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Indomethacin – Induced Enteropathy and Its Prevention with the Probiotic Bioflora in Rats

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1. Introduction

It is already proved that chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs) produce multiple small intestine erosions (SI) with a higher prevalence in the terminal ileum (1). This new condition is called NSAIDs induced enteropathy. In long term NSAIDs administration studies, almost 60 to 70% of patients were diagnosed through endoscopic capsules as bearing an asymptomatic enteropathy (2); characterized by increased intestinal permeability and mild mucosa inflammation, with hypoalbuminemia and deficient iron anemia (3). It was hypothesized that NSAIDs could act as liposoluble acids interacting with superficial membrane phospholipids, inducing a direct damage on the enterocyte mitochondria during the absorption. The mitochondrial damage could lead to an intracellular energy depletion, calcium efflux and generation of free radicals. The intercellular integrity is disrupted increasing the intestinal permeability, thus making the enterocytes more vulnerable in the lumen content, such as bacteria, bile, enzymes and neutrophil activation (5).

In this hypothesis no prostaglandins are effective, where the NSAIDs COX-1/ COX-2 inhibitors produce gastrointestinal necrosis (6) besides, we were able to prove that COX-3 inhibition with paracetamol simultaneously with COX-1, produce multiple erosions in the small intestine (7), and that paracetamol aggravated the intestinal erosions produced by diclofenac (8). Anyway, the selective COX-1, COX-2 or COX-3 inhibition does not produce gastrointestinal lesions (9).

bioflora is a well known probiotic containing 4 bacteria, i.e., *Lactobacillus casei*, *Lactobacillus plantarum*, *Streptococcus faecalis* and *Bifidobacterium brevis*, with anti-inflammatory effect given either orally or sc, with live or dead bacteria (10, 11); that in stressed rats hindered the bacterial overgrowth, blocking neutrophils without intestinal bacterial translocation and in

other organs, and increase of t lymphocytes (cd4+) (12) the aim of the present study was to study prevention yielded by bioflora in indo induced enteropathy, its probable mechanism induced by the bacterial overgrowth, the neutrophiles, the bacterial translocation and de cd4+ intestinal immunodeficiency.

2. Material and methods

Randomized female Sprague-Dawley rats groups (n=10 each one), 200g, 24h fast, water ad libitum, avoiding coprophagy were submitted to the following experiments: I. 30 mg/kg Indo, SC each 12h; 2 days (control). II. 1 ml Bio (1.3×10^7 live bacteria), by orogastric gavage in bolus each 12h for 2 days and Indo. The rats were sacrificed by ether overdose, performing laparotomy, total gastrectomy and enterectomy, stomach aperture and small intestine to tabulate the macroscopic necrotic percentage by computerized planimetry. The number of intestinal erosions (mm^2) was quantified, obtaining gastric and intestinal mucosa samples for histochemical examination (myeloperoxidase (MPO)). Bacteriological cultures were performed on mesenteric lymph nodes. Four cm terminal ileum was removed to quantified CD4+ T lymphocytes utilizing immunohistochemical techniques; anti-rat human antibody (Dakko, USA) evaluating each sample through Madsen scale. (13)

Statistics: Student's *t* test and ANOVA; for the microbiological evaluation of mesenteric lymph nodes exact Fisher's test, and Man-Whitney's test for intestinal cultures; $p < 0.05$ significance was accepted. Drugs: Indomethacin (Sigma Chemical Co. St. Louis, Missouri) and Bioflora probiotic (Laboratorios Sidus).

3. Results

Percentage of macroscopic gastric lesional area is presented in table 1, demonstrating that the Bio-Indo Group provided a marked gastric mucosa protection ($p < 0.001$), and MPO showed also a decrease of neutrophile infiltrate ($p < 0.02$).

In table 2, are shown the erosive intestinal area were Bioflora avoid the occurrence of Indo induced erosions ($p < 0.01$) and MPO reverted also the neutrophile infiltrate.

In table 3 can be observed the significant decrease of the intestinal bacterial overgrowth produced by Bio ($p < 0.01$), as well as the bacterial translocation to the intestinal mesenteric lymph nodes ($p < 0.02$) and the immunohistochemistry of the ileum mucosa. Bio restored the immunity showing a marked increase of T lymphocytes (CD4+). (Figure 1).

4. Discussion

Our results confirmed that the NSAIDs such as Indo produced marked decrease of small intestine immunity due T lymphocytes (CD 4+) effect, that might lead to a secondary bacterial overgrowth, intestinal bacterial translocation with altered intestinal permeability and finally occurrence of intestinal erosions. This could lead to a new

hypothesis since the increase of T (CD4+) that impede the bacterial overgrowth and the neutrophile infiltration might protect the defensive barrier avoiding the onset of NSAID enteropathy.

Reuter (14), demonstrated the importance of the enteropathic circulation of NSAIDs, where sulindac, without effect, does not produce a damage to the small intestine; there could be also altered absorption of biliary salts by NSAIDs, and which is most important, loss of integrity of COX-1 and COX-2 (15).

The cyclooxygenase inhibition could affect the blood flow of intestinal villi, since it was observed microvascular injury in the jejunal villi as a previous event to the erosion occurrence (16). The eNOS could be administered associated with NSAIDs, since it provides gastrointestinal protection, but not INOS that aggravates ulceration. (17, 18)

Misoprostol in high doses showed a mild increase of the intestinal permeability to Indo (19) although other works do not show such effect (20). Metronidazol that attenuate the intestinal inflammation and hemorrhage was also studied, although it did not modified the intestinal permeability (24). Sulphasalazine was also evaluated showing a slight improvement of the intestinal permeability (22).

There is important to differentiate the NSAIDs induced enteropathy from others such as the one produced in the espondiloarthrosis, especially if NSAIDs are administered, in Crohn's disease (23). Patients with NSAIDs enteropathy must suppress as a first option NSAIDs, since the disease could persist up to a year after therapy discontinuation (24) and any kind of NSAIDs is forbidden, COX-2 included (25) except in patients with chronic joint pain and gastroduodenal ulcer risk that could be treated with naproxen, without cardiovascular risks and with a proton pump blocker such as esomeprazol (26). Briefly, NSAIDs enteropathy presents in its physiopathology a similarity with Chron's disease (27), although attenuated, where the theory of the inflammatory intestinal disease is actually an immunodeficiency with bacteria proliferation on the intestinal mucosa crypts and penetration of the intestinal defensive barrier. This observation is supported by the fact that a-defensines production is not correlated with the disease severity(28); finally in the NSAIDs mucosa enteropathy a good defense of the intestinal mucosa to avoid bacterial penetration is to treat immunodeficiency, through probiotics prescription Live bacteria could theoretically prevent the damage induced by NSAIDs altering the microbial alteration induced by NSAIDs in the intestinal microbial ecology (30) and by immune function modulation (31). Anyway there were different probiotics that exacerbated the intestinal ulceration, confirmed with the same model of induced Indomethacin enteropathy (32). The Bioflora probiotic provided a marked protection of the gastrointestinal mucosa in the same indomethacin model. The efficacy of the drugs under study, probiotics included, depends also on the inhibition of the pro-inflammatory cytokines activated by the TLR4/D88 mediators, that are important in the intestinal pathology of Crohn's disease and NSAIDs enteropathy development (33, 34).

5. Conclusion

We postulated that NSAID induced lesion in stomach and small intestine, by two mechanism different, in stomach the NSAID inhibited both COX1 and COX2 and provokes depletion of Prostaglandins and gastric necrosis; in contrast, the NSAID in small intestine produced marked decrease of the immunity due T Lymphocytes (CD4T) effect, that lead to a secondary bacterial permeability with the neutrophile infiltration in mucosa intestinal and formation of mesenteric lymph nodes; besides, the inhibition COX3 induce multiple erosions in small intestine. The cyclooxygenase inhibition affect the blood flow of intestinal villi as a previous event to the erosions occurrence. The Probiotics its increased T lymphocytes (CD4T), inhibited the bacterial overgrowth, the neutrophiles, the bacterial translocation and erosions in all the small intestine.

	% gastric necrotic area	MPO mg / protein
INDO	65 ±7 P	410 ±31 P
BIO-INDO	7.5 ±1.3 < 0.001	30 ±7 <0.01

Table 1. Table 1. Gastric necrotic area percent and MPO in the INDO Group (Control) and in the Bio-Indo treated one.

	Erosions in SI mm²	MPO mg / protein
INDO	380 ±31 P	435 ± 45 P
BIO-INDO	41 ± 6 <0.001	55 ± 11 <0.001

Table 2. Table 2. Number of erosions on the small intestine and MPO, with marked remission in the BIO-INDO group.

	SI Culture CFU		Mesenteric lymph node cultures		CD4+ Ileum	
INDO	7,5 ±3,5 x10 ¹⁰	P	9 (+) 1 (-)	P	0,5 ±0.1	P
BIO-INDO	2,3 ±0,8 x 10 ⁵	<0.01	8 (-) 2 (-)	< 0.01	4 ± 1	< 0.01

Table 3. Prevention of intestinal bacterial overgrowth, bacterial translocation and increased immunity through T lymphocytes T (CD 4+) by Indo and Bio-Indo.

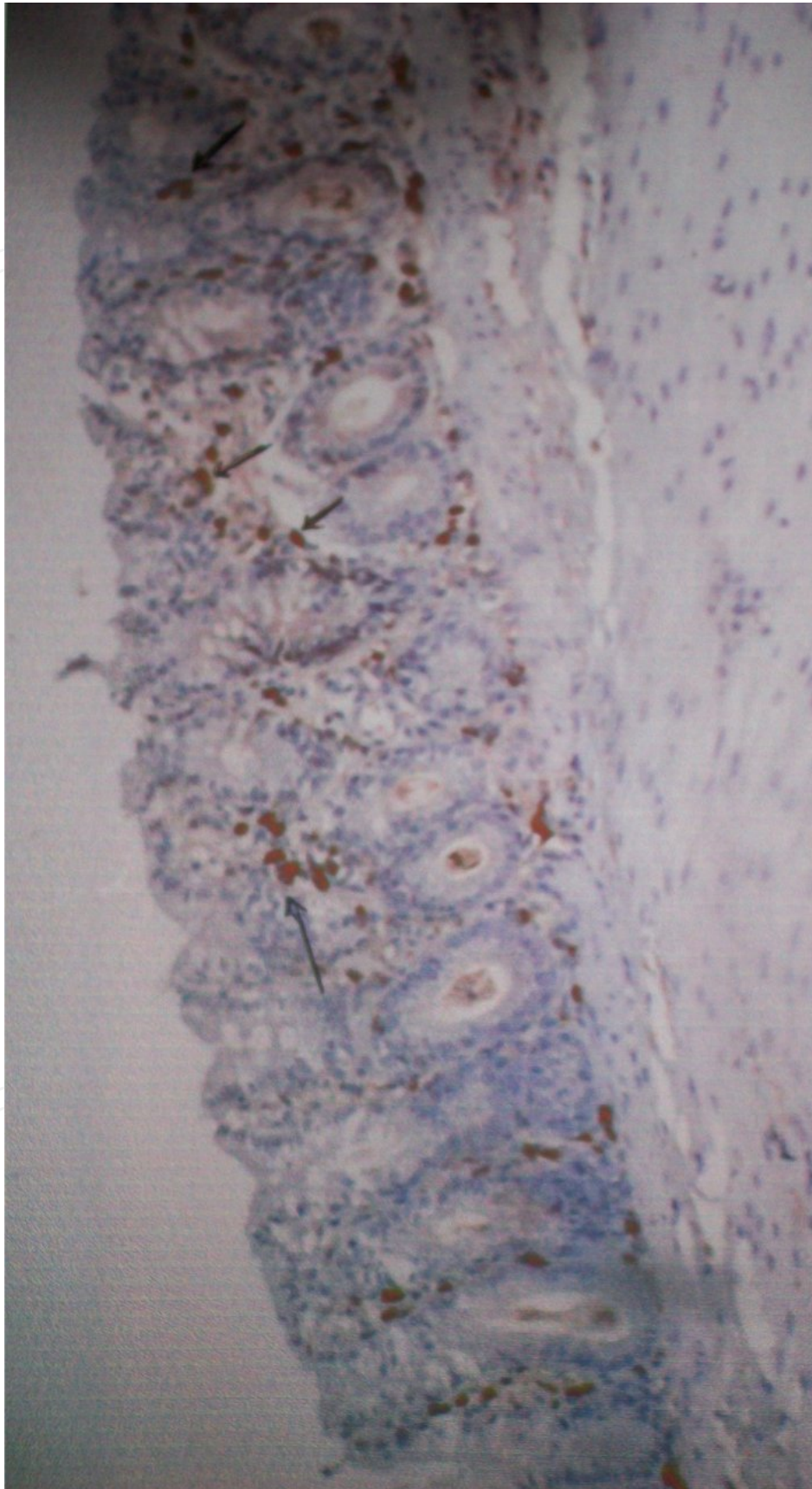


Figure 1. Bioflora Restored the immunity showing a marked increase of T lymphocytes (CD4t)

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