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Antagonism of the gastrointestinal ulcerogenic effect of some non-steroidal anti-inflammatory agents by sodium salicylate

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Gastrointestinal side-effects of anti-inflammatory agents appearing in the form of microhaemorrhages or ulcers are well documented (Hurley & Crandall, 1963; Somogyi, Kovács & Selye, 1969, Robert, 1974).

In the process of investigating the combined effects of sodium salicylate and other non-steroidal anti-inflammatory drugs we found that in rats the salicylate antagonized the ulcerogenic side effects of the other drugs without modifying the main effects of the compounds.

On the basis of this finding we suppose that an antagonism exists between sodium salicylate and other non-steroidal anti-inflammatory drugs towards the ulcerogenic side-effect. To prove this antagonism the interaction of salicylate with well-known non-steroidal drugs was examined. The drugs were indomethacin (Chinoin), nifluminic acid (Squibb), phenylbutazone (Richter) and acetylsalicylic acid (Polfa).

Wistar rats of either sex, 120–150 g were fasted for 24 h. Gastric ulceration was then induced according to Lee, Mollison & Cheng (1971). The animals were killed 4 h after receiving the drug. The stomachs were immediately removed, opened along the greater curvature, rinsed with water and the number of ulcers counted. Only lesions exhibiting dark brown colorations were counted. Indomethacin when administered as single dose, produces lesions (ulcers) in the small intestine after a few days of treatment (Somogyi & others, 1969; Robert, 1974). The ulcerogenic processes decrease the tensile strength of the small intestine wall (in lesion areas) and perforation may occur. These perforated ulcers produce massive peritonitis which kills the animals. For the evaluation of indomethacin-induced intestinal ulceration the method of Ezer & Szporny (1975) was used.

The tensile strength of the intestine wall was measured by ligating the small intestine at one end and inserting into the other end a polyethylene cannula connected to a U-shaped mercury manometer and a Griffin-type hand bulb inflator. The tissue was placed in saline at 37° and pressure was gradually increased until the wall was torn or a bubble appeared in the solution. The tensile strength value was determined as the pressure, in mm Hg, required to rupture the intestinal wall. Animals having spontaneous perforation were scored zero tensile strength value. The results were analysed using Student's *t*-test.

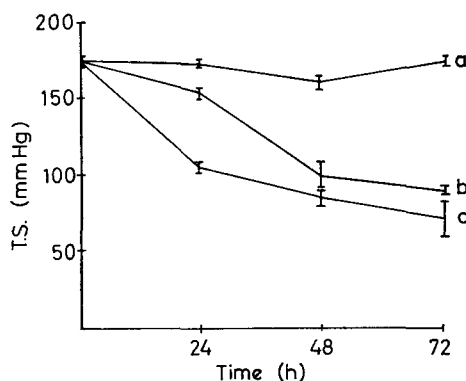A single administration of indomethacin, dose-

Fig. 1. Decrease of tensile strength of small intestine after a single oral dose of indomethacin a–5, b–10, c–20 mg kg⁻¹. Each point represents the mean ± s.e. of 20 rats.

dependently decreased the tensile strength value (Fig. 1). The inhibitory effect of sodium salicylate on drug-induced gastric ulceration is shown in Table 1 From these data it can be seen that sodium salicylate has a dose-dependent inhibitory effect on indomethacin-induced ulceration. Table 2 shows that the oral administration of indomethacin (3×10 mg kg⁻¹ on three consecutive days) produces a low tensile strength value, and causes 25% mortality but when sodium salicylate was also given the tensile strength had a normal value and there were no deaths.

The effect of sodium salicylate was more pronounced when the animals were treated with indomethacin in an oral dose of 3×20 mg kg⁻¹. Then the mortality was 73%. The simultaneous oral administration of

Table 1. *Inhibition of various drug-induced gastric ulceration by simultaneous administration of sodium salicylate.*

Treatment	n	Dose mg kg oral	No. of ulcer per stomach, (s.e.m.)
Indomethacin (Ind)	120	20	15·0 ± 0·5
Aspirin	120	100	6·5 ± 0·7
Nifluminic acid	50	25	8·6 ± 0·1
Phenylbutazone	50	100	9·7 ± 1·2
Sodium salicylate (SS)	64	200	1·2 ± 0·4
Ind + SS	18	20 + 50	7·5 ± 1·4
Ind + SS	22	20 + 100	4·0 ± 0·8
Ind + SS	58	20 + 200	0·27 ± 0·1
Aspirin + SS	40	100 + 200	0·3 ± 0·1
Nifluminic acid + SS	30	25 + 200	1·4 ± 0·5
Phenylbutazone + SS	22	100 + 200	0·2 ± 0·1

* Correspondence.

$P < 0.01$ when compared to corresponding control groups.

Table 2. *Prevention of indomethacin-induced small intestine ulceration by sodium salicylate.*

Treatment	n	Dose mg kg⁻¹, oral	Tensile strength (mm Hg)
Normal animals	40	—	181 ± 4·5
Indomethacin	20	3 × 10	38 ± 7·6†
Ind + SS	20	3 × (10 + 25)	156 ± 7·4*
Ind + SS	10	3 × (10 + 50)	176 ± 4·7
Ind + SS	10	3 × (10 + 100)	181 ± 5·4

* $P < 0.01$ when compared to indomethacin group.
† 25% mortality.

sodium salicylate (3×200 mg kg⁻¹) completely eliminated the toxic effect of indomethacin and there were no deaths.

An essential role in the main and side-effects of the aspirin-like anti-inflammatory drugs is attributed to prostaglandins (Vane, 1971). That the intestinal lesions produced by these agents could be due to a local prostaglandin deficiency is a possibility (Robert, 1974). Sodium salicylate and aspirin have almost equal anti-inflammatory actions on animals (Vane 1971; Smith, Ford-Hutchinson & Elliott 1975), but salicylic acid, in contrast to aspirin, is practically ineffective as a prostaglandin synthetase inhibitor.

To explain the antagonism described we suppose that certain types of prostaglandins play a role in maintaining the integrity of the gut and that aspirin-like drugs separately induce ulcers by unbalancing prostaglandin equilibrium. When salicylic acid and aspirin-like drugs are combined disturbances in the equilibrium are not strong enough to cause damage to the gut wall.

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Stimulation by hydrocortisone of the rate of collagen synthesis in cultured fibroblasts

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Nakagawa, Ikeda & Tsurufuji (1975) recently reported that dexamethasone sodium phosphate produced an apparent stimulation of collagen synthesis in granulation tissue *in vitro*. We wish to present the results of some experiments we have made using another water soluble anti-inflammatory steroid, hydrocortisone sodium succinate, in which stimulation of collagen synthesis by cultured human fibroblasts was observed.

Early passage human foetal skin fibroblasts were obtained by trypsinization of the skin from 3 to 5 month old human embryos, all experiments were performed on cells which had been passaged between 5 and 10 times. The cells were maintained in monolayer culture in Dulbecco's modification of Eagle's minimal essential medium (Gibco-Biocult) containing 10%

foetal calf serum (Flow Laboratories), 100 μg ml⁻¹ penicillin and 50 μg ml⁻¹ streptomycin sulphate in an atmosphere of 5% CO_2 in air at 37°. For experiments cells were trypsinized and suspended in medium at a concentration of $2 \cdot 5 \times 10^5$ cells ml⁻¹ and 2 ml of this suspension was added to plastic culture tubes with a growth area of 5 cm². Three to 5 days later when the cells had formed a dense confluent monolayer, the medium was removed and replaced by 2 ml of fresh medium containing 50 μg ml⁻¹ ascorbic acid plus the drug. The sodium succinate salt of hydrocortisone was used since it is freely soluble in water and so does not require the addition of organic solvents to the medium. One h after the addition of the drug 5 μCi of tritiated proline ([L-G-³H]proline, 653 mCi mmol⁻¹, Radiochemical Centre, Amersham) was added in 100 μl of phosphate buffered saline. To terminate the incubation the tubes were transfered to crushed ice and 2 ml of ice cold 0·9% NaCl containing 0·1% proline and 0·2%