The comparative prophylactic effects of sulphasalazine, prednisolone and azathioprine in experimental colonic ulceration

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Food quality carrageenan extracts of the red seaweed *Eucheuma spinosum* can be readily degraded yielding a product which, when fed in the drinking fluid to guinea-pigs, produces ulcerative disease of the colon in 100% of animals in a relatively short period of time (Watt et al 1979), thus providing an experimental model which can be used for the screening of drugs of potential therapeutic or prophylactic value.

We have used this model to investigate the comparative prophylactic effects of three drugs, sulphasalazine, prednisolone and azathioprine, currently used in the management of ulcerative colitis and Crohn's disease in man.

Animals. Adult male albino guinea-pigs (Dunkin-Hartley), 450–500 g, were randomly grouped into one control and three experimental groups each of 12 animals. The animals were housed in pairs and fed a vitamin C-enriched cube diet (RGP Dixon and Sons Ltd.). All four groups received a 3% degraded carrageenan solution as drinking fluid over 1 week. The average daily intakes of degraded carrageenan were 0·33 g/100 g control and 0·30 g/100 g experimental groups respectively.

Degradation of carrageenan. Food quality carrageenan extract (*Eucheuma spinosum*) obtained commercially (British Ceca Company Ltd., London) was degraded by exposure of the dry powder to concentrated HCl for 1 h (Watt et al 1979). A 3% degraded solution was prepared by admixing 2 ml concentrated HCl diluted with 1 ml H_2O with 3 g of the dry powder carrageenan extract; water was added, the mixture stirred until dissolved, the acidified solution neutralized with 2M NaOH to pH 7 to 8 and the volume then adjusted to 100 ml. Supplied to guinea-pigs as drinking fluid, this 3% degraded carrageenan solution causes ulcerative disease of the colon in 100% of animals in less than 1 week (Watt & Marcus 1980).

Table 1. Effect of sulphasalazine, azathioprine and prednisolone on the incidence and severity of ulceration of the large bowel in guinea-pigs fed 3% degraded carrageenan as drinking fluid.

Treatment (Dose)	No. of animals with ulceration of graded severity*					No. with ulcers in ascending colon
	1+	2+	3+	4+	5+	
Sulphasalazine (50 mg kg^{-1})	8	1	1	0	2	3/12 ($P < 0.02$)
Azathioprine (30 mg kg^{-1})	1	7	3	0	1	4/12
Prednisolone (1 mg kg^{-1})	7	2	1	0	2	6/12
Controls	2	6	0	1	3	9/12

* Severity assessed arbitrarily according to the number of ulcers found in the caecum and colon as follows: 1+ = 1–50, 2+ = 51–200, 3+ = 201–350, 4+ = 351–500, 5+ = over 500 ulcers.

* Correspondence.

Drug administration. All drugs were administered by gastric intubation beginning 48 h before and continuing throughout the experimental period. One group received sulphasalazine 50 mg kg^{-1}, a second group prednisolone 1 mg kg^{-1}, and a third azathioprine 30 mg kg^{-1} per day, sulphasalazine being supplied in divided doses twice daily, prednisolone and azathioprine once daily. The control group received corresponding volumes of water by gastric intubation.

Assessment of ulcerative damage. At the end of the 7 day experimental period, the animals were killed by a blow on the head and the large bowel removed, moderately distended with 4% solution of formaldehyde in 0·9% NaCl and, after fixation, opened, emptied of faeces, and examined by transmitted light. Each specimen was coded, so that the assessor was unaware of the group to which the specimen belonged. The ulcers in the caecum and colon were counted, and the severity of damage in each specimen was assessed on an arbitrary 1 to 5 plus scale according to the number of ulcers present (Table 1, footnote).

The incidence and severity of ulceration in the four groups of animals at the end of the period are shown in Table 1. Ulceration occurred in all of the animals. The severity of damage in the caecum and ascending colon was least in the sulphasalazine- and prednisolone-treated animals; in the azathioprine-treated group, the ulcerative damage was marginally but not significantly less than in the control group. The incidence of ulcers in the ascending colon was reduced in all experimental groups, particularly so in the group treated with sulphasalazine.

Experimental colonic ulceration induced by 3% degraded carrageenan solution in the drinking fluid is acute and severe in comparison with damage caused by undegraded carrageenan. The three drugs investigated were administered over a relatively short time viz. 48 h before inducing ulceration. The results would seem to indicate some measure of protection in relation to all

three drugs, particularly so with sulphasalazine, and appear to reflect fairly closely the relative prophylactic value of the drugs as used clinically.

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The effect of overcrowding stress on the development of adjuvant-induced polyarthritis in the rat

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Activation of the pituitary-adrenal axis with subsequent adrenal hypertrophy and the accompanying marked elevation of 17-hydroxy corticosteroids are long known physiological responses to stressful situations (Selye 1946, 1950; Maickel et al 1961). One such stressful situation is overcrowding. The effect of this on the development of a laboratory animal model of chronic inflammation has been examined using adjuvant-induced polyarthritis (AIP) in rats, which is a method of assessing the potential usefulness of drugs in various types of arthritis (Swingle 1974).

Male, albino Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts), 100 to 140 g at the start of the experiment, were housed in groups of five (in gang cages) for five days for acclimatization to a controlled laboratory environment of temperature (20–22 °C), humidity (35–65%) and lighting (12 light: 12 dark). Food and water were freely available. The rats were randomly assigned to the following groups (n = 10 each): non-arthritic—10 rats/cage; non-arthritic—1 rat/cage; arthritic—10 rats/cage; and arthritic 1 rat/cage. AIP was induced by injection of heat killed *Mycobacterium tuberculosis* (Newbould 1963). Each rat received 0·1 ml of a 0·5% (5 mg ml^{-1}) adjuvant suspension in heavy mineral oil injected subcutaneously into the plantar surface of the right hind paw. The rats were immediately placed in their respective housing environment which consisted of a cage 42·5 cm wide × 18·0 cm high × 25·0 cm long constructed of stainless steel and having a wire mesh floor, top and front panel and solid sides. Food and water were freely available. The volume (ml) of the injected (primary lesion) and uninjected (secondary lesion) hind paw were measured by water displacement to the lateral malleolus (ankle) just before adjuvant injection and again at weekly intervals during the 42 days of the experiment. The animals were weighed weekly. Mean values and their standard errors were evaluated using Student's *t*-test.

The response of the AIP injected paws was dramatic by day 7 when compared with control paws (Fig. 1), the rate of oedema formation was similar for both crowded and single groups during the first 21 days but by day 28 there was a clear separation of effect with crowded animals having a reduced mean volume (5·78 ml vs 6·45 ml). In addition, paw volumes in both groups reached their respective peaks by day 28 and remained at that value until the end of the experiment. Involvement of the uninjected hind paw did not become apparent until day 14 (Fig. 1), thus confirming earlier findings (Sofia et al 1975). By this time paw volumes in both groups were significantly increased compared with non-arthritic controls but the volume was significantly less in crowded rats. The rate of development of the secondary lesion in both groups of rats was parallel until day 28 when in the crowded rats a maximal mean value of 3·60 ml was reached and remained unchanged to day 42 but in the singly housed rats the volume continued to increase, reaching a mean volume of 4·78 ml by day 42.

AIP had a significant retardant effect on body weight gain (Fig. 2), rats in either housing condition gaining weight to a lesser degree than non-arthritic controls throughout the study. However, by day 28 the effect was

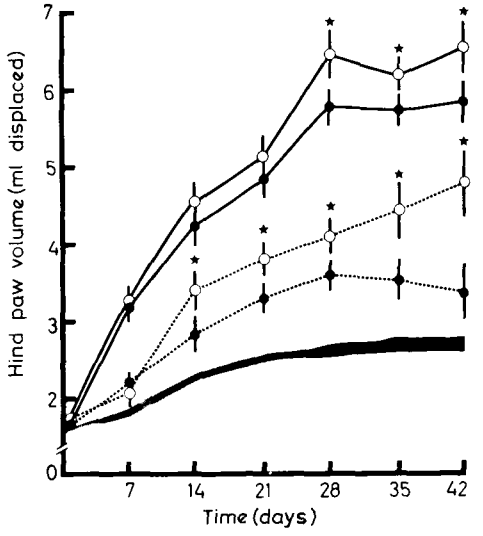

FIG. 1. Mean (±s.e.m.) volume of the injected hind paw or primary lesion (——) and uninjected hind paw or secondary lesion (- - -) of AIP in rats housed 1 per cage (○) or 10 per cage (●). Shaded area: mean range of paw volumes of each hind paw of untreated non-arthritic control rats. n = 10 for each point. $*P < 0.05$ for comparison of each housing condition.