United States Patent [19]

Brown et al.

[11] **4,279,906**

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[54] **INDOMETHACIN-ANTIHISTAMINE COMBINATION FOR GASTRIC ULCERATION CONTROL**

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[21] Appl. No.: **850,504**

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[51] **Int. Cl.**[3] **A61K 31/40;** A61K 31/54; A61K 31/445

[52] **U.S. Cl.** **424/247;** 424/267; 424/274

[58] **Field of Search** 424/247, 267, 274

[56] **References Cited**

PUBLICATIONS

Chem. Abst., 71–59356q (1969).
Chem. Abst., 73–33796z (1970).
Chem. Abst., 66–27467g (1961).

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[57] **ABSTRACT**

An anti-inflammatory and analgesic composition containing indomethacin and an H_1 or an H_2 histamine receptor antagonist in an amount sufficient to reduce gastric distress caused by the indomethacin. Usable antagonists include pyrilamine, promethazine, metiamide and cimetidine.

8 Claims, 5 Drawing Figures

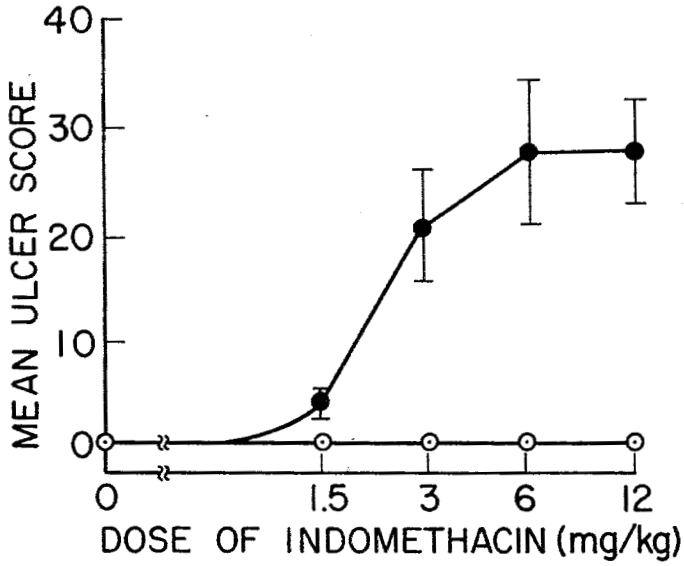
MEAN ULCER SCORE
40
30
20
10
0
0
1.5
3
6
12
DOSE OF INDOMETHACIN (mg/kg)

FIG. 1

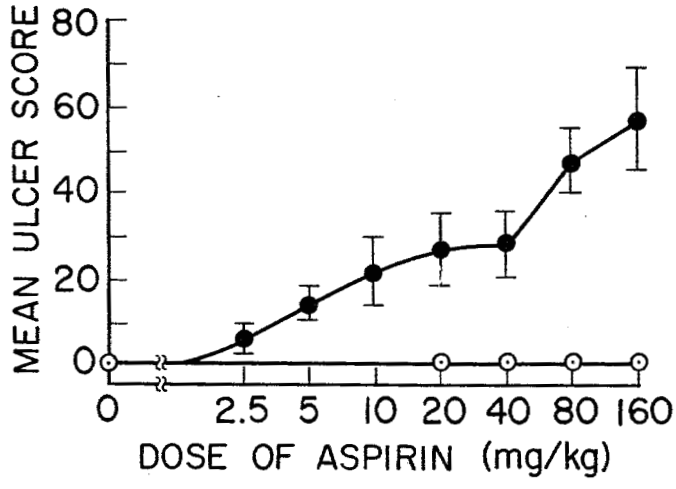
MEAN ULCER SCORE
80
60
40
20
0
0
2.5
5
10
20
40
80
160
DOSE OF ASPIRIN (mg/kg)

FIG. 2

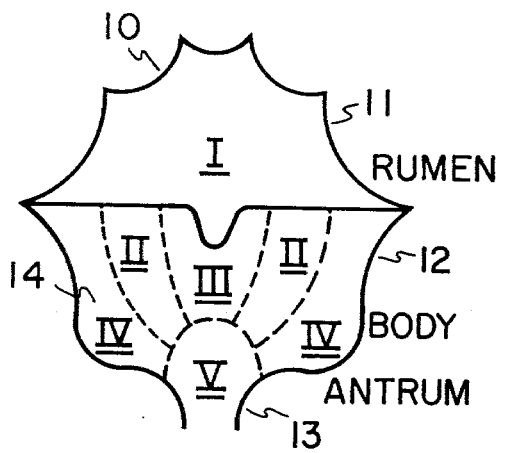

FIG. 3

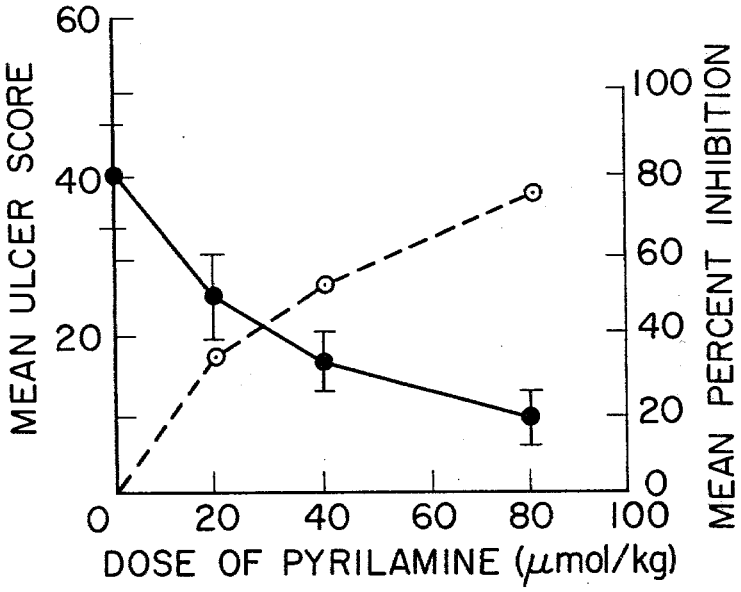
60
40
20
0
MEAN ULCER SCORE
100
80
60
40
20
0
MEAN PERCENT INHIBITION
0
20
40
60
80
100
DOSE OF PYRILAMINE (μmol/kg)

FIG. 4

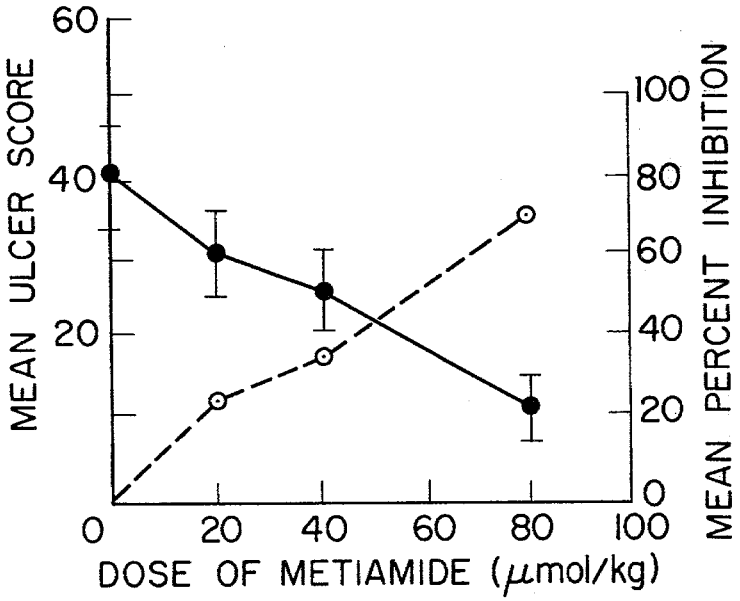
60
40
20
0
MEAN ULCER SCORE
100
80
60
40
20
0
MEAN PERCENT INHIBITION
0
20
40
60
80
100
DOSE OF METIAMIDE (μmol/kg)

FIG. 5

cially available plexiglas devices placed in an environmental chamber maintained at 5° C. [See R. J. Levine, *Peptic Ulcer,* (Ed. C. J. Pfeiffer), pages 92–97, Lippincott (1971); E. C. Senay and R. J. Levine, *Proc. Soc. Exp. Biol. Med.* 124, 1221–1223 (1967)]. Within each condition, the animals received orally one of several doses of aspirin or indomethacin. Thirty minutes after drug administration, the animals were subjected either to two hours of cold restraint (stress condition) or returned to their home cages (non-stress condition) for the same duration of time.

At the end of the two-hour period, the animals were sacrificed by decapitation and blood was collected from the trunk in chilled heparinized tubes and centrifuged. The plasma was separated and frozen for subsequent fluorometric assay of corticosterone [Vernikos-Danellis J. et al. Changes in adrenal corticosterone concentration in rats. Method of bioassay for ACTH. Endocrinology, 1966, 79, 624–630]. The stomach then was removed from the animal, cut along the greater curvature, opened, rinsed with tap water and pinned in a standard position for microscopic examination and scoring of ulcers. Ulcers measuring less than 1 mm were not scored. For each animal, the overall ulcer score was defined as the sum of the maximum continuous lengths (in mm) of each ulcer. This scoring method was found previously to be highly reliable (Brown et al. 1976). Representative stomachs were fixed in 10% formol saline and 8μ paraffin sections from tissue blocks taken from ulcerated areas were stained with hematoxylin and eosin and examined microscopically.

The dosages of aspirin and indomethacin and the consequences of such dosages are shown in FIGS. **1** and **2** of the drawings.

As FIG. **2** indicates, there was a dramatic synergism between aspirin and environmental stress in the production of gastric ulceration. Control stressed animals that received only the methylcellulose vehicle (zero dose of aspirin) essentially did not ulcerate (ulcers generally were less than 1 mm) and even very high doses of aspirin failed to produce appreciable gastric damage in the absence of stress. However, in conjunction with stress, the administration of low doses (2.5 mg/kg) did result in minor ulceration (FIG. **1**). Increasing the dose to 20 mg/kg resulted in substantial ulceration in these animals with a mean score of 27.4. Maximum ulceration was obtained with 160 mg/kg of aspirin, yielding a mean ulcer score of 56.9. Still higher doses produced no further increase in gastric ulceration. The dose-response relationship was highly significant as tested with a one-way analysis of variance ($F=4.88$; $df=6,63$; $p<0.01$).

Similarly, in the absence of stress, high doses (12 mg/kg) of indomethacin failed to produce gastric ulcers as shown in FIG. **1**. However, the lowest dose of indomethacin administered (1.5 mg/kg) produced appreciable gastric damage in stressed rats. Increasing the dose to 12 mg/kg resulted in a mean ulcer score of 28.2. Still higher doses produced no further increase in ulceration. The dose-response relationship was highly significant as tested with a one-way analysis of variance ($F=7.35$; $df=4.45$; $p<0.001$). The difference between the maximum ulcer scores produced by indomethacin and aspirin was also significant ($p<0.05$).

The dose-response relations for aspirin and indomethacin appear to differ in several respects. First, the maximum response to aspirin is more than twice that for indomethacin. Second, the dose-response relation for aspirin rises gradually over about a 2 log unit range, whereas that for indomethacin rises abruptly over less than 1 log unit.

Since both stress and high doses of aspirin are known to individually elevate the level of corticosteroids and since corticosteroids are known to be ulcerogenic, an attempt was made to determine whether the ulceration attributed to either indomethacin or aspirin could have been caused in part by an augmented adrenocortical response.

Mean plasma corticosterone levels were therefore determined on the two-hour blood samples earlier mentioned and the results of the tests are shown in the following table.

TABLE 1

EFFECT OF ANTI-INFLAMMATORY AGENTS ON MEAN PLASMA CORTICOSTERONE LEVELS[a] IN STRESSED AND NONSTRESSED RATS

Dose (mg/kg)	Experimental Condition: Aspirin (n = 9)	Aspirin + Stress (n = 9)
0	32.0 ± 3.7	134.1 ± 5.3
20	27.3 ± 7.2	138.9 ± 4.4
40	26.9 ± 5.0	137.2 ± 3.9
80	34.7 ± 5.9	137.3 ± 7.1
160	63.9 ± 7.7	126.0 ± 9.2
	Indomethacin (n = 10)	Indomethacin + Stress (n = 10)
0	21.1 ± 3.7	134.3 ± 5.0
1.5	23.6 ± 4.9	132.4 ± 5.7
3.0	21.1 ± 5.2	138.5 ± 3.9
6.0	29.0 ± 5.9	136.5 ± 3.5
12.0	32.9 ± 6.5	139.4 ± 8.7

[a]Mean ± S.E. (μg/100 ml of plasma)

From the table, it can be readily seen that corticosterone levels at the end of the two-hour period were markedly elevated in rats under stress. However, neither indomethacin nor aspirin produced any significant additional increase in corticosterone in these animals. Thus, increasing gastric damage produced by the drugs in the stressed rats was not associated with an increase in circulating corticosterone. The mean corticosterone levels of nonstressed animals were also unaffected by the drugs, except at the highest dosage tested. This latter rise in corticosterone was not accompanied by any corresponding increase in gastric ulceration.

EXAMPLE 2

In this example the size and regional distribution of ulceration produced by indomethacin and aspirin are compared.

For the experiment, animals were assigned randomly to groups receiving either 12 mg/kg of indomethacin or 20 mg/kg of aspirin. The doses administered were selected on the basis of results of the first experiment (FIGS. **1** and **2**) to give approximately the same mean ulcer score. Thirty minutes after drug administration, the animals were put in cold restraint for two hours. At the end of the stress period, the animals were sacrificed and ulcers were scored as described in Example 1.

The gastric ulcers produced by aspirin and indomethacin in stressed rats appeared as black or brown punctate or elongate regions with sharply defined edges. Microscopic examination of hematoxylin and eosin stained paraffin sections taken from regions of ulceration revealed necrosis of the gastric mucosa sometimes extending to, but never through, the muscularis mucosa.

In stressed animals, high doses of aspirin (≧80 mg/kg) produce very large ulcers (see FIG. 1*b*, Brown et al., 1976) generally not seen with very high doses (≧24 mg/kg) of indomethacin. However, at lower doses, for a given mean ulcer score, the size as well as the regional distribution of the ulcers were similar for aspirin and indomethacin. At the doses selected, both agents produced numerous small ulcers in the range of 1.0 to 2.9 mm and only occasionally ulcers 3.0 mm or larger. Following the scoring method of Tagaki et al. [Chemical and Pharmaceutical Bulletin, 1964, 12, pages 465 to 472], the number of ulcers in each of five zones of the stomach was determined. FIG. **3** shows the anatomical divisions of a stomach which for better visualization has been opened along the greater curvature and pinned out. As can be seen from the drawing, the stomach is divided into three main areas, i.e., the rumen (**11**), the body (**12**), and the antrum (**13**), with each area comprising the anatomical divisions indicated (I, II, III, IV, and V). The distribution of gastric ulcers observed is shown in the following table.

TABLE 2

REGIONAL DISTRIBUTION OF GASTRIC ULCERS PRODUCED BY ASPIRIN AND INDOMETHACIN IN STRESSED ANIMALS

	Mean Number of Ulcers	
Zone of Stomach	Aspirin (20 mg/kg) N = 8	Indomethacin (12 mg/kg) N = 8
I	0.0	0.0
II	23.5	12.3
III	1.6	2.5
IV	15.0	18.9
V	0.9	1.3

As can be seen in Table 2, neither indomethacin nor aspirin caused ulcers in Zone I, the rumen of the stomach, but both drugs induced numerous ulcers in Zones II and IV of the body. On the other hand, few ulcers were produced by either agent in Zone III of the body or Zone V, the antrum.

EXAMPLE 3

In these tests to determine the effect of antihistamines on ulceration caused by the combined action of indomethacin and stress, animals again were assigned randomly to groups receiving either pyrilamine maleate or metiamide at the dosages indicated in FIGS. **4** and **5**. Additionally each animal received 6 mg/kg of indomethacin. Both the antihistamine and the anti-inflammatory agent were delivered orally in the same 2 ml of methycellulose solution. Thirty minutes after drug administration each animal was subjected to two hours of cold restraint. At the end of that stress period, all animals were sacrificed by decapitation and the stomachs were removed, pinned, and scored as in Example 1.

To determine whether the antiulcerogenic properties of pyrilamine is specific to that drug, other animals were treated in the same manner with a structurally different H_1 receptor blocker, promethazine.

The effectiveness of the antihistamines in inhibiting gastric ulceration produced by indomethacin in rats under stress in shown in FIGS. **4** and **5**, and in Table 3.

As can be seen from the curves plotted, the dose-response curves for pyrilamine and metiamide are very similar. In the absence of antihistamine, the mean ulcer score in both control groups was somewhat higher than expected from the results of the first experiment, probably reflecting monthly variations to susceptibility to gastric ulceration [Wilson, T. R., Monthly Variations in the Severity of Experimental Stress Ulcers in Rats. In C. J. Pfeiffer (Ed.) Peptic Ulcer, Philadelphia: J. C. Lippincott, 1971]. At the lowest dose of pyrilamine administered, the mean ulcer score was reduced 38%. Similarly the lowest dose of metiamide reduced the mean ulcer score 24%. Increasing the dose of both antihistamines produced further reductions in ulcer severity resulting in a mean score of 10.1 and 11.2 for pyrilamine and metiamide, respectively. The dose-response relationship for these antihistamines was highly significant as tested by a one-way analysis of variance (Pyrilamine: $F=6.47$; $df=3,53$; $p<0.001$; Metiamide: $F=4.65$; $df=3,52$; $p<0.01$). The ID_{50} was roughly 35 μmol/kg for pyrilamine and 55 μmol/kg for metiamide.

TABLE 3

THE ANTIULCER EFFECTS OF METIAMIDE AND PROMETHAZINE ON INDOMETHACIN PRODUCED ULCERS IN STRESSED RATS

Condition	Mean ± SEM	% Reduction
IND	46.2 ± 6.8	
IND + MET	14.3 ± 2.5	69.0
IND ± PROM	19.6 ± 4.9	57.0

*$t(15,15) = 4.25$, $p<.005$
**$t(15,15) = 3.06$, $p<.005$

As can be seen further from Table 3, the H_1 receptor antagonist, promethazine, achieved reductions in gastric ulceration that are comparable to those produced by metiamide. The latter tests were carried out with high doses of antihistamine (80 μmol/kg) in stressed rats receiving 6 mg/kg of indomethacin.

As the present results clearly demonstrate, a dramatic synergism occurs between acute stress and moderate dosages of anti-inflammatory agents in the production of gastric ulceration (FIGS. **1** and **2**). The difference between the effects of aspirin and indomethacin cannot be attributed, however, to any additional effects in plasma corticosterone levels. The results do indicate that the level of plasma corticosterone was greatly increased by the stress procedure, but neither anti-inflammatory agent was found to produce any additional increase in corticosterone level.

As to the effect of antihistamines on gastric ulceration caused by a combination of anti-inflammatory agents and stress, it was rather surprising to discover that not only metiamide could reduce ulceration, but that the H_1 receptor antagonist, pyrilamine and promethazine, also did. Earlier published data (Brown et al. 1976) showed that the latter drugs do not function in that capacity with aspirin. These data suggest generally that the mechanism of action of indomethacin differs, in an unknown manner, from that of aspirin.

Although the invention has been described in terms of certain preferred embodiments, it is contemplated that the man skilled in the art may carry out modifications that will remain within the spirit and the scope of the following claims.

What is claimed is:

1. A process for reducing gastric distress in a mammal caused by the administration of indomethacin, which comprises administering to said mammal an antihistamine substance selected from the group consisting of promethazine and pyrilamine in a quantity sufficient to reduce gastric distress caused by the indomethacin.

2. The process of claim **1** wherein the antihistamine substance is promethazine.

3. The process of claim **1** wherein the antihistamine substance is pyrilamine.

4. The process of claim **1** wherein there is administered about 0.025 to 0.400 gram of indomethacin and from about 0.2 to 2.0 grams of the antihistamine.

5. A pharmaceutical composition comprising indomethacin and an antihistamine substance selected from the group consisting of promethazine and pyrilamine in a quantity sufficient to reduce gastric distress caused by the indomethacin.

6. The composition of claim **5** wherein the antihistamine substance is promethazine.

7. The composition of claim **5** wherein the antihistamine substance is pyrilamine.

8. The composition of claim **5** comprising about 0.025 to 0.400 gram of indomethacin and about 0.2 to 2.0 grams of the antihistamine.

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