# INTERNATIONAL JOURNAL OF LEPROSY And Other Mycobacterial Diseases

VOLUME 44, NUMBER 4 OCTOBER-DECEMBER 1976

## Activity of Ascorbic Acid in Inhibiting the Multiplication of *M. leprae* in the Mouse Foot Pad<sup>1</sup>

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Matsuo et al (1) have postulated that hyaluronic acid is a major nutrient for *M. lep*rae and that ascorbic acid (vitamin C) inhibits bacterial  $\beta$ -glucuronidase and thereby interferes with bacterial utilization of this substrate. They present anecdotal evidence of histopathologic response of a lepromatous leprosy patient to 1.5 grams of ascorbic acid daily for 4.5 months in the absence of specific antileprosy chemotherapy. This report prompted us to study the effect of ascorbic acid on the multiplication of *M. leprae* in the son Coleman & Bell, Norwood, Ohio)—feed (Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri) mixtures were prepared fresh weekly. The bacteria isolated from the skin biopsy and inoculated into the mouse foot pads did not grow on Lowenstein-Jensen medium at 37° or 32°C. In addition to ascorbic acid, other animals were treated with dapsone (Sigma Chemical Co., St. Louis, Mo.), clofazimine (Ciba-Geigy Co., Summit, N.J.), and rifampin (Dow Chemical Co., Zionsville, Indiana).

mouse foot pad utilizing standard methodol- $ogy(^2)$ .

#### **MATERIALS AND METHODS**

*M. leprae* was isolated from a skin biopsy specimen from a newly diagnosed, previously untreated, lepromatous patient and  $5 \times 10^3$  acid-fast bacilli were inoculated into the foot pads of locally-bred BALB/c mice. At the times indicated mice were sacrificed and bacterial enumeration made of each individual foot pad. Body weights and gross autopsies of selected animals were recorded. Ascorbic acid (L [+] Ascorbic Acid, Mathe-

#### RESULTS

Groups of three control animals were sacrificed at four, five, and five and a half months after inoculation and growth was observed in 0, 2, and 1 of the foot pads respectively. At six months after inoculation, as indicated in the tables, all control animals were positive for bacterial growth, and ascorbic acid treated animals were harvested for the first time. Additional harvests of ascorbic acid treated animals were made at seven and eight months after inoculation. The results of these harvests are presented in Table 1 and Figure 1.

Six animals from each of the groups fed 0.01% dapsone, 0.001% dapsone, 0.001% dapsone, 0.001% dapsone, 0.001% clofazimine, and 0.01% rifampin w/w in diets were harvested six months after inoculation. No growth ( < 7.79

<sup>&</sup>lt;sup>1</sup>Received for publication 28 July 1976.

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Time after inoculation		Ascorbic acid concentration (w/w) in feed				
(months)	Control	0.05%	0.15%	0.45%		
6	1.35 (±0.62) × 10 <sup>5</sup>	7.88 (±0.90) × 104	5.21 (±1.27) × 104 <sup>a</sup>	$4.44 (\pm 1.45) \times 10^{4^{a}}$		
	[6]	[4]	[6]	[6]		
7	1.43 (±1.08) × 10 <sup>5</sup>	9.73 ( <u>+</u> 6.59) × 10 <sup>4</sup>	9.21 (±4.91) × 10 <sup>4</sup>	6.36 (±5.15) × 10 <sup>4</sup>		
	[12]	[6]	[6]	[6]		
8	2.09 (±1.50) × 10 <sup>5</sup>	1.70 (±1.11) × 10 <sup>5</sup>	9.48 (±4.78) × 104	7.66 (±5.09) × 104		
	[6]	[5]	[6]	[6]		

TABLE 1. The effect of ascorbic acid on the multiplication of M. leprae in the mouse foot pad.

Data presented as mean (± S.D.) acid-fast bacilli per foot pad. Number of mice examined (N) given in brackets.

 $^{a}p < 0.01$ , Student's t test, compared to controls.

Month of	Ascorbic acid concentration (w/w) in feed				
feeding	Control	0.05%	0.15%	0.45%	
0	24.40 ± 1.86 gm	22.70 ± 3.25 gm	24.92 ± 1.91 gm	24.81 ± 1.31 gm	
	[20]	[20]	[20]	[20]	
1	24.30 ± 1.84	23.43 ± 2.20	23.61 ± 1.78	23.84 ± 1.58	
	[20]	[19]	[20]	[20]	
2	23.33 ± 1.44	23.07 ± 1.90	22.67 ± 1.89	22.57 <u>+</u> 1.71	
	[20]	[18]	[20]	[20]	
3	22.83 ± 1.81	23.44 ± 2.00	23.07 ± 2.20	22.08 ± 1.66	
	[20]	[16]	[20]	[20]	
4	25.80 ± 2.14	26.29 ± 1.55	27.42 ± 2.70	26.62 ± 1.94	
	[20]	[15]	[20]	[20]	
5	28.37 ± 3.18	28.07 ± 2.37	30.09 ± 2.43	29.62 ± 2.37	
	[20]	[15]	[20]	[20]	
Mean monthly weight gain	0.60	1.00	1.00	0.01	
(grams) <sup>a</sup>	0.68	1.02	1.08	0.91	

TABLE 2. Body weights of mice fed ascorbic acid.

Data presented as mean (± S.D.) body weight. Number of mice examined (N) given in brackets. <sup>a</sup>Calculated by linear regression.

		Ascorbic acid concentration (w/w) in feed			
	Controls	0.05%	0.15%	0.45%	
Number of animals	4	6	6	6	
Body weight (gm)	$26.53 \pm 3.34$	$25.98 \pm 0.96$	$29.70 \pm 1.24$	$28.43 \pm 1.63$	
Heart (mg)	$204.80\pm22.07$	$234.92 \pm 19.92$	$201.48 \pm 32.95$	$208.07 \pm 18.87$	
Lungs (mg)	$305.95 \pm 21.46$	$287.40 \pm 35.39$	$277.65 \pm 37.00$	$270.22 \pm 36.92$	
Thymus (mg)	$29.80 \pm 11.17$	$32.32 \pm 13.05$	$32.37\pm6.00$	$24.48 \pm 3.66$	
Spleen (mg)	$133.35 \pm 21.20$	$69.27 \pm 10.93^{\mathrm{a}}$	$137.90 \pm 27.26$	$126.05 \pm 33.92$	
Liver (mg)	$1777.03 \pm 303.42$	1261.57 ± 99.17 <sup>b</sup>	$2030.18 \pm 187.99$	1876.63 ± 164.62	
Testes (mg)	118.60 ± 34.10	103.80 ± 16.39	$131.53 \pm 32.26$	113.57 ± 10.97	
Kidneys (mg)	$539.78 \pm 38.04$	$439.02 \pm 51.35^{\circ}$	$502.97 \pm 20.90$	$480.28 \pm 33.55$	

TABLE 3. Organ weights of mice fed ascorbic acid.

Animals fed ascorbic acid in the concentrations indicated for seven months. Data presented as mean ± S.D.

a p < 0.001, Student's t test, compared to controls.

 ${}^{b}p < 0.01$ , Student's t test, compared to controls.  ${}^{c}p < 0.02$ , Student's t test, compared to controls.



FIG. 1. The effect of ascorbic acid on the multiplication of *M. leprae* in the mouse foot pad. Ascorbic acid was fed in the diet in the concentrations indicated (w/w). The data are derived from Table 1. The number of animals (N) is indicated at the base of each bar.

 $\times 10^3$  acid-fast bacilli per foot pad) was ob-

mg daily. Thus the ascorbic acid concentrations in the mouse feed of 0.05%, 0.15% and 0.45% presumably approximate human doses of 0.5, 1.5 and 4.5 grams daily respectively. As indicated, there was statistically significant activity of ascorbic acid in inhibiting the multiplication of *M. leprae* in concentrations of 0.15% and 0.45% in the diet at the time of the six month harvest. An inspection of the data suggests that there is a doseresponse relationship of ascorbic acid on bacterial multiplication at all three time intervals the figure. On the other hand, dapsone, clofazimine, or rifampin in the diet resulted in uniformly negative harvests at six months (  $< 7.79 \times 10^3$  acid-fast bacilli per foot pad in each animal). Thus although ascorbic acid appears to have activity, its effect is quantitatively minimal compared to standard chemotherapeutic agents.

In a small number of animals, no apparent toxic effects of these doses of ascorbic acid were observed. The long-term toxicity of high doses of ascorbic acid clinically are, however, not known with certainty.

In view of the facts that a) high doses of ascorbic acid appear necessary to inhibit the multiplication of *M. leprae* in mice, b) that leprosy is a chronic disease requiring many years of therapy, c) that the long-term toxicity of high doses of ascorbic acid are not clear, and d) that the effect of ascorbic acid is minimal even in the mouse foot pad; legitimate reservations are in order regarding the risk-benefit ratio of a clinical trial of high doses of ascorbic acid in leprosy. The present findings do not shed light on the mechanism of action of ascorbic acid but do provide support for the claim of Matsuo et al (1) that vitamin C may have antibacterial activity in leprosy.

served in any of these foot pads.

The results of monthly body weight determinations in the mice fed ascorbic acid are given in Table 2. No detrimental effect of these doses of ascorbic acid on the rates of weight gain was observed.

No gross pathologic changes were seen in the animals receiving ascorbic acid. The results of the gross autopsies (organ weights) in these animals are given in Table 3. Other than some quantitatively minor changes in the weights of spleens, livers, and kidneys in mice receiving the lowest dose of ascorbic acid, no changes were apparent.

#### DISCUSSION

Ascorbic acid concentrations in the diet were selected on the assumption that, by analogy to dapsone, 0.01% w/w of a drug in the powdered diet of a mouse is approximately equivalent to a human dosage of 100

#### SUMMARY

Ascorbic acid was fed to mice in concentrations of 0.05%, 0.15%, and 0.45% w/w in the diet. Six months after inoculation of M. *leprae* into the foot pads, there were significantly fewer acid-fast bacilli harvested from animals receiving 0.15% and 0.45% w/w ascorbic acid than from control mice. On the other hand, M. *leprae* did multiply in mice fed ascorbic acid while no multiplication at all was observed in animals fed dapsone, clofazimine or rifampin. No toxic effects of ascorbic acid were noted in these mice.

#### RESUMEN

Se alimentaron ratones con una dieta conteniendo 0.05%, 0.15%, of 0.45% (peso/peso) de acido ascorbico. Los animales mantenidos bajo estas condiciones se inocularon en los cojinetes plantares con una suspensión de M. leprae. Seis meses después de la inoculación, los ratones alimentados con las dietas conteniendo 0.15% y 0.45% de ácido ascórbico presentaron un menor número de bacilos ácido-alcohol resistentes que los animales usados como control. Por otro lado, el M. leprae se multiplico en los ratones alimentados con ácido ascórbico pero no en aquéllos animales alimentados con una dieta conteniendo dapsona, clofazimina o rifampina. No se observaron efectos tóxicos del ácido ascórbico en los animales estudiados.

### RÉSUMÉ

On a ajouté de l'acide ascorbique au régime de donné à des souris à des concentrations de 0,05pour cent, 0,15 pour cent et 0,45 pour cent, par comparaison au poids. Six mois après l'inoculation de *M. leprae* dans les coussinets plantaires, on a observé une réduction significative du nombre de bacilles acido-résistants qui pouvaient être récoltés chez les animaux ayant reçu 0,15 pour cent et 0,45 pour cent d'acide ascorbique par rapport à leur poids, par comparaison aux souris témoins. Par ailleurs, *M. leprae* s'est multiplié chez les souris auxquelles on avait donné de l'acide ascorbique, alors qu'aucune multiplication n'a été observée chez des animaux auxquels on avait ajouté de la dapsone, de la clofazimine ou de la rifampine dans la nourriture. Aucun effet toxique de l'acide ascorbique n'a été observé chez ces souris.

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