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Indomethacin: Relationship between Ulcerogenic and Anti-Inflammatory Properties (40108)

I. Effects of an Intestinal Lesion-Preventing Fat-Free Diet on Anti-Edema and Anti-Granuloma Properties of Indomethacin in the Rat

G. VOLTERRA, P. DEL SOLDATO, AND A. MELI

Pharmacology Department, Research Laboratories, A. Menarini Pharmaceuticals, 50131 Florence, Italy

The activity and side effects of nonsteroidal anti-inflammatory agents (NSAI) are attributed to mechanism(s) involving common factors (1, 2). Therefore, it seemed worthwhile to determine whether or not a fat-free diet (FFD), shown to reduce indomethacin-induced intestinal lesions (3) and toxicity (4), would affect its anti-inflammatory activity. Our preliminary data indicated that FFD does not reduce, but rather increases, this activity. However, our initial experimental design did not include the prostaglandin phase of inflammation, shown to be reduced in rats fed an essential fatty acid-deficient diet (5).

In view of the above, we thought it worthwhile to determine the possible effects of FFD on the essential fatty acid pattern and to extend our observation period to include FFD effects on the prostaglandin phase of inflammation.

Materials and methods. Male albino rats, Wistar strain, weighing 110–130 g, were divided into groups of eight or more animals each and fed either a regular or a fat-free diet over a 10-day period before commencing the experiments, unless otherwise specified. Indomethacin was administered by gavage suspended in an aqueous vehicle (NaCl, 0.9%; Tween 80, 0.4%; CMC, 0.5%; and benzyl alcohol, 0.9%).

The regular diet (RD) consisted of 33.3% yellow corn, 12.7% soybean flour, 12.7% meat meal, 14.8% wheat bran, 4.5% barley flour, 4.5% oatmeal, 7.4% nonfat milk solids, 2.8% linseed flour, 3.7% yeast, and 3.6% vitamins and minerals. The fat-free diet (FFD) (6) consisted of 30% vitamin-free casein, 64% starch, 2% cellulose, and 4% vitamins and minerals.

(A) Effect of diet on growth curve. Two groups of 20 animals each were fed either a regular (RD) or a fat-free (FFD) diet over a 31-day period. The growth curve was recorded at several time intervals up to the end of the observation period.

(B) Carrageenin-induced paw edema. Immediately before the administration of indomethacin or the aqueous vehicle, each animal received 5 ml of water by gavage. One hour later, 0.1 ml of a 0.5% suspension of carrageenin (Rex 7205, Marine Colloids Inc., Springfield, N.J.) was injected through a 20-gauge needle into the plantar aponeurosis of the right hind paw (7). Foot volume was measured immediately following carrageenin administration and again 3 hr later by means of a mercury plethysmometer. This was done by immersing the paw of the unanesthetized animal up to the point where mercury was at the level of the lateral malleolus. The increase in paw volume was calculated from the difference in the values at 0 and 3 hr after the injection of the phlogistic agent.

(C) Effects on the essential fatty acid (EFA) pattern and the prostaglandin phase of inflammation. Animals were handled as described in B, with the exceptions that: (i) Paw edema was measured at 3 and 6 hr [prostaglandin phase (5)] after carrageenin administration; and (ii) groups of five animals each were used to determine plasma free fatty acids (FFA) and indomethacin, as well as the arachidonic (20:4) to eicosatrienoic (20:3) acid ratio in erythrocytes as a measure of the EFA pattern (8). Blood samples were obtained under ether anesthesia by heart puncture using a heparinized syringe. After centrifugation (30 min, 950*g*), plasma FFA were determined according to Ducombe (9), except that chloroform–heptane–methanol (49:49:2) was used as the extraction mixture. Plasma indomethacin was determined according to Kwan *et al.* (10). Arachidonic and eicosatrienoic acids

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were determined in erythrocytes' total fat through extraction (11) and alkaline isomerization (12).

(D) Cotton pellet-induced granuloma. Under light ether anesthesia, one cotton pellet was introduced subcutaneously on each side of the back through a midline incision. The pellets were cut from dental cotton rolls (No. 1, Johnson & Johnson) and paired to a combined dry weight of 100 ± 1 mg. Prior to implantation, the pellets were dipped for 1 min in a 1% suspension of carrageenin, placed on filter paper, and allowed to dry overnight under a lamp. Thereafter the pellets were autoclaved at 121°C for 1 hr (13). At the time of implantation, the pellets were individually soaked in a 1% solution of Pfizer Combiotic. Treatment started at the same time as implantation and lasted for 8 consecutive days. On the morning of the eighth day, immediately before the last treatment, each animal received 5 ml of water by gavage. One hour later, 0.1 ml of a 0.5% carrageenin suspension was injected through a 20-gauge needle into the plantar aponeurosis of the right hind paw. Thereafter the procedure is similar to that described in B. Immediately after measuring the paw volume, the animals were killed by CO_2 asphyxiation and the pellets with the surrounding granuloma were excised and dried overnight at 60°C. The amount in excess of 100 mg represents the weight of the granuloma, which was expressed either in absolute values or in mg/100 g body weight (14).

(E) Three-day carrageenin paw edema (15). This modification of the method outlined in B was carried out as follows.

(i) Animals were divided into two groups of 20–22 rats each and were fed either a regular (RD) or a fat-free (FFD) diet. Fourteen to fifteen animals of each group received oral indomethacin (18 mg/kg) in three divided doses of 6 mg each, at 42, 30, and 1 hr before carrageenin. Seventeen hours after measuring the degree of swelling, the animals were autopsied and the intestine was examined for the presence of ulceration by an observer who was unaware of the treatment.

(ii) Animals were divided into six groups of 8 rats each and fed a regular diet (RD). Indomethacin was given orally in single doses of 6 mg/kg each at different time intervals before carrageenin, as specified in Table VI. This technique of dosing, which differs from that outlined in (i), was aimed at elucidating to what extent the anti-edema properties of indomethacin were due to a specific (anti-inflammatory) or to a nonspecific (ulcerogenic) effect. Seventeen hours after measuring the degree of swelling, the animals were autopsied and the intestine was carefully and thoroughly examined for the presence of inflammation site(s) and/or ulceration by an observer who was unaware of the treatment. For a better evaluation of the specific to nonspecific anti-edema ratio, the degree of intestinal pathology was graded according to the scale shown in Table VI.

(F) Statistical procedures. The data relative to carrageenin-induced paw edema were calculated as adjusted values derived from an analysis of covariance, where the variates x and y were the paw volume before carrageenin and the increase in paw volume at 3 and 6 hr after the injection of the phlogistic agent, respectively. Contrasts among adjusted means were calculated according to Snedecor and Cochran (16).

The data relative to granuloma weight, plasma concentration of FFA, or indomethacin and arachidonic acid content in erythrocytes were calculated by means of an analysis of variance. When the test indicated significant F values, an inspection of all differences between pairs of means was made according to the LSD method (17).

Percentage inhibition values as well as average body weight data were subjected to Student's t test unless otherwise specified in the tables. The data relative to incidence and degree of intestinal ulcers were analyzed by means of the χ^2 test (corrected for continuity) and the Wilcoxon test (18), respectively, unless otherwise specified in the tables. The correlation between indomethacin-induced body weight changes, anti-edema activity, and degree of intestinal pathology was calculated according to the Spearman rank coefficient (19).

Results. (A) Effect of diet on the growth curve. The data in Table I indicate that, from Day 10 on, FFD significantly reduced the growth rate in the male rat.

(B) Carrageenin-induced paw edema. Statistical analysis of the data in Tables II and III indicates that: (i) Indomethacin significantly reduces carrageenin-induced paw

TABLE I. EFFECT OF DIET ON THE GROWTH CURVE OF MALE RATS.

Experimental design	No. of animals	Body weight in grams (mean ± SE) on Days: 0	10	17	24	31
Regular diet (RD)	20	135.3 ± 1.8	193.0 ± 4.3	227.2 ± 6.2	259.1 ± 6.4	284.5 ± 5.8
Fat-free diet (FFD)	20	135.3 ± 1.8	179.0 ± 6.1	198.3 ± 6.5*	213.1 ± 7.8*	232.9 ± 10.5*

* $P < 0.01$.

TABLE II. EFFECT OF DIET AND ORAL INDOMETHACIN (5 mg/kg) ON CARRAGEENIN-INDUCED PAW EDEMA.

Experimental design	No. of animals	Increase in paw volume after carrageenin: Adjusted mean	Percentage inhibition
Regular diet (RD)			
Indomethacin	8	13.2	30
Controls	8	19.0	
Fat-free diet (FFD)			
Indomethacin	8	8.0	52.1*
Controls	8	17.1	
Least significant difference			
$P < 0.05$		5.9	
$P < 0.01$		8.0	

* $P < 0.05$ compared to RD group.

edema in both RD and FFD rats; (ii) the degree of inhibition is significantly greater in FFD as compared to RD animals.

(C) Effects on the essential fatty acid (EFA) pattern and the prostaglandin phase of inflammation. Statistical analysis of the data in Table III indicates that: (i) Six hours after carrageenin, the paw edema volume is identical in RD and FFD control rats. (ii) Six hours after carrageenin, the paw edema volume of indomethacin-treated RD and FFD rats is significantly lower than that of control animals. (iii) The arachidonic acid content in erythrocytes is independent of the nature of the diet, carrageenin, or indomethacin treatment. There is no detectable amount of eicosatrienoic acid in either RD or FFD rats. (iv) Independently of diet and indomethacin, the plasma FFA concentration is significantly increased at 3, but not at 6, hr after carrageenin. This increase is significantly greater in FFD as compared to RD rats. (v) There is no detectable difference in plasma concentration of indomethacin between RD and FFD rats at either 3 or 6 hr after carrageenin.

(D) Cotton pellet-induced granuloma. Statistical analysis of the data in Table IV indicates that indomethacin significantly reduces granuloma formation and carrageenin-induced paw edema in both RD and FFD rats, the degree of inhibition being significantly greater in FFD as compared to RD animals.

(E) Three-day carrageenin paw edema. (i) Statistical analysis of the data in Table V indicates that: (a) Indomethacin significantly reduces carrageenin-induced paw edema in both RD and FFD animals, the degree of inhibition being significantly greater in RD as compared in FFD rats. (b) Decrease in body weight (limited to RD rats) is associated with greater anti-inflammatory activity, as indicated by the close relationship ($P < 0.001$) between body weight changes determined immediately before the last dose of indomethacin and anti-edema activity. (c) Both the incidence and degree of indomethacin-induced intestinal lesions are significantly greater in RD as compared to FFD rats.

(ii) Statistical analysis of the data in Table VI indicates that: (a) Indomethacin administered 42 hr before carrageenin does not influence paw edema formation. (b) Indomethacin administered either at 42 and 30, at 30 and 1, or at 1 hr before carrageenin produces a significant but similar antiedema effect. Such an effect is significantly lower than that observed following indomethacin administered at 42, 30, and 1 hr before carrageenin. (c) There is a close relationship (Fig. 1) between anti-edema activity, degree of intestinal pathology, and body weight changes. The latter were determined immediately before the last dose of indomethacin.

Discussion. Reduction and/or prevention of indomethacin-induced intestinal lesions has been achieved by a variety of experimental conditions, such as fasting (3, 21), fat-free diet (3, 4), various catatoxic steroids (22, 23), prostaglandins (24), cholestyramine (21), low-residue diet, (25) and antibiotics (26).

With the exception of spironolactone, which has been shown to reduce both intes-

TABLE III. EFFECT OF DIET AND ORAL INDOMETHACIN (5 mg/kg) ON CARRAGEENIN INDUCED PAW EDEMA, PLASMA CONCENTRATION OF FREE FATTY ACIDS (μmole/ml), AND INDOMETHACIN (μg/ml) AND ARACHIDONIC ACID CONTENT IN ERYTHROCYTES (PERCENTAGE OF TOTAL LIPIDS).

Experimental design	Time after carrageenin (hr)	No. of animals	Paw volume increase after carrageenin: Adjusted mean	Paw volume increase after carrageenin: Percentage inhibition	No. of animals	Plasma free fatty acids[b]	Erythrocyte arachidonic acid[b]	Plasma indomethacin[b]
Regular diet (RD)								
Indomethacin	3	14	8.8	45.6	5	2.64 ± 0.57	7.7 ± 1.9	14.2 ± 1.1
	6	9	10.6	36.0	5	1.08 ± 0.28	9.2 ± 2.0	8.3 ± 1.4
	0	—	—	—	5	0.58 ± 0.15	8.0 ± 1.4	—
Controls	3	15	16.1	—	5	2.85 ± 0.42	11.7 ± 3.6	—
	6	10	16.6	—	5	1.09 ± 0.57	9.0 ± 1.8	—
Fat-free diet (FFD)								
Indomethacin	3	15	7.8	60.1*	5	4.1 ± 0.57	12.3 ± 2.3	14.3 ± 1.6
	6	10	12.2	28.0	5	1.01 ± 0.25	6.6 ± 2.0	10.7 ± 1.5
	0	—	—	—	5	0.76 ± 0.22	9.9 ± 0.2	—
Controls	3	15	19.7	—	5	4.12 ± 0.46	10.4 ± 2.4	—
	6	10	16.5	—	5	1.1 ± 0.28	5.2 ± 1.1	—
Least significant difference[a]								
$P < 0.05$			2.5 (4.0)			1.17		2.6
$P < 0.01$			3.3 (5.3)			1.57		3.5

[a] Those relative to paw volume increase at 6 hr are in parentheses.
[b] Mean ± SE.
* $P < 0.05$ as compared to paw volume increase of RD group at 3 hr.

TABLE IV. EFFECTS OF DIET AND ORAL INDOMETHACIN (2.5 mg/kg/day) ON GRANULOMA FORMATION AND CARRAGEENIN-INDUCED PAW EDEMA.

Experimental design	No. of animals	Granuloma weight (mean ± SE): Absolute wt (mg)	Granuloma weight (mean ± SE): Milligrams per 100 g body wt	Percentage inhibition: Absolute wt (mg)	Percentage inhibition: Milligrams per 100 g body wt	Increase in paw volume after carrageenin: Adjusted mean	Increase in paw volume after carrageenin: Percentage inhibition
Regular diet (RD)							
Indomethacin	8	107.1 ± 7.9	56.2 ± 3.6	30.5	26.0	8.7	42.4
Controls	8	154.1 ± 19.8	75.9 ± 9.0			15.1	
Fat-free diet (FFD)							
Indomethacin	8	93.4 ± 6.2	53.9 ± 4.6	43.2	41.2*	4.1	72.4*
Controls	8	164.4 ± 9.8	91.6 ± 7.0			14.9	
Least significant difference							
$P < 0.05$		36.9	18.5			2.9	
$P < 0.01$		49.8	25.0			3.9	

* $P < 0.05$ compared to RD group.

tinal lesions and anti-inflammatory activity (27), no effort has been made to determine whether or not a reduction in intestinal lesions is accompanied by a concomitant modification in anti-inflammatory activity.

Our data indicate that FFD prevented and/or reduced intestinal ulcers without reducing, but rather enhancing, the anti-inflammatory properties of indomethacin. This enhanced activity, observed in models of both chronic and acute inflammation, but particularly evident in the latter, could be attributed to differences in body distribution of indomethacin between RD and FFD rats, due to a dissimilarity in protein binding. This hypothesis is consistent with data indicating that:

(i) There is a correlation (28, 29) between protein-bound indomethacin (30, 31) and anti-inflammatory activity.

(ii) Any increase in plasma FFA concentration ranging from 2 to 4 m*M* (32), like that observed in FFD animals, has been associated with the displacement of protein-bound NSAI (32–34).

(iii) A direct involvement of catecholamines (35) and/or corticosterone in enhancing the anti-inflammatory activity of indomethacin in FFD rats seems unlikely, since increase in paw volume of the untreated animals was independent of the nature of the diet.

(iv) An indirect involvement of corticosterone in enhancing the anti-inflammatory activity of indomethacin in FFD animals through an indomethacin-induced modification in the free to protein-bound corticosterone ratio (36) seems likewise unlikely in view of Winter's findings (37).

The fact that we were unable to detect any variation in total plasma concentration of indomethacin between RD and FFD rats is not at variance with this hypothesis, since total drug concentration in plasma appears to

TABLE V. EFFECT OF ORAL INDOMETHACIN (18 mg/kg IN THREE DIVIDED DOSES) ON CARRAGEENIN-INDUCED PAW EDEMA AND INCIDENCE AND DEGREE OF INTESTINAL ULCERS.

Experimental design	No. of animals	Mean body weight (g)[a]	Increase in paw volume after carrageenin: Adjusted mean	Increase in paw volume after carrageenin: Percentage inhibition	Intestinal ulcers: Percentage incidence	Intestinal ulcers: Degree[b] (mean score)
Regular diet (RD)						
Indomethacin	14	−11.1 ± 1.8	2.2	86.2	100	1.9
Controls	8	—	16.4		—	
Fat-free diet (FFD)						
Indomethacin	12	+3.5 ± 1.8	9.5	53.7*	25*	0.4*
Controls	8	—	21.0		—	
Least significant difference						
$P < 0.05$			3.3			
$P < 0.01$			4.5			

[a] From Day 1 to Day 3 (before the third administration of indomethacin); mean ± SE.

[b] 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.

* $P < 0.01$ compared to RD group.

TABLE VI. EFFECT OF DOSING SCHEDULE OF ORAL INDOMETHACIN ON CARRAGEENIN-INDUCED PAW EDEMA AND INCIDENCE AND DEGREE OF INTESTINAL LESIONS.

Indomethacin (mg/kg) at the following hours before carrageenin: 42	30	1	No. of animals	Δ body weight (g)[a]	Increase in paw volume after carrageenin: Adjusted mean	Increase in paw volume after carrageenin: Percentage inhibition	Intestinal pathology: Percentage incidence of ulcers	Intestinal pathology: Degree[b] Mean score	Intestinal pathology: Degree[b] Mean rank
—	—	—	8	+18.3 ± 1.2	20.7				
6	—	—	8	+17.3 ± 1.0	19.1	9.3	0	0.25	12.0
6	6	—	8	−2.9 ± 2.6	9.4	54.6	62.5	3.25	25.6
6	6	6	8	−0.4 ± 2.2	2.8	86.6	100	5.13	33.8
—	6	6	8	+13.8 ± 3.0	10.4	49.7	50	2.0	19.1
—	—	6	8	+16.7 ± 1.5	11.6	44.0	12.5	0.5	11.9
Least significant difference									
$P < 0.05$				5.8	4.5		40		16
$P < 0.01$				7.8	6.0		55		19

[a] From Day 1 to Day 3 (before the third administration of indomethacin); mean ± SE. Data were analyzed by means of a one-way analysis of variance. Multiple comparisons among groups were made by means of the LSD method (17).

[b] Degree of intestinal pathology was graded according to an arbitrary scale: 0 = normal, 1 = irritation, 2 = marked irritation, 3 = one or two small ulcers, 4 = several primary ulcers, 5 = many advanced ulcers, 6 = many ulcers, with adhesions, 7 = death. Scores were reduced to ranks and analyzed by means of a Kruskal–Wallis one-way analysis of variance (20). Multiple comparisons among groups were made by means of the critical range method (20).

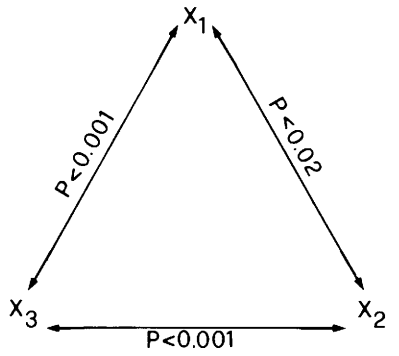

FIG. 1. Spearman rank coefficients of the values shown in Table VI relative to body weight changes (X_1, increase in paw volume (X_2), and degree of intestinal pathology (X_3).

be a relatively poor index of changes in drug distribution (38). For example, if indomethacin is 99% protein-bound (37) and is 5% displaced, this will result in a negligible and undetectable variation in total plasma concentration, but in a significant sixfold increase in the amount of drug free to enter the sites of action.

Further indirect evidence for this hypothesis is provided by the fact that FFD failed to enhance the anti-inflammatory activity of indomethacin during the prostaglandin-mediated phase of inflammation. Such a failure cannot be attributed to a modification in prostaglandin availability as indicated by the similarity in EFA pattern and paw edema response between untreated RD and FFD rats during this phase, but rather to restoration of plasma FFA to normal levels.

In view of the data obtained thus far, the finding that, in the 3-day carrageenin paw edema, indomethacin was significantly more effective in RD as compared to FFD animals was rather unexpected. However, the almost-complete inhibition of paw edema formation observed in RD rats following the three doses of indomethacin has to be attributed to the combined anti-inflammatory effect of specific and nonspecific factor(s). That is, the effect (specific) of the last dose of indomethacin superimposes on the effect (nonspecific) produced by the preexisting inflammatory processes at intestinal levels apparent in RD, but not FFD, animals.

The nonspecific role played by the preexisting inflammatory processes produced by the administration of the first two doses of indomethacin is substantiated by the following:

(i) The presence of indomethacin in the body, following single or repeated administrations (42 and 30 hr before the injection of the phlogistic agent), at the time of carrageenin injection is against kinetic considerations (39, 40).

(ii) Paw edema formation is significantly reduced in the presence of preexisting inflammatory processes, as indicated by our own data and previous findings (15, 41). This nonspecific anti-edema effect does not seem to be due (42) to the production, at preexisting inflammatory sites, of a factor(s) capable of inhibiting inflammatory processes at distant sites upon release into the blood stream. It appears more likely to be attributable, at least in part, to a reduction in serum kinins and complement (15) rather than to a deficiency in prostaglandin availability (24), since this effect is evident in the non-prostaglandin-mediated phase of inflammation (5).

In view of the above, it appears legitimate to conclude that FFD prevents and or reduces intestinal lesions without reducing, but rather enhancing, the specific anti-inflammatory activity of indomethacin. This is at variance with the hypothesis that anti-inflammatory activity and side effects have a common denominator (1, 2).

This observation is of clinical relevance, and it is tempting to speculate that feeding a low-fat diet might reduce NSAI-induced gastrointestinal disturbances without reducing therapeutic efficacy.

Summary. The anti-edema properties of ulcerogenic doses of indomethacin were shown to be due to the concomitant influence of specific (anti-inflammatory) and nonspecific (presence of intestinal lesions) effects. The fat-free diet (FFD) prevented and/or reduced both the incidence and degree of indomethacin-induced intestinal lesions without reducing, but rather enhancing, anti-inflammatory activity through a specific effect.

These findings are at variance with the hypothesis that anti-inflammatory activity and side effects of indomethacin have a common denominator. The greater anti-inflammatory activity displayed by indomethacin in FFD as compared to regular diet (RD)-fed rats might be attributed to differences in body distribution of the drug. It is tempting to

speculate that feeding a fat free or a low fat diet might reduce NSAI-induced gastrointestinal disturbances without reducing therapeutic efficacy.

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