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INFLUENCE OF FASTING AND CIMETIDINE ON THE RELATIONSHIP BETWEEN ULCEROGENIC AND ANTI-INFLAMMATORY PROPERTIES OF INDOMETHACIN

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1 Further studies have been carried out on the relationship between ulcerogenic and anti-inflammatory properties of indomethacin in the rat.

2 Fasting, which increases gastric and reduces intestinal lesions, enhances the anti-oedema properties of indomethacin.

3 The presence of intestinal lesions, greatly increases the anti-oedema properties of indomethacin through a mechanism(s) unrelated to the specific pharmacological properties of this drug.

4 Studies with cimetidine, have shown that the enhancement of anti-oedema effects produced by fasting are due to specific pharmacological properties of indomethacin rather than to non specific effects related to the presence of gastric ulcers.

5 The greater anti-inflammatory effects of indomethacin in fasted as compared to fed animals must be attributed to the greater amount of indomethacin available for tissue distribution rather than other mechanisms associated with free fatty acid mobilization.

6 In view of its ineffectiveness in preventing intestinal lesions, the use of cimetidine for prevention or reduction of indomethacin-induced gastrointestinal disturbances in humans is contraindicated.

Introduction

It has been shown (Volterra, Del Soldato & Meli, 1978) that the activity and side effects of non steroidal anti-inflammatory agents cannot always be attributed to mechanism(s) involving common factors and that the presence of pre-existing inflammatory processes at intestinal level enhances the anti-oedema effects of indomethacin in the rat.

Fasting, which increases gastric (Shriver, White, Sandor & Rosenthale, 1975) and reduces intestinal lesions (Volterra, Pisanti & Meli, 1974; Del Soldato & Meli, 1978) and toxicity (Del Soldato & Meli, 1978) enhances the anti-oedema effects of indomethacin. This prompted us to determine whether or not this phenomenon should be attributed to the presence of gastric lesions or to modifications in indomethacin kinetics. The first of the two hypotheses has been tested by the use of cimetidine which inhibits indomethacin-induced gastric ulceration (Okabe, Takeuchi, Urishidani & Takagi, 1977), while the second was tested by determination of plasma levels of indomethacin and free fatty acid (FFA), the rise in which is elicited by fasting (Brodie, Krishna & Hynie, 1969). Changes in plasma FFA have been associated with the displacement of protein-bound non steroidal anti-inflammatory drugs (Solomon, Schrongie & Williams, 1968; Rudman, Bixler & Del Rio, 1971) and subsequent interference with their distribution and pharmacological activity (Volterra *et al.*, 1978).

Methods

Male albino rats, Wistar-Nossan strain, weighing 170 to 180 g were divided into groups of 8 or more animals and housed in plastic cages with wire bottoms to minimize coprophagy. Indomethacin and cimetidine were 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. Rats were fasted from 17 h before administration of indomethacin until the end of the experiment unless otherwise specified.

Carrageenin-induced paw oedema following three days treatment with indomethacin

Fed animals Fourteen out of 22 rats in this group received oral indomethacin (18 mg/kg) in 3 doses of 6 mg/kg, at 42, 30 and 1 h before the injection of carrageenin. The remaining animals (controls) received the suspending vehicle. Paw oedema was in-

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duced as described by Winter, Risley & Nuss (1962): 0.1 ml of 0.5% suspension of carrageenin (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 carrageenin and again 3 h later by means of a mercury plethysmometer.

Fasted animals Thirteen rats were treated with indomethacin and eight with vehicle alone. Administration was preceded and followed by fasting periods as follows: Monday: 15 h 00 min food removal, 19 h 00 min indomethacin; Tuesday: 07 h 00 min indomethacin, 15 h 00 min access to food; Wednesday: 09 h 00 min food removal, 13 h 00 min indomethacin, 14 h 00 min carrageenin, 17 h 00 min determination of paw volume; Thursday: 10 h 00 min autopsy: the intestine was examined for presence of ulceration by an observer who was unaware of the treatment.

Influence of fasting and cimetidine on indomethacin-induced gastric ulcers and anti-oedema activity

In this modification of the above method, 8 groups of 8 rats were either fed or fasted for 17 h. They received orally indomethacin (6 mg/kg) and/or cimetidine (100 mg/kg) and/or the aqueous vehicle. Animals were autopsied 5 h after carrageenin and the stomach and intestine carefully examined for presence of ulceration by an observer who was unaware of the treatment. The degree of gastric lesion was graded according to an arbitrary scale: 0 = normal; 1 = small sized ulcer; 2 = medium sized ulcer; 3 = large sized ulcer. Scores were reduced to ranks and analyzed by means of a Kruskal Wallis non-parametric one way analysis of variance (Colquhoun, 1971, pp. 193–195). Multiple comparisons among groups were made by means of the critical range method (Colquhoun, 1971, pp. 208–209).

Carrageenin-induced paw oedema and effects on plasma concentration of free fatty acid and indomethacin

Four groups of 15 rats were either fed or fasted for 17 h. They received orally indomethacin (5 mg/kg) or the aqueous vehicle. Paw oedema was induced as described above. Five animals of each group were used to determine plasma concentration of FFA and indomethacin. Blood samples were obtained under ether anaesthesia by heart puncture with a heparinized syringe. After centrifugation (30 min at 950 *g*), plasma FFA were determined according to Ducombe (1973) with slight modifications (Volterra *et al.*, 1978). Plasma indomethacin was determined according to Kwan, Breault, Umbenhauer, McMahon & Duggan (1976).

Influence of cimetidine on incidence and degree of indomethacin-induced intestinal lesions

Unfasted rats received oral cimetidine in two doses of 100 mg/kg each at 30 min and 12 h after oral indomethacin (12 mg/kg). This dose was selected as it had been shown to induce intestinal ulcers in 90 to 100% of the animals (Volterra *et al.*, 1974). Animals were killed 72 h after indomethacin and the intestine was carefully examined for presence of ulceration by an observer 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.

Statistical procedure

The data relative to carrageenin-induced paw oedema were calculated as adjusted values derived from the analysis of covariance where the variates X and Y represent paw volume before carrageenin and the increase 3 h later respectively. Contrasts among adjusted means were calculated according to Snedecor & Cochran (1972, p. 423). The data relative to to plasma concentration of FFA or indomethacin were calculated 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 LSD method (Snedecor & Cochran, 1972, pp. 271–273). Percentage inhibition values as well as degree of gastro-intestinal lesions were analyzed by means of Student's *t* test, unless otherwise specified in the tables. The correlations between indomethacin anti-oedema properties and degree of gastric or intestinal lesions were calculated according to the Spearman rank coefficient (Colquhoun, 1971, pp. 274–276).

Results

Carrageenin-induced paw oedema following three days treatment with indomethacin

Analysis of data in Table 1 indicates that indomethacin significantly reduced paw oedema formation in both fed and fasted animals, the degree of inhibition being significantly greater in fed than in fasted rats. There is a close relationship between anti-oedema properties and degree of intestinal lesions ($P < 0.001$). Both incidence and degree of indomethacin-induced intestinal lesions were significantly greater in fed than in fasted animals.

Influence of fasting and cimetidine on indomethacin-induced gastric ulcers and anti-oedema activity

Analysis of data in Table 2 indicates that indomethacin significantly reduced paw oedema formation in both fed and fasted animals, the degree of inhibition being significantly greater in fasted as compared to fed rats. Cimetidine did not influence anti-oedema properties of indomethacin in fed or fasted rats. The degree of indomethacin-induced gastric lesions was significantly greater in fasted than in fed animals and markedly reduced by cimetidine. Paw oedema development is unaffected by either incidence or degree of indomethacin-induced gastric lesions (Spearman rank coefficient with $P > 0.7$).

Carrageenin-induced paw oedema and effects on plasma concentration of free fatty acid (FFA) and indomethacin

Analysis of data in Table 3 indicates that indomethacin significantly reduced paw oedema formation in fed as well as fasted rats, the degree of inhibition being significantly greater in fasted than in fed animals. Plasma concentration of FFA was independent of feeding schedule and indomethacin ($F < 1$, $P > 0.025$). Plasma concentration of indomethacin was significantly greater in fasted than fed animals.

Influence of cimetidine on incidence and degree of indomethacin-induced intestinal lesions

Results in Table 4 show that cimetidine did not influence indomethacin-induced intestinal lesions.

Table 1 Effects of fasting and oral indomethacin (18 mg/kg in three divided doses, see Methods) on carrageenin-induced paw oedema and incidence and degree of intestinal lesions

Experimental design		No. of animals	Paw volume increase after carrageenin: Adjusted mean	Paw volume increase after carrageenin: % inhibition	Intestinal ulcers: % incidence	Intestinal ulcers: Degree (mean score)
Fed	Controls	8	17.48			
	Indomethacin	14	2.96	83.1	100	1.9
Fasting	Controls	8	20.92			
	Indomethacin	13	9.90	52.7*	46	0.92*
Least significant difference at the following probability level.	$P < 0.05$		2.96			
	$P < 0.01$		3.96			

* $P < 0.01$ as compared to fed animals.

Table 2 Effects of oral cimetidine (100 mg/kg), fasting and oral indomethacin (6 mg/kg) on gastric ulcers and anti-oedema activity in the rat

Experimental design		No. of animals	Paw volume increase after carrageenin: Adjusted mean	Paw volume increase after carrageenin: % inhibition	Gastric ulcers: % incidence	Gastric ulcers: Degree: Mean score	Gastric ulcers: Degree: Mean rank
Fed rats	Controls	8	17.0	—	—	—	—
	Cimetidine	8	17.2	0	—	—	—
	Indomethacin	8	9.7	38.9	12.5	0.13	12.5
	Indomethacin + cimetidine	8	10.0	41.4	0	0	11
Fasted rats	Controls	8	19.0	—	—	—	—
	Cimetidine	8	16.7	12.6	—	—	—
	Indomethacin	8	8.0	58.0*	100	16	28.5
	Indomethacin + cimetidine	8	7.5	60.6*	25	0.25	14
Least significant difference	$P < 0.05$		3.3				12.6
	$P < 0.01$		4.4				14.1

* $P < 0.01$ as compared to fed rats.

Discussion

Our aim was to ascertain whether or not reduction in gastro-intestinal lesions is accompanied by a concomitant modification in anti-inflammatory activity. The results indicate that, as in the case of a fat-free diet (Volterra *et al.*, 1978), fasting prevented or reduced intestinal ulcers but enhanced the anti-oedema properties of indomethacin. The observation that indomethacin, administered over three days in doses which cause intestinal lesions, was more effective in inhibiting carrageenin-induced paw oedema in fed as compared to fasted animals appears to be at variance with these findings. This phenomenon may be due to a nonspecific effect, such as the existence of intestinal ulcers which are present to a greater extent in fed than in fasted rats, superimposed on the specific anti-oedema effects (Volterra *et al.*, 1978). The possibility that the results are due to differences in metabolism or distribution of indomethacin, associated with different dosage regimens has not been excluded; however, we have previously observed (Volterra *et al.*, 1978) that indomethacin administered 42 and 30 h before carrageenin almost completely inhibits paw oedema formation at a time when the anti-inflammatory agent is virtually absent (Yesair, Remington, Callahan & Kensler, 1970).

Fasting inhibits the production of intestinal but favours that of gastric lesions. Evidence that the presence of gastric lesions does not affect the anti-oedema properties of indomethacin is provided by the observation that cimetidine, administered in doses which prevented or reduced gastric ulceration, did not affect anti-oedema activity. The fact that cimetidine did not interfere even with anti-oedema effects of non-ulcerogenic doses of indomethacin (unpublished observations) indicates that the former does not mask the non specific effects of the latter through a reduction of its specific properties. Therefore it would appear that oedema formation is unaffected by the presence of gastric lesions. In view of the above, the relatively greater anti-oedema activity in fasted rats is probably related to a specific anti-inflammatory effect, due to the greater amount of indomethacin available for tissue distribution rather than to an involvement of other factors such as a modification in plasma concentration of FFA, whose increase is known to affect the distribution (Solomon *et al.*, 1968; Rudman *et al.*, 1971) as well as the pharmacological activity (Volterra *et al.*, 1978) of non-steroidal anti-inflammatory drugs.

In man, fasting produces changes in indomethacin kinetics (Rothermich, 1966; Champion, Paulus, Mongan, Okun, Pearson & Sarkissian, 1972; Wallusch, Leopold & Netter, 1978) and side effects (Huskisson,

Table 3 Effects of fasting and oral indomethacin (5 mg/kg) on carrageenin-induced paw oedema, plasma concentration of free fatty acids (μmol/ml) and indomethacin (μg/ml)

Experimental design		*No. of animals*	*Increase in paw volume after carrageenin* *Adjusted mean*	*% inhibition*	*No. of animals*	*Plasma free fatty acids* *Mean ± s.e.*	*Plasma indomethacin* *Mean ± s.e.*
Fed	Controls	15	19.6	—	5	1.41 ± 0.35	—
	Indomethacin	15	14.9	24	5	2.13 ± 0.81	14.3 ± 1.0
Fasting	Controls	15	20.3	—	5	0.95 ± 0.13	—
	Indomethacin	15	12.2	39.7*	5	1.74 ± 0.26	22.7 ± 2.2
Least significant difference at the following probability level		$P < 0.05$	2.5				3.9
		$P < 0.01$	3.4				5.4

* $P < 0.01$ as compared to fed animals.

Table 4 Effect of cimetidine (200 mg/kg in two divided doses) on incidence and degree of intestinal ulcers after oral indomethacin (12 mg/kg) in the rat

Treatment	*No. of animals*	*% incidence of ulcers*	*Ulcer degree (mean score)*
Indomethacin	10	100	2.1
Indomethacin + cimetidine	10	100	2.2

Taylor, Burston, Chuter & Hart, 1970) similar to those observed in rats, suggesting a possible similarity in the sequence of events leading to gastro-intestinal pathology; that is, gastric irritation and ulcers, followed by intestinal involvement, ulceration and peritonitis. The fact that in man, intestinal involvement is not very frequent, is not at variance with this, for indomethacin treatment is usually stopped at the first signs of abdominal pain (index of gastric irritation). Okabe *et al.* (1977) suggested that cimetidine might be used to prevent gastro-intestinal side effects of drugs. However, its inability to inhibit intestinal lesions in rats in doses which prevent gastric erosions, suggests that the use of cimetidine to reduce gastro-intestinal disturbances caused by indomethacin is contraindicated.

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