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Sulphasalazine fails to prevent development of mucosal ulceration and 5-lipoxygenase activity in guinea-pigs with chronic inflammatory bowel disease induced by combined bacterial immunization and oral carrageenin

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Abstract—A model of inflammatory bowel disease in guinea-pigs involving a 14 day initial treatment with formalin-killed *Bacteroides vulgatus* subcutaneously and oral carrageenin plus live *B. vulgatus* for 10 days was used to determine the effects of sulphasalazine 100 mg kg^{-1} day^{-1} b.i.d., p.o. given for 4, 7 and 10 days after cessation of the bacterial/carrageenin treatment on the morphological and histological states of the established disease and on the production of the principal 5-lipoxygenase products, 5-hydroxyeicosatetraenoic acid and leukotriene B_4. The drug treatment did not cause any significant changes in this established disease as measured by these parameters.

Sulphasalazine (salicylazosulphapyridine) is used extensively for the treatment of ulcerative colitis and other inflammatory bowel diseases (IBDs) (Klotz et al 1980; Hoult 1987; Peppercorn 1990; Ruderman 1990). In a variety of acute and subacute animal models, sulphasalazine has been shown to inhibit intestinal inflammation and ulceration (Pfeiffer 1986). This effect of sulphasalazine is possibly related to its diverse effects on eicosanoid metabolism (Hoult 1987), inhibition of the production of oxygen radicals (Miyachi et al 1987; Kanerud et al 1990), inhibition of interleukin-1 production (Remvig & Andersen 1990), and effects on cytotoxic lymphocyte functions (MacDermott et al 1986). The drug is often associated with the development of certain side-effects which limit its clinical applications and utility (Taffet & Das 1983; Hoult 1987). Moreover, while undoubtedly the drug of choice and effective in reducing the symptoms associated with IBD, sulphasalazine does not always reverse or 'cure' these diseases.

This limited effectiveness of sulphasalazine in human disease thus contrasts with its potent actions in most acute laboratory animal models (Pfeiffer 1986). Indeed, the latter, while useful, are a limitation in representing the human disease because of their acute nature. In an attempt to obtain a model of human IBD which mimics that where patients have had conditions such as ulcerative colitis fully established as a chronic disease, we have modified a model of colitis developed by Onderdonk et al (Onderdonk 1985; Onderdonk et al 1983, 1984, 1987), the conditions for optimal development of which are described elsewhere (Oestreicher et al 1991). Carrageenin alone gives a relatively weak inflammatory response in the intestinal tract of guinea-pigs (Onderdonk 1985) and the addition of an immunological reaction to endogenous bacterial species as provided in the model used here gives immuno-inflammatory reactions which are both potent and mimic those in human ulcerative colitis (Oestreicher et al 1991). Here we describe negative effects of sulphasalazine in this model, both on the development of intestinal ulcers and on mucosal products of the 5-lipoxygenase pathway which are considered important mediators of inflammatory reactions in IBD (Broughton-Smith & Whittle 1985; Sharon & Stenson 1985).

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Materials and methods

Ulcerative colitis was induced in male Hartley guinea-pigs (approx. 400–500 g initial body wt) by two subcutaneous injections per week of 0·1 mL formalin-killed *Bacteroides vulgatus* in phosphate buffered saline with a further injection of the bacteria suspended in Freund's complete adjuvant, once weekly, for a period of 14 days. A booster injection of the same quantity of killed *B. vulgatus* suspended in phosphate buffered saline was given subcutaneously one week following the last injection. Following the first immunization the animals were given 5% w/v acid-degraded *iota*- carrageenin and viable *B. vulgatus* (2×10^9 CFU) orally daily for 10 days. The animals were then divided into groups of 19 controls, which received two daily doses of 5 mL 0·25% w/v aqueous methylcellulose, and a total of 17 animals which were given two 5 mL doses of 50 mg kg^{-1} sulphasalazine (i.e. 100 mg kg^{-1} day^{-1}) morning and late afternoon. Body weights and the appearance of stools and animals' physical appearance were noted daily. Approximate equal numbers of the animals were killed by CO_2 asphyxiation at 4, 7 and 10 days, respectively. Gross pathological observations of the intestinal tract were recorded and sections of the ulcerated and non-ulcerated regions of the caecum, colon and rectum were selected for microscopic examination. These were fixed in 4% formaldehyde in phosphate buffered saline, dehydrated in a graded concentration series of ethanol, embedded in paraffin and the sections stained with haematoxylin and eosin. The percentage of animals with mucosal changes comprising oedema, inflammation (with white cell infiltrate), erosions and haemosiderin deposits was recorded.

Assays of 5-hydroxyeicosatetraeonic acid (5-HETE) and leukotriene B_4 (LTB_4) were performed in incubated mucosal scrapings of the colon and caecum of animals killed on days 4 and 7 of sulphasalazine control treatments. The mucosal tissues were incubated at 37°C for 5 min in 2 mL 50 μM Tris-HCl buffer, pH 7·4, with 1 mg mL^{-1} glucose and 1 mM $CaCl_2$ in the presence and absence of 2 μM calcium ionophore, A23187 (Sigma, St Louis, MO, USA). The tissues were then homogenized in 1 vol of 0·1 M citric acid, pH 3·0, and the organic acids extracted into 2 vol of diethylether (peroxide-free). The ether extraction was then repeated 2–4 times and the combined ether layers were evaporated to dryness over N_2 gas. The recoveries of a standard of [^{3}H]LTB_4 (33 128 counts min^{-1}, n = 3) extracted by this procedure averaged 72%. The concentrations of 5-HETE and LTB_4 were determined in the dried ether extracts by radioimmunoassay (RIA) (Seragen, Boston, MA, USA); the extracts being first dissolved in the respective RIA buffers. This method has been validated by HPLC following addition of known quantities within the assay limits of a full range of leukotrienes and HETEs and the amounts in each peak quantified by UV absorbance and RIA (for 5-HETE and LTB_4, respectively).

Table 1. Summary of incidence of pathological changes in *Bacteroides* carrageenin model of IBD and responses to sulphasalazine.

	Time (days) 4		7		10	
Dose group	Control	Sulphasalazine	Control	Sulphasalazine	Control	Sulphasalazine
Caecum % with ulcers*	100	100	100	100	100	100
Rectum % with ulcers	16·7	33·3	16·7	20	60	40
No. with inflammation	1/6	2/6	1/7	0/7	1/5	2/6
Colon % with ulcers	16·7	16·7	14·3	0	40	0
No. with inflammation	2/6	2/6	1/7	0	1/5	0/6

* Histopathology of this region of the intestinal tract detailed in Fig. 1. Ulcers in the colon and rectum appear uniformly throughout the entire length of these organs and are approximately 0·5–1·3 cm in diameter. Thus ulceration is both substantial and extensive.

For the preparation of degraded carrageenin, sodium *iota*-carrageenin, 50 g (Type X-5852, Copenhagen Pectin Factory, Lille Skensved, Denmark DK-4623) was added to 900 mL distilled H_2O and 25 mL 1 M HCl and heated for 3·5 h at 70°C. Sucrose (25 g) was then added to improve the taste and the solution was neutralized to pH 7·0 by addition of approximately 1·5 mL 1 M NaOH. The final volume was adjusted to 1L with H_2O.

Bacteroides vulgatus strain TISVM 40G2-22, generously donated by Dr A. Onderdonk, was grown in brain HEMPT infusion broth in 4 mL NUNC cryotubes gassed with N_2. The bacteria were prepared in sterile phosphate-buffered saline for injection. The bacteria used for immunization were killed by addition of 10% formalin and placed at 4°C overnight. The cells were then centrifuged (300 *g*, 10 min) and washed with sterile phosphate-buffered saline.

Results and discussion

The results in Table 1 show the gross morphological changes in the caecum, colon and rectum of animals which received the combined treatments comprising immunization with killed *B. vulgatus* and oral carrageenin/live *B. vulgatus*. Histological gradings of cellular injury and inflammation in the caecum of these animals (the region of the intestinal tract most affected) are shown in Fig. 1. Extensive ulceration and inflammation was also evident in the colon and rectum (Table 1). Treatment with sulphasalazine (100 mg kg^{-1} day^{-1} in 2 divided doses per day) for 4, 7 and 10 days post-induction of the IBD failed to affect the development of the condition whether judged by overall morphological appearance (Table 1) or from histological examination of the caecum (Fig. 1). The only significant changes in body weights that was evident was a decline in the sulphasalazine-treated groups on days 2, 9 and 10 (Fig. 2); it was also noted that there was a trend towards lower body weights in this group on the other days. Since all animals had diarrhoea (data not shown) it is presumed that the slight overall decline in body weight was due to this condition. The sulphasalazine treatment did not affect the occurrence of diarrhoea, thus the drug treatment was ineffective in controlling the progress of all parameters of the disease.

The results in Fig. 3 show that there was a marked elevation in 5-HETE produced in the caecal tissue on day 7, with somewhat less being evident in the colonic scrapings, irrespective of these being incubated with or without the calcium ionophore, A23187. The production of 5-HETE in these two regions of the intestinal

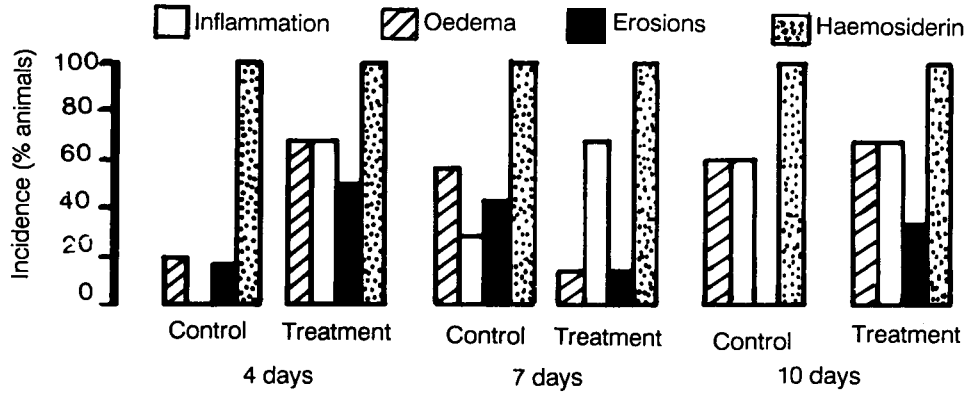

FIG. 1. Effects of sulphasalazine (100 mg kg^{-1} day^{-1}, given orally as divided doses daily) for 4, 7, and 10 days on the presence of mucosal changes (oedema, inflammation, erosions, haemosiderin deposition assessed histologically) in the caecum of guinea-pigs with established colitis.

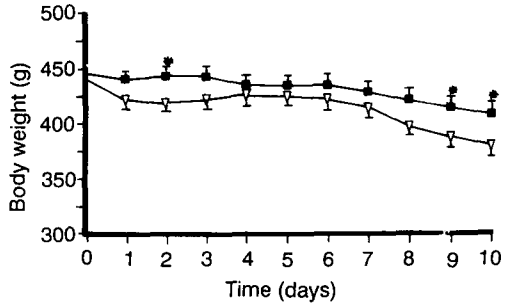

FIG. 2. Changes in body weights of guinea-pigs with colitis established following the 2-week immunization schedule developed in the present study and receiving either 100 mg kg^{-1} day^{-1} sulphasalazine (▽), or 0·25% methylcellulose vehicle alone (control ■) for 10 days. *$P<0.05$ (Student's t-test).

tract appeared to parallel the appearance of ulceration and inflammation in that both 5-HETE levels and the latter pathological reactions were more abundant in the caecum than in the colon. LTB_4 production by these tissues was less than that of 5-HETE and overall did not appear to be influenced by the addition of the calcium ionophore. No obvious conclusions can be drawn from the latter observations other than the possibility that maximal activation is evident in the non-Ca^{2+} ionophore treated tissues. LTB_4 most likely originates from inflammatory cells infiltrated into the mucosa. The results in Fig. 3 clearly show that the sulphasalazine treatment does not influence either LTB_4 or 5-HETE production.

The model of colitis employed in the present study has characteristics which are possibly more like those in the established chronic states resembling those in patients who have had ulcerative colitis for a relatively long period of time in contrast with other more acute animal models (Oestreicher et al 1991). It is possible that the lack of effects of sulphasalazine on morphological, histological and biochemical properties in this disease is indicative of limitations of the effectiveness of sulphasalazine. While the single dose of 100 mg kg^{-1} of sulphasalazine was employed here, other studies with higher doses (250 mg kg^{-1}) were also without effect in this model (Oestreicher et al 1991). Higher doses were, however, not used in the present study because of the possibility of excessive diarrhoea and consequent loss of body weight and health of the

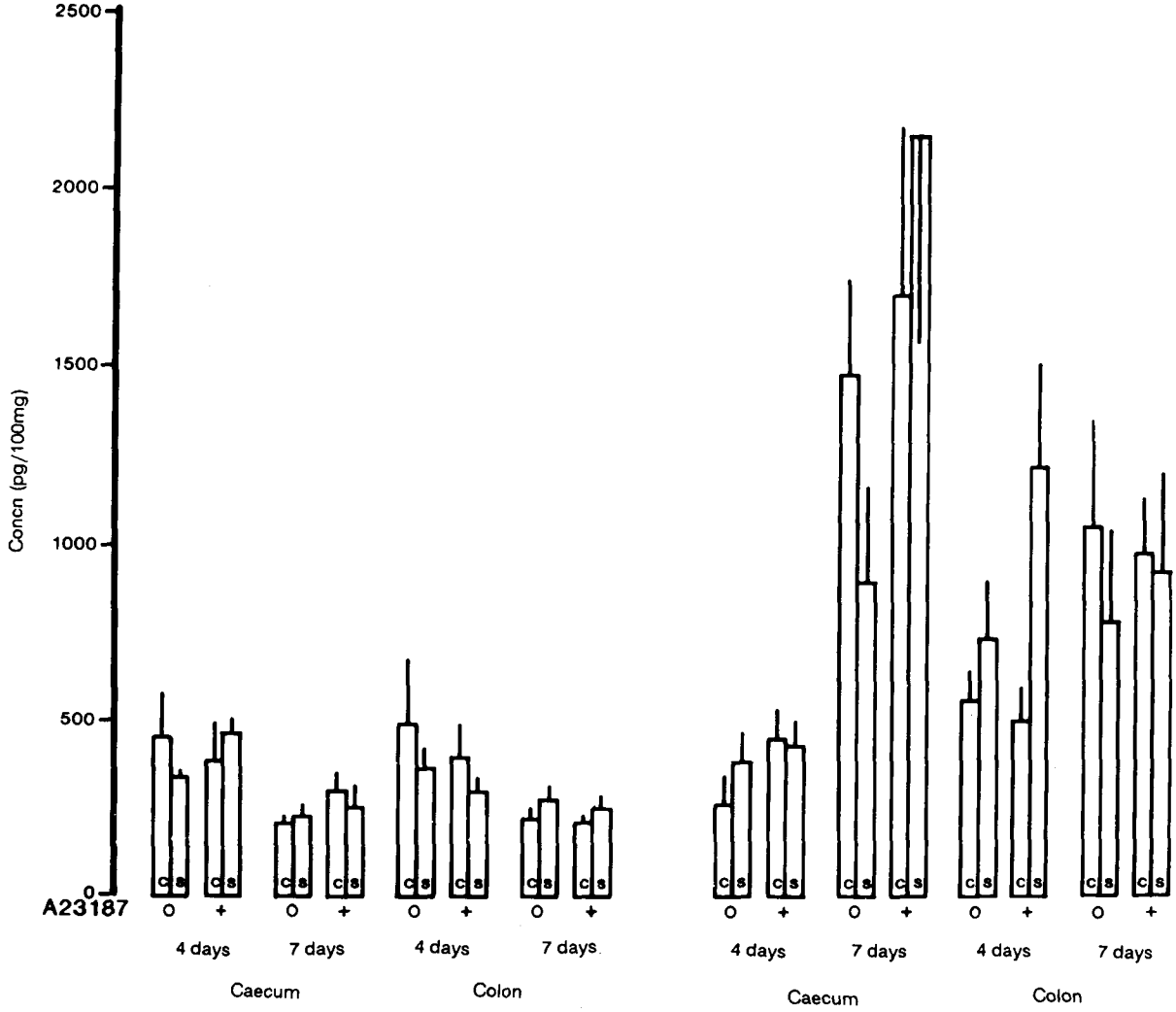

FIG. 3. Concentrations of LTB_4 (left panel) and 5-HETE (right panel) in the colonic and caecal mucosal homogenates of control (c) and sulphasalazine (s, 100 mg kg^{-1} day^{-1} × 10 days)-treated guinea-pigs incubated in the presence or absence of calcium ionophore, A23187 (to fully activate release and metabolism of endogenous arachidonate). No statistically significant differences were observed between the sulphasalazine- and control-treated groups (Student's t-test for unpaired samples, $P<0.05$, n = 3–4 per group).

animals. This model of IBD might thus be described as a sulphasalazine-resistant type.

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Influence of changes in protein binding on the central activity of antidepressants

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Abstract—The central effect (expressed as analgesic response), protein binding and brain uptake of mianserin were measured in mice receiving drug intraperitoneally. A significant decrease of the central effect of mianserin (30 mg kg^{-1}) was seen in mice with experimental inflammation when compared with control animals (reaction time (s) = $12{\cdot}12 \pm 1{\cdot}22$ vs $25{\cdot}56 \pm 2{\cdot}92$; $P < 0{\cdot}001$) and the dose-analgesia response curve (10–60 mg kg^{-1}) was significantly shifted to the right in mice with inflammation. In serum of mice with inflammation, unbound concentration of mianserin was decreased from $19{\cdot}37 \pm 0{\cdot}73$ to $17{\cdot}83 \pm 0{\cdot}30\%$ ($P < 0{\cdot}05$) and seromucoid levels were significantly increased ($P < 0{\cdot}001$). Following the intraperitoneal administration of 30 mg kg^{-1} of mianserin, brain uptake decreased in diseased mice when compared with control animals ($P < 0{\cdot}02$), suggesting that the decrease in analgesia was secondary to a decrease in drug delivery to the brain because of increased protein binding.

Although the effects of binding on pharmacokinetics have been investigated (Dayton et al 1973; Gibaldi et al 1978; Wilkinson 1983), the effect of binding on pharmacodynamics is a relatively unexplored area; the relationship between free drug concentration and pharmacological effects is difficult to investigate, and is particularly complex for drugs that cross the blood-brain barrier to enter the central nervous system.

However, information is already available on the increased effect of central agents in several disease states, clearly identified as a cause of an increase of free fraction of acidic drugs in plasma (Greenblatt & Koch-Wesser 1974; Greenblatt & Allen 1978; Halliday et al 1985). In contrast, there is an apparent lack of clinical and experimental studies regarding the effect of augmented serum binding on the central effect of basic drugs (e.g. tricyclic antidepressants, neuroleptics, opiates). Basic drugs are avidly bound by α_1-acid glycoprotein (AAG) (Piafsky 1980; Abranson 1982; Tierlynck et al 1982). It is known that levels of AAG in serum show an important interindividual variability and increases in inflammatory conditions, such as rheumatoid arthritis or metastatic cancer (Piafsky 1980); consequently the drug response could be affected.

Thus we have set out to evaluate whether in mice with increased AAG plasma concentrations, there is an increase in binding of mianserin, thereby affecting its delivery to the brain. The central effect of mianserin was evaluated by its analgesic effect (Reichenberg et al 1985).

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