A STUDY OF THE POTENTIAL PROTECTIVE THERAPEUTIC EFFECT OF THE ANTI OBESITY DRUG BRI 37344 IN AN EXPERIMENTAL MODEL OF INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Background: Because the aetiology of inflammatory bowel disease (IBD) is unclear, no causative therapy is available. However, pathophysiology of the disease offers a lot of possibilities to disrupt the inflammatory cascade that maintains the inflammatory process. Hyperleptinemia has been reported in acute inflammation especially during the early stage of intestinal inflammation. This study was done to investigate the potential therapeutic effect of B3 adrenoreceptor agonist BRI 37344 (BRI) and compare it with the traditional used prednisolone in an experimental model of inflammatory bowel disease.

Methods: The present study was done on 40 rats. They were divided into 2 main groups: Group I: 10 rats, as control group, received distilled water. Group II: 30 rats, subdivided into 3 equal subgroups as follow: Subgroup IIa: indomethacin only treated group, received 7.5mg/kg indomethacin for 2 doses separated by 24 hours, subcutaneously. Subgroup IIb: indomethacin treated rats, received 0.5 mg/kg prednisolone intragastrically, with the 1st dose of indomethacin and for 4 consecutive days. Subgroup IIc: indomethacin treated rats, received 1 mg/kg BRI intragastrically at the same time with the 1st dose of indomethacin and for 4 con-
chronic inflammatory enteritis seen in Crohn's disease (5,6).

Prednisolone, is known to improve flare ups and permeability changes in Crohn’s disease (7). It induces a general reduction in the inflammatory activity by stabilizing lysosomal-membranes and free radical activity (8).

BRI 37344 is a B3 adrenoreceptor agonist that plays an important regulatory role in adipose tissue metabolism through stimulation of thermogenesis and lipolysis. It also reduces body weight and fat content as well as increases resting metabolic rate and energy expenditure (9).

The aim of the work is to assess the inflammatory changes in rat model of NSAID induced enteropathy and also the effect of BRI in indomethacin induced enteropathy and comparing its effects with traditionally used prednisolone in indomethacin-induced enteritis.

MATERIALS & METHODS

Drugs used:
- Indomethacin: indomethacin ampoule 50 mg supplied by the Nile Co for pharmaceuticals.
- B3 adrenoreceptor agonist: BRI 37344 sodium salt powder, 5 mg supplied by Sigma.
- Prednisolone: prednisolone tablet, 5 mg supplied by Kahira Pharm.

Animals used:
Fourty male Sprague dawley rats were used, weighing (140-160 gm each) and obtained from animal house (Mansoura Faculty of Medicine, Egypt). They were housed individually in plastic cages.

Experimental protocol:
1- Indomethacin was dissolved in a pathogen free water to make a concentration of 25 mg/ml.
2- Prednisolone was dissolved as indomethacin to make a concentration of 1 mg/ml.
3- BRI 37344 was dissolved as the previous drugs to make a concentration of 1 mg/ml.
4- All chemicals were used, were obtained from Sigma Chemicals.

Treatment:
Fourty (40) rat groups were randomly divided into 2 main group:
Group I: Consisted of 10 rats,
mogenized and centrifuged. The supernatant was assayed for MPO spectrophotometrically (Jenway 6405 uv/vis).

B) Histopathological examination:
Small specimens from the jejunum of the rats of the control group, indomethacin group and groups treated with prednisolone and or BRI 37344 were obtained, fixed in 10% formalin for 24 hours and processed to obtain paraffin sections that were stained by Hematoxylin and Eosin and PAS stains for routine histological examination.

C) Isolated intestinal (ileum) preparation:
1- According to Ghosh (14): Proximal 3 cm of ileum from freshly killed rat (140-180 g) is dipped in salt solu-
tion at 4 to 6°C for two to three hours before use.

The duration of each drug contact used in this preparation was 30 seconds at intervals of five minutes. The next figure showed the assembly used for recording the contraction of isolated ileum. Perfusion fluid: Dejalon, Temperature: 30-31°C. Gas: 5% CO2 in O2, Lever: Isotonic frontal

Apparatus used to record isolated ileum contraction of rat
10.053±0.4 respectively; P<0.05).

Effect of prednisolone on indomethacin induced biochemical changes (group IIb) : As shown in table (2) & fig. (2), simultaneous prednisolone treatment produced a significant reduction in serum leptin (0.859±0.04, P<0.05) as compared to indomethacin only treated group (18.255±0.6, P<0.05). It also produced a significant decrease in serum nitrite (35.600±3.5, P<0.05) versus indomethacin only treated group (86.700±4.9). Moreover, prednisolone resulted in a significant decrease in serum MDA level (30.612±2.4, P<0.05) and tissue myeloperoxidase activity (10.814±0.2, P<0.05) versus indomethacin only treated group (77.649±1.1, 20.255±0.53, respectively)

Effect of BRI 37344 on indomethacin induced biochemical changes:

Table (2) & fig. (2) showed that BRI simultaneous treatment produced a significant reduction in serum leptin (0.884±0.08, P<0.05) versus indomethacin only treated group (18.255±0.6, P<0.05). It also produced a significant decrease in both serum nitrite and MDA levels (35.000±2.9, 28.209±1.5, respectively P<0.05), versus indomethacin only treated group (86.700±4.9, 77.649±1.1, respectively) Furthermore, BRI treatment induced a marked reduction in myeloperoxidase activity (10.698±0.3, P<0.05) versus group IIa (20.255±0.53)

Comparison between the effect of prednisolone and BRI on indomethacin induced biochemical changes:

Table (3) & fig. (3) showed that there was no significant difference between the effect of prednisolone and BRI on serum leptin, serum nitrite, MDA and myeloperoxidase activity as compared to indomethacin only treated group.

B) Histopathological findings:

In comparison to control normal group(figs. 4 ,5), indomethacin produced abnormal desquamation of the epithelium covering villi of jejunum with some necrotic debris. There were congested blood vessels and in-
ministration produced relaxation on sustained contraction (plateau) produced by acetylcholine (100 uM).

On the other hand, in ileitis, BRI (0.1 uM) failed to relax the sustained contraction produced by acetylcholine (100 uM).

Table (1): Effect of indomethacin (7.5 mg/kg S.C.) on serum leptin level (ng/ml), serum nitrite level (uM), serum malonaldehyde (MDA) level (nmol/ml) and tissue myeloperoxidase (MPO) (milli Unit (mU)/mg tissue). (means ± SE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Indomethacin treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum leptin</td>
<td>1.294±0.012</td>
<td>18.255±0.6 P&lt;0.05</td>
</tr>
<tr>
<td>Serum nitrite</td>
<td>33.500±2.6</td>
<td>86.700±4.9 P&lt;0.05</td>
</tr>
<tr>
<td>Serum MDA</td>
<td>24.383±1.1</td>
<td>77.649±1.1 P&lt;0.05</td>
</tr>
<tr>
<td>Tissue MPO</td>
<td>10.053±0.4</td>
<td>20.255±0.53 P&lt;0.05</td>
</tr>
</tbody>
</table>

*P = Significance of difference between indomethacin treated group versus control group (non treated group).*
Fig. (1): Effect of indomethacin (7.5mg/kg S.C.) on serum leptin level, serum nitrite level, serum MDA level, and tissue myeloperoxidase activity.

Fig. (2): Effect of prednisolone (0.5 mg/kg orally) and BRI (1 mg/kg orally) on serum leptin level, serum nitrite level, serum MDA level and tissue myeloperoxidase activity.
Fig. (5): A photomicrograph of a section in the jejunal mucosa of a control rat (group I) showing numerous PAS-positive goblet cells lining the crypts (arrows) and covering the villi (crossed arrows) (PAS x 250).

Fig. (6): A photomicrograph of a section in the jejunum after induction of ulcer (group 1la) showing villi which are abnormal in shape with complete desquamation of the covering epithelium (arrow heads). Some necrotic debris (d) appear in the lumen. The corium of the villi shows congested blood vessels (arrows) and excessive mononuclear cellular infiltrate (cross arrows). Note: parts of the crypts (C) are seen. (Hx & E x 100).
Fig. (9): A section in the jejunum of prednisolone treated rats showing normal appearance of villi with PAS +ve goblet cells over the villi (arrow) and lining the crypts (crossed arrows) (PAS x 400).

Fig. (10): A photomicrograph of a section in the jejunum of group (IIc), after induction of ulcer and treatment with BRI 37344 showing normal shape of villi (arrows) and crypts (crossed arrows). (Hx & E x100).
Effect of BRL on rat ileitis precontracted with acetyl choline

- Dejalon Solution.
- Frontal lever
- Time marker 1/8 m.
- O₂+CO₂ aeration.
Effect of BRL on sustained contraction produced by cholinomimetic drug on rat ileum

- Dejalon Solution.
- 31°C.
- Frontal lever
- Bath 20 ml
- O₂+CO₂ aeration.
- Time marker 1/8 m.

Cholinomimetic acetylcholine 100 nM normal

Cholinomimetic acetylcholine 100 nM ileitis

Cholinomimetic acetylcholine 100 nM ileitis

BRL 0.1 uM

BRL 0.1 uM

Wash

BRL >10 folds

Wash
In the present work, myeloperoxidase was assayed because it's a sensitive specific marker released from the inflammatory cells within the mucosa of the inflammatory bowel diseases. It is a useful way of quantifying the early inflammatory response and it is an index of granulocyte infiltration. It has been shown that rats treated with indomethacin showed a significant increase of myeloperoxidase activity and this finding is in agreement with the study made by Takeuchi et al., and Mchuge et al., who stated that indomethacin administration increases the extent of myeloperoxidase activity.

Inflammatory changes induced by indomethacin in this study as evidenced by biochemical parameters are confirmed by histopathological examination. This appeared in the form of mucosal inflammation, epithelial desquamation, congestion of the blood vessels and excess cellular infiltrations. (Fig. 6)

**Effect of prednisolone on indomethacin-induced inflammatory changes:**

In the present study, it was found that prednisolone administration prevented the inflammatory lesion induced by indomethacin. This is evidenced by significant reduction in serum leptin, serum nitrite and serum MDA and tissue activity of myeloperoxidase enzyme. These findings are in consistent with that of Williams and Hallett. They showed that prednisolone induces a general reduction in the inflammatory activity by stabilizing lysosomal membranes, decreases migration of inflammatory cells to the site of inflammation and is a free radical scavenger. Prednisolone also produces an inhibition of intestinal arachidonic acid metabolism resulted in inhibition of the inducible cyclooxygenase enzyme II and the inducible nitric oxide production. Furthermore administration of prednisolone resulted in a decrease in macrophage induced tumor necrosis factor alpha (TNF-α) that may accelerate the inflammatory process.

This is confirmed with histopathological examination (fig.8,9) that showed prednisolone treated rats normal appearance of villi and crypts. The villi have normal columnar absorbing cells with intact brush border.
In ileitis, a greater concentration of BRI is needed to relax acetylcholine induced contractions; nearly 10 fold higher than in control. This is confirmed by study of Zhao et al., (29) who investigated the inflammation-induced changes in adrenergic regulation of smooth muscles and stated that experimental ulcerative colitis in rats induces a down regulation of the inhibiting B3 adrenergic control of colonic smooth muscle function and this loss of adrenergic regulation may contribute to the diarrhea in this inflammatory bowel disease. In addition, Anthony (28) considered B3 adrenoceptor agonists-future antiinflammatory drugs and they are spasmyolytic and potent inhibitors of non-steroidal antiinflammatory drugs induced gastric and small intestinal ulcers and this is evidenced by their role in enhancement of mucosal blood flow.

Furthermore, in this study, it has been shown that BRI 37344 ability to relax pre-contracted smooth muscle is attenuated in ileitis and it has needed a nearly 10 fold increase in the concentration of BRI required to relax pre-contracted inflammed ileum. The maximal relaxations of BRI on pre-contracted smooth muscle are also decreased in ileitis and also, there is a loss of cyanopindolol effect that augment the amplitude of spontaneous contractions in ileitis. These data are supported by the results of Zhao et al., (29) who proved that B3 receptors are preserved in both acute and chronic colitis, but cyanopindolol ability to enhance spontaneous contractions is reduced, and BRI ability to relax carbachol induced contractions was blocked completely by cyanopindolol. While in inflamed colon, a greater concentration of BRI is needed for relaxation. Furthermore, the predominant adrenergic regulation of spontaneous contractions that is mediated by B3 adrenoreceptors; down regulates in indomethacin induced ileitis and this loss of regulation is similar to that in Crohn’s disease. (29)

CONCLUSION

In the present study, it has been concluded that, comparing the effect of BRL versus the effect of prednisolone on indomethacin induced enteritis in rats; BRI ameliorates the indomethacin induced lesion to a similar extent to that of prednisolone. However-


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