ORIGINAL ARTICLE

ADAPTATION OF RAT GASTRIC MUCOSA EXPOSED TO INDOMETHACIN: A HISTOLOGICAL STUDY

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ABSTRACT

Background: Indomethacin is still used in the remote areas because of its low cost. It relieves pain, reduces swelling and tenderness. It induces gastric ulceration both in experimental animals and humans. This study was conducted to observe the reversible changes in the damaged gastric mucosa during its prolonged administration.

Material & Methods: In this study intrapertioneal injections of indomethacin were given to rats in maximum therapeutic dose (4mg/kg body weight) to three experimental groups B, C and D for one, two and three weeks respectively. Group A was the control group. Effects were observed in the stomach pylorus.

Results: There were well-defined superficial ulcers during initial two weeks of drug administration. During third week, there was minor damage in the form of focal necrosis. Morphometric analysis initially showed increase in the number of goblet cells, columnar cells, and mitotic figures, which was part of the tissue response to an injury. These findings were significantly reduced with continued use of the drug. The unusual phenomenon observed was that when the duration of the drug was prolonged, the ulcerogenic effects of the drug were reduced.

Conclusion: Indomethacin given in a maximum therapeutic dose initially induces lesions in stomach but almost no effects are noted when the duration of drug administration is prolonged. This unusual response is due to adaptation of gastric mucosa to the continued use of the drug.

Key words: Indomethacin, Gastric mucosa, Mucosal adaptation.

INTRODUCTION

Indomenthacin, a methyl indole derivative was introduced in 1963 and approved by federal drug agency in 1965. It is a synthetic non-steroidal anti-inflammatory drug with analgesic and antipyretic activity. It is a potent inhibitor of postaglandins synthesis which are important mediators of the inflammatory response.[1] The anti-inflammatory action of indomethacin is due to inhibition of vasodilator prostaglandin E2 and prostaglandin I,[2] synthesized from arachidonic acid through cyclo-oxygenase pathway by inhibiting cyclo-oxygenase I and cyclo-oxygenase-II.[2] The inhibition of cyclo-oxygenase, change in the mitochondrial function and free radical induced oxidative changes, all contribute to its anti-inflammatory action. Deficiency of cyclooxygenase-I is of pivotal importance in anti-inflammatory response of non-steroidal anti-inflammatory drugs. Only long-term deficiency of cyclooxygenase-II is associated with significant pathology.[3] Decrease in mucosal prostaglandin (PGE2) content, inhibition of cyclo-oxygenase-I subregulation of cyclo-oxygenase-II are responsible for its anti-inflammatory action.[2] The antipyretic action of indomethacin is due to inhibition of prostaglandin synthesis released in response to inflammatory pyrogen interlenkin-I. This inhibition causes elevation of set point for temperature in hypothalamus. The analgesic action of indomethacin is due to decrease in the production of prostaglandin that sensitizes nociceptors to inflammatory mediators such as bradykinin and 5-hydroxytryptamine.[4]

Indomethacin is readily absorbed from the gastrointestinal tract almost completely after oral ingestion. It is 90% bound to plasma proteins and also extensively bound to tissues. Its concentration in synovial fluid is equal to that in the plasma within 5 hours of administration. It is inactivated by the formation of metabolites in the liver. Some of the metabolites undergo entero-hepatic cycling and are eliminated through bile.[5] About 10-20% of the drug is eliminated unchanged in the urine.[6] Its half life in plasma is variable perhaps because of enterohepatic cycling, which is 7-10 hours.[7] Indomethacin is used in musculoskeletal disorders such as rheumatoid arthritis, osteo-arthritis, acute gouty arthritis and ankylosing spondylitis. It is also

used for the closure of patent ductus arterious in premature infants.[8] The initial oral dose of indomethacin is 25-50 mg 2-3 times a day which can be increased upto 150-200 mg/24 hours. Injectable dose is 1-2 mg/Kg/24 hours in 2-4 divided doses, which can be increased upto 4 mg/kg/24 hours. Indomethacin is contra-indicated in pregnant women, nursing mothers, patients with ulcerative lesions of the stomach and intestine, renal disorders and epilepsy.[4] The adverse effects of indomethacin especially on gastrointestinal tract are its systemic effects and not local. Therefore, oral and parental indomethacin have similar untoward effects.[9] Its untoward effects are nausea, vomiting, anorexia, epigastric distress, diarrhea, gastrointestinal ulcers and perforation.[10]

MATERIAL AND METHODS

In this study, 42 adult rats of Albino Wistar strain, weighing 250.8-256.6 grams were used. They were randomly divided into 4 groups A, B, C and D. Group A was control while B, C and D were experimental groups. Each group comprised of 12 rats. Injection indomethacin 50 mg in powder form was dissolved in 50 ml distilled water to get 1 ml solution containing 1 mg indomethacin. Intraperitoneal injection of indomethacin 1mg/kg/24 hours in two divided doses at 12 hours interval was given to rats of experimental groups B, C and D for one, two and three weeks respectively. Rats of each group were anesthetized by cotton soaked in ether. After 2–3 minutes, while rats were still breathing normally, each rat was fixed on a wooden block with the help of paper pins. Dissection was performed, abdomen opened and gross observations noted. Stomach body and pylorus were preserved in neutral buffered formalin. For microscopic observations, Harris Hemotoxylin stained slides were prepared and studied under light microscope at 10x, 20x, 40x and 100x magnifications. For morphometry, an ocular micrometer was used at a magnification of 40x. The ocular micrometer was already graduated with a stage micrometer and changes were recorded in the proformas. Data was collected from proformas and appropriately compiled. Mean of all values was expressed as a mean $\pm$ standard deviation. Difference in the mean of all the values of control and experimental groups was analyzed using the two-tailed student 't' test.

RESULTS

The damaging effects of the drug as evident on naked eye examination were, decrease in weight and change in the behaviour of rats of experimental groups. The rats became lethargic, drowsy and stopped fighting in 12-24 hours following the drug administration. At the end of first week, the rats of group B showed slight improvement in their general condition. At the end of sec-

Table 1: Comparison of histological observations of stomach pylorus in control and experimental groups.

Parameters	Stomach Pylorus			
	Control Group	Experimental Groups		
	Group A	Group B	Group C	Group D
Ulcer	Absent	Present	Present	Absent
Shape of ulcer	Nil	Flat	Flat / Punched out	Focal necrosis
Depth of ulcer (mm)	Nil	357.5	316	79.5
Diameter of ulcer (mm)	Nil	362.5	256	81
Perforation	Absent	Absent	Absent	Absent
Inflammatory cells	Absent	Present	Present	Absent
Haemorrhages	Absent	Absent	Absent	Absent
Number of mitotic figures in a gastric gland/ HPF	5.0$\pm$0.9	**9.3$\pm$1.6	*-7.0$\pm$1.3	$^{\times}$6.0$\pm$0.9

Values are expressed as mean $\pm$ standard deviation.

$\times$ P>0.05 Insignificant

* P<0.05 Significant

** P<0.001 Highly significant

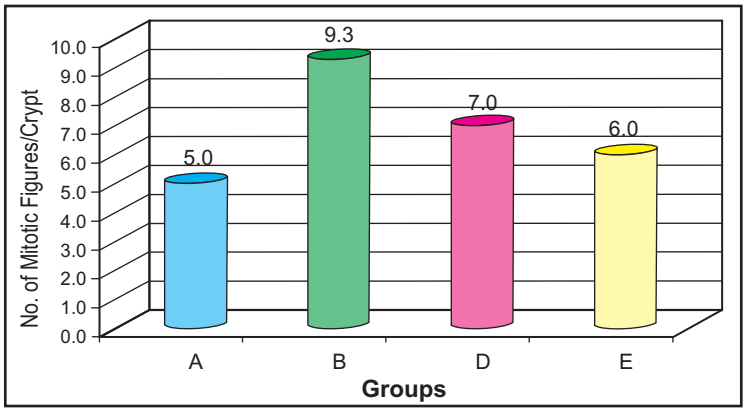

Fig. 1: Comparison of mean values of number of mitotic figures in pylorus of control and experimental groups.

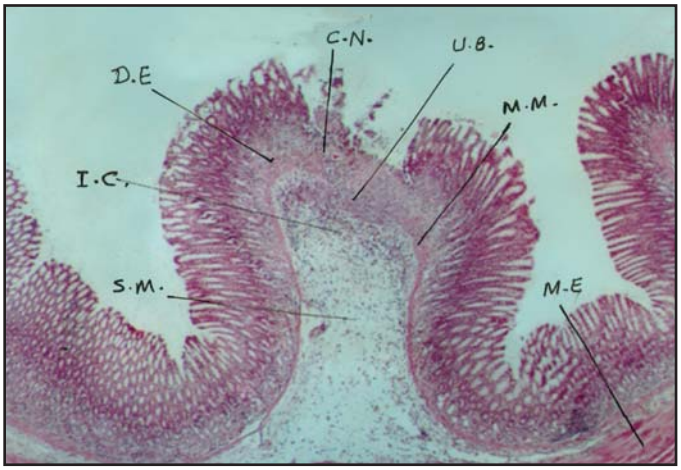

Fig. 2: A photomicrograph of histological section of stomach pylorus of rat of experimental group B, showing superficial ulcer. DE: Distorted Epithelium, UB: Ulcer Bed, CN: Coagulative Necrosis, IC: Inflammatory Cell MM: Muscularis mucosae, S.M: Sub mucosa ME: Muscularis externa. (H & E stain. Magnification 54x)

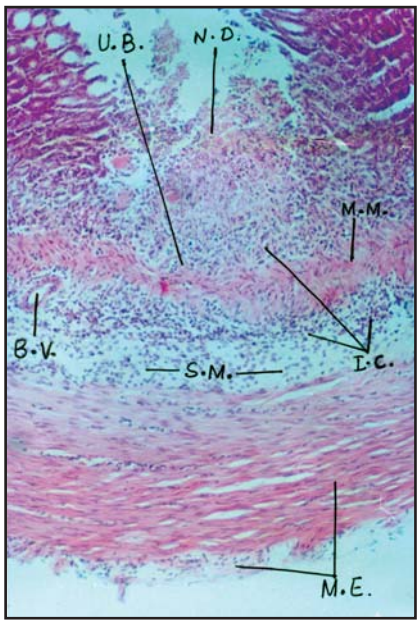

Fig. 3: Photomicrograph of the histological section of Stomach Pylorus of rat of Experimental Group C, showing punched out superficial ulcer. The ulcer bed is filled by necrotic debris. U.B: Ulcer Bed, N.D: Necrotic Debris, M.M: Muscularis Mucosae, S.M: Sub Mucosa, I.C: Inflammatory Cell, B.V: Blood Vessel, M.E: Muscularis Externa. H & E stain. Magnification 145x.

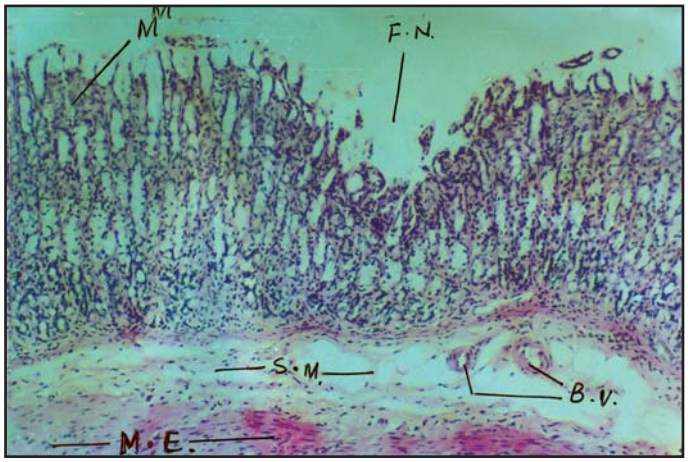

Fig. 4: Photomicrograph of the histological section of stomach pylorus of rat of experimental Group D, showing focal necrosis of epithelium. M: Mucosa, F.N: Focal Necrosis. S.M: Sub Mucosa, M.E: Muscularis Externa. B.V: Blood Vessels H & E stain. Magnification 54x.

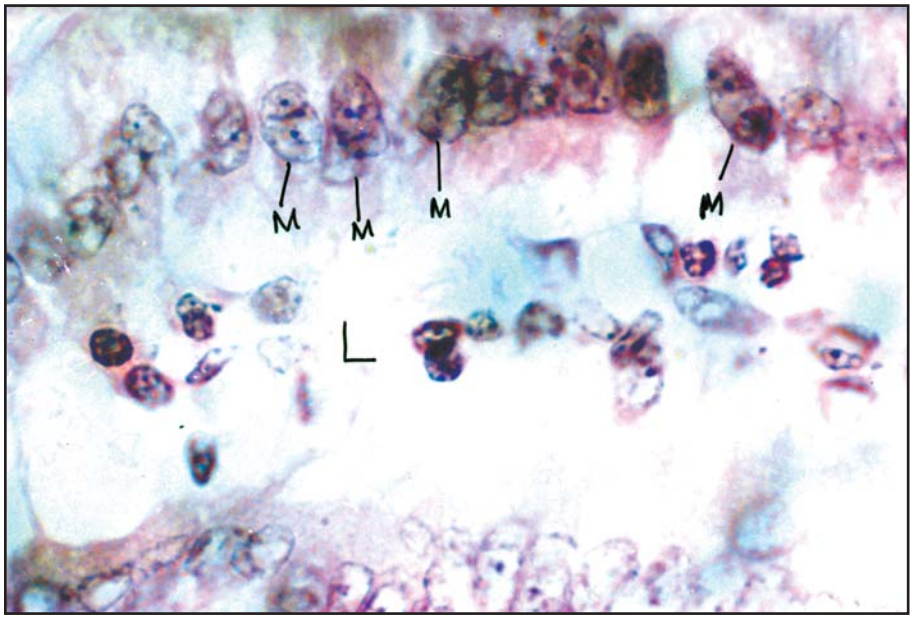

Fig. 5: Photomicrograph of histological section of pyloric gland of experimental group B, showing increased mitotic activity. L: Lumen of Pyloric gland. M: Mitotic figure. H & E stain. Magnification 1143x.

ond week, the rats of group C became active and their general condition was better than group B rats. At the end of third week, the rats of group D were as active as control group A, despite the drug administration. This improvement in general behavior was in favour of internal regenerative changes.

Microscopic observation: At the end of first week the stomach body of experimental animals showed normal architecture while stomach pylorus showed superficial ulcers. There was loss of mucosa and denuded mucosa was lying in the lumen. The ulcers were flat with irregular margins and penerated as far as muscularis mucosae. The ulcer bed was formed mainly by necrotic debris. Inflammatory cells were present in the deeper part of ulcers and also infiltrated the submucosa. There was no haemorrhage or perforation. The mean depth of ulcers was 357.5 μm, mean diameter 362.5 μm and mean number of mitotic figure 9.3 ± 1.6 ($p<0.001$).

In group C, the observations were noted at the end of second week of drug administration. Stomach body had normal appearance like group B whereas pylorus showed flat and punched out superficial ulcers. Ulcer bed was filled with necrotic debris and inflammatory cells. Granulation tissue was also observed indicating healing process. The mean depth of ulcer was 316 μm, mean diameter was 256 μm and mean number of mitotic figure was 7.0 ± 1.3 ($p<0.01$). The depth and diameter of ulcer indicated less severe damage than group B.

In group D, observations at the end of third week showed normal stomach body while pylorus had minor changes in the form of focal necrosis involving only superficial half to one third of mucosa. The mean depth of necrosed area was 79.5 μm while its mean diameter was 81 μm. Increase in the number of mitotic figure was insignificant.

DISCUSSION

Indomethacin given subcutaneously to rats for 7 days in a dose of 4 mg/kg in two divided doses caused serofibrinous exudates in the abdomen, blackish food debris and erosive gastritis inside the stomach.[11,12] The exact mechanism that stomach body was not affected by indomethacin was not known. Ulceration of stomach body can be produced by giving toxic doses to fasted rats instead of re-fed rats.[11,13,14] No lesion was observed in the stomach corpus when indomethacin was given in toxic doses of 10 and 85 mg/kg respectively to rats and mice.[15] When toxic doses cannot produce lesion, it is less likely that therapeutic dose will cause damage. Stomach pylorus is the most vulnerable part to NSAID related gastropathy. The effect of indomethacin in group B, in which the drug was given for one week, was in the form of a superficial ulcer that penetrated as far as submucosa. The ulcer bed had coagulative necrosis and inflammatory cells in abundance. There was significant increase in the number of mitotic figures in the adjacent normal pyloric glands. The rapid turn over was due to acute damage and responsible for re-epithelization. The regeneration is in response to acute damage. The antral lesion reached a maximum size in 6-10 hours, penetrated the muscularis mucosae within 3 days and did not diminish for at least 7 days.[16] Stom-

ach showed antral ulcer and full thickness mucosal coagulative necrosis.[11,13]

In group C the ulcer was superficial and penetrated as far as muscularis mucosae. It seems that the ulcergenic effects of the drug are less severe than group B. Superficial ulcer was observed in rats when indomethacin was given orally for fifteen days.[16]

In group D, the drug given for 3 weeks showed mild damage in the form of focal necrosis. There was no ulcer and inflammatory changes were also absent. Mitotic figures in the pyloric glands were not increased significantly. Considering all the three groups, one should expect more severe damage in group C and D than group B, as the duration of drug administration was more prolonged in group C and D than group B. The cause of this unexpected result is mucosal adaptation involving the process of regeneration evident by increased number of mitotic figures so that new cells replace the damaged ones. There was superficial ulcer in stomach pylorus at 20 hours but no ulcer at day 73.

Acute gastric erosions and haemorrhages were resolved despite continued administration of the drug for 28 days in human volunteers. Gastric adaptation to non steroidal anti-inflammatory drugs in man occurs. In this phenomenon visible gastric mucosal injury lessens or resolves completely despite continued administration of an injurious substance. Adaptation has at least two elements, lessening damage and more rapid healing.[17] Migration of neutrophils into ulcerated lesion and increased number of mitotic cells were also evident.[17]

Indomethacin given orally to human volunteers for three weeks in a dose of 50 mg TID revealed 6 mm gastric antral ulcer by 24 hours but normal antrum at the end of third week in majority of subjects' inspite of continued use of the drugs. The process of mucosal adaptation is observed as a result of regenerationof antral mucosa.[18]

Adaptation of rat gastric mucosa occurs to the repeated doses of non-salicylate non-steroidal anti-inflammatory drugs. There was a highly significant reduction in the amount of deeper mucosal damage.[19] Treatment with indomethacin in humans induces gastric adaptation, which entails the increase in mucosal blood flow, the rise in neutrophil activation and the enhancement in mucosal growth.[20] Indomethacin given orally to man for 28 days caused acute gastroduodenal damage,which was maximal at 24 hours of administration but with continued intake mucosal adaptation occured,resulting in resolution of endoscopic mucosal damage. This recovery was associated with a return of a blood flow to normal, though PGE2 in mucosal homogenate was still significantly reduced compared to that before drug intake.[21]

CONCLUSION

Indomethacin produces lesions in stomach pylorus when given in maximum therapeutic dose. However, the ulcerogenic effects are less marked when duration of the drug administration is prolonged. These unexpected results are due to mucosal adaptation of the stomach to the continued use of the drug.

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