

The Effects of Indomethacin of the Rat Gastric Mucosa

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Indomethacin is used widely in clinics nowadays and the side effect of ulceration is well known. This experiment was performed to study the morphological and histochemical changes on gastric mucosa after indomethacin treatment.

The microscopic finding of the mucosa was observed following oral administration of 10 mg/kg indomethacin in alcohol as solvent. The histological changes were observed from 6 hours after administration of indomethacin and the maximal injury was found at 24 hours. Structural changes of injury included hemorrhage, epithelial desquamation and inflammatory cell infiltration.

From the 3 day specimens, regeneration signs had started and in the 6 day specimens almost complete recovery of the mucosal epithelium was noted. The histochemical changes of the mucus were also observed from the 6 hr specimens. As far as neutral glycoprotein was concerned, the decrease was most significant in the 3 day old group, and besides, they showed minimal reaction to PAS stain.

For acidic mucus, the decrease was significant in the 24 hr group and the 3 day group showed minimal reaction to Alcian blue stain. It was noted that these changes of the mucus had recovered 6 days after the administration of indomethacin.

Key Words: Indomethacin, Gastric mucosal change.

Indomethacin is a nonsteroidal drug with antiinflammatory, antipyretic, and analgesic properties. Its side effects were studied by many authors and they reported gastrointestinal bleeding and ulceration as the main untoward effect of indomethacin treatment (Lovgren & Allander, 1964; Hart, 1965; Djahanguiri, 1969).

The structural formula of indomethacin is shown in figure 1. Since acetylsalicylic acid was introduced to therapeutics in 1899, many studies of gastromucosal injury by aspirin have

been published, and clinical and physiological investigations have clearly shown that aspirin injures the gastric mucosa of man and various laboratory animals (Anderson, 1964; Davenport, 1964).

In recent years, it was reported by Corell *et al.* (1979) that concurrent administration of sodium salicylate or salicylic acid and indomethacin significantly reduces the ulcerogenicity of indomethacin. Also, antagonism of the gastroucerogenic effect of indomethacin by salicylic acid has been shown after simultaneous oral administration in rats by Ezer *et al.* (1976 a & b).

Therefore indomethacin is used often in

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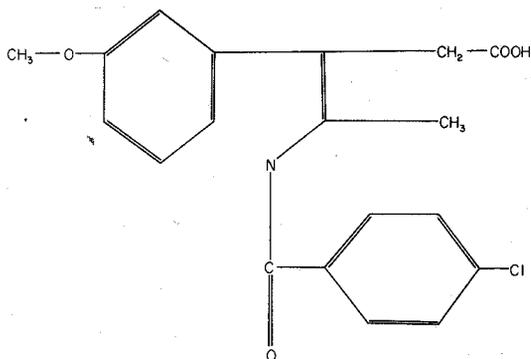


Fig. 1. Structural formula of indomethacin.

combination with salicylic acid or sodium salicylate.

Although there have been so many physiological studies on the effect of indomethacin, few studies have taken into account the structural changes of the mucosal epithelium during the course of the injury. So a need existed for a morphological study of it.

The main purpose of our investigation is to study the structural change due to indomethacin administration at different time intervals and the surface epithelium was studied with special emphasis on the mucous cells in order to define better the relationship between indomethacin induced damage and mucus production.

MATERIALS AND METHODS

Sprague-Dawley rats, weighing 150-200 gm were used throughout the study. The animals were fasted for 36 hr prior to the experiment but were allowed water. Indomethacin (Sigma) was dissolved in 4% ethanol plus 0.1 N NaOH, and distilled water, and was administered using esophageal intubation under light ether anesthesia in volumes of 0.5 ml of the mixture (10mg/kg indomethacin). Control rats received 0.5 ml of solvent. Rats were not fed until 2 hr after treatment. At predetermined times, the rats were killed by cervical dislocation and abdominal

cavities were rapidly opened.

The stomach was excised from each rat and opened along the greater curvature and the mucous membranes were cleaned of food residues under gently running tap water.

These tissues were fixed overnight in neutral buffered formalin and then dehydrated, cleared, and embedded in paraffin in the routine manner. Sections were cut from different levels of each block in order to obtain adequate sampling of each specimen. Sections were stained with hematoxylin-eosin for routine morphological study, with the PAS stain for neutral glycoproteins, and with Alcian blue at pH 2.5 for acidic glycoproteins.

To compare the degree of the structural change, the level of impairment was expressed from + to +++, doubtful finding as \pm and no change as -.

RESULTS

1) Histological findings

The microscopic findings are summarized in Table 1. Damaged mucosal epithelium and hemorrhage into the mucosal lamina propria were the most common changes. These were found most marked at 24 hours after indomethacin administration.

- (1) Six hr group: Sections taken 6 hours after administration of indomethacin revealed not too significant changes but slight hemorrhage in the lamina propria was noted and the vessels in the submucosa were generally engorged. Acute inflammatory cell infiltration was considerable but the chief and parietal cells were not particularly changed.
- (2) Twenty-four hr group: After 24 hours, a variable histological picture was noted. Epithelial desquamation was the most prominent finding of these specimens

Table 1. Major histological findings in gastric mucosa at different intervals after indomethacin administration.

	hemorrhage	epithelial desquamation	inflammatory cell aggregation	regenerating epithelium
6 hr	+	+	+++	-
24 hr	++	+++	++	-
3 days	++	+	-	++
6 days	-	-	-	+++

* Intensity graded from - (no change) to +++ (most severe change)

and hemorrhage became very severe, especially in the upper part of the mucosa, that is, where the epithelial desquamated part. Acute inflammatory cell infiltration was also noted and crypt cells including chief and parietal cells were necrotic.

(3) **Three day group:** Specimens obtained 3 days after indomethacin administration revealed widespread patches of cuboidal, regenerating type epithelium and it was found throughout the whole surface. Crypt cells also showed regeneration with cytoplasmic basophilia. The regenerating parietal cells were smaller than mature parietal cells (so it could be called immature parietal cells) and their cytoplasm was less eosinophilic than that of mature cells. No chief cells could be identified until this period and no inflammatory cell infiltration was found at this time, but hemorrhagic evidence was still noticeable.

(4) **Six day group:** After 6 days, epithelial regeneration was considerable and the general histological feature was quite normal, but no chief cells could be identified until this period.

2) Histochemical findings

These results are summarized in Table 2. For the degenerating epithelium there were

Table 2. Histochemical changes of mucus in the gastric mucosa after indomethacin administration.

	neutral glycoprotein (PAS stain)	acidic mucus (Alcian blue stain) pH 2.5
control	++++	+++
6 hr	+++	+++
24 hr	++	+
3 days	+	±
6 days	++++	+

* Intensity of staining properties of mucus ± (insignificant reaction) to +++ (strongest reaction)

decreased amounts of mucus and decreased staining intensity with the PAS and Alcian blue.

(1) **PAS stain finding:** The PAS positive materials began to decrease and progressively from the 6 hr specimen. As a result, the 3 day specimens showed the most significant decrease of the neutral mucosubstances. After 6 days, the neutral mucosubstances had recovered and the degree of recovery was almost complete. The degree of stainability was more intense than that of the normal.

(2) **Alcian blue stain finding:** Acidic mucus did not show a change in the 6 hr specimens and the decrease of the amount was prominent in the 24 hr specimens.

The 3 day specimens showed minimal reaction to Alcian blue stain and from 6 days it began to recover but the degree of recovery was not as prominent as the neutral mucosubstances.

DISCUSSION

Indomethacin is known as a nonsteroidal drug with antiinflammatory, antipyretic, and analgesic properties. The therapeutic value of the drug has been reported in a variety of rheumatic diseases.

Its serious and frequent side effect is reported as gastrointestinal bleeding and ulceration by many authors (Lovgren & Allander, 1964; Hart, 1965; Djahanguiri, 1969; Kent *et al*, 1969; Brodie *et al*, 1970; Corell & Jensen, 1979). Corell & Jensen reported that indomethacin had a potent ulcerogenic activity (ulcerogenic % effects 82.8-100%). The present study showed that administration of indomethacin caused no distinct localized ulceration, but showed generalized destructive effects. Inflammation, edema and hemorrhage were frequently observed and this phenomenon could be explained by Johnson's report (1966). He suggested that when the rat gastric mucosa is damaged by acetic or salicylic acid, its histamine concentration falls and that the histamine content of the gastric lumen rises.

From this fact, they inferred some of the histamine liberated from the mucosa is likewise present in the interstitial fluid of the mucosa. Since histamine causes both vesodilatation and increased capillary permeability, its presence in interstitial fluid would probably result in the flow of a large volume of plasma like fluid from the mucosal capillaries into the interstitial spaces and through the mucosa, rendered more permeable by damage, into the gastric lumen. We can

assume that this could account for the generalized change of the gastric mucosa.

There has been an interest in the theory that defective mucigenesis may contribute to gastrointestinal ulcer formation. Indomethacin (Menguy & Desbaillets, 1967), aspirin (Menguy & Masters, 1965) and steroids (Hirschowitz *et al*, 1955) are said to depress the amount of gastric mucus or certain of its constituents.

In recent years, however, Lev *et al* (1970) showed that steroids increase rather than decrease cytoplasmic mucus synthesis by canine gastric surface epithelium, using radioautographic and histological study.

But in this work, the results showed reduction in the amount and synthesis of cytoplasmic mucus in the degenerating epithelium and it is consistent with the work of Menguy *et al* (1967). This observation supposedly has great significance because, as has been pointed out by others, epithelial mucus plays an important role in protecting the mucosa of the digestive tract from peptic ulceration by forming a barrier between the underlying epithelial cells and the proteolytic enzymes present in the digestive juices. Of signal importance in this protective function of epithelial mucus is its ability to resist enzymatic hydrolysis. Obviously, gastric mucus which is predominantly protein in nature would not be a very effective barrier if it were readily digested by pepsin. Mucus owes this property of resisting enzymatic hydrolysis to its carbohydrate moieties. The latter form polysaccharide prosthetic groups which branch from the polypeptide core of the mucoprotein molecule in such a way as to hinder sterically the action of proteolytic enzymes on the susceptible peptide bonds of the protein core (Menguy & Masters, 1965).

Lev *et al* (1976) have worked on stress ulcer and there were similarities between their findings and our findings. They reported the acute

inflammatory cells appeared as early as 6 hours after application of stress and we could find also inflammatory cells in the 6 hr specimens and the peak of the inflammatory response was in the 24 hr specimens in both cases.

Lev *et al* (1971) described the implication concerning the pathogenesis of stress ulcer and one of these is the reduction of cytoplasmic mucus in degenerating and regenerating surface epithelium may be significant in view of the evidence that mucus protects against mucosal ulceration. In the case of degenerating epithelial cells this reduction may contribute to the development of acute erosions or ulcers. Mucus depleted regenerating epithelium on the other hand, may be prone to reulcerate or heal poorly and thus be predisposed to chronic ulcer formation.

Dipasquale & Welaj (1973) and Shriver *et al* (1977) have worked on the ulcerogenic potential of indomethacin in arthritic and nonarthritic rats. They reported that it appears that arthritic rats during the later stages are more sensitive to the ulcerogenic potential of indomethacin.

The effects of indomethacin on small intestine, reported by Fang *et al*, (1977) showed similar reaction as our results but they reported that light microscopic observation of intestinal tissue reveals no changes at 2 and 6 hrs after indomethacin alone. However, by 24 hrs after administration of indomethacin, there was diffuse and acute inflammation in the villi.

From our experiments, the distinct localized gastric ulceration by indomethacin administration was not observed but generalized destructive picture was present, however, mucigenesis was reduced and the decrease of the amount of mucus was considerable in both neutral glycoproteins and acidic mucus. Both types of mucus had started to recover by 6 days after indomethacin administration. The reason why these results showed less severe damage than others,

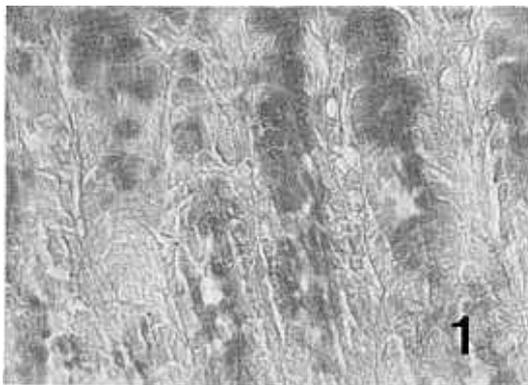
could be assumed on the basis that the dosage was underthreshold. Djhanguriri (1969) reported that the dosage was related to ulceration and their 10 mg/kg administration of indomethacin showed an 80% ulcerogenicity after 5 hrs. Our results are not consistent to theirs. Menguy & Desbaillets (1967) observed decreases of mucus production by the 5 mg/kg of indomethacin, therefore, we could conclude that change of mucigenesis is more sensitive than the generalized histological changes. Some authors (Ezer *et al*, 1976 b; Hayden *et al*, 1978; Corell & Jensen, 1979) reported that sodium salicylate and salicylic acid reduce the gastric ulcerogenicity of indomethacin after concurrent administration in rats. Corell & Jensen (1979) suggested that the ulcerogenic interaction might be explained partly by the reduced indomethacin plasma concentration and partly by a weaker inhibition by sodium salicylate on the prostaglandin system in the rat stomach. The morphological confirmation of this phenomenon should be investigated in continuity.

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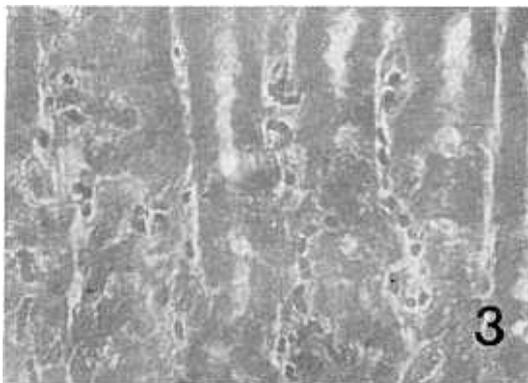
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Picture 1. Gastric mucosa, 6 hours after indomethacin administration. Gastric mucosal cells contain quite a large amount of acidic mucus. Alcian blue stain, x 450



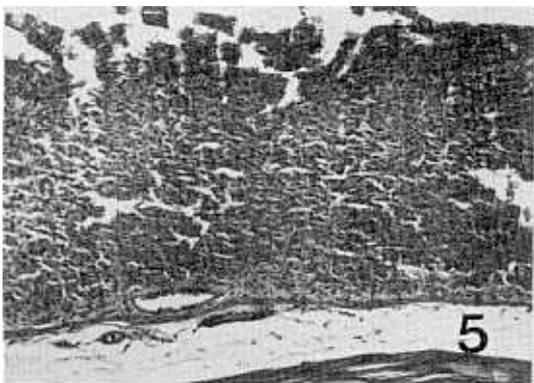
Picture 2. Gastric mucosa, 3 days after indomethacin administration. There is no acidic mucus in the gastric mucosa. Alcian blue stain, x 450



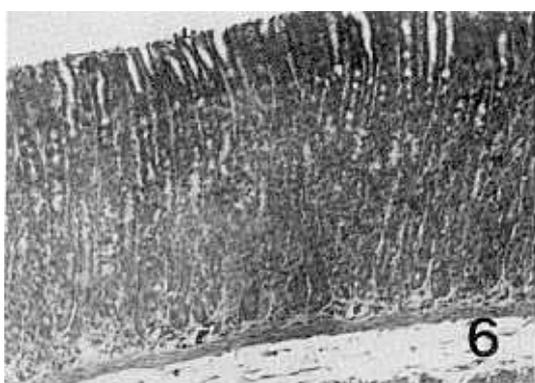
Picture 3. Gastric mucosa, 3 days after indomethacin administration. Very small amounts of neutral glycoproteins are found in the gastric mucosa. PAS stain, x 450



Picture 4. Gastric mucosa, 6 days after indomethacin administration. Gastric mucosal cells contain very large amounts of neutral glycoproteins. PAS stain, x 450



Picture 5. Severe damage 24 hours after indomethacin administration. H-E stain, x 100



Picture 6. Recovered gastric mucosa 6 days after indomethacin administration. H-E stain, x 100