The Effect of Octreotide on Bacterial Translocation in Obstructive Jaundice

TIKANMA SARILIĞINDA OCTREOTİD'İN BAKTERİYEL TRANSLOKASYON ÜZERİNE OLAN ETKİSİ

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Summary

Today, obstructive jaundice is known to cause bacterial translocation. In this study, the protective effect of Octreotide, a synthetic analogue of somatostatin on the bacterial translocation, was studied in an experimental obstructive jaundice model in rats.

Forty-five rats were divided into three groups. Group I (n: 15) was sham operated group. Rats in Group2 (n: 15) and Group3 (n: 15) were administered 3ml / day saline SC and 20 micrograin/kg Oclreotid intra muscular every 8 hour respectively. Obstructive jaundice was created in Group! and Group3 by ligation of common bile ducts in second day of treatment which continued for 9 days.

All rats were sacrificed at post operative 7th day (9th day of treatment). Liver, spleen, mesenteric lymph node bacterial translocation rate and ceacal bacterial contents were evaluated. In addition terminal ileum and liver samples were examined h istopa th o logically.

In Group2 ceacal bacterial contents were significantly higher than Group I and Group3 ($p<0.()5$) In Group 2 there was also statistically significant bacterial translocation to the mesenteric lymph nodes. Pathological changes were found in liver and terminal ileum samples in Group2 which seemed to improve in Group 3.

In this experimental study, it was shown that Octreotide is effective in preventing bacterial translocation.

Key Words: Obstructive jaundice. Bacterial translocation, Octreotide

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Özet

Bugün artık tıkanma sarılığının, bakteriye! translokasyonun nedenlerinden birisi olduğu bilinmektedir. Bu çalışmada Somatostatinin sentetik bir analoğu olan Octreotidin, deneysel olarak tıkanma sarılığı modeli oluşturulmuş rotlarda bakteriye! translokasyon üzerine olan etkisi araştırıldı.

Kırkbeş rat üç gruba ayrıldı. Grup 1 'e (n:15) sham operasyonu uygulandı. Grup 2 'ye (n:I5) 3 ml/giin %0.9 NaCl SC ve Grup 3 'e (n:i5) 20 meg/kg Octreotid IM hergiin 8 saatele bir uygulandı. İkinci ve üçüncü gruba tedavinin ikinci gününden dokuzuncu gününe kadar sürecek şekilde koledok bağlandı.

Dokuzuncu günde bütün raflar sakrifiye edilerek karaciğer, dalak ve mezenterik lenf nodunda bakteriye! translokasyon hızı ve çekıımdaki ba/eteri miktarı araştırıldı. Ayrıca terminal ilenin ve karaciğerden alınan örnekler histopatolojik olarak incelendi.

İkinci grıupda çekıımdaki bakteri miktarı, üçüncü ve birinci grıuptakinden belirgin olark daha fazla bulundu ($p<0.05$). Aynı zamanda ikinci grupta belirgin şekilde mezenter lenf nodunda bakteriye! translokasyon izlendi. Grup 2 'de izlenen karaciğer ve terminal i/eıumdaki patolojik değişikliklerin, üçüncü grupta belirgin düzelme gösterdiği izlendi.

Bu deneysel çalışma, Octreotidin bakteriye! translokasyonıı (inlediğini göstermektedir.

Anahtar Kelimeler: Tıkanma sanlığı, Bakteriycl translokasyon, Octreotid

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Bacterial translocation may be defined as the passage of especially gram negative bacteria from intestinal lumen to mesenteric lymph nodes, liver, spleen, and systemic circulation (1).

There are three main causes of bactcrial translocation. Thèse are, altered intestinal mucosal **baiTİer,** changes in intestinal bactcrial flora, and altered host's defence mechanisms. Diseascs that may cause bactcrial translocation are listed as

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mesenteric vascular disorders (2), ileus (3), cellular immune deficiency (4), trauma (5), bums (6), usage of broad spectrum antibiotics(7), total parenteral nutrition (8), liver parenchyma disease (9), and obstructive jaundice (10). Deitch et al found that of bacterial translocation rate to mesenteric lymph nodes (M L N)in experimental rats with obstructive jaundice is 33% (11). Infection and endotoxemia are the two main causes of morbidity and mortality in obstructive jaundice.

There are two sources of bactcriemia in obstructive jaundice: one of them is micro-organisms in the biliary tract, and the other and the most important one is bacterias that may translocate from intestinal lumen to M L N , portal system, and blood circulation (10,11).

Reticulo endothelial systcm(RES) plays an important role in the prevention of bacterial translocation. One of the most important function of the RES is to remove micro-organisms and endotoxins from the blood, thus providing an important defensive mechanism against infection. The phagocytic activity of the RES is impaired in ciiThosis mainly due to depression of the hepatic RES which comprises the largest fraction of the total RES (12).

Octreotide, an analogue of somatostatin with a long half life, enabled somatostatin to be usable in clinical situations. Octreotide might modulate kupffer cells and possibly other macrophages and these effects may have a role in the physiologic or pharmacological actions of this peptide (13). In addition to these, the protective effect of octreotide against intestinal mucosal injury was observed (14).

For above reasons, we planned an experimental smdy to evaluate the possible effect of octreotide in preventing bacterial translocation in obstructive jaundice.

Materials and Methods

Healthy 45 Wislar albino rats weighing 250-350 g. and from both sexes were randomly divided into 3 groups. A11 rats were kept at standard temperature (22C), humidity, and lighting. A11 rats were fed by standard laboratory chow , and acidified water (0.001 N hydrochloric acid) ad libitum.

Rats were divided into 3 groups. In group 1 (n=15) , only median laparotomy and porta hepatis manipulation (sham ligation) , in group 2 (n=15), laparotomy and common bile duct (CBD) ligation, and in group 3 (n=15), in addition to laparotomy and CBD ligation octreotide (sandostalin=sandoz) treatment was given.

Group 1 and 2 rats received 3 ml saline subcutaneusly, and group 3 received 20 microgram/kg octreotide intramuscularly in 3 divided doses during 9 days.

At the second day of treatment, all rats went laparotomy in sterile conditions, under IM ketaminc hydrochloride (35mg/kg) anaesthesia. In group 1 only porta hepatis was manipulated, but in the 2 and 3rd group, CBD ligation with 5/0 prolen was pcrfonned. In the postoperative 7th day (9th day of treatment) all rats were killed by excessive ether anaesthesia. Tissue culUires were obtained from liver, spleen, M L N and terminal ileum, and liver and terminal ileal biopsies were taken for histopathological examination.

Testing for bacterial translocation

in sterile conditions paramedian laparotomies parallel to previous midline incisions were made. Different sterile equipments were used for skin, liver and each organ. Tissue samples obtained from liver, spleen, and M L N were put into tiyptic soy broth (TSB). After weighing tissue samples, they were homogenised in 0.5 ml TSB by Teflon grinders, and after this 0.1 cc of this homogenate were put into Me Conkey's agar for the culture of gram negative enteric bacillus and also to blood agar for gram positive coccus. The exposed viscera were swabbed with sterile cotton tipped applicator - sticks, which were cultured in tryptic soy broth to detect any accidental bacterial contamination. Caecal contents which were kept in a sterile tube were diluted with 0.085 NaCl until reaching a 105 concentration, and 0.1 ml of this mixture was cultured in Mc conkey's and blood agar.

A11 cultures were incubated at 37C for 24-48 hours and then evaluated. Growing bacterias were identified and measured as CFU (colony forming unit). :

Histological evaluation

Tissue samples of terminal ileum and liver were kept in %35-40 formaldehit solution for 6 hours at room temperature. The tissues were sliced into 4-6 mm pieces, dehydrated with %95 ethanol and embedded in JB-4. Sections, 1-2 micrometre were examined and stained with hcmatoxylene eosine and photographed with the use of an Olympus research microscope. Statistical analysis:

A11 datas were evaluated with PC-statistics program by using the I B M computer. The incidence of bacterial translocation was determined by Chi-square. For each group caccal bacterial population level was measured by M A N O V A (multiple analysis of variance) and Schaffc tests.

Results

Caecal bacterial content is shown at table 1. Caecal bacterial population levels in group 2 (obstructive jaundice group) were higher than group 1 (sham ligated group) and group 3 (obstructive jaundice model treated with octreotide) (P< 0.05) (multiple range tests = Schcffe test with significance level 0.05). In obstructive jaundice model there was a statistically significant bacterial translocation to M L N (P=0.0098). Translocating bacteria to M L N was E.coli. Cecal E.coli content was 10-30 times higher in rats with bacterial translocation than the rats with no bacterial translocation, in the same group (group 2) . In octreotide group there was a slight increase in the count of enteric bacillus, but this was not statistically significant when compared to control group. [All groups had a statistically meaningful mean bilurubin levels them (p=0.0000)].

Table 1. Ceacal bacterial population levels

Groups	n	Enteric Bacilli(CFU)
I (control / sham operated)	15	5.41 ± 3.38 x 105
II (CBD ligated)	15	16.34 ± 3.89x105
III (CBD Ligated+Octreotide)	15	6.85 ± 2.51x105

CBD: Common bile duct
CFU:Colony forming unit

Histological evaluation

Group 1 had (sham ligated) a normal liver and terminal ileum samples on histological evaluation. But in group 2 there was inflammatory cell infiltration and fibrosis starting from portal tractus and advancing through lobular parenchyma that led to bridging necrosis and fibrosis (Figure 1). In group 3, in contrast to group 2 inflammatory cell infiltration and fibrosis were confined to the portal tractus (Figure 2).

Tissue samples obtained from ileum in group 1 and 2 showed similar results as there were mucosal oedema, blunting and shortening of the mucosal villuses in both of them. In group 2 there were also manifest ulceration of the mucosa and dense lymphoplasmocytic infiltration in the entire thickness of intestinal wall (Figure 3). But in octreotide group (group 3), there was only mild to moderate inflammatory cell infiltration at tunica propria (Figure 4).

Discussion

Today it is well known that functions of the intestine are not limited to food transport and absorption, but there are also other important complex functions of intestine, such as endocrine, immunological, metabolic and barrier functions. If this functions are destroyed, not only gastrointestinal

Table 2. Bacterial translocation incidence in mesenteric lymph node

Group	Bacterial translocation n	incidence %	C F U / MLN	Bilirubin (mg/dl)
I (control/sham operated)	15	0	0	0.4± 0.14
II (CBD ligated)		6	15±3.6	15.4±4.17
III (CBD ligated+ Octreotide)	15	0	0	8.43± 2.29

CBD: Common bile duct
CFU.Colony forming unit
M L N : mesenteric lymph node

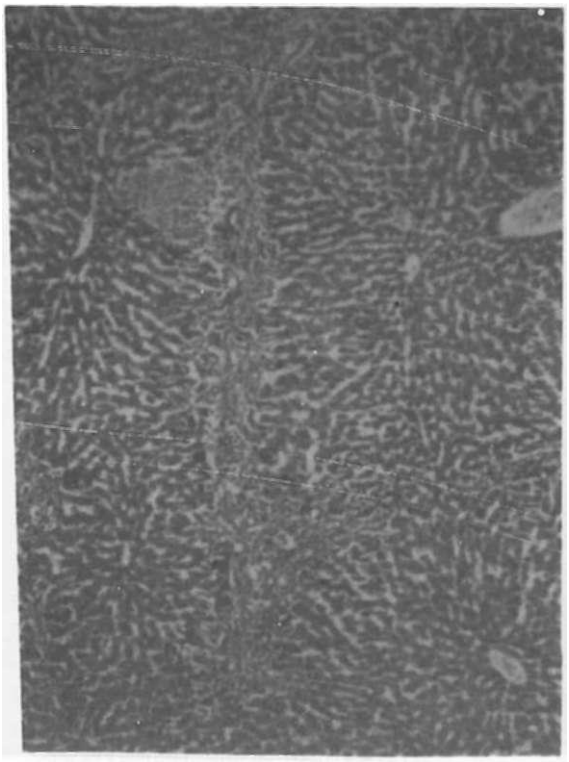

Figure 1. The liver slides of group2, it was observed that inflammatory cell infiltration and fibrosis being from portal tractus and advancing towards lobular parenchyma, cause bridging necrosis and fibrosis in a lot of areas.(Eosine methylene blue;orginal magnification x 100)

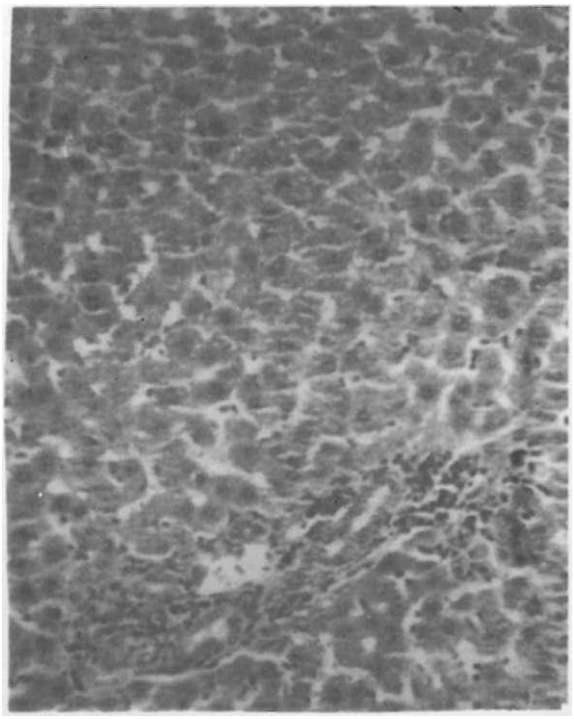

Figure 2. The liver slides of group3, it was observed that inflammatory cell infiltration and the fibrosis were limited to portal tractus.(Eosinc methylene blue; orginal magnification x200)

but also other systems will be affected. Especially, when different kinds of bacteria and toxins existing in gastrointestinal system pass from lumen to lymphatic system, peritoneum and portal system, they can cause many problems (15). Thirty years ago, for the first time, Ravin accused bacterias in the intestinal lumen, as the source of endotoxemia(16). Then many researchers reported that, endotoxemia may become evident in either portal system or peripheral circulation in obstructive jaundice. In different reports, portal endotoxemia was shown to be 55-73% and systemic endotoxemia 15-64% in obstructive jaundice (17-19).

One of the most important function of intestinal mucosa is to form a barrier against bacteria and endotoxins. The mucosal barrier of the intestine is so strong that, the dense bacterial population of the intestine can not pass from lumen to systemic circulation and organs to cause infection (20). In obstructive jaundice, as a result of bacterial overgrowth and diminished bile salts and slgA that inactivate bacterial endotoxins in intestinal lumen, intestinal mucosa can be injured (21,22).

In our study, it is possible to show the different ways by which octreotide can prevent bacterial translocation in cholestasis. Cecal bacterial content that increased in obstructive jaundice, was found to be decreased in octreotide treatment group, and this finding supports the protective effect of octreotide on mucosa.

The protective effect was accompanied by a significant reduction in platelet activating factor activity, leukotrienc B4 and vasoactive intestinal peptide concentrations (14).

There was neither mucosal ulceration nor lymphoplasmocytic infiltration in octreotide treatment group, and these findings support that octreotide has a protective effect on intestinal mucosa. However although some rats had mucosal injury

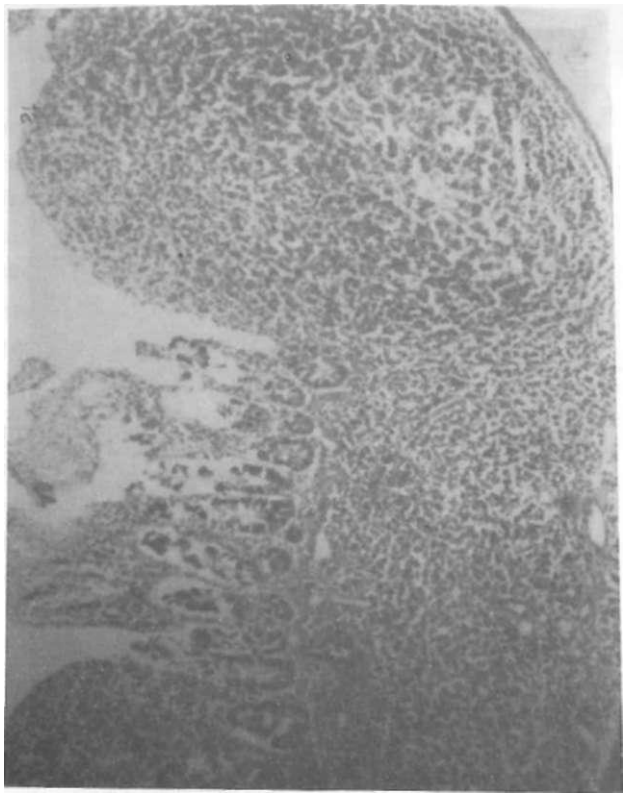

Figure 3. In terminal ileum specimens of group2 , manifest ulceration in mucosa and dense lymphoplasmocytic infiltration throughout whole intestinal wall were observed.(Eosine methylene blue; orginal magnification x400)

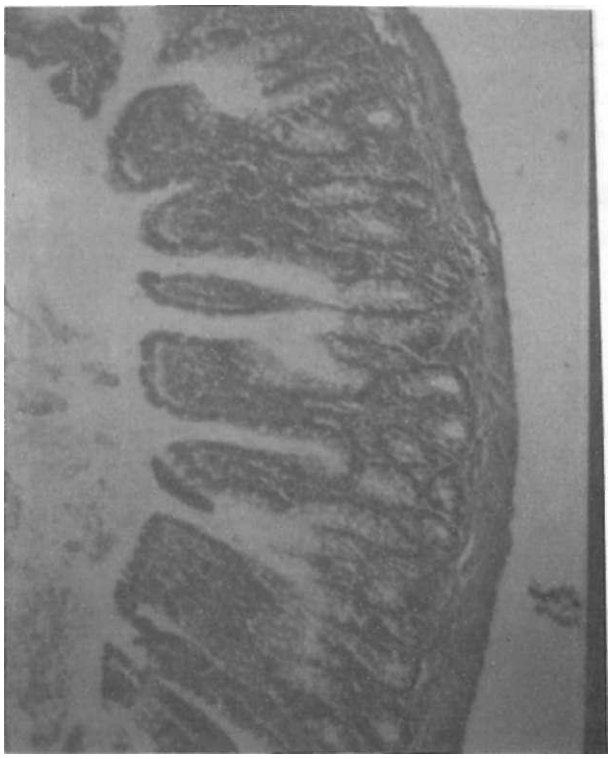

Figure **4.** In terminal ileum samples of Octreotide treatment treatment group (group3), mild or middle inflammatory cell infiltration was observed in tunica propria of intestine.(Eosine methylene blue; orginal magnification xlOO)

and increased population of bacteria in their lumen, there was no bacterial translocation, and this shows that there are some other factors that modulate bacterial translocation.

In some situations, even though the intestinal wall integrity is intact, such as shock, burns, immune deficiency, total parenteral nutrition and systemic chemotherapy, bacterial translocation may develop. The most important causes are,'changing intestinal flora, cellular immune deficiency, impaired RES functions, and increased absorption from intestinal lumen (1,2,23)

Bile inhibits bacterial invasion of entcrocyte. For this reason, bacterial translocation is facilitated in obstructive jaundice (24). As a matter of fact, it was shown that bacterial translocation may be prevented by administration of bile salts per orally or by providing biliary decompression in rats with obstructive jaundice (25,26).

In our opinion impaired RES function is the most important factor causing bacterial translocation in obstructive jaundice. In obstructive jaundice, depressed cellular immunity, impaired bacterial clearance, bacterial capture and impaired RES functions were shown by previous studies (15,23,27-29). Ding et al. reported that macrophage stimulator liposomal muramyl tripeptide phosphatdylethanolaminc inhibits bacterial translocation to M L N in rats with obstructive jaundice (30).

Octreotide administration to rats significantly increases the plasma clearance of colloidal carbon and this shows that octreotide has a stimulating effect on RES activity (29,31). In an experimental study, octreotide was shown to improve survival in rats that received intraperitoneal injection of E. coli, by the way of RES activation (13).

Extrahepatic biliary obstruction leads to bile duct epithelial cell proliferation and periportal fibrosis (32). Somatostatin and its analogue, oc-

treotide, have been shown to inhibit DNA synthesis and proliferation in hepatocytes (33). Hepatocyte was preserved with octreotide treatment, which also significantly decreased bile duct proliferation and periportal extracelluler matrix deposition in response to biliary obstruction compared with saline treated, duct ligatcd animals (34). Also in our study, we observed that periportal fibrosis and hepatocyte injury were decreased in octreotide treatment group. These results show that octreotide prevents the morphological changes that accompany extrahepatic biliary obstruction. In our study it was clearly shown that octreotide prevented bacterial translocation by activating the RES, decreasing cecal bacterial content and maintaining intestinal wall integrity.

New studies are required to confirm these effects of octreotide.

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