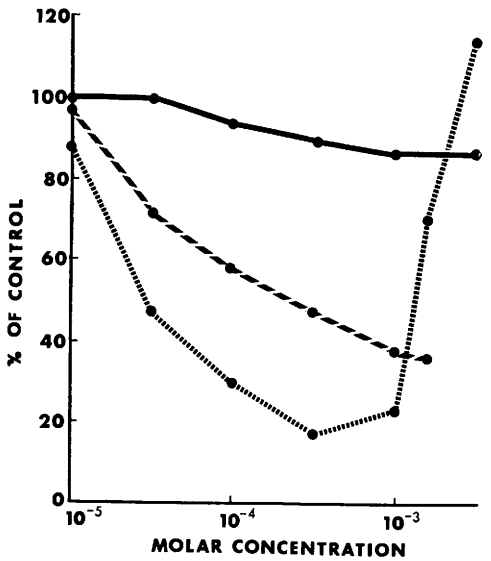

FIG. 1. The inhibition of lysosomal β-glucuronidase by ascorbic acid (●——●), aspirin (●▬▬●), and phenylbutazone (●▬▬●). The drugs were added in 50 μl aliquots to 1 ml of dilute liver lysosome suspension in KRP.

to the method of Pearson (12) with the injection of 0.25 mg *Mycobacterium tuberculosis* in 0.05 ml of mineral oil into the right hind foot pad. Paw volumes were determined at intervals of 0, 10, and 15 days. Animals were dosed po for 15 days.

Peritonitis–polyarthritis (PPA) was produced by the method of Dolbeare (13). A sterile peritonitis was induced by injecting 1 mg heat-killed *Mycobacterium tuberculosis* (Difco) in 0.2 ml of mineral oil. Paws begin to show first signs of swelling around day 8. Animals were dosed *po* daily for 10 days. Paw volumes were measured every 2 days for 22 days.

Results. Ascorbic acid between 0.01 and 1 m*M* incubated with lysosomal suspensions inhibited β-glucuronidase activity (Fig. 1). Higher ascorbic acid concentrations not only did not inhibit but enhanced the enzyme activity above that for controls. Phenylbutazone was less effective as an inhibitor at the lower concentrations but was more effective above 1 m*M*. Aspirin at 1 m*M* produced less than 20% inhibition of the enzyme.

Table I compares the anti-inflammatory actions of ascorbic acid, aspirin, and phenylbutazone in the carrageenan, erythema blocking, and adjuvant tests. Ascorbic acid did not inhibit the UV erythema response in guinea pigs. Aspirin (100 mg/kg) and phenylbutazone (50 mg/kg) produced almost complete inhibition of the erythema at 1 hr after irradiation. In the carrageenan test ascorbic acid inhibited the edema 12% compared to 29% for aspirin and 50% for phenylbutazone ($p < .05$). In adjuvant arthritis ascorbic acid (50 mg/kg) showed significantly greater activity (21.4% inhibition) than in the carrageenan edema. Aspirin (100 mg/kg) inhibited the swelling 6% and phenylbutazone (50 mg/kg) 33% ($p < .05$).

In the PPA model ascorbic acid (20 mg/kg) inhibited swelling to the same extent as phenylbutazone (50 mg/kg) during the 10–22 day period of the experiment (Fig. 2). Aspirin (100 mg/kg) was approximately 50% as effective as either ascorbic acid or phenylbutazone.

Discussion. We have demonstrated that ascorbic acid inhibits β-glucuronidase in lysosomal suspensions. Our earlier study showed that the inhibition was not due to membrane stabilization since ascorbic acid

TABLE I. The Comparative Effects of Ascorbic Acid, Aspirin, and Phenylbutazone in Animal Models of Inflammation.

Test	Percent of Inhibition		
	Ascorbic Acid	Aspirin	Phenylbutazone
UV Erythema	0	80 ± 1.5	90 ± 1.3
Carrageenan	12 ± 1.1	29 ± 2.5	49 ± .6
Adjuvant Arthritis			
7 days	21 ± .1	12 ± .2	33 ± .1
14 days	25 ± .2	0	43 ± .1

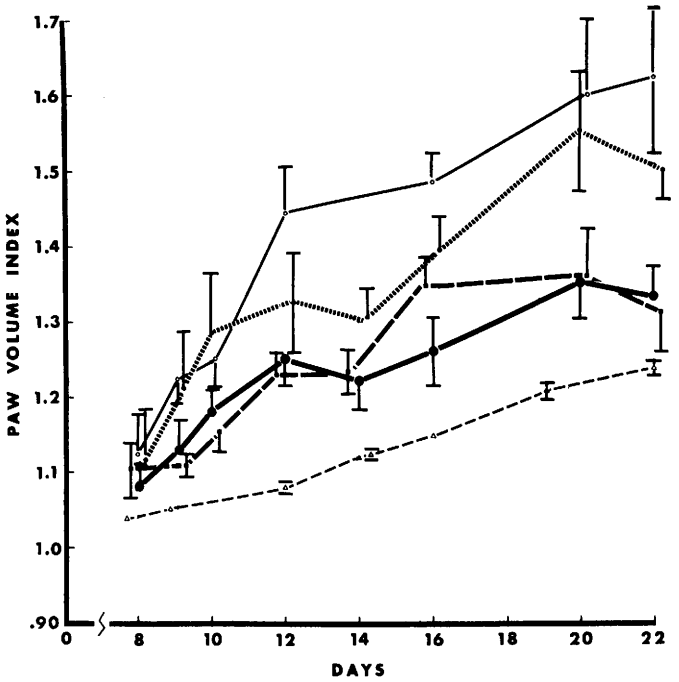

FIG. 2. Inhibition of swelling by ascorbic acid (●▬▬▬●), aspirin (●,,,,,,,,,,●), and phenylbutazone (▬ ▬ ▬ ▬) in peritonitis–polyarthritis. Control, noninjected, (△▬ ▬ ▬△). Control, TB injected (○▬▬▬○). Paw volume index—paw volume/initial paw volume. Standard error of the mean is presented.

inhibits the enzyme in sonicated and nonsonicated suspensions almost equally (8). Synovial levels of this enzyme increase during arthritis in humans (14) and the blood of rats during the development of polyarthritis (13, 15). Since many of the anti-inflammatory compounds inhibit lysosomal hydrolases (16), an anti-inflammatory role is suggested by the ascorbic acid inhibition of β-glucuronidase.

The lack of activity in the UV erythema and the good activity in the polyarthritis in the rats suggest that ascorbic acid may act as an inhibitor in the immune process. Dawson *et al.* (4) showed that ascorbic acid had synergistic effects when administered with antihistamines against antigen-induced bronchospasms in sensitized guinea pigs. In the same paper these authors proposed a possible anti-bradykinin activity for the ascorbic acid. In still another study Dawson and coworkers (17) demonstrated the inhibition of histamine induced bronchospasms in guinea pig. Other investigators have shown similar effects with the mouse (5), and rat (6).

Ascorbic acid may have important membrane action because of its reducing properties. It may behave similarly to the antioxidant, α-tocopherol, which stabilizes lysosomes at low concentrations (18) but causes hydrolase release at high concentrations (9). Chayen *et al.* (19) have shown that there is a relationship between free to bound acid hydrolase and free to total sulfhydryl groups in cultured synovial fibroblasts. Inagaki (20) has shown that ascorbate and cysteine inhibit a membrane bound K^+-Na^+-ATPase which may be involved in ion transport through plasma membrane. Tsen and Collier (21) and Jacob and Jandl (22) have shown that sulfhydryl reagents react with membrane -SH sites, lead to K^+ loss, Na^+ gain, and ultimately cell lysis. Of interest is the finding of Ignarro (23) that lysosomes in heavy mitochondrial

liver fractions were unstable in the presence of Na^+. Alteration in the oxidation state of the membrane may lead to increases in Na^+ in the cytoplasm around lysosomes. The lysosomes release hydrolases which further the damage to the plasma membrane.

Receptor sites may be affected through the adenyl cyclase system. Fassina (24) and Hynie *et al.* (25) have shown that ascorbic acid can potentiate the action of epinephrine and ACTH on fat cell lipolysis. This action appears to be via phosphodiesterase inhibition and not a direct effect on adenyl cyclase. May *et al.* (26) have shown that cyclic 3′, 5′-AMP inhibits the release of lysosomal β-glucuronidase. Other investigators (27–29) have shown that epinephrine exhibits anti-inflammatory action. Ascorbic acid may potentiate this effect.

Summary. Ascorbic acid was compared with aspirin and phenylbutazone for inhibitory action against lysosomal β-glucuronidase, UV erythema, carrageenan paw edema, adjuvant arthritis, and peritonitis–polyarthritis. The vitamin was a very effective inhibitor of β-glucuronidase activity and peritonitis–polyarthritis swelling, but a moderately effective inhibitor of adjuvant arthritis swelling. Ascorbic acid was weakly inhibitory in the carrageenan edema, and not inhibitory against UV erythema.

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