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Further Studies on the Mechanism of Fat-Free Diet (FFD)—Protection against Indomethacin-Induced Intestinal Ulcers in the Rat (42072)

S. EVANGELISTA,*,[1] R. CIAPPI,† C. ORTOLANI,† A. BARZANTI,† AND A. MELI*

Pharmacology and †Microbiology Departments, Research Labs, A. Menarini Pharmaceuticals, 50131 Florence, Italy

Abstract. A fat-free diet (FFD) reduces bacterial flora when compared to a regular diet (RD) in rats. FFD reduces the degree and incidence of indomethacin-induced intestinal ulceration concomitantly with a reduction in bacterial flora overgrowth produced by the drug. Present and previous findings indicate that diminished deconjugation of indomethacin and reduction of intestinal flora overgrowth play a major role in the ulcer-preventing properties of FFD.

Indomethacin-induced overgrowth of intestinal flora appears to play a role in the development of indomethacin-induced intestinal lesions (1–3). This is substantiated by the observation that (a) prevention of bacterial overgrowth by antibiotics reduces both the incidence and degree of intestinal lesions (1, 2, 4) and (b) indomethacin does not induce intestinal lesions in male germ-free rats (5).

Del Soldato and Meli (6) reported that a significant decrease in β-glucuronidase activity occurs in rats fed an indomethacin-treated FFD (fat-free diet) as compared with that in rats fed a regular diet (RD) and suggested that a diminished deconjugation of recycled indomethacin glucuronide might play a role in reducing intestinal ulcers. Since β-glucuronidase appeared to be of bacterial rather than mammalian origin (6) it appeared worthwhile to determine whether or not FFD prevention of indomethacin-induced intestinal flora overgrowth was a mechanism responsible for reduction or prevention of intestinal ulceration.

Materials and Methods. Male albino rats, Sprague–Dawley–Morini strain, 200–240 g body wt, were fed either RD or FFD over a 10-day period before the experiments were commenced. Indomethacin, suspended in an aqueous vehicle containing 0.9% NaCl, 0.5% carboxymethylcellulose, and 0.5% Tween 80 was administered orally at the dose of 12 mg/kg in a volume of 5 ml/kg. This dose was selected because it had been shown to induce intestinal ulcers in 90–100% of the animals (7). Animals were killed by CO_2 asphyxiation 72 hr after indomethacin administration, and the intestine was carefully examined for the 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 ulcers and intestinal adhesions (8). The lumenal content of a 12-cm segment of mid-small intestine was quickly removed by sterile technique, weighed, and homogenized in 10 ml saline according to the procedure described by Kent *et al.* (3). Serial 10-fold dilutions were prepared and 0.1-ml aliquots of appropriate dilutions were spread on selective media for bacteriological analysis (Table I).

The results are expressed as $\log_{10}$ of the number of organism per milligram stools, and the differences of the means were analyzed by the Student t test for unpaired data.

Percentage incidence and degree of intestinal ulcers were analyzed by means of the χ^2 test.

Results. Data in Table II indicate that in animals fed FFD the incidence and degree of indomethacin-induced intestinal ulcers were markedly reduced as compared to RD animals.

FFD significantly reduced mid-small intestine microflora as compared to RD (Table III).

[1] To whom all correspondence should be addressed: Pharmacology Dept., Research Labs, A. Menarini Pharmaceuticals, v. Sette Santi 3, 50131 Florence, Italy.

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TABLE I. MEDIA, ATMOSPHERE, TEMPERATURE, INCUBATION TIME, AND ORGANISMS ENUMERATED FROM MID-SMALL INTESTINE SEGMENT

Media	Atmosphere and temperature	Time (hr)	Organisms
Bacto-tergitol 7 agar (Difco) and Levine eosin methylene blue agar (BBL)	Air, 35–37°C	24	Coliforms
Bacto-tergitol 7 agar (Difco) and violet red bile agar (Merck)	Air, 35–37°C	24	Proteus
Bacto-rogosa SL agar (Difco)	Air, 35–37°C	48	Lactobacilli aerobes
Bacto-rogosa SL agar (Difco)	Gas pak, $H_2 + CO_2$ 35–37°C	48	Lactobacilli anaerobes
Bacto-TPEY agar base (Difco)	Air, 35–37°C	48	Cocci
Vogel-Johnson agar (BBL)	Air, 35–37°C	72	Staphylococci
Bacto-KF streptococcus agar (Difco)	Air, 35–37°C	72	Streptococci

Likewise an indomethacin-induced increase in mid-small intestinal microflora was significantly reduced in FFD-fed as compared to RD-fed animals (Table III).

Discussion. Among the factors claimed to favor the formation of indomethacin-induced intestinal ulcers, changes in bile flow and/or composition do not appear to play any role in this phenomenon or the protective effects exerted by FFD (6, 7).

On the other hand, indomethacin-induced intestinal ulcers have been reported to depend upon endogenous prostaglandins deficiency (9) although there is no temporal correlation between inhibition of prostaglandins synthesis and development of intestinal lesions (10). Moreover, it is widely accepted that prostaglandin deficiency reduces the defensive mechanisms of the intestinal mucosa to make it more sensitive to further noxious factors such as bacterial overgrowth leading to ulceration. Our studies provide further evidence that indomethacin induces intestinal bacterial flora overgrowth and that bacterial flora plays a major role in the development of intestinal ulcers. In fact it has been hypothesized that development of indomethacin-induced intestinal lesions requires the formation of secondary bile acids by the intestinal flora (5). In support of this hypothesis it has been shown that *Escherichia coli* transforms primary into secondary bile acids (11) although microorganisms other than *E. coli* may contribute to the formation of these acids which have been held responsible for mucosal damage.

TABLE II. EFFECT OF FFD ON ORAL INDOMETHACIN (12 mg/kg)-INDUCED INTESTINAL ULCERS

		Intestinal ulcers	
Type of diet	*n*	% Incidence	Degree (mean score)
Regular (RD)	15	100	3.0
Fat free (FFD)	15	46.6*	0.8*

* $P < 0.001$ as compared with RD group.

However, prostaglandin deficiency and intestinal bacterial flora overgrowth do not appear to be the only factors responsible for indomethacin-induced intestinal ulcerations. In fact in the rat, in which extensive enterohepatic circulation of indomethacin occurs (12), development of intestinal lesions has been ascribed to persistence of free indomethacin within the gut due, to a certain extent, to enzymatic deconjugation of recycled indomethacin glucuronide (13, 14) producing disruption of the mucosal barrier.

The observation that intestinal β-glucuronidase activity was markedly reduced in indomethacin-treated FFD-fed rats was interpreted as an indication that a decrease in

TABLE III. EFFECT OF DIET AND ORAL INDOMETHACIN (12 mg/kg) ON MID-SMALL INTESTINE BACTERIAL FLORA

Organisms	Control RD	Control FFD	Indomethacin-treated RD	Indomethacin-treated FFD
Coliforms	1.35 ± 0.16 (20)[a]	0.72 ± 0.26 (20)*	5.42 ± 0.14 (20)**	3.13 ± 0.24 (16)***
Proteus	1.10 ± 0.16 (20)	0.63 ± 0.13 (20)*	4.57 ± 0.13 (20)**	2.35 ± 0.22 (16)***
Cocci	2.10 ± 0.13 (20)	1.12 ± 0.19 (20)**	4.56 ± 0.13 (20)**	2.12 ± 0.19 (20)***
Staphylococci	0.21 ± 0.20 (20)	−0.39 ± 0.13 (20)*	1.74 ± 0.24 (20)**	0.59 ± 0.26 (20)***
Streptococci	1.16 ± 0.17 (20)	0.26 ± 0.21 (20)**	4.79 ± 0.12 (19)**	1.88 ± 0.30 (16)***
Lactobacilli aerobes	5.00 ± 0.16 (20)	2.89 ± 0.35 (17)**	4.02 ± 0.12 (19)**	3.30 ± 0.20 (17)***
Lactobacilli anaerobes	5.46 ± 0.15 (20)	3.96 ± 0.17 (19)**	5.20 ± 0.14 (20)	3.97 ± 0.11 (19)***

[a] Results are expressed as mean ± SE of $\log_{10}$ of the number of microorganisms/mg of intestinal content. Number of rats in parentheses.

* $P < 0.05$ and ** $P < 0.01$ as compared to RD-fed animals. *** $P < 0.01$ as compared to RD-fed indomethacin-treated animals.

enzymatic deglucuronidation of recycled indomethacin glucuronide was a possible mechanism responsible for FFD-induced reduction in intestinal lesions (6). In view of the above and of our present and past studies (6), experimental evidence strongly indicates that reduced formation of β-glucuronidase, with diminished deglucuronidation of the drug, and reduction in indomethacin-induced bacterial overgrowth are the likeliest mechanisms responsible for FFD protection from indomethacin-induced intestinal ulceration.

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