PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 146–152 (1974)

Factors Influencing the Development of Indomethacin-Induced Intestinal Ulcers in the Rat[1] (38060)

G. VOLTERRA, N. PISANTI, AND A. MELI

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

It is known that administration of large doses of steroidal as well as non steroidal antiinflammatory agents causes gastrointestinal ulceration.

Recently it has been reported that, unlike gastric ulcers, the formation of indomethacin-induced intestinal lesions in the rat is prevented by starvation most likely through a starvation-induced decrease in bile flow with a concomitant reduction in enterohepatic circulation of indomethacin and/or its metabolites (1).

Since bile flow seems to play such a key role, we thought it worthwhile to determine whether or not substances which increase bile flow would enhance ulcer formation. A choleretic agent [1-Phenylpropanol (PP)] was selected which had been shown to enhance significantly bile flow in unanesthetized animals (2). Preliminary experiments indicated that the use of a lipid (sesame oil) instead of an aqueous vehicle was "conditio sine qua non" for the choleretic agent to increase the incidence of intestinal lesions.

This observation prompted us to investigate the influence of sesame oil as well as that of a fat free diet on indomethacin-induced intestinal ulcers.

Materials and Methods. (a) *General.* Male and female albino rats, Wistar-Morini strain, weighing 125–175 g were used. Rats were divided into groups of 10–30 animals each and had free access to food and water. On the evening before testing, the animals were individually caged in makrolon cages with a wire bottom to minimize coprophagy. Food was either left in place or removed according to the experimental design.

The feeding schedule, selected on the basis of preliminary experiments which indicated that it afforded maximum protection from ulceration, consisted of a fasting period of 17 hr before and 7 hr after indomethacin.

Indomethacin (suspended in an aqueous vehicle consisting of sodium chloride 0.9%, polysorbate 80 0.4%, CMC 0.5% and benzyl alcohol 0.9%) was administered by gavage at several dose levels in a constant volume of 5 ml/kg.

In all studies the animals were sacrificed by CO_2 asphyxiation 72 hr after indomethacin and the intestine was examined carefully for the presence of ulceration by an observer who was unaware of the treatment.

(b) *Influence of the choleretic agent.* 1-Phenylpropanol (PP)[1] was solu-suspended either in sesame oil or in an aqueous vehicle (see paragraph a).

PP (400 mg/kg in a constant volume of 5 ml/kg) as well as an equivalent volume of sesame oil or aqueous vehicle were administered 4 hr after indomethacin.

(c) *Influence of a fat free diet* [*FFD* (*3*)]. The animals were fed either a regular (RD) or a fat free diet (FFD) over a 10 day period before commencing the experiments outlined in paragraph a and b.

(d) *Statistics.* Statistical analysis of the data was performed by computing Chi^2 for $m \times n$ contingency table according to Snedecor and Irwin (4) using an Olivetti Programma 101.

Results. Our data clearly indicate that starvation significantly reduces the incidence of indomethacin-induced intestinal ulcers in

[1] 1-Phenylpropanol was kindly supplied by Bieffe Biochimici, Firenze (Italy).

TABLE I. Effect of Feeding Schedule and Nature of the Diet on Indomethacin Induced Intestinal Ulcers in the Rat.

Type of diet	Feeding schedule	Treatment	Dose/kg	Oral indomethacin (mg/kg) 6	8	10	12	14
Regular	Fasting	Aqueous vehicle	5 ml	—	1/20[a] (5%) [a]	3/10 (30%)	6/10 (60%)	17/20 (85%)
	Fed	Aqueous vehicle	5 ml	1/20 (5%)	5/20 (25%)	6/10 (60%)	18/20 (90%)	—
Fat free	Fasting	Aqueous vehicle	5 ml	—	—	0/10 (0%)	1/10 (10%)	8/20 (40%)
	Fed	Aqueous vehicle	5 ml	—	1/20 (5%)	2/20 (10%)	4/10 (40%)	10/20 (50%)

Values of Chi²

Group	FFD fed	FFD fasting	RD fed
RD fasting	6.19 $P < 0.025$	14.68 $P < 0.005$	9.85 $P < 0.005$
RD fed	21.01 $P < 0.005$	27.00 $P < 0.005$	
FFD fasting	1.00 P = N.S.		

[a] Number of animals with intestinal lesions/total number of animals and percent value.

TABLE II. Effect of Feeding Schedule, Nature of Diet, Sesame Oil and 1-Phenylpropanol (PP) on Indomethacin-Induced Intestinal Ulcers in the Rat.

Type of diet	Feeding schedule	Treatment	Dose/kg	Oral indomethacin (mg/kg)				
				6	8	10	12	14
Regular	Fasting	—	—	—	2/20[a] (10%) [a]	6/20 (30%)	10/20 (50%)	16/20 (80%)
		Aqueous vehicle	5 ml	—	1/20 (5%)	3/10 (30%)	6/10 (60%)	17/20 (85%)
		Sesame oil	5 ml	—	8/20 (40%)	13/20 (65%)	19/20 (95%)	20/20 (100%)
		PP aqueous vehicle	400 mg	—	2/20 (10%)	3/10 (30%)	6/10 (60%)	18/20 (90%)
		PP sesame oil	400 mg	—	7/20 (35%)	7/10 (70%)	19/20 (95%)	—
	Fed	—	—	0/10 (0%)	6/20 (30%)	12/20 (60%)	19/20 (95%)	20/20 (100%)
		Aqueous vehicle	5 ml	1/20 (5%)	5/20 (25%)	6/10 (60%)	18/20 (90%)	—
		Sesame oil	5 ml	1/10 (10%)	7/20 (35%)	9/10 (90%)	—	—
		PP aqueous vehicle	400 mg	—	1/10 (10%)	6/10 (60%)	19/20 (95%)	—
		PP sesame oil	400 mg	1/10 (10%)	15/20 (75%)	19/20 (95%)	—	—
Fat free	Fasting	—	—	—	—	2/30 (6.7%)	2/30 (6.7%)	6/20 (30%)
		Aqueous vehicle	5 ml	—	—	0/10 (0%)	1/10 (10%)	8/20 (40%)
		Sesame oil	5 ml	—	5/20 (25%)	5/10 (50%)	7/10 (70%)	9/10 (90%)
		PP aqueous vehicle	400 mg	—	—	—	0/10 (0%)	4/10 (40%)
	Fed	PP sesame oil	400 mg	—	2/10 (20%)	3/10 (30%)	9/10 (90%)	—
		—	—	1/10 (10%)	2/20 (10%)	4/30 (13.3%)	10/30 (33%)	15/30 (50%)
		Aqueous vehicle	5 ml	—	1/20 (5%)	2/20 (10%)	4/10 (40%)	10/20 (50%)
		Sesame oil	5 ml	0/10 (0%)	11/30 (36%)	18/20 (90%)	—	—
		PP aqueous vehicle	400 mg	—	—	1/10 (10%)	2/10 (20%)	12/20 (60%)
		PP sesame oil	400 mg	2/10 (20%)	19/30 (63%)	19/20 (95%)	—	—

[a] = Number of animals with intestinal lesions/total number of animals and percent value.

TABLE III. Chi² Values Related to Data Shown in Table II (Fasting Animals).

		FFD				RD		
	Group	PP sesame oil	PP Aqueous vehicle	Sesame oil	Aqueous vehicle	PP sesame oil	PP Aqueous vehicle	Sesame oil
RD	Aqueous vehicle	3.57 P = N.S.	15.51 $P < 0.005$	0.53 P = N.S.	14.68 $P < 0.005$	14.97 $P < 0.005$	0.13 P = N.S.	13.12 $P < 0.005$
RD	Sesame oil	3.33 P = N.S.	39.44 $P < 0.005$	7.27 $P < 0.01$	41.54 $P < 0.005$	O P = N.S.	10.53 $P < 0.005$	
RD	PP aqueous vehicle	2.74 P = N.S.	17.53 $P < 0.005$	0.14 P = N.S.	16.36 $P < 0.005$	13.18 $P < 0.005$		
RD	PP sesame oil	2.89 P = N.S.	25.91 $P < 0.005$	4.97 $P < 0.05$	32.22 $P < 0.005$			
FFD	Aqueous vehicle	13.79 $P < 0.005$	0.62 P = N.S.	15.79 $P < 0.005$				
FFD	Sesame oil	0.12 P = N.S.	14.40 $P < 0.005$					
FFD	PP aqueous vehicle	16.36 $P < 0.005$						

TABLE IV. Chi² Values Related to Data Shown in Table II (Fed Animals).

		FFD				RD		
	Group	PP sesame oil	PP aqueous vehicle	Sesame oil	Aqueous vehicle	PP sesame oil	PP aqueous vehicle	Sesame oil
RD	Aqueous vehicle	19.92 $P < 0.005$	20.41 $P < 0.005$	6.91 $P < 0.01$	21.01 $P < 0.005$	21.24 $P < 0.005$	0.46 $P =$ N.S.	3.48 $P =$ N.S.
RD	Sesame oil	5.72 $P < 0.025$	12.80 $P < 0.005$	0.33 $P =$ N.S.	18.21 $P < 0.005$	6.89 $P < 0.01$	1.62 $P =$ N.S.	
RD	PP aqueous vehicle	10.46 $P < 0.005$	22.74 $P < 0.005$	3.02 $P =$ N.S.	24.89 $P < 0.005$	15.40 $P < 0.005$		
RD	PP sesame oil	0.14 $P =$ N.S.	21.67 $P < 0.005$	5.26 $P < 0.025$	48.32 $P < 0.005$			
FFD	Aqueous vehicle	42.04 $P < 0.005$	0.30 $P =$ N.S.	24.73 $P < 0.005$				
FFD	Sesame oil	4.13 $P < 0.05$	18.37 $P < 0.005$					
FFD	PP aqueous vehicle	21.67 $P < 0.005$						

RD fed animals (Table I); on the other hand, feeding schedule does not appear to play a significant role on ulcer formation in FFD fed animals. Regardless of feeding schedule, the incidence of indomethacin-induced intestinal lesions is significantly lower in anlmals fed the FFD as compared to those on RD (Table I).

Independently of feeding schedule and nature of the diet PP administered as an aqueous suspension failed to enhance the ulcerogenic properties of indomethacin. On the other hand, regardless of the nature of the diet, when given as an oily solution PP promoted ulcer formation over and above that observed following the administration of the sole sesame oil. However, this effect could be demonstrated only in unfasted animals (Table II). Within the FFD fed groups, sesame oil potentiated ulcer formation both in fasted and unfasted animals as compared to controls. This effect was significantly greater in unfasted as compared to fasted rats. On the other hand, within the RD fed groups, sesame oil significantly potentiated ulcer formation only in fasted animals, although there was a tendency toward such a potentiation even in unfasted rats (Table II). Among the fasting animals sesame oil is more ulcerogenic in the RD fed animals than in those on FFD whereas such a difference could not be demonstrated in unfasted rats (Table II). The results of the statistical analysis related to data shown in Table II are reported in Tables III–V.

Discussion. In agreement with the results of Brodie *et al.* (1), our data show that starvation significantly reduces the incidence of indomethacin-induced intestinal ulceration only in RD-fed rats. In addition, evidence is presented indicating that feeding schedule does not appear to play a significant role on ulcer formation in FFD-fed rats (Table I).

Brodie *et al.* (1) have suggested that starvation-induced reduction in bile flow might be responsible for the lower incidence of intestinal lesions observed in fasting animals. Therefore it was rather surprising that, regardless of feeding schedule or nature of the diet, the choleretic agent administered as aqueous suspension failed to enhance the incidence of intestinal ulcers even when administered in doses well above those which were shown to increase significantly bile flow (2).

Even when administered as an oily solution, presence of food was "conditio sine qua non" for PP to promote ulcer formation over and above that observed in sesame oil treated animals. This could be interpreted as a further indication of the ineffectiveness of the choleretic agent "per se."

The mechanism(s) responsible for the facilitating role played by sesame oil is not quite clear. Although it could be attributed to the sesame oil-induced increase in bile flow resulting in an increased entero-hepatic circulation of indomethacin, and/or its metabolites, it would be rather difficult to explain the lack of effectiveness of the choleretic agent, unless composition of bile rather than bile flow was a determining factor. However the observation that the incidence of intestinal ulcers was significantly reduced in animals fed a fat free diet, as compared with those fed a regular diet, casts some doubts on this hypothesis. It is rather

TABLE V. Statistical Analysis of Data Shown in Table II (Fasting vs Fed Animals).

Type of diet	Treatment	Chi² and P values	
Regular	Aqueous vehicle	9.85	$P < 0.005$
	Sesame oil	0.004	P = N.S.
	PP aqueous vehicle	11.31	$P < 0.005$
	PP sesame oil	11.69	$P < 0.005$
Fat free	Aqueous vehicle	1.00	P = N.S.
	Sesame oil	4.56	$P < 0.05$
	PP aqueous vehicle	3.70	P = N.S.
	PP sesame oil	15.68	$P < 0.005$

unlikely that decrease in bile flow or a difference in bile composition could be held responsible for the reduced incidence of intestinal lesions observed in the FFD-fed animals. In fact, dietary constituents, other than lipids, such as those present in our FFD are known to stimulate bile flow.

In view of the above, it seems likely that the sesame oil-facilitating as well as the FFD preventing effects on indomethacin induced intestinal ulcers are independent of bile flow and/or composition.

Summary. The influence of various experimental conditions, such as feeding schedule, composition of the diet, administration of a choleretic agent and vehicle used for its administration, on the development of indomethacin-induced intestinal ulcers in the rat has been studied.

Independently of diet composition, incidence of ulcer formation is reduced by starvation and favored by the presence of food. Regardless of feeding schedule, animals on a regular diet are significantly more susceptible to ulceration than those on the fat free diet.

The choleretic agent failed to enhance the ulcerogenic properties of indomethacin unless solubilized in sesame oil. Sesame oil alone significantly increased ulcer formation. These findings are discussed.

---

1. Brodie, D. A., Cook, P. G., Bauer, B. J., and Dagle, G. E., Toxicol. Appl. Pharmacol. **17**, 615 (1970).

2. Meli, A., and Riva, M., Il Farmaco, Ed. Pr. **26**, 269 (1971).

3. Kaunitz, H., Slanetz, C. A., Johnson, R. E., and Babayon, V. K., J. Nutr. **73**, 386 (1961).

4. Snedecor, G. W., Irwin, M. R., in Snedecor, G. W. & Cochran, W. G. "Statistical Methods" p. 240, The Iowa State University Press (1972).

Received Dec. 19, 1973. P.S.E.B.M., 1974, Vol. 146.