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-

secutive days in a dose of Histological examination, myeloperoxidase (MPO) enzyme, malonldialdehyde (MDA), nitric oxide (NO) and leptin levels were detected. Effect of BRI on isolated intestinal tissue was also examined.

Results : Indomethacin treatment produced a significant increase in MDA, NO leptin levels and MPO content prednisolone and BRI produced a beneficial effect on indomethacin-induced intestinal lesions

Conclusion : BRL 37344 is as effective as prednisolone in inflammatory bowel disease indicating some possible therapeutic application for gastrointestinal tract disorders.

INTRODUCTION

Inflammatory bowel diseases (IBD) are commonly characterized by flare up phases. Anorexia, increased resting energy expenditure and body weight loss are prominent features of these diseases [1]. Because the aetiology of inflammatory bowel diseases 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 [2].

Leptin, a hormone primarily secreted from adipocytes, plays a key role in controlling body weight-homeostasis and it is also implicated in immune response. Hyperleptinemia has been reported in acute inflammation especially during the early stage of intestinal inflammation in rats [3]. It has been suggested that leptin plays a role in the anorexia associated with inflammation [3].

Enteropathy is a recognized lesion of the alimentary tract characterized in its chronic form by strictures and a chronic enteritis. These lesions are the end result of a disease process of which local permeability changes are the earliest detectable abnormalities and involve alterations in intestinal wall blood flow [4].

NSAIDs administered chronically to humans and acutely to the experimental animals produces an enteropathy with striking clinical functional and pathological similarities to the

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 ,

considered as control group and received a pathogen free water.

Group II : Consists of 30 animals, subdivided into 3 equal subgroups (10 rats each) as follow.

Subgroup IIa : served as indomethacin treated group (enteritis group). They were treated with indomethacin for two doses separated by 24 hour in a dose of 7.5 mg/kg subcutaneously (10).

Subgroup IIb : Indomethacin treated rats, received prednisolone at the same time with the 1st dose of indomethacin and for 4 consecutive days in a dose of 0.5 mg/kg intragastrically by gavage (4).

Subgroup IIc : Indomethacin treated rats, received BRI, at the same time with 1st dose of indomethacin and for 4 consecutive days in a dose of 1 mg/kg intragastrically by gavage (9).

Animals were killed in the 5th day after the 1st dose of indomethacin, Fasting rats were sacrificed by cervical decapitation. Fasting blood was obtained for measurement of serum leptin, MDA, nitric oxide levels and also, a piece of small intestine was obtained 20 cm from ileocaecal valve for biochemical analysis of MPO enzyme content, isolated preparation and histopathological examination.

A) Biochemical analysis :

1- Serum leptin level was measured according to the method of Blum et al.(11) by enzyme immunometric assay kit for rat.
2- Serum nitrite level was measured according to the method of Green et al. (12), nitrate in the sample is reduced by cadmium column to nitrite which reacts with a Griess reagent to form a purple azo dye and its absorbance at 546 nm is detected by spectrophotometer (Jenway6405uv/vis).
3- Serum Malondialdehyde (MDA) level was measured according to the method of Draper and Hadley (13), (serum is precipitated by addition of trichloroacetic acid. Then thiobarbituric acid reacts with malondialdehyde Which is a product of lipid peroxidation to form thiobarbituric acid, a reactive product ,which is measured at 532 nm by spectrophotometer (Jenway 6405 uv/vis).
4- Myeloperoxidase (MPO) content in intestinal homogenate was measured according to the method of Banerjee and Peters, (4)(the sample was weighed, suspended in hexadenyltrimethylammonium ho-

mogenized and centrifuged. The supernatant was assayed for MPO spectrophotometrically (Jenway 6405 uv/vis).

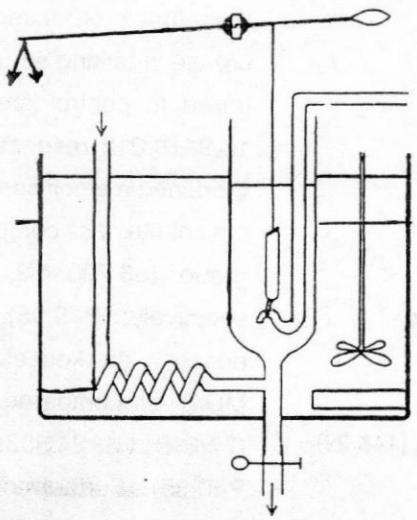
B) Histopathological examination :

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 (140-160 g) is dipped in salt solution at 4 to 6oC 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

2- Reagents for isolated preparation :

Dejalon solution contained (mg/ litre) 0.5 glucose, 9.0 Nacl, 0.42 Kcl, 0.03 $Cacl_2$ and 0.5 NaHCO3. Stock solutions 1 milli mole(mM) of acetylcholine, norepinephrine, dobutamine, salbutamole, BRI 37344 and propranolol were prepared, stored at 4°C and diluted in water. On the day of the experiment, Cyanopindolol (B3 antagonist) was dissolved in dimethyl sulfoxide (1mM), and Butoxamine hydrochloride (B2 antagonist) was dissolved in a pathogen free water .

3- Doses used for isolated preparation(microMole(uM)/ Bath :

- Acetylecholine 100 uM
- Norepinephrine 1 uM
- Dobutamine 1 uM
- Salbutamole 10 uM
- BRI 37344 0.1 uM
- Cyanopindolol 10 uM
- Butoxamine HCL 10 uM
- Atenolol 10 uM
- Propranolol 10 uM

Doses were according to [14& 29].

Statistical Analysis :

1- Analysis was done by SPSS program (statistical package for social science) version 10, 1999.
2- Multiple comparisons were performed by ANOVA statistical analysis between groups.
3- Post hoc test (Scheffe test) was done between 2 groups; P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean ± SE for ten animals in each group.

RESULTS

A) Biochemical findings :

Effect of indomethacin on biochemical parameters (group IIa) :

As shown in table (1) & fig. (1), indomethacin produced a significant increase in fasting serum leptin as compared to control group (18.255±0.6, 1.294±0.012 respectively; P<0.05). It produced a significant increase in serum nitrite as compared to control group (86.700±4.9, 33.500±2.6 respectively; P<0.05), and also produced a marked elevation in serum MDA as compared to control group (77.649±1.1, 24.383±1.1 respectively; P<0.05). Furthermore, there was a marked elevation in tissue myeloperoxidase enzyme activity as compared to control group (20.255±0.53,

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-

flammatory cellular infiltration (fig. 6,7)

Treatment with either prednisolone (figs. 8,9) or BRI (figs.10) effectively decreased the inflammatory ulcerative effect of indomethacin and normalized villi and crypts. Moreover, there was normal number and shape of goblet cells and intact Brush borders of enterocytes.

C) Isolated rat ileum preparation : Spontaneous contractions (curve 1) :

to determine the effect of B3 adrenoreceptor agonist on spontaneous ileum contractions isolated from normal rat. The effect of BRI (0.1 uM) was observed and indicated that B3 action was inhibitory on smooth muscle, and the inhibitory action was blocked by administration of B3 receptor blocker (Cyanopindolol 1 uM).

While, salbutamol (B2 adrenoreceptor agonist, 10 uM) administration produced an inhibitory action with less in amplitude than that of BRI on smooth muscle, and this action was blocked by butoxamine Hcl (1 uM).

On the other hand noradrenalin administration (1 uM) produced an inhibitory action on the ileum that blocked completely after administration of propranolol (1 uM).

Effect of BRI 37344 on rat ileum precontracted with acetylcholine (curve 2) :

BRI effect on smooth muscle function isolated from normal rat and or indomethacin treated rat :

Maximum responses to acetylcholine (100 nM) were the same in controls, and in ileitis.

In control ileum, BRI (0.1 uM) relaxed acetylcholine (100 uM) induced contractions and this effect was abolished completely by cyanopindolol (1 uM).

In ileitis, a greater concentration of BRI was needed to relax acetylcholine induced contractions (nearly 10 fold higher than in control; but it was not a complete relaxant effect).

Effect of BRI 37344 on sustained contractions produced by acetylcholine on rat ileum (curve 3) :

In normal ileum, BRI (0.1 uM) ad-

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), while larger dose BRI (1 uM) produced relaxation in 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).

Groups / Parameter	Control group	Indomethacin treated group
Serum leptin	1.294±0.012	18.255±0.6 P<0.05
Serum nitrite	33.500±2.6	86.700±4.9 P<0.05
Serum MDA	24.383±1.1	77.649±1.1 P<0.05
Tissue MPO	10.053±0.4	20.255±0.53 P<0.05

P= Significance of difference between indomethacin treated group versus control group (non treated group).

Table (2): Effect of prednisolone (0.5 mg/kg orally) , BRL (1 mg/kg orally) on serum leptin (ng/ml) level, serum nitrite (uM) level, serum malonaldehyde (MDA) level (nmol/ml) and tissue myeloperoxidase (MPO) (mU/mg tissue). (means ± SE).

Groups / Parameter	Control group (group I)	Indomethacin treated rats (group II)		
		Distilled water treated group (group IIa)	Prednisolone treated group (group IIb)	BRLtreated group (group IIc)
Serum leptin	1.294±0.012	18.255±0.6 P<0.05	0.859±0.04 P1<0.05	0.884±0.08 P2<0.05
Serum nitrite	33.500±2.6	86.700±4.9 P<0.05	35.600±3.5 P1<0.05	35.000±2.9 P2<0.05
Serum MDA	24.383±1.1	77.649±1.1 P<0.05	30.612±2.4 P1<0.05	28.209±1.5 P2<0.05
Tissue MPO	10.053±0.4	20.255±0.53 P<0.05	10.894±0.2 P1<0.05	10.698±0.3 P2<0.05

P= Significance of difference between indomethacin only treated rats versus non treated rats.

P1= Significance of difference between prednisolone treated rats versus indomethacin treated rats.

P2= Significance of difference between BRI treated rats versus indomethacin treated rats.

Table (3): Comparison between the effect of prednisolone (0.5 mg/kg orally) and BRI (1 mg/kg orally) on biochemical changes induced by indomethacin (7.5mg/kg S.C.) (means ± SE).

Groups / Parameter	Indomethacin treated group (group II)		
	Distilled water (D.W.) treated group (group IIa)	Prednisolone treated group (group IIb)	BRL treated group (groupIIc)
Serum leptin	18.255±0.6	0.859±0.04 P1<0.05	0.884±0.08 P2<0.05 P3>0.05
Serum nitrite	86.700±4.9	35.600±3.5 P1<0.05	35.000±2.9 P2<0.05 P3>0.05
Serum MDA	77.649±1.1	30.612±2.4 P1<0.05	28.209±1.5 P2<0.05 P3>0.05
Tissue MPO	20.255±0.53	10.894±0.2 P1<0.05	10.698±0.3 P2<0.05 P3>0.05

P1= Significance of difference of prednisolone treated rats versus non treated (indomethacin) treated rats.
P2= Significance of difference of BRI treated rats versus non treated (indomethacin) rats.
P3= Significance of difference of prednisolone treated rats versus BRI treated rats.

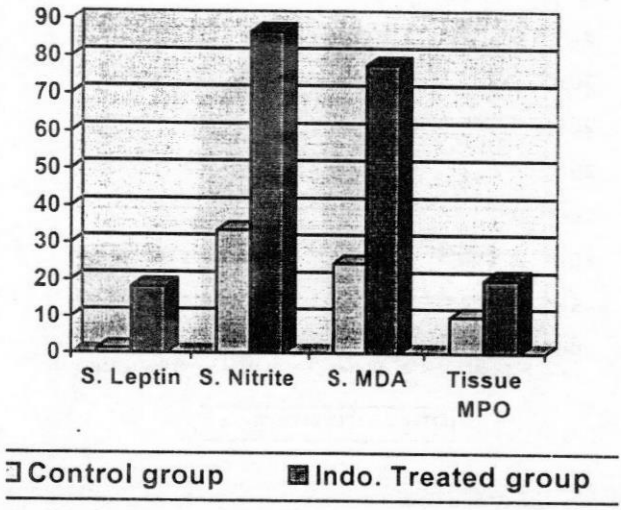


Fig. (1): Effect of indomethacin (7.5mg/kg S.C.) on serum leptin level. serum nitrite level, serum MDA level, and tissue myeloperoxidase activity

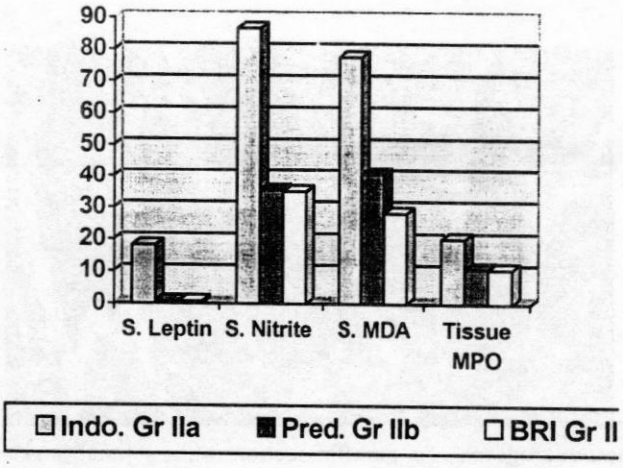


Fig. (2): Effect of prednisolone (0.5 mg/kg orally) and BRI (1 mg/kg orally) on serum leptin level, serum nitrite level, serum MDA level and tissue myeloperoxidase activity.

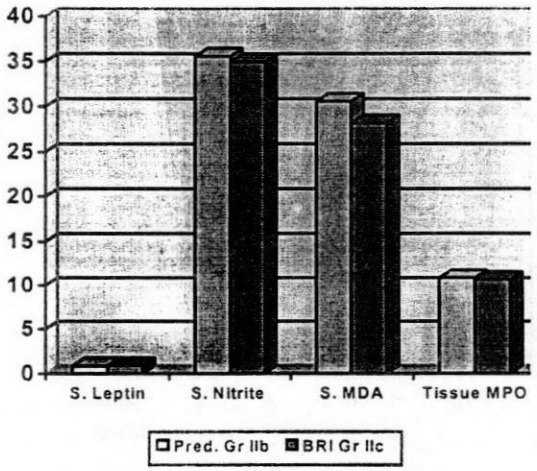


Fig. (3): Comparison between the effect of prednisolone and BRI on biochemical changes induced by indomethacin

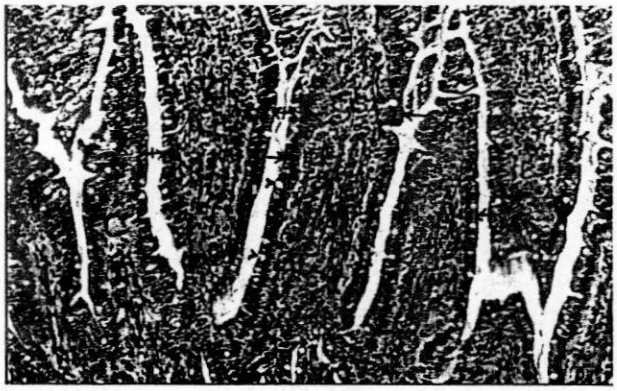


Fig. (4): A photomicrograph of a paraffin section in the jejunal musoca of a control rat (group I) demonstrating the presence of long villi (arrows). There are numerous goblet cells with unstained cytoplasm (arrow heads), enterocytes (crossed arrows) and connective tissue (C.T.) corium (C). (Hx & E x 100).

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 IIa) 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. (7): A photomicrograph of the rat jejunal mucosa after induction of ulcer (group IIa) demonstrating jejunal villi with nearly complete loss of goblet cells (arrows). Only very few goblet cells (crossed arrows) are seen lining the bases of the villi. (PAS x 250).

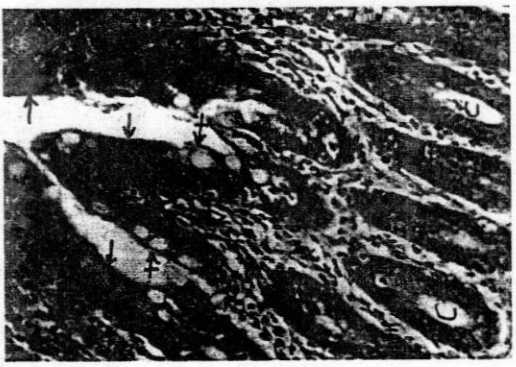


Fig. (8): A photomicrograph of a paraffin section in the jejunum of prednisolone treated rats showing normal appearance of villi (V) and crypts (C). The villi have normal columnar absorbing cells with intact brush border (arrows) and normal goblet cells (crossed arrows).

(Hx & E x 400).

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).

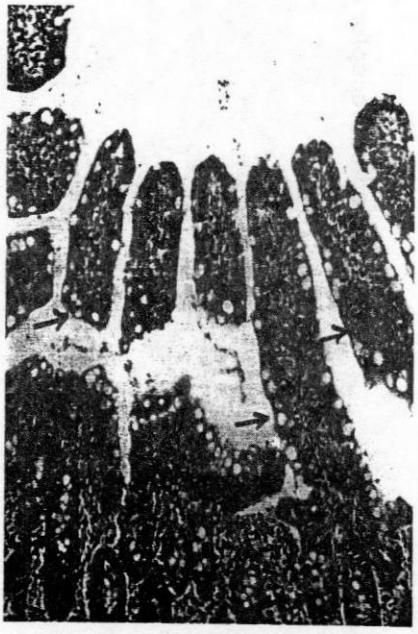
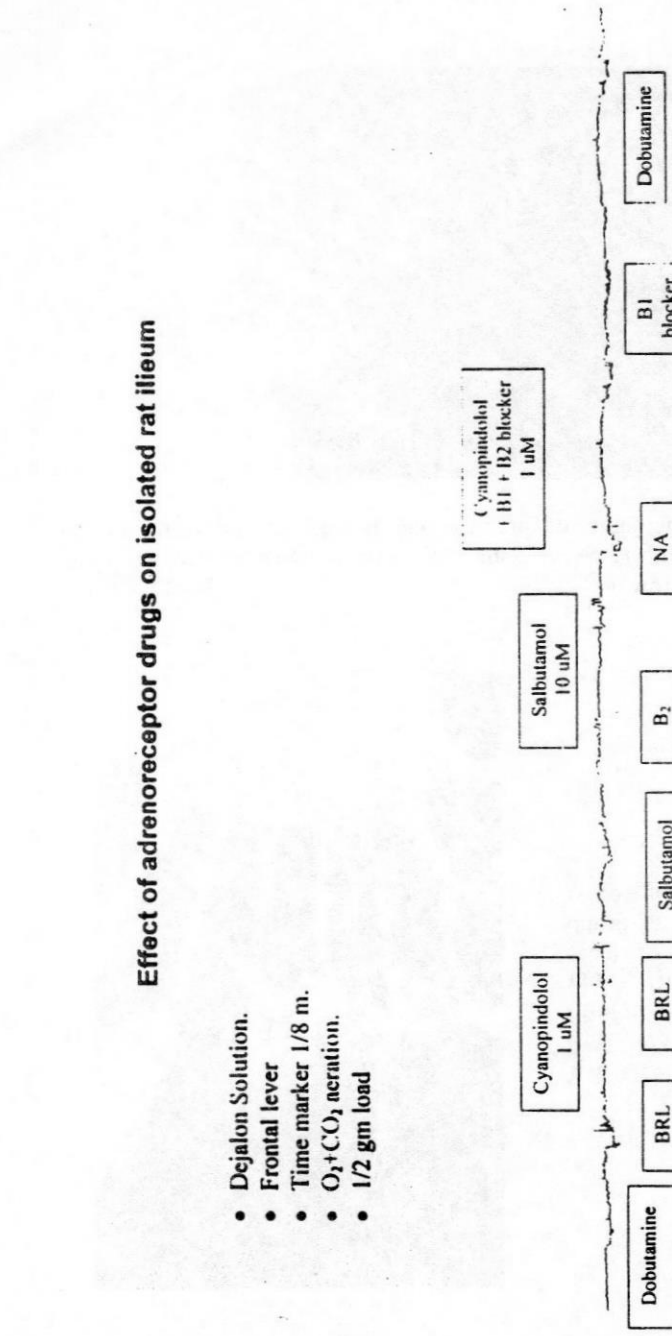
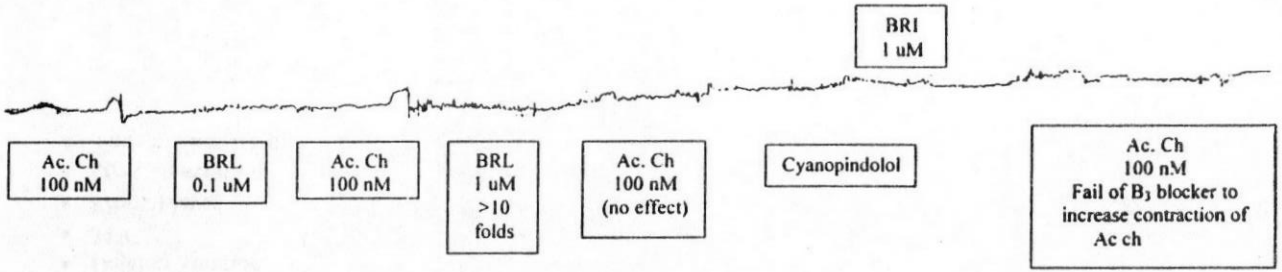


Fig. (10): A photomicrograph of a section in the jejunm of group (IIc), after induction of ulcer and treatment with BRI 37344 showing normal shape of villi (arrows) and crypts (crossed arrows). (Hx & E x100).


Effect of adrenoreceptor drugs on isolated rat ilieum
Dejalon Solution.
Frontal lever
Time marker 1/8 m.
O_2+CO_2 aeration.
1/2 gm load
Dobutamine
BRL 0.1 uM
Cyanopindolol 1 uM
BRL 0.1 uM
Salbutamol 10 uM
Salbutamol 10 uM
B_2 Blocker
NA 1 uM
Cyanopindolol + B1 + B2 blocker 1 uM
B1 blocker
Dobutamine

Effect of BRL on rat ileitis precontracted with acetyl choline

- Dejalon Solution.
- Frontal lever
- Time marker 1/8 m.
- O_2+CO_2 aeration.



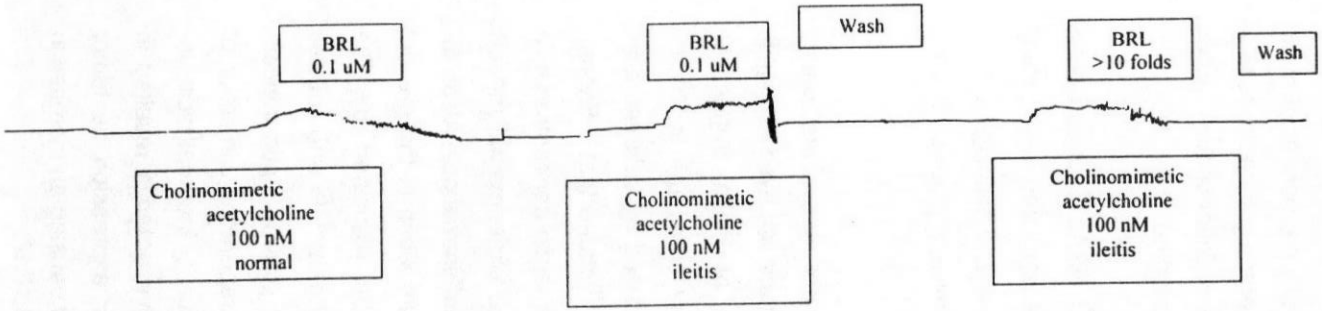
Effect of BRL on isolated rat ilieum precontracted with acetylcholine in presence of cyanopindolol

(Normal ilieum)

- Dejalon Solution.
- 31^0C.
- Frontal lever
- O_2+CO_2 aeration.
- Time marker 1/8 m.
-

Effect of BRL on sustained contraction produced by cholinomimetic drug on rat ilieum

- Dejalon Solution.
- 31^0C.
- Frontal lever
- Bath 20 ml
- O_2+CO_2 aeration.
- Time marker 1/8 m.



DISCUSSION

In this study , the beneficial effect of BRL and prednisolone in experimental model of inflammatory bowel disease were studied.

A model of NSAID induced enteropathy in rats was used in this study as it has clinical and histological similarities to inflammatory bowel disease (15).

In the present study, rats treated with indomethacin showed a significant increase of leptin, suggesting that leptin may contribute in the inflammation induced by indomethacin. Barbier et al. (3)stated that Expression of plasma leptin concentration in the established experimental models of intestinal inflammation. Also, increase in serum leptin in the present study could be explained by The study of Sarraf et al. (16) who showed an increase in plasma leptin concentration after intraperitoneal injection of cytokines. Thus , indomethacin induced ulcerative enteritis resulted in cytokines over expression as tumor necrosis factor causing an increase in serum leptin (3,16,17).

In the present study , indomethacin produced an increase in serum nitrite. Serum nitrite reflects the inducible nitric oxide synthase activity. This finding is in consists with the study of Kane and Vincenti (18) that showed similar increase in serum nitric oxide activity in NSAIDs treated rats. Furthermore, Sitaraman et al. (19) proved that leptin in lumen of small intestine and colon may act as a proinflammatory cytokine which activates the nuclear transcription factor (NF-KB) implicated in the pathogenesis of inflammatory bowel disease and regulate the expression of inducible nitric oxide synthase (20) and so excessive production of nitric oxide in small intestine, colon (21) which is reflected in the serum level (22).

In addition, the present study showed an increase in serum MDA level that correlate with the harmful effects of the reactive oxygen free radicals involved in indomethacin induced ulcerative enteritis and resulted in injury of the membrane lipids of intestinal tissue and MDA release in the circulation (22).

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 [16] and it is an index of granulocyte infiltration [3]. 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., [23] and Mchuge et al., [24] 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 [8]. 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 [25].

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

and normal goblet cells

Effect of BRI 37344 on indomethacin-induced inflammatory changes :

In the present study, it was found that BRI 37344 ameliorates the indomethacin induced enteritis which is evidenced by a significant reduction in hyperleptinemia . This is confirmed with the results of (3) who found that hyperleptinemia is involved in acute inflammation of indomethacin induced enteritis in rats., Sitaraman et al. (19) mentioned that lumen leptin of the intestine is a source of proinflammatory cytokines involved in inflammatory bowel disease and disseminates the inflammatory stimuli to the non inflamed areas. He also suggested that leptin in the intestinal lumen activated NF-KB that implicated in pathogenesis of inflammatory bowel disease and modulates the inducible nitric oxide in rat small intestine (26). A study done by Barbier et al. (27) supported our results but in another model of inflammatory bowel disease in rats, which is trinitrobenzene sulfonic acid (TNBS) induced colitis in rats, they showed that beta-3 agonist (BRI 37344) produced an inhibition of leptin secretion in experimental colitis that markedly reduced colonic inflammation, histologically and or biochemically changes. So, these results provide direct evidence for an important deleterious role of leptin in the pathogenesis of experimental intestinal inflammation.

Furthermore, BRI 37344 produced areduction in leptin of the intestine and a significant reduction in serum nitrite , serum MDA and tissue activity of myeloperoxidase enzyme (19.26) .

Effect of BRI 37344 on Isolated rat ileum preparation :

In the present study , ileum isolated from normal rat, showed the response to the relaxant effects of B1 agonist, B2 agonist, B3 agonist (BRI and noradrenaline). While noradrenaline relaxant effect is abolished by propranolol administration, indicating that adrenergic inhibition of the ileum is mediated by B receptors . This is in agree with studies of Barbier etal., (27), Anthony, (28) and Zaho etal., (29). Also, the inhibitory effect of BRI through B3 receptors is predominant than that of B1 agonist and/or of B2 receptors in ileum Barbier etal., (27) and Zaho etal., (29)

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 adrenoreceptor agonists-future antiinflammatory drugs and they are spasmolytic and potent inhibitors of nonsteroidal 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. Howev-

er, BRL has a less side effects .Thus, BRI may be provided as a new treatment possibility for reversing changes of indomethacin induced enteritis which is a valid model of IBD.

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دراسة التأثير الوقائى المحتمل لدواء ب ر ل ٣٧٣٤٤ (منبه لمستقبلات بيتا-٣) فى نموذج لمرضى الإلتهاب المعوى الحاد فى فئران التجارب البيضاء

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- أجريت هذه الدراسة التجريبية لبحث التأثير الوقائى لدواء ب ر ل المنبه لمستقبلات بيتا-٣ وهى دراسة عياريه حيث تمت مقارنة الدواء بدواء عيارى وهو دواء البريدنيزولون فى الفئران البيضاء المصابة بالإلتهاب المعوى الحاد.

- إستخدم فى إجراء هذا البحث ٤٠ فأرا أبيضا وقسمت الى ٤ مجموعات متساوية كل منها يتكون من ١٠ فئران كالتالى:
  - المجموعة الأولى: مجموعة ضابطة لا تأخذ دواء وتعطى ماء مقطر عادى.
  - المجموعة الثانية: مجموعة ضابطة تعطى دواء الإندوميثاسين بجرعة ٧,٥ مجم/كجم حقنة تحت الجلد يوميا ولمدة يومين غير متتاليين كنموذج لمرض الإلتهاب المعوى الحاد.
  - المجموعة الثالثة: مجموعة مصابة بالإلتهاب المعوى الحاد وتعطى دواء البريدنيزولون (½ مجم/كجم) مرة واحدة بالفم مع أول حقنة إندوميثاسين ولمدة أربعة أيام.
  - المجموعة الرابعة: مجموعة مصابة بالإلتهاب المعوى الحاد وتعطى دواء ب ر ل (١ مجم/كجم) مرة واحدة بالفم مع أول حقنة إندوميثاسين ولمدة أربعة أيام.

- بعد إنتهاء الأربعة أيام تم أخذ عينات دم صائم من الفئران لقياس مستويات هرمون اللبتن والمالونالدهيد وأكسيد النيتريك. وكذلك تم أخذ عينة من النسيج المعوى من الفئران المصابة والضابطة:
  ١- لقياس مستوى إنزيم الميلوبيروأكسيديز.
  ٢- للفحص الهستوباثولوجى.
  ٣- لإجراء دراسة معزولة.

ويمكن تلخيص نتائج هذا البحث كما يلى:

- إعطاء دوائى البريدنيزولون و ب ر ل فى الدراسة الحية فى الفئران البيضاء المصابة بالإلتهاب المعوى الحاد نتج عنه نقص ذو دلالة إحصائية فى مستويات هرمون اللبتن والمالونالدهيد وأكسيد النيتريك فى الدم بالإضافة الى الدراسة الهستوباثولوجية المتفقة مع النتائج الكيميائية. كذلك وجد أن ب رل له تأثير منفصل فى الدراسة المعزولة التى أجريت على الأمعاء مما يؤكد وجود مستقبلات بيتا-٣ بصورة سيادية فى الأمعاء الدقيقة. وهذا يؤدى إلى أن مستقبلات بيتا-٣ لها دور فى مرضى الإلتهاب المعوى الحاد.
- وعلى ضوء هذا البحث يمكن أن نؤكد أن دواء ب ر ل المنبه لمستقبلات بيتا-٣ المنتشرة فى القناة الهضمية له تأثير وقائى علاجى مساوى لدواء البريدنيزولون مما يثبت أهمية هذا الدواء الجديد فى حالات الإلتهاب المعوى الحاد.