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Preventive Effect of Zaprinast and 3-Isobutyl, 1-Methylxanthine (Phosphodiesterase Inhibitors) on Gastric Injury Induced by Nonsteroidal Antiinflammatory Drugs in Rats

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Cyclic GMP plays an important role in maintaining homeostasis in the gastric mucosa. NSAIDs damage the mucosa by mechanisms that may be mediated by alterations in the intragastric concentration of cyclic GMP. To test this hypothesis we studied the effects of the oral administration of acetylsalicylic acid (100, 300, and 500 mg/kg), piroxicam (5, 10, and 20 mg/kg) and sodium diclofenac (10, 25, 50, and 100 mg/kg), and of their interaction with zaprinast (5 mg/kg) and IBMX (10 mg/kg), on intragastric concentrations of cyclic GMP and the gastric erosive index in rats. All determinations were done 3 hr after the NSAID was given. All NSAIDs induced dose-dependent decreases in mucosal concentrations of cyclic GMP, which correlated inversely with the surface area showing mucosal injury. In contrast, cyclic GMP concentrations remained normal, and no intragastric damage was seen in rats given zaprinast (cyclic GMP-specific phosphodiesterase inhibitor) or IBMX (non-specific phosphodiesterase inhibitor) or in combination with NSAIDs. These findings are in line with the hypothesis that cyclic GMP is involved in the biochemical mechanisms of NSAID-induced gastric injury.

KEY WORDS: gastric injury; nitric oxide; nonsteroidal antiinflammatory drugs; phosphodiesterase inhibitors.

The induction of gastric lesions is the most frequent toxic effect of nonsteroidal antiinflammatory drugs (NSAIDs). Although the biochemical basis for their toxicity has not been fully explained, inhibition of prostaglandin (PG) synthesis is accepted as the main mechanism responsible for this effect. Work done in the previous decade has established that nitro-NSAID derivatives capable of donating nitric oxide (NO) inhibit PG synthesis to the same degree as do their parent NSAIDs, but without damaging the gastric mucosa (1, 2). This observation suggested that NO might also be involved in the the mechanisms of NSAID-induced gastropathy. In addition, several studies have shown that NO has protective effects on the mucosa (3–6) and prevents the gastric toxicity associated with NSAIDs (7, 8). This effect is thought to be mediated by the second messenger cyclic guanosine monophosphatase (cGMP) (4, 9–13).

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The effects on the gastric mucosa of NSAIDs are the opposite of those of cGMP: the former alter blood flow in the mucosa (14–16), diminish mucus synthesis (14, 17) and bicarbonate synthesis (6, 14), decrease mucosal hydrophobicity (15, 17, 18), and decrease cell proliferation and angiogenesis (6). These drugs also decrease NO synthesis induced by inducible NO synthase (iNOS) (19).

Because NSAIDs have the opposite effect of cGMP, and because the ability of NO to prevent gastric lesions is mediated by cGMP, we suspected that phosphodiesterase inhibitors (PDI), might be able to prevent NSAID-induced gastric lesions by impeding cGMP catalysis. To test this hypothesis we investigated the effects of two PDIs [zaprinast (ZAP) and 3-isobutyl, 1-methylxanthine (IBMX)] on gastric lesions induced by NSAIDs.

MATERIALS AND METHODS

We used 256 male Wistar rats that weighed 200–250 g. The animals were housed in individual Macrolon cages under a 12-hr light–dark cycle (lights on at 08:00), with free access to water. Food was withheld from 16 hr before the start of the experiments. All experiments began between 08.00 and 10:00 with the subcutaneous administration of vehicle (saline solution) or a PDI. Ten minutes later either the vehicle alone or one of the NSAIDs suspended in 1 ml 1% gum arabic was given. Three hours later the animals were anesthetized with sodium pentobarbital (20 mg/kg) intraperitoneally The abdominal cavity was opened to remove the stomach, which was opened along the major curvature to expose the gastric mucosa. The mucosa was washed with ice-cold phosphate buffer and the erosive index (EI) was measured under a stroboscopic lens and recorded as square millimeters of affected mucosal surface. The mucosa was then scraped off, weighed, frozen in liquid N_2, and stored at -80°C for later biochemical analysis.

Drugs. The drugs used in these experiments were as follows: acetylsalicylic acid (ASA) at 100 ($N = 13$), 300 ($N = 16$), and 500 ($N = 15$) mg/kg; sodium diclofenac (DICLO) at 10 ($N = 9$), 25 ($N = 7$), 50 ($N = 7$), and 100 ($N = 6$) mg/kg; piroxicam (PIRO) at 5 ($N = 6$), 10 ($N = 6$), and 20 ($N = 7$) mg/kg; type V (cGMP-specific) PDI ZAP at 5 mg/kg ($N = 7$); and the nonspecific PDI IBMX at 10 mg/kg ($N = 8$). We also tested the following associations of NSAIDs and PDI: ZAP + ASA at 100 ($N = 6$), 300 ($N = 6$), and 500 ($N = 8$) mg/kg; IBMX + ASA at 100 ($N = 6$), 300 ($N = 10$), and 500 ($N = 10$) mg/kg; ZAP + DICLO at 25 ($N = 6$), 50 ($N = 8$), and 100 ($N = 7$) mg/kg; IBMX + DICLO at 25 ($N = 5$), 50 ($N = 6$), and 100 ($N = 6$) mg/kg; ZAP + PIRO at 5 ($N = 6$), 10 ($N = 7$), and 20 ($N = 6$) mg/kg; and IBMX + PIRO at 5 ($N = 9$), 10 ($N = 13$), and 20 ($N = 10$) mg/kg. All drugs were from Sigma Aldrich Chemical Co (St. Louis, Missouri, USA). A control group of 14 rats was treated with vehicles alone.

cGMP Measurement. The concentration of cGMP in the gastric mucosa was measured with a commercial kit from DRG (Marburg, Germany). Samples of mucosa were homogenized (5 cycles of 10 sec each in a Virtis-Hear Tempest, New York, New York, USA) in ice-cold phosphate buffer (pH 7.4) with 6% trichloracetic acid at a proportion of 1:10 w/v. Homogenates were sonicated in a Virtis Virsonic 300 apparatus (5 pulses of 5 sec each) set at 70%, then centrifuged at 4°C for 20 min at 20,000 *g* in a Heraeus Suprafuge 22 apparatus (Hanau, Germany). The supernatant was treated with three cycles of water-saturated ether at a proportion of 1:5 v/v and then lyophilized. The residue was treated in accordance with the manufacturer's instructions provided with the kit. The concentration of cGMP was measured with an LKB-Wallace 1282 Compugamma CS gamma scintillation counter (Turku, Finland).

Statistical Analysis. The results were subjected to one-way analysis of variance (ANOVA) with the nonparametric Kruskal-Wallis test; when the results indicated a significant difference, the Mann-Whitney U test was used. All correlation calculations were done with the Spearman test. $P \leq 0.05$ was considered significant. All results are expressed as the mean and 95% confidence interval (95% CI) in picomols per gram of tissue for cGMP and in square millimeters for EI.

RESULTS

The administration of NSAIDs induced lesions in the gastric mucosa. The extent of the lesions correlated inversely with the concentration of cGMP ($r = -0.565$, $N = 106$, $P < 0.001$) (Figure 1).

Influence of NSAIDs on cGMP Concentration and Erosive Index in Gastric Mucosa. The administration of ASA led to a dose-dependent decrease in the concentration of cGMP ($r = -0.777$, $N = 44$, $P < 0.001$) and an increase in EI ($r = 0.620$, $P < 0.001$). The decrease in cGMP was statistically significant in comparison to the control group at all three doses (100, 300, and 500 mg/kg (Figure 2). The results for EI were also significant at all three doses of ASA (Figure 3).

Like ASA, DICLO induced a dose-dependent decrease in cGMP concentration in the gastric mucosa ($r = -0.782$, $N = 29$, $P < 0.001$) and an increase in EI ($r = 0.844$, $P < 0.001$). The effect of DICLO on cGMP concentration was significant at doses of 25, 50, and 100 mg/kg (Figure 4), and the effect on EI was also significant at these doses (Figure 5).

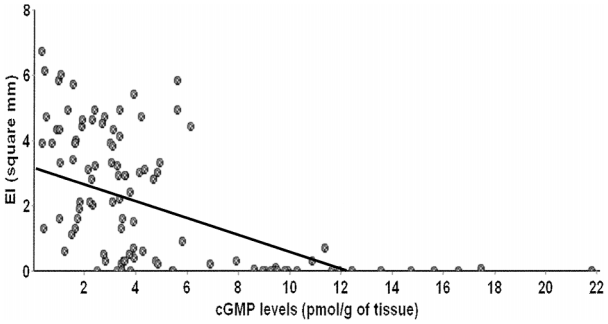

Fig 1. Correlation between cGMP levels and erosive index in gastric mucosa ($r = -0.565$, $N = 106$, $P < 0.001$).

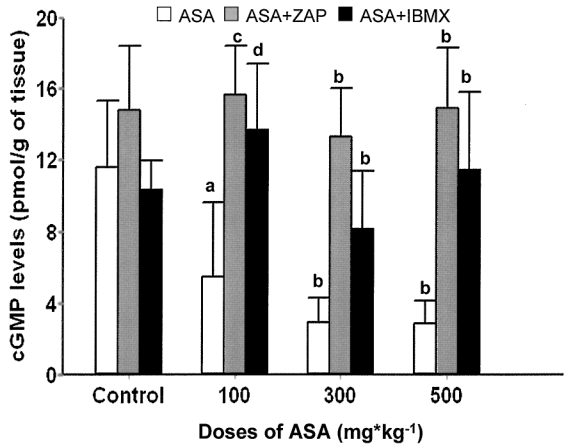

Fig 2. Effect of ASA administration (100, 300, and 500 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on cGMP levels in the gastric mucosa. Significant effects of ASA are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding ASA group. a: $P = 0.0056$; b: $P < 0.0001$; c: $P = 0.0047$; d: $P = 0.0167$.

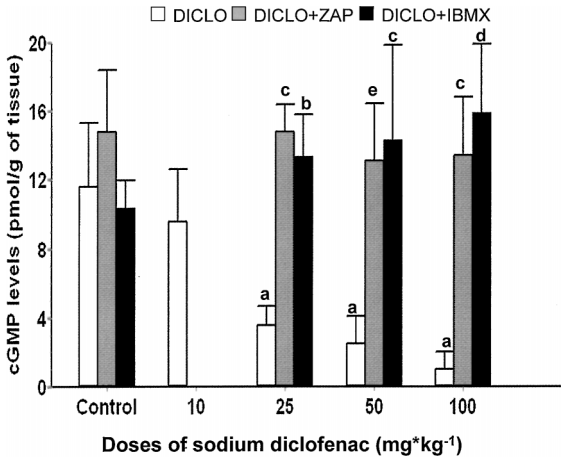

Fig 4. Effect of DICLO administration (10, 25, 50, and 100 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on cGMP levels in the gastric mucosa. Significant effects of DICLO are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding DICLO group. a: $P < 0.0001$; b: $P = 0.0025$; c: $P = 0.0012$; d: $P = 0.0022$; e: $P = 0.003$.

Like the other NSAIDs, PIRO led to a dose-dependent decrease in cGMP concentration ($r = -0.741$, $N = 19$, $P < 0.001$) and an increase in EI ($r = 0.873$, $P < 0.001$). Although all the doses had this effect, the decrease seen with 5 mg/kg was not significant (Figure 6). In contrast, the increase in EI was significant at all doses (Figure 7).

Influence of Zaprinast and 3-Isobutyl, 1-Methylxanthine on NSAID-Induced Changes in cGMP Concentration and Erosive Index. The administration of 5 mg/kg ZAP led to a nonsignificant increase in cGMP concentration, in comparison to the control group. Zaprinast did not cause lesions in the gastric mucosa. This drug prevented the NSAID-induced decrease in cGMP (Figures 2, 4, and 6), and decreased the extent of EI induced by NSAIDs (Figures 3, 5, and 7).

As with ZAP, the administration of IBMX led to a nonsignificant increase in cGMP concentration in the gastric mucosa, but did not induce the appearance of

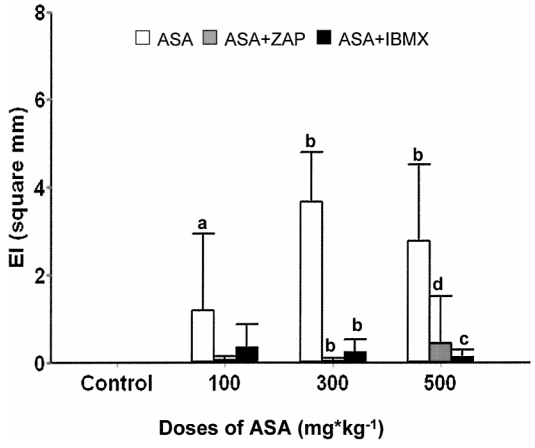

Fig 3. Effect of ASA administration (100, 300, and 500 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on erosive index (EI) in the gastric mucosa. Significances are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding ASA group. a: $P = 0.0015$; b: $P < 0.0001$; c: $P = 0.0002$; d: $P = 0.0025$.

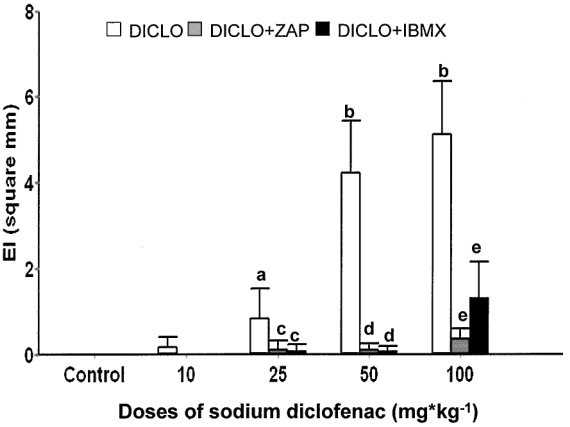

Fig 5. Effect of DICLO administration (10, 25, 50, and 100 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on erosive index (EI) in the gastric mucosa. Significant effects of DICLO are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding DICLO group. a: $P = 0.0005$; b: $P < 0.0001$; c: $P = 0.0303$; d: $P = 0.0012$; e: $P = 0.0022$.

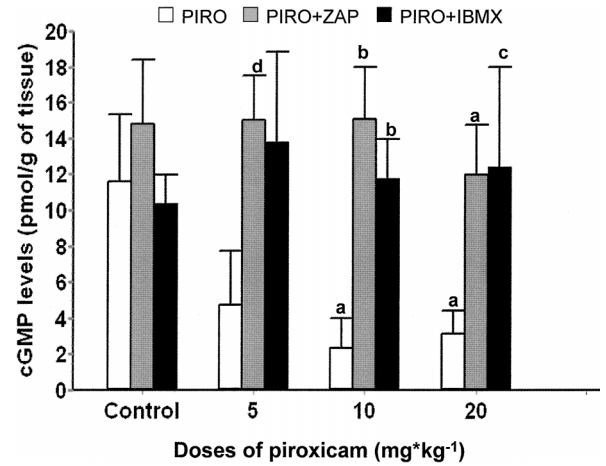

Fig 6. Effect of PIRO administration (5, 10, and 20 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on cGMP levels in the gastric mucosa. Significant effects of PIRO are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding PIRO group. a: $P < 0.0001$; b: $P = 0.0002$; c: $P = 0.0025$; d: $P = 0.0426$.

lesions. This drug, like ZAP, prevented the NSAID-induced decrease in cGMP concentration (Figures 2, 4, and 6) and markedly, although not completely, inhibited the formation of NSAID-induced lesions (Figures 3, 5, and 7).

DISCUSSION

Earlier studies have documented the protective effects of NO on the gastric mucosa. (20–26). In fact, NO donors prevent the appearance of NSAID-induced lesions (7, 8), an effect that appears to be mediated by the second messenger cGMP (4, 9–13). Studies of the influence of cGMP have found that this substance is able to diminish acid secretion (27, 28), decrease the extent of lesions induced by ethanol (27) and cold (29) (which coincided course with decreased cGMP production), increase bicarbonate synthesis, and decrease pepsinogenesis (30). Cyclic GMP is also able to inhibit apoptosis in gastric cells (26, 31). Thus, if cGMP is able to exert these protective effects on the gastric mucosa, PDI, by increasing the concentration of cGMP or preventing the breakdown of this messenger, would be expected to enhance the effects of cGMP.

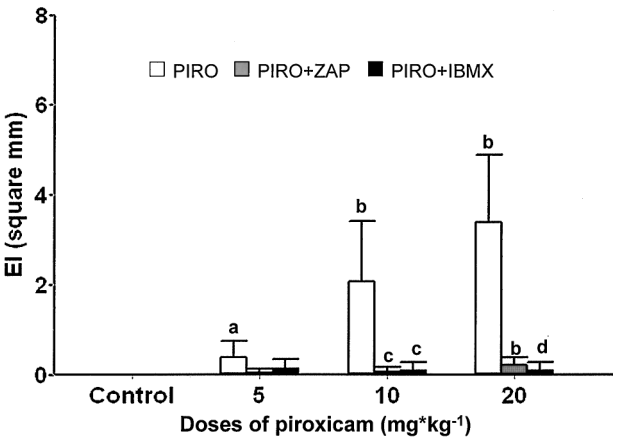

Fig 7. Effect of PIRO administration (5, 10, and 20 mg/kg) and its interaction with ZAP (5 mg/kg) and IBMX (10 mg/kg) on erosive index (EI) in the gastric mucosa. Significant effects of PIRO are shown with respect to the control group. Significant interactions with ZAP or IBMX are shown with respect to the corresponding PIRO group. a: $P = 0.02$; b: $P < 0.0001$; c: $P = 0.0005$; d: $P = 0.0025$.

Studies of NO-releasing NSAIDs showed that these substances have the same antiinflammatory, analgesic, and cyclooxygenase inhibiting effects as their parent compounds. However, they do not induce lesions in the gastric mucosa (1, 2, 32–34), and in fact accelerate the healing of existing lesions (1, 35). These findings, together with our results, show that NO plays an important role in preventing gastropathy. Because the effects of NO on the gastric mucosa are mediated by cGMP, our results support the hypothesis that it is the nucleotide that is responsible for preventing the appearance of NSAID-induced lesions. Like cold (29), NSAIDs induce lesions and decrease cGMP concentration in the gastric mucosa. In contrast, PDI—which impedes the decrease of in cGMP in the mucosa—prevents the formation of NSAID-induced lesions.

Because NO increases PG synthesis in the gastric mucosa (36), it may be that PGs are ultimately responsible for the preventive effects of NO and cGMP. However, this possibility is ruled out by the fact that PG synthesis is inhibited by NSAID administration (37). We must therefore assume that the absence of toxicity and the accelerated healing of existing lesions are related with the concentration of NO and cGMP. It is thus possible that NSAID-induced gastric injury may result (at least in part) from the inhibition of NO and cGMP synthesis. In this connection, NSAIDs have been shown to reduce NO production in the gastric mucosa (38) and to inhibit inducible NO synthase (iNOS) (19). However, it is not known whether this inhibitory effect is responsible for gastric toxicity. Our findings nevertheless show that cGMP is involved in the appearance of gastric lesions and that inhibition of cGMP synthesis may mediate the toxic effect of NSAIDs in the mucosa.

It could be posited that the decrease in cGMP is unrelated to the physiopathology of gastropathy, but instead is a consequence of NSAID-induced damage to the gastric mucosa. Although intuitively attractive, this hypothesis is ruled out by a number of considerations. First, the eroded surface comprises only a small portion of the total area of

the gastric mucosa, despite the marked decrease in cGMP. Second, NO donors prevent the appearance of lesions and favor the healing of existing lesions, and the effects of NO are mediated by cGMP. Third, if cGMP were not involved in the physiopathological mechanism of damage, the recovery of normal cGMP concentrations would not be expected to have any protective effect. Our findings show that both ZAP and IBMX prevented the appearance of lesions and maintained intragastric concentrations of cGMP within normal limits.

This observation might be contested by arguing that the effect of ZAP and IBMX results from a protective mechanism that is unrelated to cGMP. Although this is a possibility that deserves attention, it seems unlikely that both substances, which differ in chemical structure and which have in common only their PDI action, would exert the same specific action via unknown mechanisms unrelated to cGMP.

Critics of the use of PDI have noted that despite its antiinflammatory properties (39), the therapeutic usefulness of PDI is limited by the incidence of undesirable side effects such as nausea and increased gastric secretion. The administration of nonspecific, type III or type IV (cAMP-specific) PDI leads to toxicity; however, this effect is not seen with ZAP, a type V PDI (40, 41). Thus, the undesirable side effects may be related to cAMP; in fact, the effect of ZAP on acid secretion is the opposite of that of other PDIs. Zaprinast, because it increases cGMP, also favors mucus production (9) and potentiates the effect of NO donors (42). It also has antioxidant effects by preventing superoxide synthesis (43).

The results reported here show that neither ZAP nor IBMX was able to increase cGMP concentration in the gastric mucosa under our experimental conditions in healthy rats: the increases we recorded did not reach statistical significance. However, it should be recalled that NOS can be inhibited by its product NO and its messenger cGMP (44). This inhibition may have accounted for the fact that the increase in cGMP concentration failed to reach statistical significance in the present study.

In conclusion, our findings confirm that cGMP mediates the prevention and healing of lesions and suggest that influencing cGMP concentration might be a potentially useful approach to therapy in the future. Manipulating the mechanisms related to NO and cGMP production thus offers new possibilities for the treatment of gastropathy with NSAIDs.

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