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On the relationship between ulcerogenic and anti-inflammatory properties of indomethacin

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It has been shown (Volterra et al 1978; Del Soldato et al 1979) that indomethacin-induced intestinal lesions, inhibit the development of carrageenan-induced paw oedema in the rat. Preliminary kinetic studies suggested that in rats with intestinal ulcers, plasma levels of indomethacin were too low to account for any specific (indomethacin related) anti-oedema effect. Therefore we have examined the anti-oedema potency of indomethacin in relation to its plasma levels in presence or absence of intestinal lesions.

Materials and methods

Male albino rats, Sprague-Dawley-Morini strain, 160–190 g, were housed in plastic cages with wire bottoms to minimize coprophagy. Indomethacin was administered orally suspended in an aqueous vehicle containing NaCl 0·9% Tween 80 0·4%, carboxymethylcellulose 0·5% and benzyl alcohol 0·9% and administered by gavage at different time intervals before carrageenan (as shown in Table 1). Paw oedema was induced as described by Winter et al (1962): 0·1 ml of 0·5% suspension of carrageenan (Rex 7205, Marine Colloids Inc., Springfield, N.J.), was injected into the plantar aponeurosis of the right hind paw. Foot volume was measured immediately following carrageenan and again 3 h later by means of a mercury plethysmometer. Animals were autopsied 5 h after carrageenan and the intestine carefully examined for presence of ulceration by an observer who was unaware of the treatment. The degree of intestinal ulceration was graded according to an arbitrary scale: 0 = normal; 1 = primary ulcers; 2 = advanced ulcerative processes; 3 = perforating ulcer and intestinal adhesions (Del Soldato et al 1979). Blood samples were collected by cardiac puncture by means of a heparinized syringe under CO_2 anaesthesia. Plasma concentration of indomethacin was determined according to a slight modification of the method described by Wahlin-Boll et al (1981) using a Hewlett-Packard HPLC 1084B.

Data relative to two groups were analysed by Student's *t*-test. When more than two groups were compared, data were analysed by means of the analysis of variance. When the test indicated a significant F value, inspection of all differences between pairs of means was made according to the LSD method (Snedecor et al 1972). The linear regression was calculated according to the method of least squares.

Results and discussion

Statistical analysis of data in Table 1 indicate that in the presence of intestinal pathology of moderate degree or in absence of it, the anti-oedema effectiveness of indomethacin was proportional to its plasma concentration (Fig. 1a) and therefore ascribable to the specific

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Table 1. Effects of plasma concentration of indomethacin and degree of intestinal pathology on carrageenan-induced paw oedema in the rat.

Group	Indomethacin (mg kg^{-1}) and time (h) before carrageenan	Increase in paw volume 3 h after carrageenan (mean ± s.e.)	% inhibition	Intestinal ulcers		Plasma indomethacin[a] μg ml^{-1} Mean ± s.e.
				% incidence	Degree (mean score)	
Control	—	13·8 ± 0·35 (128)	—	—	—	—
A	0·5 mg 1h	11·0 ± 1·33 (10)	14	—	—	0·93 ± 0·06 (16)
B	1·0 mg 1h	10·2 ± 0·89 (10)*	26	—	—	1·43 ± 0·16 (16)
C	2·0 mg 1h	8·5 ± 1·01 (10)**	38	—	—	3·15 ± 0·31 (16)
D	6·0 mg 1h	6·2 ± 0·71 (10)**	55	—	—	11·5 ± 1·06 (16)
E	6·0 mg 30h	10·4 ± 0·65 (30)**	25	60	0·63	2·2 ± 0·18 (16)
F	6·0 mg 42h	14·0 ± 0·61 (30)	—	6·6	0·06	0·79 ± 0·16 (16)
G	6 + 6 mg 42 & 30h	2·1 ± 0·27 (30)**	85	100	2·83	2·25 ± 0·24 (16)
H	6 + 6 mg 66 & 54h	4 0 ± 0·38 (38)**	71	100	2 76	0·6 ± 0·11 (16)
Least significant difference:				$P < 0·05$	0·47	
				$P < 0·01$	0·62	

(a) Determined at the time carrageenan should have been injected.
In parentheses number of animals.
* $P < 0·05$.
** $P < 0·01$.

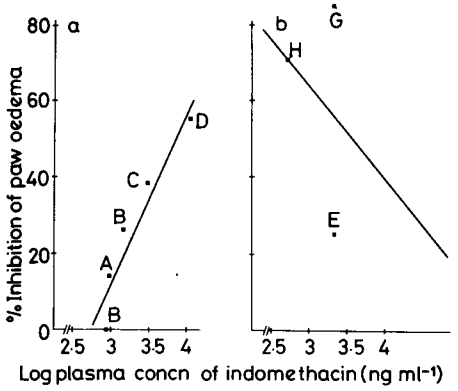

FIG. 1. Correlation between plasma concentration of indomethacin and inhibition of paw oedema in groups having pathologic mean score minor (a) and greater (b) than 0·47 (see Table 1). (a): n = 5, r = 0·968 and $P < 0{\cdot}01$. (b): n = 3, r = 0·282 and P not significant.

anti-inflammatory properties of the drug. On the other hand, the presence of marked inflammation in the intestine produced a considerable reduction in oedema development (Table 1) unrelated to the plasma concentration of drug (Fig. 1b) and therefore conceivably attributable to factor(s) other than its specific anti-inflammatory properties (Del Soldato et al 1979).

The non-specific role played by pre-existing inflammatory processes is substantiated by the observation that the plasma concentration of indomethacin that produced marked anti-oedema inhibition in presence of intestinal ulceration was below that which did not influence oedema formation in absence of intestinal pathology.

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Acetylcholine stimulates the release of prostacyclin by rabbit aorta endothelium

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Acetylcholine causes relaxation of isolated blood vessels in-vitro. Furchgott & Zawadski (1980) have shown that removal of the endothelium from the isolated blood vessels of different species abolishes the relaxation induced by the cholinergic transmitter. Since prostacyclin (PGI_2) is a potent vasodilator (Bunting et al 1976) and the major product of arachidonic acid metabolism in vascular endothelial cells (Weksler et al 1977), the effect of acetylcholine on the release of prostacyclin by rabbit aorta endothelium was studied. For this purpose, the 'well' or 'template' technique, originally described by Eldor et al (1981) was modified to limit the damage to the endothelial cells.

Methods

Rabbits were heparinized (100 iu kg^{-1} i.v.) before being killed by a blow on the head and exsanguination. The thorax was opened and the aorta was freed from fat and connective tissue. The aorta was cannulated and continuously perfused with oxygenated Krebs solution to remove the blood. The aorta was then removed to a petri dish, filled with Krebs solution. The remaining fat and connective tissue was further removed and the aorta was cut open longitudinally, in between the intercostal branches. During the entire procedure, the aorta was perfused with Krebs solution, to prevent the aorta from collapsing and the endothelium being damaged. Prepared in this way, the aortic intima remained completely covered with endothelium cells, as shown by silver nitrate staining. In experiments in which the aorta was not prevented from collapsing during the preparation, large areas of the aortic intima were denuded of endothelial cells.

The aorta was then placed between two lucite plates, held together with 4 lateral screws. The upper plate contained six holes (Ø 7 mm) narrower than the aortic width which served as incubation chambers in which the aortic luminal surface formed the chamber base. No leakage occurred from one chamber to another, and no diffusion out of the well of a solution of Evans blue or cyanocobalamin took place. The chambers were filled with 100 µl isotonic Hepes buffer and the aorta was allowed to equilibrate for 40 min. After the preincubation, the buffer was discarded and the chambers were refilled with 100 µl Hepes buffer. After incubation for

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