Effect of Indomethacin on colon of albino rat histopathological study

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Abstract
Background and Objective: Non steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used of all therapeutic agents. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) that reduces fever, pain and inflammation. It is similar to ibuprofen and naproxen. Indomethacin works by reducing the production of prostaglandins. Prostaglandins are chemicals that the body produces to cause fever and pain that are associated with inflammation. Indomethacin blocks the enzymes that make prostaglandins (cyclooxygenase 1 and 2) and thereby reduces the levels of prostaglandins.

Methods: Adult albino rats were treated with Indomethacin in a dose of 10 mg/kg/day orally. The rats were sacrificed at the end of the 1st, 2nd and 3rd week. Colon was preserved in 10% neutral formalin for 72 hours and processed for histological studies.

Result: Microscopic examination revealed Increase in the number of columnar cells, goblet cells, and mitotic figures may be due to rapid regeneration following damage of epithelium.

Conclusion: Indomethacin causes injury to the epithelium of colon of albino rat.

Keywords: Blunt abdominal trauma, pancreatic injury, pancreatic transection, conservative management.

1. Introduction

The classic signs of inflammation have long been recognized, the tissue becomes red, swollen, tender or painful, there is local heat and the patient may be febrile1. Indomethacin is a synthetic non-steroidal anti-inflammatory drug with analgesic and antipyretic activities. It is a potent inhibitor of prostaglandin synthesis.

The analgesic action of indomethacin is due to a decrease in the production of prostaglandins that sensitizes nociceptors to inflammatory mediators such as bradykinin and 5-hydroxytryptamine. Indomethacin is readily absorbed from the gastrointestinal tract almost completely after oral ingestion. 90% of it is bound to tissue proteins and are also the mediators of inflammatory response. Indomethacin is used in musculoskeletal disorders such as rheumatoid lesions. It is widely used in the treatment of arthritis (osteoarthritis and acute gouty arthritis) and ankylosing spondylitis. The oral dose of Indomethacin is 25–50 mg 2-3 times a day and injectable dose is 1–2 mg/Kg/24 hours in two divided doses. The adverse effects of indomethacin especially on the gastrointestinal tract are due to its systemic effects and not due to its local actions. Ingestion of indomethacin has been shown, in man, to be associated with NSAID gastropathy. Its untoward effects are
nausea, vomiting, anorexia, epigastric distress, diarrhoea, gastrointestinal ulcers and perforation. The traditional drugs like indomethacin are still widely used in the remote areas because of its low cost \(^2,3,4\).

2. Material and Methods

This study was carried out in the Department of Anatomy, Himalayan Institute of Medical Sciences, Swami Ram Nagar, Dehradun over a period of twelve months.

In this study 16 adult albino rats of Charles Foster strain (8 males and 8 females) weighing 120gms (+10gms) obtained from the Central Animal House, HIMS, Dehradun (Uttarakhand) were used as experimental animals after obtaining the approval of IAEC (Institutional Animal Ethical Committee). The rats were housed in cages with a 12hr: 12hr light-dark cycle. They were allowed to access food and water ad libitum.

The rats were randomly divided into 4 groups i.e. Group I, II and III and IV for the study. Each group was comprised of 4 rats out of which group I was taken as control and the remaining II, III, and IV were taken as the experimental groups. Each experimental group was subjected to oral administration of indomethacin (Cap Indocap-25mg manufactured by Jagsonpal). They were fed with the help of a rat-feeding tube. The drugs were administered for 1, 2 and 3 weeks in Group II, III and IV respectively.

Indomethacin (Cap 25mg) was dissolved in 10 ml of distilled water so that the 1 ml of the solution of the drug had a concentration of 2.5 mg/ml. The above drugs were given orally twice daily at the interval of 12 hours to the respective groups & subgroups. The controls were administered distilled water according to their weight.

The rats of each group were sacrificed at the end of one, two, and three week intervals after giving ether anesthesia. They were immediately fixed on a wooden block with the help of paper pins. Dissection was done to open the abdomen for gross observations. The rats were infused with normal saline to wash out the blood. Colon was preserved in neutral buffered formalin. Staining was done with Harris’ Hematoxylin and Eosin. 3-5 \(\mu\)m thick paraffin sections of the colon were studied under light microscope and the histological and morphometric changes were studied.

The processing was carried out in the following steps:

2.1. Fixation

The fixative used was 10% formal-saline containing 100 ml of formalin, 8.5 grams of sodium chloride and 900 ml of tap water. The fixation was carried out for 24-48 hours. The purpose of fixation was to preserve the morphology and chemical composition of the tissue, to prevent autolysis and putrefaction, to harden the tissue for easy manipulation and to solidify colloidal material and influence staining.

2.2. Dehydration

The preserved tissues were thoroughly washed under running tap water for 4-6 hours. They were then passed through ascending concentrations of alcohol as follows:

- 50% alcohol (50 cc of absolute alcohol + 50cc of distilled water) for 1 hour
- 70% alcohol (70 cc of the absolute alcohol + 30 cc of distilled water) for 1 hour
- 90% alcohol (90 cc of the absolute alcohol + 10 cc of distilled water) for 1 hour
- Absolute alcohol - 1 (first change) for 1 hour
- Absolute alcohol -2 (second change) for 1 hour

As paraffin wax does not penetrate the tissue in the presence of water so dehydration was an essential step in this process. This was affected by immersion of the tissue in ascending concentrations of alcohol to prevent the distortion of cellular constituents that would accompany the direct transference of tissue from an aqueous medium such as 10% formalin to absolute alcohol.
2.3. Clearing

The clearing of the tissue was done in xylene, giving three changes of one hour each. As alcohol is scarcely miscible with paraffin wax so after dehydration, it was necessary to treat the tissue blocks with a reagent that mixes with both alcohol and water and which might in turn be eliminated in the process of wax impregnation. Clearing was considered complete when the tissue became more or less translucent due to increase in refractive index.

2.4. Embedding

The cleared tissue was put in xylene wax mixture (50% v/v) for one hour and then in molten paraffin wax for 12-14 hrs (m.p. 56-58°C) in the oven. At the end of the process, the sections were made into paraffin blocks by using Leuckhart's metallic blocks, which were then cooled, to a temperature of 0 – 4°C for at least 24 hours.

2.5. Sectioning

Sections of 3-5µ thickness were cut by Rotatory Microtome and floated in a water bath having a temperature of 5-10°C less than the melting point of the wax. The sections were then spread on the slide, which were smeared with an adhesive solution (mixture of equal amount of glycerol and egg albumin) and then left to dry in the air. The prepared sections were labelled for the sex, experimental or control group and the region to which it belonged. The slides were dried on a hot plate having a temperature of 5-10°C above the melting point of paraffin wax.

2.6. Deparaffinisation of sections

The slides were put in xylene. Three changes each for 5-10 minutes were given in order to remove the extracellular and intracellular wax. The sections were then dried by means of blotting paper.

2.7. Hydration

The sections were dipped in descending grades of alcohol i.e. absolute alcohol (2 changes), 90% alcohol, 70% alcohol and 50% alcohol. The slides were then washed under very slow running tap water for 2 minutes. As paraffin wax is poorly permeable to stains i.e. haematoxyline & eosin, which are water soluble stains, its removal with a solvent is necessary and xylene is used for this purpose.

2.8. Staining

1. The deparaffinised slides were treated with Harris' haematoxylin for 5-7 minutes.
2. Blueing of the stained sections was done by passing the sections through a gentle stream of tap water for 5-10 minutes.
3. Over stained slides were treated with 1% hydrochloric acid in 70% alcohol for five seconds to remove excess stain. The blueing of the sections was done again in running tap water.
4. The sections were then stained with 1% aqueous eosin for 30 seconds to 1 minute and rinsed rapidly in slow running tap water for 10-12 minutes.
5. The stained slides were dipped into xylene (2 changes) for clearing after passing through ascending grades of alcohol i.e. 50% alcohol, 70% alcohol, 90% alcohol and 2 changes of absolute alcohol.
6. The sections were finally mounted with D.P.X. mountant and covered with the cover slip for observation.

The data collected was subjected to standard statist

3. Observation and Result

Microscopic examination was done under low (100x) and high (400x) magnification of the control and experimental groups on the sections prepared from the colon of albino rats. Ten randomly selected areas were taken from each control and experimental groups to study any particular aspects, the mean and standard deviation were calculated. The measurements were done with the help of an eyepiece micrometer. All numerical data were subjected to student’s t-test before deriving any conclusion.
3.1 Microscopic observations after administration of Indomethacin

(4) Large Intestine (Proximal Part)

Table 1. Mean number of goblet cells, columnar cells, and mitotic figures per crypt per high power field in the large intestine after administration of indomethacin.

<table>
<thead>
<tr>
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<th>Control Group (Mean ± SD)</th>
<th>Experimental Group (Mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>1st week</td>
<td>2nd week</td>
</tr>
<tr>
<td>No. of goblet cells/ crypt/ HPF</td>
<td>13.1 ± 2.1</td>
<td>19.7 ± 5.7</td>
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<tr>
<td></td>
<td>p&lt;0.01(HS)</td>
<td>p&lt;0.01(HS)</td>
</tr>
<tr>
<td>Number of columnar cells/ crypt/ HPF</td>
<td>20.5 ± 7.6</td>
<td>40.9 ± 9.3</td>
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<tr>
<td></td>
<td>p&lt;0.01(HS)</td>
<td>p&lt;0.01(HS)</td>
</tr>
<tr>
<td>Number of mitotic figure/ Crypt/HPF</td>
<td>4.1 ± 0.7</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05(S)</td>
<td>p&lt;0.05(S)</td>
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p < 0.01 – Highly Significant
p < 0.05 – Significant
p > 0.05 – Not significant

There was normal architecture of the tissue. It was observed that the number of the goblet cells which was 13.1 ± 2.1 in the control group, increased to 19.7 ± 5.7, 20.6 ± 5.3 and 17.3 ± 5.8 at the end of the 1st, 2nd and 3rd weeks respectively. The p values were significant in all the treated groups. It was observed that the number of columnar cells was 20.5 ± 7.6 in the control group. It increased to 40.9 ± 9.3, 38.6 ± 11.3, 32.2 ± 12.3 at the end of the 1st, 2nd and 3rd weeks respectively. The p values were significant in all the treated groups. It was noted that the mean number of mitotic figures in the control group was 4.1 ± 0.7. It increased to 7.7 ± 0.6, 10.4 ± 0.9, 12.7 ± 1.4 at the end of the 1st, 2nd and 3rd weeks respectively. The p values were significant in all the treated groups. Increase in the number of columnar cells, goblet cells, and mitotic figures may be due to rapid regeneration following damage of epithelium or increase in the number of columnar cells, goblet cells, and mitotic figures may be due to a consequence of loss of epithelium or.

4. Discussion

The present study deals with the histological and morphological effects of indomethacin on colon of albino rats. After administration of indomethacin it was observed that all the parameters were increased at the end of the 1st, 2nd and 3rd weeks respectively. Demetre M studied the effects of indomethacin in adult mongrel dogs each weighing about 10-12 kg, after oral administration of the drug in a dosage of 5 mg/kg of body weight for 30 days. He observed melaena in the 1st week of administration. Antral ulcerations were noted in all cases. Superficial ulcers were observed in the body and fundus of stomach. Satoh H et al studied the effects of subcutaneous injection of indomethacin in male JcL: Sprague-Dawley rats weighing about 180-220 gm in doses of 30 mg/kg of body weight. The experimental animals were divided into three groups. The first group which was sacrificed 6 hours after administration of the drug showed antral lesions, in which the outer half of mucosa had sloughed with evidence of damage in the inner half of mucosa with pyknosis and cytoplasmic shrinkage of parietal and chief cells. Infiltration of mono or polymorphonuclear leukocytes was also seen at the base of the lesion and the adjacent mucosa. The second group which was sacrificed 24 hours after administration of the drug depicted necrotic lesions involving muscularis mucosae in the same area while the submucosa was oedematous and infiltrated with inflammatory cells. The third group (3 days post administration) showed disappearance of the mucosal layer with destruction of the muscularis mucosa completely.
Brodie DA et al studied the effects of the indomethacin in rats after oral administration of the indomethacin in a dose of 16 mg/kg of the body weight. They divided the rats into two groups. The first group which was sacrificed 24 hours after a single dose administration showed small white nodules palpable from the serosal surface of the small intestine. Microscopically, the lesions showed perforating ulcers surrounded by oedema with infiltration of polymorphonuclear cells extending up to the serosa and mesentery. Non-perforating ulcers were both of discrete and coalescing varieties found along the mesenteric border of the small intestine. The second group sacrificed after 3 days of oral administration of Indomethacin revealed perforated ulcers in the small intestine while none were found in the large intestine.

References