Gastric mucosal cellular changes induced by indomethacin (NSAID) in male albino rats

Vedavyasa Sagar & R. Nazeer Ahamed
Department of Zoology, Karnataka University, Dharwad, 580 003, India

Received 3 July 1998; revised 22 December 1998

Indomethacin (2 mg/100 g body weight), induces haemorrhagic gastric ulcers in albino rats. The incidence and severity of ulceration increased with starvation period. Indomethacin caused little or no effect on the cellular and the nuclear diameter of parietal and chief cells while reduction was observed in mucus and endocrine cells. The effect was enhanced with increased duration of starvation. Both mucous and endocrine cells decreased in their number after 72 hr of starvation. Thus prolonged starvation enhanced the gastric mucosal damage induced by indomethacin.

There are several drugs usually stated to be ulcerogenic in experimental animals and man. These include adrenal corticosteroids, aspirin, phenylbutazone, indomethacin, ethanol, caffeine and tobbaco.

Various views have been put forward to explain the possible causative factors for indomethacin induced gastric ulcers such as prostaglandin inhibition, release of catecholamines, reduced blood flow starvation and increased gastric motility.

Norcross was the first to report indomethacin to be a useful drug for the relief of symptoms in rheumatoid arthritis. Its therapeutic effect was accepted by Lockie and Dick. The drug has now become one of the most commonly used therapeutic agents in rheumatology. It is also reported to exhibit side effects, the peptic ulcers being major concern among these. The present study has been undertaken to investigate the effects of indomethacin on the morphology and morphometry of mucosal cell types of corpus in male albino rats with a view to find out the possible role of starvation on the effect of indomethacin in causing damage to the gastric mucosa. Among the non-steroidal antiinflammatory drugs, indomethacin has been widely used in medical therapy and therefore, it has been chosen in the present study.

Materials and Methods

Adult male albino rats of Wistar strain (2-3 months old) weighing 160-200 g were used. The animals were divided into 4 groups and housed in metabolic rat cages with wire mesh bottoms to minimize coprophagy. They were fasted prior to the administration of drugs with water ad libitum.

Preparation of drug suspension—Indomethacin (LA No.1-7378, Anhydrous mol. wt. of 35-98, C_{19}H_{20}CIN0_{4}, St. Louis USA), pale yellow crystalline powder (2 mg/100g body weight) was suspended in a trace of Tween 80 and 1 ml of 0.9 % saline. After recording the initial body weight of the animals, the drug was administrated orally in a single dose of 1 ml/100g body weight by gastric incubation as per the following experimental protocol:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/ treatment (mg/100g body weight)</th>
<th>Starvation Duration (hr)</th>
<th>Autopsy After (hr)</th>
<th>Total duration of expt (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2</td>
<td>24</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>24</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>48</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>48</td>
<td>24</td>
<td>72</td>
</tr>
</tbody>
</table>

Suitable controls were maintained. The controls received saline.

The animals were killed by cervical dislocation. The stomach was removed, opened along the greater curvature, washed and a gross examination was performed for ulceration using an occular magnifier.

Histology and histometry—Immediately after gross examination, the stomach was cut and tissue samples were taken from the corpus, fixed in Bowin's fluid,
dehydrated in ethanol grades, cleared in benzene and
eMBEDED in paraffin and sectioned (5-6, μm thick)
LONGITUDINALLY to the long axis of the glandular region,
sections were stained with Harris-haematoxylin and
eosin for histological studies. Fresh tissue sections
were also cut in cryostat and stained with PAS for
observations. Parietal, chief, mucous and endocrine
cells were identified by their morphological
appearance. Evaluation of morphology, morphometry
and population of cell types was carried out by
randomly selecting different histological zones using
a micro ocoulometer (Erma, Japan). The cell and
nuclear diameters were measured using the scale on
the micro ocoulometer. The cell population was
calculated in an unit area of the ocoulometer.
The data were statistically analysed and all values
were expressed as means ± SE. Student's t test was
applied for significance of c~
differences.

Results
In control group, the stomach was free from
ulceration. The entire thickness of the mucosa in all
parts of the stomach is occupied by a multitude of
glands (Fig.1).

In group I and II, treatment induced very few
superficial haemorrhagic gastric ulcers, which were
generally confined to the crests of rugae. Histological
observation of group II animals showed that the cells
of the gastric glands were haphazardly dispersed and
few small vacuoles/cavities were observed at certain
places in the mucosa (Figs 2-4). More gastric lesions
were observed in the animals starved for 48 hr and
autopsied 7/24 hr after treatment (group III and IV).
The nuclear and cellular diameter of both parietal and
chief cells were not affect~ much except nuclear
diameter of parietal cells after 72 hr starvation with
treatment. However, significant changes in the above
parameter were observed with mucus and endocrine
cells after 72 hr of starvation and treated with
indomethacin. (Tables, 1 and 2; Figs 5-8).

In some of the group IV rats, chief cells are
aggregated in groups of 4 to 15 numbers at the base

| Table 1—Effects of treatment of indomethacin on cellular diameter (μm) of parietal cells chief cells, mucus cells and endocrine
cells in corpus of male albino rats |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Parietal cell</td>
<td>Chief cell</td>
<td>Mucus cell</td>
<td>Endocrine cell</td>
</tr>
<tr>
<td>Control</td>
<td>12.97±0.38</td>
<td>9.25±0.21</td>
<td>9.40±0.33</td>
<td>8.76±0.85</td>
</tr>
<tr>
<td>I</td>
<td>13.55±0.46</td>
<td>9.62±0.40</td>
<td>8.54±0.29</td>
<td>6.84±0.54 a</td>
</tr>
<tr>
<td>(+ 4.47)</td>
<td>(+ 4.00)</td>
<td>(- 9.15)</td>
<td>(- 21.98)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>13.02±0.27</td>
<td>10.27±0.34 a</td>
<td>8.37±0.22</td>
<td>8.70±0.83</td>
</tr>
<tr>
<td>(+ 0.38)</td>
<td>(+ 11.02)</td>
<td>(- 10.96)</td>
<td>(- 0.69)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>12.52±0.22</td>
<td>8.92±0.37</td>
<td>7.72±0.24 b</td>
<td>8.52±0.15</td>
</tr>
<tr>
<td>(- 4.47)</td>
<td>(- 3.57)</td>
<td>(- 7.88)</td>
<td>(- 2.74)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>11.82±0.55</td>
<td>9.50±0.62</td>
<td>7.75±0.33 b</td>
<td>—</td>
</tr>
<tr>
<td>(- 8.87)</td>
<td>(+ 2.70)</td>
<td>(- 7.56)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P values *<0.05; **<0.01

Fig. 1—T.S. of corpus of saline treated albino rat showing parietal, chief, mucus and endocrine cells.
Fig. 2—T.S. of corpus of 24 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 7 hr later. Note the
shrinkage of mucus and endocrine cells.
Fig. 3—T.S. of corpus of 24 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 24 hr later. The mucus
cell size is further reduced.
Fig. 4—T.S. of corpus of 24 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 24 hr later. Note the
cavities/vacuoles formed in the mucosa.
Fig. 5—T.S. of corpus of 48 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 7 hr later. The parietal
and chief cells appear normal.
Fig. 6—T.S. of corpus of 48 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 7 hr later. The mucus cell
size is further reduced.
Fig. 7—T.S. of corpus of 48 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 7 hr later. The Chief
cells aggregate in groups.
Fig. 8—T.S. of corpus of 48 hr starved rat treated with 2 mg/100 g body weight of indomethacin and autopsied 24 hr later. The Chief
cells aggregate in groups.

Figs 1-8 : ×400
of the gastric glands. Endocrine cells completely disappeared in the group IV animals (Table 3). The intensity of the PAS reaction also was reduced in the treated animals compared to controls.

Further, the effect on number of different mucosal cells in the corpus showed a tendency to increase in mucus and endocrine cells upto 48 hr of starvation and a tendency to decrease afterwards in the number was observed. The decrease was more marked after 72 hr fasting (Group IV) (Table 3).

Discussion

Indomethacin is known to produce erosions and ulcers in the gastrointestinal tract of experimental animals such as rats, dogs, guinea pigs and mini pigs. Indomethacin also causes gastrointestinal damage in the humans. The gastric mucosa is covered by a layer of mucus which appearantly forms a barrier. The gastric mucus production is stimulated by prostaglandins. Prostaglandin deficiency has been regarded to be primarily responsible for ulceration. Indomethacin is a known prominent inhibitor of prostaglandin synthesis. Past several years’ work has shown that gastric mucosal barrier has an important role in protecting the stomach from injury and that its function can be impaired in many circumstances. When the barrier is broken, acid contained in the lumen can diffuse rapidly into the mucosa and sodium ions can diffuse from the mucosa into the lumen. Back diffusion of acid causes the Patho-physiological consequences.

The present study suggests effect of indomethacin on the gastric cells in general and mucus cells in particular as indicated by the shrinkage and decrease in the number of mucus cells and reduced PAS reaction intensity, probably, resulting in reduced mucus secretion leading to reduced mucosal resistance and subsequent damage of the gastric mucosa.

The observations that starvation of animals after 48 hr lead to further increase in the shrinkage and decrease in the number of mucus cells, indicates the role played by starvation in the cytotoxic effect of indomethacin. The increase in the number of mucus

| Table 2—Effects of treatment of indomethacin on the nuclear diameter (μm) of parietal, chief, mucus, and endocrine cell in corpus of male albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Parietal cell</th>
<th>Chief cell</th>
<th>Mucus cell</th>
<th>Endocrine cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.01±0.13</td>
<td>4.29±0.24</td>
<td>6.03±0.19</td>
<td>5.63±0.12</td>
</tr>
<tr>
<td>I</td>
<td>5.60±0.12*</td>
<td>4.80±0.17</td>
<td>5.49±0.31</td>
<td>4.26±0.24</td>
</tr>
<tr>
<td></td>
<td>(+ 11.77)</td>
<td>(+ 11.88)</td>
<td>(− 8.96)</td>
<td>(− 5.82)</td>
</tr>
<tr>
<td>II</td>
<td>5.12±0.25</td>
<td>4.12±0.14</td>
<td>5.40±0.15*</td>
<td>3.90±0.28*</td>
</tr>
<tr>
<td></td>
<td>(+ 2.19)</td>
<td>(− 3.91)</td>
<td>(− 10.45)</td>
<td>(− 22.93)</td>
</tr>
<tr>
<td>III</td>
<td>5.72±0.25*</td>
<td>4.35±0.22</td>
<td>4.18±0.57*</td>
<td>4.62±0.12*</td>
</tr>
<tr>
<td></td>
<td>(+ 14.17)</td>
<td>(+ 1.39)</td>
<td>(− 10.68)</td>
<td>(− 8.70)</td>
</tr>
<tr>
<td>IV</td>
<td>4.55±0.17</td>
<td>4.00±0.23</td>
<td>4.97±0.47*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(− 9.19)</td>
<td>(− 6.96)</td>
<td>(− 7.58)</td>
<td>—</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;0.05;</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Table 3—Effect of treatment of indomethacin on the percentage of parietal, chief, mucus, and endocrine cells of corpus of albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Parietal cell</th>
<th>Chief cell</th>
<th>Mucus cell</th>
<th>Endocrine cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.42±1.40</td>
<td>55.00±2.23</td>
<td>19.28±2.23</td>
<td>4.28±0.28</td>
</tr>
<tr>
<td>I</td>
<td>22.72±1.80</td>
<td>50.90±1.80</td>
<td>20.90±1.49</td>
<td>5.45±0.12</td>
</tr>
<tr>
<td></td>
<td>(+ 6.06)</td>
<td>(− 7.46)</td>
<td>(+ 8.40)</td>
<td>(+ 27.33)</td>
</tr>
<tr>
<td>II</td>
<td>19.38±1.45</td>
<td>50.00±1.95</td>
<td>26.53±2.49</td>
<td>4.08±0.24</td>
</tr>
<tr>
<td></td>
<td>(− 9.53)</td>
<td>(− 9.10)</td>
<td>(+ 37.60)</td>
<td>(− 4.68)</td>
</tr>
<tr>
<td>III</td>
<td>24.76±2.49</td>
<td>50.47±2.01</td>
<td>21.90±1.45</td>
<td>2.85±0.12</td>
</tr>
<tr>
<td></td>
<td>(+ 15.59)</td>
<td>(− 8.24)</td>
<td>(+ 13.58)</td>
<td>(− 33.42)</td>
</tr>
<tr>
<td>IV</td>
<td>22.27±1.64</td>
<td>60.10±2.23</td>
<td>18.80±1.16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(+ 3.96)</td>
<td>(+ 9.27)</td>
<td>(− 6.33)</td>
<td>—</td>
</tr>
</tbody>
</table>
cells up to 48 hr of starvation could be due to enhanced defensive cell proliferation in response to cytotoxic effect of indomethacin (group I and II), while the tendency to decrease in number (group IV) could be due to dampening of defense mechanism, because of low nutritional status of the animals after 72 hr of starvation.

Thus the results of the present study indicates the cytotoxic effect of indomethacin on rat gastric corpus cells and prolonged starvation further enhances the effect of indomethacin.

References
10 Norcross B M, Arthritis Rheumatism, 6 (1963) 920.
26 Devenport H W, Progress in gastroenterology (Grune and Storation, New York) 1970.