HISTOLOGICAL CHANGES IN PARTS OF FOREGUT OF RAT AFTER INDOMETHACIN ADMINISTRATION

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Background: Indomethacin, a non-steriodalanti-inflammatory drug, is used mainly for the treatment of painful joints such as rehumatoid arthritis, osteo–arhtritis, gout, ankylosing spondylitis etc. It relieves pain, reduces swelling and tenderness of the joints. It also induces ulceration of stomach and small intestine both in experimental animals and humans. **Material and Methods:** In this study indomethacin was given intrapertioneally in maximum therapeutic dose (4 mg/Kg body weight) to three experimental groups B, C and D for one, two and three weeks respectively. Group A was the control group. **Results:** Effects were observed in stomach pylorus and proximal duodenum. In the stomach pylorus, well defined superficial ulcers were identified during initial two weeks of drug administration. The ulcer penetrated as for as muscularis mucosae and ulcer bed had coagulative necrosis and inflammatory cells. During third week, stomach pylorus showed minor damage in the form of focal necrosis. Duodenum was affected less than stomach and showed villi with lost tips, tilted and distorted villi. Morphometric analysis showed changes in stomach pylorus and in duodenum. The number of mitotic figure was significantly increased in stomach pylorus. Duodenum showed insignificant to significant decrease in the height of villi. Increase in the number of goblet cells, columnar cells, and mitotic figure was also noted; which was undoubtedly part of the tissue response to an injury. **Conclusion:** These observations suggested that indomethacin given in a maximum therapeutic dose, initially induces lesions in stomach pylorus and proximal duodenum but almost no effects were noted when duration of the drug administration was prolonged.

Key Words: Indomethacin, Stomach, Duodenum.

INTRODUCTION

Indomethacin is a synthetic non-steroidal anti-inflammatory drug with analgesic and antipyretic activity. It is a methylated indole derivative introduced in 1963 and approved by Federal drug agency in 1965. It is a potent inhibitor of prostaglandins 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 I2, 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 mitochondrial function and free radical induced oxidative changes, all of which contribute to its anti-inflammatory action.[3] 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.[4] 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 the elevation of set point for temperature in hypothalamus. The analgesic action of indomethacin is due to decrease in the production of prostaglandin that sensitize nociceptors to inflammatory mediators such as bradykinin and 5-hydroxytryptamine.[5] 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 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.[6] About 10–20% of the drug is eliminated unchanged in the urine.[7] Its half life in plasma is variable perhaps because of enterohepatic cycling, which is 7–10 hours.[8] 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.[9] 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 stomach and intenstine renal disorders and epilepsy.[5]

The adverse effects of indomethacin especially on gastrointestinal tract are its systemic effects and not local. Therefore, oral and parentral indomethacin have similar untoward effects.[10] Its untoward effects are nausea, vomiting, anorexia, epigastric distress, diarrhea, gastrointestinal ulcers and perforation.[11]
Though the use of newly introduced non-steroidal anti-inflammatory drugs is increasing day by day; the traditional drugs like aspirin and indomethacin are still widely used in the remote areas because of their low cost.[7]

MATERIALS AND METHODS

In this study, 42 adult rats of Albino Wistar strain, weighing 250.8–256.6 grams were used. These 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 done, abdomin opened and gross observations noted. Stomach body, pylorus and proximal duodenum 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 magnification. For morphometry, an occular micrometer was used at a magnification of 40X. The occular micrometer was already graduated with a strage 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 students "t" test.

RESULTS

Macroscopic Observations

Decrease in weight and change in the behaviour of rats of experimental groups was noted. The rats became lethargic, drowsy and stopped fighting in 12–24 hours following drug administration. At the end of first week, the rats of group B showed slight improvement in their general condition. At the end of second week, the rats of group C became active and their general conditions 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.

MICROSCOPIC OBSERVATIONS

Stomach

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 the 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 ulcer and also infiltrated the submucosa. There was no haemorrhage or perforation. The mean depth of ulcer was 357.5 µm, mean diameter 362.5 µm and mean number of motitic figure was 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 stomach 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 stomach 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.

Duodenum

In group B, majority of villi were intact and upright but distorted, tilted villi and villi with lost tips were also frequently observed. There was no ulcer, haemorrhage and inflammatory cells. The villi showed very significant to insignificant decrease in height which was 367 ± 59.6 µm to 383.3 ± 10.8 µm respectively as compare to normal height of dudenal villi which was 483 ± 17.8 µm. Insignificant rise in the number of goblet and columnar cells and mitotic figures in crypts was noted. The mean number of goblet cells was 11.0 ± 1.3, columnar cells was 48.0 ± 1.8 and mitotic figure was 7.2 ± 1.0 as compared to normal goblet cells 8.0 ± 1.4, normal columnar cells 39.0 ± 1.8 and normal mitotic figures as 5.5 ± 1.0. In group C and D, the duodenum had normal architecture and was as normal as control group.

Table– 1: Comparison of mean values of histological observations of stomach pylorus in control group 'A' and experimental groups (Values are expressed as mean ± standard deviation)

		Stomach Pylorus			
	Parameters	*Control Group*	*Experimental Groups*		
		Group A	*Group B*	*Group C*	*Group D*
			B	C	D
1	Ulcer	Absent	Present	Present	Absent
2	Shape of ulcer	Nil	Flat	Flat / Punched out	Focal necrosis
3	Depth of ulcer (μm)	Nil	357.5	316	79.5
4	Diameter of ulcer (μm)	Nil	362.5	256	81
5	Perforation	Absent	Absent	Absent	Absent
6	Inflammatory cells	Absent	Present	Present	Absent
7	Haemorrhages	Absent	Absent	Absent	Absent
8	No. of mitotic figures in a gastric gland/ HPF	5.0±0.9	*** 9.3±1.6	** 7.0±1.3	× 6.0±0.9

× P>0.05, * P<0.05, ** P<0.01, *** P<0.001

Table-2: Comparison of mean values of histological observations of duodenum in control group 'A' and experimental groups (Values are expressed as mean ± standard deviation)

	Duodenum				
	Parameters	*Control Group*	**Experimental**		
		Group A	*Group B*	*Group C*	*Group D*
1	Shape of villi	Leaf/finger shaped	Leaf/Finger shaped	Leaf/Finger shaped	Leaf/Finger shaped
2	Position of villi	Upright	Lost Distorted, tilted, Upright	Upright	Upright
3	Height of villi (μm)	483±17.8	*** 367.5±59.6	× 482.5±7.2	× 465.0±6.1
4	Ulcer	Absent	Absent	Absent	Absent
5	Shape of ulcer	Nil	Nil	Nil	Nil
6	Depth of ulcer (μm)	Nil	Nil	Nil	Nil
7	Diameter of ulcer(μm)	Nil	Nil	Nil	Nil
8	Perforations	Absent	Absent	Absent	Absent
9	Inflammatory cells	Absent	Absent	Absent	Absent
10	Haemorrhage	Absent	Absent	Absent	Absent
11	No. of goblet Cells/crypt/HPF	8.0±1.4	** 11.0±1.3	× 9.5±1.9	× 7.0±0.9
12	No. of columnar Cells/crypt/HPF	39.0±1.8	** 48.0±1.8	× 40.5±3.1	× 38.2±2.5
13	No. of mitotic figures/crypt/HPF	5.5±1.0	* 7.2±1.0	× 6.0±1.3	× 5.0±0.9

× P>0.05, * P<0.05, ** P<0.01, *** P<0.001

DISCUSSION

Particular care was taken to collect the specimen of duodenum. As duodenal villi can only be preserved when specimen of duodenum is collected from alive and normally breathing rat. When abdomin of animals of group B was opened, stomach had blackish food debris and erosions clearly visible through a magnifying lens. Indomethacin given subcutaneously to rats for 6 days in a dose of 4mg/kg in two divided doses caused serofibrinous exudates in the abdomen, blackish food debris and erosive gastritis inside the stomach.[12,13] The exact mechanism that stomach body was not affected by indomethacin was not known. Anthony et al.,[12] Karatoni et al.,[14] and Satoh and Guth,[15] observed ulceration of stomach body by giving toxic doses to fasted rats instead of refed rats. No lesion was observed in the stomach corpus when indomethacin was given in toxic doses of 10mg and 85mg/kg respectively to rats and mice.[16,17] When toxic doses cannot produce lesion, it is less likely that therapeutic dose will cause damage. Stomach pylorus was 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. These findings are consistent with the results of Anthhony et al.[12] 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.[18] Stomach showed antral ulcer and full thickness mucosal coagulative necrosis.[12,14]

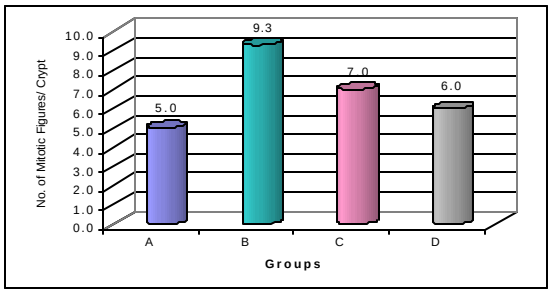

Fig-1: Comparison of mean values of number of mitotic figures of stomach pylorus in control group A and experimental groups.

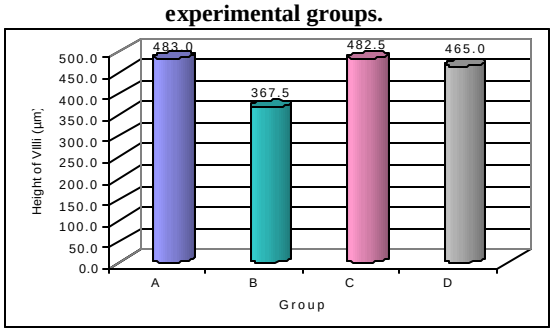

Fig-2: Comparison of mean height of villi of duodenum in control group A and experimental groups

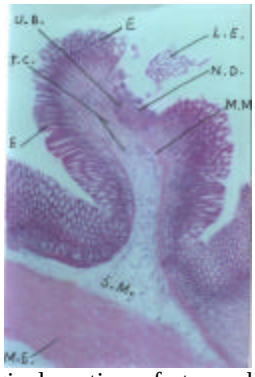

Fig-3: Histological section of stomach pyloris of rat of experimental group B, showing superficial ulcer. E: epithelium LE: lost epithelium, UB: ulcer bed, ND: necrotic debris, IC: inflammatory cell MM: muscularis mucosae, SM: submucosa ME: muscularis externa. H&E stain. Magnification 54x.

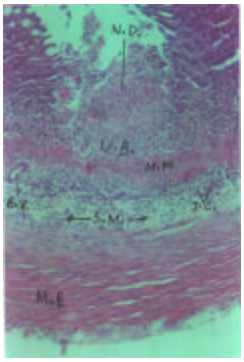

Fig-4: Histological section of stomach pylorus of rat of experimental group C, showing punched out superficial ulcer. The ulcer bed is filled by the necrotic debris. UB: ulcer bed, ND: necrotic debris, MM: muscularis mucosae, SM: sub mucosa, IC: inflammatory cell, BV: blood vessel, ME: muscularis externa. H&E stain. Magnification 145x.

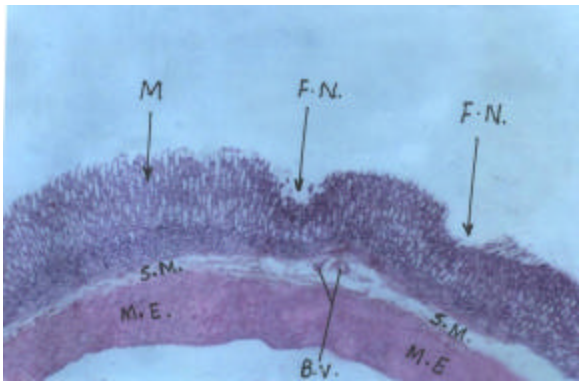

Fig-5: Histological section of stomach pylorus of rat of experimental group D, showing focal necrosis of epithelium. M: mucosa, FN: focal necrosis. SM: sub mucosa, ME: muscularis externa. BV: blood vessels, H&E stain. Magnification 54x.

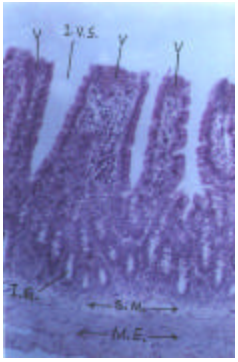

Fig-6: Histological section of duodenum of rat of experimental group b, showing lost tips of villi. V: villous having lost tip, i.g: intestinal gland, s.m: sub mucosa, m.e: muscularis externa, i.v.s: inter villous space. H & e stain. Magnification 145x.

In group C the ulcer was superficial and penetrated as far as musculars mucosae. It seems that the ulcergenic effects of the drug is less severe than Group B. Mahendran et al.[18] observed similar superficial ulcer in rats when indomethacin was given orally for fifteen days. In group D, the drug given for three weeks showed mild damage in the form of focal necrosis. There was no ulcer and accompanying 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 yet to be sorted out. One of the probable answer is the process of regeneration, evident by increased number of mitotic figures in group B and new cells replace the damage cells. There was superficial ulcer in stomach pylorus at 20 hours but no ulcer at day 7.[4] Acute gastric erosions and haemorrhages were resolved despite continued administration of the drug for 28 days in human volunteers. Migration of neutrophils into ulcerated lesion and increase number of mitotic cells were also evident.[19]

The ulcerogenic effects of indomethacin on duodenum of group B were minor and included a decrease in the height of villi, lost and distorted villi. Increase in the number of columnar, goblet and mitotic cells in the crypts of Lieberkuhn were the consequences of lost epithelium of villi. Ettarh and Carr[17,20] showed similar microscopic findings by giving 85 mg/kg of the drug given twice to mice. This tolerance is probably due to the difference of animal species. Mice are known to be resistant animal and it requires higher dose than that for rats to achieve the same results. The absence of any abnormality in group C and D was probably due to healing process which began in group B, and completed in group C and D. Indomethacin given orally to human volunteers for three weeks in a dose of 50 mg TID revealed 6mm gastric antral ulcer by 24 hours but normal antrum at the end of third week in majority of subjects inspite of continued used of the drugs.[21] In this experiment, the gastric damage observed is more than duodenal damage. The relative risk of damage associated with NSAID use were slightly greater for gastric than for duodenal damage.[22]

CONCLUSION

Indomethacin produced lesions in stomach and duodenum when given in maximum therapeutic dose. However, the ulcerogenic effects were less marked when duration of the drug administration was prolonged. The cause of these unexpected results need further studies.

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