Original article

The effect of non-absorbable antibiotics on intestinal bacterial translocation and endotoxemia in experimental obstructive jaundice

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SUMMARY

Objectives: This is an experimental study in rats, examining bacterial translocation and endotoxemia in obstructive jaundice, as well as the effect of per os administration of non-absorbable antibiotics (neomycin & cefazoline).

Method: Male Wistar rats were used, divided in four groups: I=control, II=sham, III=common bile duct ligation and IV=common bile duct ligation+antibiotics. Aerobic cultures of mesenteric lymph nodes (MLN) and liver were performed and endotoxin was measured in the portal vein and aorta. The aerobic bacterial concentration in the cecum was determined and the terminal ileum was evaluated histologically.

Results: The study showed that obstructive jaundice resulted in increased bacterial translocation to MLNs and liver, increased systemic and portal endotoxemia and increased concentration of aerobic bacteria in the cecum. Treatment with neomycin and cefazoline reduced significantly endotoxemia, reduced the number of aerobic bacterial in the cecum as well as the bacterial translocation to the liver. There was no effect on bacterial translocation to MLNs. Histologic changes in intestinal morphology observed in jaundiced animals were not influenced by the administration of antibiotics.

Conclusion: Our results indicate that administration of non-absorbable antibiotics per os in jaundiced rats reduces bacterial translocation to the liver as well as portal and systemic endotoxemia and decreases the aerobic cecal flora, but has not effect on bacterial translocation to MLNs. These findings may be of clinical significance in preventing septic complications in patients with obstructive jaundice.

Key words: Non-absorbable antibiotics, Obstructive jaundice, Bacterial translocation, Endotoxemia, Intestine, Cecal flora

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INTRODUCTION

The intestinal mucosa is no more regarded only as the site for nutrient absorption but also exerts metabolic and immunological function acting, under normal circumstances, as an effective barrier, preventing enteric bacteria and endotoxins from invading extraintestinal tissues.[1] Clinical and experimental studies have shown that the ability of this barrier to prevent the migration of intraluminal pathogens depends on the anatomical integrity of the mucosa, the host immunological mechanisms and the presence of normal intestinal flora.[2] In certain pathological conditions, this anatomical and/or functional barrier may be destroved resulting in the phenomenon of "enteric bacterial translocation" (EBT), allowing the migration of enteric bacteria and their products, such as endotoxins, to remote organs and tissues.[3] Conditions related to increased bacterial and endotoxin translocation include shock, sepsis, trauma, burns, intestinal radiation, endotoxemia, antibiotic overuse, malnutrition and immunosuppression.[4-8] There is an increasing interest in the literature on the role of the gastrointestinal tract as a reservoir of pathogens that may translocate to the circulation, initiating the septic process and even-

tually leading to multiple organ failure.[9]

Surgery, in the presence of obstructive jaundice, is associated with increased morbidity and mortality, due mainly to septic and renal complications.[10] Endotoxemia is thought to be the major cause of these complications.[13] Recent data have shown that experimental obstructive jaundice is also followed by translocation of enteric bacteria.[12] The lack of bile salts and their antiendotoxin effect from the intestinal lumen may result in a significant increase in endotoxin concentration in the gastrointestinal tract and consequently in absorption into the portal circulation.[13,14] Impairment phagocytic function of Kupffer cells in obstructive jaundice may result in defective clearance leading to "escape" of the portal endotoxin into the systemic circulation.[15,16] Systemic endotoxemia, by increasing intestinal permeability and altering host defence, may also be considered as a causative mechanism for bacterial translocation in obstructive jaundice.[17] The lack of intraluminal secretory IgA, normally present in exceptionally large amounts in the bile of rodents,18 may also impair the local immunological resistance of the gut and contribute to the phenomenon of EBT.

In the present study, we examined endotoxemia and bacterial transocation in a model of experimental obstructive jaundice in rats, as well as the effect of non-absorbable antibiotics on the phenomenon. Changes in colonic microflora and disruption of the anatomical integrity of the mucosal barrier were also studied.

MATERIALS AND METHODS

Male albino Wistar rats (n=87) weighing 250-320g were used. The animals were housed in stainless-steel cages (three rats per cage), under controlled temperature (23˚C) and humidity conditions and 12 hour dark/light cycles. They were maintained on standard laboratory rat chow and tap water ad libitum throughout the experiment, except for an overnight fast on the evening before surgery.

The animals were divided randomly into four groups according to the treatment they received: Group I: controls (n=21, non-operated), Group II: sham (n=22, laparotomy and manipulation of hepatoduodenal ligament), Group III: (n=22), ligation and division of the common bile duct, Group IV: (n=22), ligation and division of the common bile duct followed by antibiotics administration.

Surgical Technique and Animal Treatment

The animals of group II, III and IV underwent laparotomy on day 0. Under light ether anesthesia and by using sterile techniques, a midline encision was performed and the porta hepatis was isolated. In-group II animals the gastroduodenal ligament was only manipulated without ligating the common bile duct. In-groups III and IV, the common bile duct was doubly ligated with a 5-0 silk suture and then was transected between the ligatures. The abdominal incision were closed in two layers with chromic 5-0 catgut and 5-0 silk. The animals of group IV received 20-mg/day neomycin sulphate (Upjohn) and 10-mg/day cefazoline (Fujisawa Pharmaceutical Co., Ltd). The antibiotics, diluted in 2ml normal saline, were administered twice daily via a nasogastric tube for 10 days. The animals from groups II, III, and I were also gavaged twice daily for the 10 subsequent days with equal volumes of normal saline. On the 10th day, the rats of group I were operated and those of group II, III, and IV were reoperated, again under general anaesthesia and sterile conditions. Blood and tissue samples were obtained according to the experimental protocol after which the rats were sacrificed by exsanguination.

Cultures and Endotoxin Measurement

Bilirubin/endotoxin measurements. By transecting the tail, 0.5ml of blood was collected of all animals for the determination of total bilirubin. Then a laparotomy was performed, the portal vein and the abdominal aorta were punctured and 1 and 2ml of blood, respectively, were obtained for the determination of endotoxin concentrations. Endotoxin concentration was measured by using the Limulus Amebocyte Lysate test, proposed in 1987 by the USA Food and Drug Administration for the determination of endotoxin in biological materials and medical prostheses.[19]

Mesenteric lymph nodes/liver cultures. The small bowel mesentery, including the MLNs, was sharply excised in all animals. It was homogenized, placed into a sterile tube containing thioglycolate broth and incubated at 37˚C. Any aerobic growth was Gram stained and further cultured on blood and MacConkey's agar for 24h at 37˚C. Different organisms were identified by using standard microbiological techniques. Cultures of liver samples were also performed applying the same methods.

Cecil bacterial population. Collection of cecal content for the determination of aerobic bacterial concentration was performed, by the following technique. The ascending colon was ligated and a 21-gauge needle mounted on a 5-ml syringe was introduced into the cecum, through the ileocecal valve, after puncturing the terminal ileum. Then, 2ml of sterile saline were infused

in the cecum, the needle was withdrawn and the terminal ileum was ligated. The cecal contents were manually mollified for 2 min and after a good mixture had been achieved, 1ml was removed of which 0.5ml was inoculated into 20ml of thioglycolate broth (dilution 1/41). Serial tenfold dilutions were performed and 0.001ml of each were inoculated into 5% blood and MacConkey's agar plates for recovery of aerobic bacteria. Coliform units per millilitre (cfu/ml) of the cecal samples were counted for each species. Micro-organisms were identified by Gram stain and biochemical tests.

Bile cultures. In the animals of group III and IV, at relaparo-tomy, the stump of the ligated common bile duct was punctured and 1ml of bile was obtained for aerobic cultures, by using routine techniques of our laboratory.

Histological Analysis

Samples from the terminal ileum and liver were removed for histological examination. The specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4μm and stained with hematoxylin-eosin. Under a light microscope, the following morphologic parameters were examined: number of villi per cm (V/cm), villus height (Vh) and number of mitoses per crypt (M/c).

Statistical Methodology

In order to assess the statistical importance of the observed differences of nominal factors among groups, we used the x2 test or Fisher's exact probability test where the expected frequency of the dichotomous variables was less than five. since the distribution of the aforementioned variables was not a normal one, we adopted the Kruskal-Wallis one-way Anova by ranks. In addition to Anova, we performed pairwise comparisons between groups, with the Mann-Whitney test. Data are expressed as means +SD, and statistical significance was accepted at the 5% significance level.

RESULTS

One animal of group II died during the operation due to ether overdose and one of group IV presented trauma infection at the reoperation. Therefore, they excluded from the study. The rest survived the whole experiment without significant problems. At reoperation, in the third and fourth group, the ligation and division of the common bile duct was checked and found to be successful in all cases, without evidence of bile leak.

Bilirubin values increased almost tenfold (12+2 mg) in-groups III and IV compared with groups I and II (1,35+0,5 mg) ($p<0,001$). In all bile samples, collected from the bile duct stumps, no aerobic bacteria were developed.

Table 1 shows the values of endotoxin, measured in portal vein and aorta. Animals of group III presented significantly higher concentrations of endotoxin when compared to groups I and II. When ligation of the common bile duct was followed by antibiotics treatment, endotoxin values were significantly reduced, in both the portal vein and the aorta, although they were still increased compared to control and sham groups. There was no difference between portal and aortic endotoxin concentration.

Table 2 shows the results of MLN and liver cultures for aerobic bacteria in all the experimental groups. Jaundiced rats (group III) were found to have 77% of their MLN cultures and 36% of their liver cultures positive. These values were statistically higher than those of groups I and II. Antibiotics did not decrease statistically the translocation of bacteria to MLNs but decreased the translocation to the liver, since animals of group IV were found to have 66% of their MLNs and 9% of their liver cultures contaminated. The same types of bacteria were identified in both lymph nodes and liver, and they were of intestinal origin (Table 3). Furthermore, the liver was contaminated only in the animals, which also presented positive MLN cultures.

A statistical significant increase of aerobic cecal bacterial population was observed in animals subjected to bile duct ligation (Table 4). After the administration of nonabsorbable antibio-tics the bacterial population de-

Table 1. Endotoxin Consetrations in Portal and Aortic Blood in the Four Groups

Groups (Endotoxin EU/ml)	I (n=21)	II (n=21)	III (n=22)	IV (n=21)
Portal	0.24+0.18	0.34+0.25	3.60+2.70	0.65+0.42
Aortic	0.10+0.11	0.28+0.20	3.68+2.80	0.68+0.45

Portal I/III: P<0.001, I/IV: P<0.001, II/III: P<0.001, II/IV: P<0.01, IV: P<0.001
AorticI/III: P<0.001, I/IV: P<0.001, II/III: P<0.001, II/IV: P<0.001, III/IV: P<0.001
(only significant differences are mentioned)

Table 2. Results from MLN and Liver Cultures in the Four Groups

Groups	I (n=21)	II (n=21)	III (n=22)	IV (n=21)
		Positive cultures		
MLN	4 (19%)	4 (19%)	17 (77%)	14 (66%)
Liver	0	0	8 (36%)	2 (9%)

MLN:I/III P<0.001, I/IV P<0.001, II/III P<0.001, II/IV P<0.001
Liver:I/III P<0.001, II/III P<0.001, III/IV P<0.05
(only significant differences are mentioned)

Table 3. Bacteria Species Isolated in MLNs and Liver Cultures in the Four Experimental Groups

	MLN	LIVER
Group I (n=21)	Escherichia coli (1)	-
	Staphylococcus sp (2)	-
	Streptococcus sp. (1)	-
Group II (n=21)	Escherichia coli (2)	-
	Streptococcus sp. (2)	-
Group III (n=22)	Escherichia coli (7)	Escherichia coli (3)
	Staphylococcus sp. (6)	Staphylococcus sp. (3)
	Streptococcus sp. (2)	Streptococcus sp. (2)
	Proteus sp. (1)	-
	Klebsiella sp. (1)	-
Group IV (n=21)	Escherichia coli (5)	Escherichia coli (1)
	Pseudomonas sp. (8)	Pseudomonas sp. (1)
	Citrobacter (1)	-

(Figures in parentheses indicate the number of positive cultures)

Table 4. Cecal Bacterial Population (mean + SD)

Groups	I (n=21)	II (n=21)	III (n=22)	IV (n=21)
Aerobic Bacteria (105 cfu/ml)	33+32.7	41.3+33	166.3+235	19.1+17.6

I/III P<0.02, II/III P<0.02, II/IV P<0.01, III/IV P<0.01
(only significant differences are mentioned)

creased, returning to normal levels.

Histologically, the intestinal mucosa in the jaundiced animals characterised by the presence of edema and stromal infiltration of inflammatory cells. This was found in both groups III and IV, with no significant effect of antibiotic treatment in improving histopathological changes. The other histopathologic parameters studied (V/cm, Vh, M/c) were not mandatory. Histologic changes typical of bile duct obstruction (cholestasis, bile duct proliferation) were observed in liver samples in all animals of group III and IV.

DISCUSSION

The majority of patients with obstructive jaundice present endotoxemia, particularly in portal blood,[20,21] resulting to significantly higher postoperative complications and death. Increased translocation of enteric bacteria to MLNs has also been reported in jaundiced mice,[12] however, the clinical significance of this phenomenon in humans seems to greatly depend on the host integrity.[22] Bile salts play an important role in the balance of enteric bacterial flora and have a trophic effect on the intestinal mucosa.[23] There is evidence that specific or non-specific antibodies contained in bile inhibit adhesion of enteric

bacteria on the intestinal wall, preventing bacterial translocation.[18] Recent reports on cellular immune dysfunction suggest that depression of T cell function have a direct effect on systemic endotoxemia.[24,25] Additionally, bile salts are thought to prevent intestinal endotoxin and bacterial translocation, by binding directly to intraluminal endotoxin or bacteria, creating poorly absorbable detergent-like complexes.[14] Oral administration of bile salts in jaundiced patients has also shown to significantly prevent absorption of endotoxin from the gut.[26,27]

In the present study we employed an experimental model for producing obstructive jaundice in rats, by ligating and dividing the common bile duct, which resulted in a significant bilirubin increase in all animals? Bacterial translocation to MLNs and liver as well as portal and systemic blood endotoxemia and aerobic cecal population were evaluated by the end of the experimental period of 10 days duration. We did not perform anaerobic cultures, since it is well established that translocating bacterial are almost exclusively aerobic.[28] Obstructive jaundice of 10 days' duration was shown to promote bacterial translocation both to MLNs and liver, as well as to increase translocation of intestinal endotoxin to portal and systemic blood. These findings are in agreement with previous reports.[11,13,14] Despite the "cleaning" effect of the hepatic reticuloendothelial system, endotoxemia of portal blood did not differ significantly from that of aortic blood in any group. The finding of sterile bile in the common bile duct stump in the jaundiced animals precludes liver contamination by ascending cholangiitis. Gut decontamination has several clinical applications such as the protection from infections in critical patients.[29] Similarly, the use of polymyxin B, amikacin and amphotericin B have been reported to reduce the incidence of bacterial translocation and early mortality in mice with induced necrotising pangreatitis.[30]

In the present study, gut decontamination was achieved by using neomycin sulphate and cefazoline twice daily for 10 days. Neomycin is a broad-spectrum antibiotic, unabsorbed when administrate orally (absorption of less than 1%), while susceptible micro-organisms are usually inhibited by concentrations of less than 5 μg/m. Cefazoline belongs to the first gene-ration Cephalosporins and is more effective against E. coli, Klebsiella species and Staphylococcus.

Our results demonstrate that after bile duct ligation, there is a significant increase of portal and systemic endotoxemia, aero-bic cecal flora, and bacterial translocation to MLN and liver. These parameters were decreased in animals receiving antibiotics with the exception of MLN positive cultures. It is most likely that gut decontamination reduces the bacterial population of cecum, so less bacteria produce less endotoxin, and obviously this reduces endotoxemia. Endotoxemia per se, on the other hand, is considered as one of the conditions predisposing to becterial translocation.[17] However, our findings suggest that the intestinal mucosal barrier is compromised in obstructive jaundice, allowing intestinal bacteria to translocate to extraintestinal tissues, with a mechanism independent to endotoxemia. Although the endotoxin levels in blood were low in the group treated with antibiotics, surprisingly bacterial translocation to MLN did nod decrease.

Counting the aerobic bacteria of the cecum an increased number, reaching statistically significant levels, was found in jaundiced animals. The lack of the bactericidal effect of bile salts in the intestinal lumen was expected to increase the bacterial population in the gut and promote translocation.[26] The fact that the bacteria found in MLNs and liver was of intestinal origin and of the same type suggests a common route and direction of dissemination. The MLNs are the first location to be reached by the translocating bacteria and therefore are the more frequently colonised sites, while the liver as the "nex" target" is"colonised to a lesser, but statistically significant, degree. Antibiotics prevent the overgrowth of enteric bacteria and this was apparent in antibiotic treated animals (group IV) where they decreased significantly in relation to the jaundiced ones (group III). This reduction seems to prevent the EBT to the liver but no to MLNs. A possible explanation to this discrepancy might be that other factors, than bacterial overgrowth, such as intestinal mucosa atrophy and/or intestinal mucosa functional impairment may play an important role for EBT.

Bile salts are considered as a potent trophic factor for the intestinal mucosa, increasing villous density and inducing hypertrophy of the intestinal epithelium.[28] Lack of the trophic effect of bile and more specifically bile salts is expected to result in intestinal mucosal atrophy, disruption of the intestinal barrier and bacterial translocation.[23] The histologic changes of the intestinal mucosa in the present study consisted of subepithelial edema and stromal infiltration of inflammatory cells. These findings support the idea of a compromised intestinal mucosal barrier in obstructive jaundice, which may explain to a certain degree the phenomenon of EBT, observed in the jaundiced rats. The possibility of an immunological effect of obstructive jaundice on the enteric barrier has not been addressed in this study. Further studies are required in order to assess the significance of the lack of secretory IgA on disrupting the mucosal barrier and inducing enteric bacterial and endotoxin translocation in jaundiced

animals.[18] However, since antibiotics have no apparent direct immunomodulatory effect, it is unlikely that an immune mechanism may interpret our findings.

In conclusion, the present study demonstrates an increased incidence of bacterial and endotoxin translocation in animals with obstructive jaundice. Gut decontamination was shown to reduce endotoxemia and EBT to the liver, but had no effect on bacterial translocation to MLNs. Increased translocation may be explained by the disruption of intestinal mucosal integrity, observed in obstructive jaundice, while changes in intestinal bacterial ecology seem to have a significant impact on this phenomenon. It is apparent that antibiotics on cecal flora decrease the bacterial population, and therefore the endotoxin production, resulting in improvement of systemic endotoxe-mia. Although the implications of experimental data should be transferred to the clinical situation with caution, our findings indicate that clinical application might be tried, in reducing septic complications in surgery for obstructive jaundice.

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