Does granulocyte-macrophage colony-stimulating factor prevent bacterial translocation in rats with surgical trauma and obstructive jaundice?

GM-CSF cerrahi travma ve tıkanma sarılıklı ratlarda bakteriel translokasyonu önler mi?

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Background/aims: *The incidence of sepsis can be decreased by preventing bacterial translocation, as the first step in enhanced host defense. The aim of this study was to prevent translocation and to increase Kupffer cell incidence by using granulocyte-macrophage colony-stimulating factor in rats with surgical trauma and obstructive jaundice.* ***Methods:*** *Seventy-five Sprague-Dawley rats were randomized into 8 groups. After calibration of laboratory conditions by Group 0, SHAM operations in Groups I, II, IIA and common bile duct ligations in Groups III, IV, IVA and V were performed. Granulocyte-macrophage colony-stimulating factor doses were 6 µg/kg/d in Groups II, IV; 1 µg/kg/d in IIA, IVA postoperatively; and 6 µg/kg/d in Group V preoperatively, for 7 days. After one week, all rats were reoperated for cecal lymph node, liver and spleen biopsies for culture and histopathology. All culture specimens were identified as positive/negative/contaminated. Survivals were recorded, and after the 21st day surviving rats were sacrificed by decapitation.* ***Results:*** *There was no translocation in Group 0 in the three specimens of liver, cecal lymph node and spleen. Group V showed minimal (10%) positivity in only liver, and other groups ranged between 20-70% in cecal lymph node, liver and spleen tissues, respectively (p<0.05). Kupffer cell incidences were higher in the granulocyte-macrophage colony-stimulating factor given groups than in controls, and lower in common bile duct ligation groups than in SHAM groups (p<0.001). Groups 0 and V showed the best (median 20 days) and Group III the worst (median 11.7 days) survival (p<0.001).* ***Conclusions:*** *Not only surgical trauma but also obstructive jaundice caused high incidence of translocation, decreased number of Kupffer cells and shortened survival. Translocation ratios were decreased by granulocyte-macrophage colony-stimulating factor in the SHAM and common bile duct ligation groups. Granulocyte-macrophage colony-stimulating factor prevented the decrease in Kupffer cell incidence caused by jaundice and prolonged the survival by preventing translocation at the first step.*

Key words: GM-CSF, obstructive jaundice, surgical trauma, bacterial translocation, Kupffer cell

Amaç: *Sepsis insidansı, ilk basamak olan bakteriel translokasyon önlenip, konakçı defansı arttırılarak, azaltılabilir. Bu çalışmanın amacı cerrahi travma ve tıkanma sarığı olan ratlarda granülosit makrofaj koloni stimule edici faktör kullanarak translokasyonun önlenebilirliği ve karaciğerdeki Kupffer hücre sıklığının ve yaşama olasılığının artırılabilirliğini değerlendirmektir.* ***Yöntem:*** *Yetmiş beş adet Sprague-Dawley rat 8 gruba randomize edildi. Grup 0 ile laboratuvar koşulları kalibre edildikten sonra Grup I ve IIA'ya SHAM operasyonları, Grup III, IV, IVA ve V'e koledok ligasyonu yapıldı. Grup II ve IV'e 6µg/kg/gün postoperatif; Grup IIA ve IVA'ya 1µg/kg/gün postoperatif, Grup V'e 6µg/kg/gün preoperatif 1 hafta süre ile granülosit makrofaj koloni stimule edici faktör, Grup I ve III'e placebo için serum fizyolojik uygulandı. Bir hafta sonra bütün ratlar (Grup 0 hariç) kültür ve histopatolojik inceleme için çekal lenf nodu, karaciğer ve dalak biyopsilerini almak amacıyla tekrar opere edildi. Bütün kültürler pozitif/negative/kontamine şeklinde tanımlandı. İkinci seri biyopsiler histopatolojik olarak değerlendirildi. Bütün gruplarda sağkalım süreleri kaydedildi. Hala yaşayan ratlar deneyin sona erdirildiği 21. gün dekapitasyon ile sakrifiye edildi.* ***Bulgular:*** *Grup 0'ın karaciğer, çekal lenf nodu ve dalak spesmenlerinin hiçbirinde translokasyona rastlanmadı. Grup V'de sadece karaciğer'de minimal (%10) pozitiflik varken, diğer gruplarda çekal lenf nodu, karaciğer ve dalak kültürlerinde %20-70 arasında pozitiflik saptandı (p<0.05). Kupffer hücreleri granülosit makrofaj koloni stimule edici faktör verilen gruplarda kontrollere göre daha yüksek, tıkanma sarılığı oluşturulan gruplarda SHAM gruplarına göre daha düşük bulundu (p<0.001). Grup 0 ve V median 20 gün ile en uzun, Grup II median 11,7 gün ile en kısa sağ kalım süresi gösterdi (p<0.00I).* ***Sonuç:*** *Hem cerrahi travma hem de tıkanma sarılığı, yüksek translokasyon oranına ve sağ kalım süresinde kısalmaya neden olmaktadır. Tıkanma sarılığının neden olduğu Kupffer hücre sıklığı azalması granülosit makrofaj koloni stimule edici faktör ile önlenmiştir. Translokasyon oranları SHAM ve tıkanma sarılığı oluşturulan gruplara granülosit makrofaj koloni stimule edici faktör kullanılması ile azaltılmıştır. Granülosit makrofaj koloni stimule edici faktör, tıkanma sarılıklı ratlarda sepsisin ilk basamağı olan translokasyonu önleyerek sağ kalım süresini uzatabilir.*

Anahtar kelimeler: GM-CSF, tıkanma sarılığı, cerrahi travma, bakteriyel translokasyon, Kupffer hücreleri

INTRODUCTION

After its first definition, bacterial translocation (BT) was revised as "the passage of both viable and non-viable microbes and microbial products such as endotoxin across an anatomically intact

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Manuscript received: 14.01.2008 **Accepted:** 03.07.2008

*Preliminary results of this study were presented as a poster presentation at EASL 2001-36th Annual Meeting of the European Association for the Study of the Liver, Prague, Czech Republic, April 18-22, 2001.

intestinal barrier" (1). Intestinal epithelium forms the intrinsic barrier that separates the internal environment of the organism from the intestinal luminal contents, and loss of barrier may enhance translocation of intestinal bacteria and toxins to local or regional tissues. Mucosal barrier function, killing capacity of the opsonin-phagocytic system, mucus secretion, proliferative epithelial cell replacement, and intercellular tight junctions decrease in thermal (burn) injury (2), bacterial overgrowth (3), general anesthesia (2), immune-suppression (3), hemorrhagic shock (4-7), intestinal obstruction (8), endotoxemia (9), intravenous hyperalimentation (10), antibiotic therapy (11), obstructive jaundice (12), hyperpyrexia (13), short bowel syndrome (14), inflammatory bowel diseases (15), radiation injury (16), chemotherapy (17), mucosal ulceration (18), liver insufficiency (19), surgical stress (19, 20), food deprivation (4, 18), malnutrition (21), blunt/multiple trauma (22), blood transfusion (23), ischemia-reperfusion injury (24), reduced blood flow to the intestine (25), and, on rare occasions, spontaneously (26). Based on these observations, high rates of translocations increase the hypermetabolic response of injured and septic patients in the absence of a defined infectious focus, and predispose or contribute to the development of multisystem organ failure (1). The number of viable bacteria in the tissue also increases in association with the translocation process (1).

Because bile does not flow into the intestines, anti-oxidant and anti-infective substances such as bile salts, bile pigments and phospholipids cannot be used by the intestinal mucosa, and the increased number of bacteria in the intestines prepares a base for translocation of bacteria and their products. This increased intestinal permeability has been postulated to be a key factor contributing to bacterial and endotoxin translocation to mesenteric lymph nodes (LNs), portal circulation and the liver. Obstructive jaundice is a cause of sepsis and multiple organ failure due to translocations, cholangitis, decreased number and clearance capacity of Kupffer cells, impaired filtration function of the liver, endotoxemia, impaired host defense, bacterial overgrowth, consecutive release of proinflammatory cytokines, renal, cardiovascular, and hepatic complications, coagulation defects, gastrointestinal bleeding, mucosal damage and delayed wound healing, potentially leading to the development of the so-called "gut derived sepsis" (11, 27). Lipopolysaccharides, which are normally cleared by Kupffer cells (cells 80% responsible for the body's host defense mechanisms) in the healthy liver, cannot be cleared in jaundice, resulting in endotoxemia and multiorgan insufficiency (17). The mechanisms implicated in this phenomenon remain unresolved, but growing research interests during the last decade include the studies on the bile and immune system [GALT, $CD4^+$ and $CD8^+$ T-lymphocytes, MAdCAM-1 (mucosal addressin cell adhesion molecule-1), Peyer's patches, secretory immunoglobulin A] and biological [bile acids have been reported to inhibit the growth of *Bacteroides, Clostridia, Lactobacillus* and *Streptococci;* absence of bile salts results in a disturbed intestinal bacterial balance with overgrowth of gram-negative bacteria] and mechanical barriers. Recent studies have shown that bile is crucial for the maintenance of the integrity of enterocyte tight junctions, regulating the expression of the essential tight junctions, regulating the expression of the essential tight junction-associated proteins occludin and ZO-1 (zonulin-1), thus preserving the intestinal paracellular barrier; bile exerts trophic effects on the intestinal mucosa, increasing villous density and inducing hypertrophy of the intestinal wall components; bile acids promote intestinal epithelial cell proliferation through a *c-myc*-dependent mechanism and protect against apoptotic cell death through activation of nuclear factor (NF-κB), and by this mechanism, bile salts regulates the mucosal growth and repair. These reports have shed light on our knowledge in the field (27).

Colony-stimulating factors are the cytokines of growth and proliferation of bone marrow progenitor cells. These cytokines are interleukin (IL)-3, IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony-stimulating factor (G-CSF), and macrophage-colony-stimulating factor (M-CSF). G-CSF and GM-CSF enhance numerous functional activities associated with host defense, including chemotactic activity (28), phagocytosis (28), neutrophil activation (28), destruction of pathogenic parasites (29), cytotoxic function (30, 31), proliferation of immature cells in fetal blood cultures (32), suppression of apoptosis (33), potentialization of resistance to local infection (34), and inhibition of BT in hemorrhagic shock (5). As a result of these effects, GM-CSF is used in patients with myelosuppression (35), bone marrow transplantation (35), sepsis/gut-derived sepsis (36), hemorrhagic shock (4, 37), neonatal sepsis (38), post-splenectomy infections (39), multiple

trauma (40), ischemia-reperfusion injury (24), diabetic infections (41), chronic liver diseases (42), BT (43), wound/intestinal/anastomotic healing (44), and vaccine development (45) in clinical/experimental studies of prophylactic/therapeutic options.

We hypothesized that individuals with surgical trauma and obstructive jaundice have a high risk of BT, decrease in the incidence of Kupffer cells and short survival, and that GM-CSF can prevent this translocation in the first steps of sepsis. To evaluate these hypotheses, we performed an experimental prospective-randomized-cohort study.

MATERIALS AND METHODS

Seventy-five female, 8-10 week, 150-200 g Sprague-Dawley rats were used in the study. All procedures were followed according to the "Recommendations for Euthanasia of Experimental Animals of the Commission of the European Communities", in accordance with the ethical standards of the National Research Council's Guide for the Care and Use of Laboratory Animals, and with the approval of the "Experimental Study and Animal Care Ethic Committee" from the Experimental Study Department of Hacettepe University. The animals were examined, weighed, identified, recorded and housed in a temperature/humidity-controlled environment with 12-hour light-dark cycle with water and commercial rat chow provided *ad libitum.* Rats were randomized into 8 groups as shown in Figure 1. Anesthesia: After 12 hours of fasting, all rats were anesthetized by isoflurane (4% for induction, 1% for maintenance), nitrogen 4% and oxygen 1.5% with mask. First operations: In the

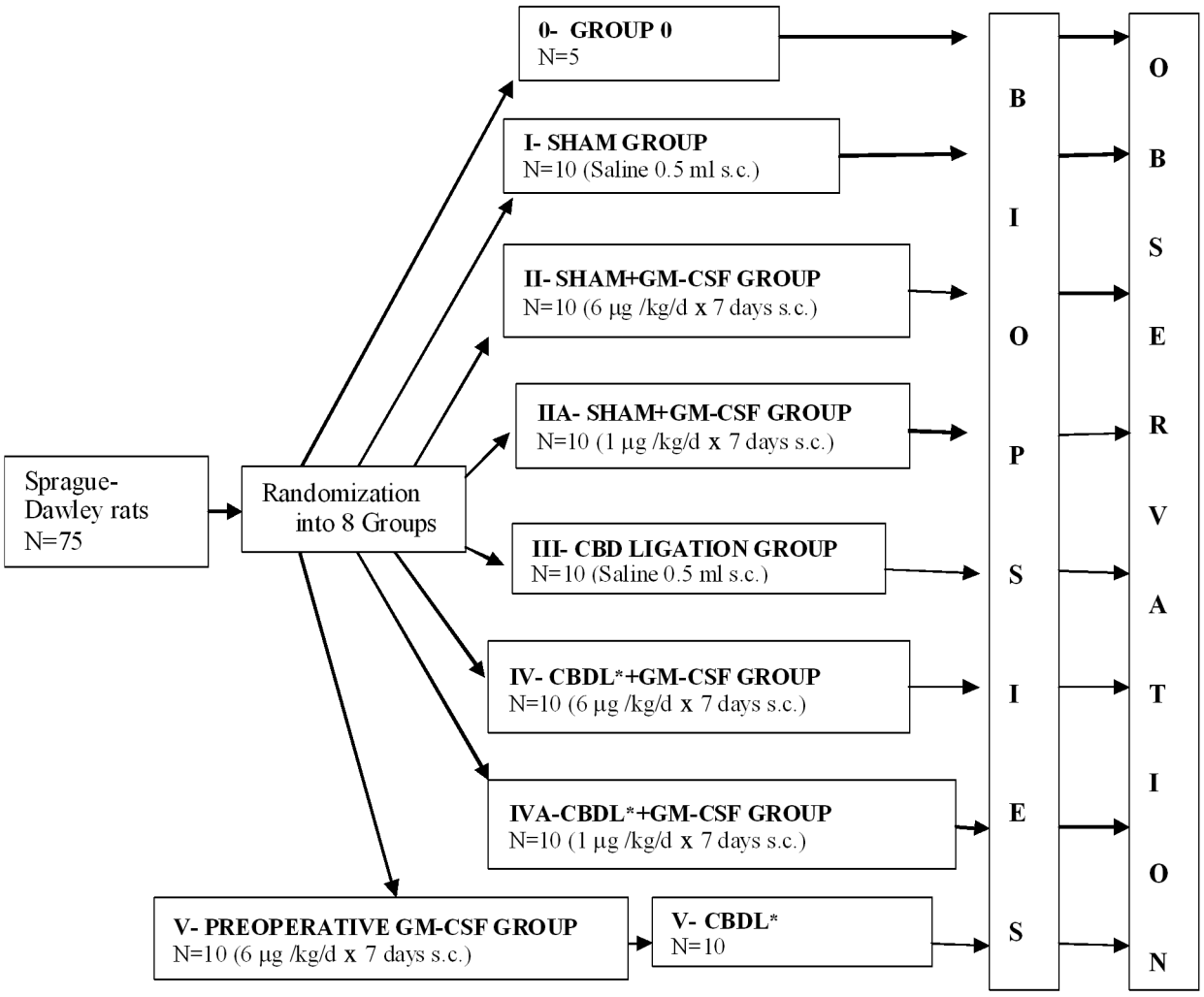

Figure 1. Experimental design. *(*CBDL: Common bile duct ligation)*

Experimental Laboratory of Ankara University, all rats were shaved and cleaned three times by povidone-iodine. A 3 cm in length-midline laparotomy was performed. In Group 0, the operation theater's conditions were arranged to prevent contamination; to calibrate anesthetic medicine/gases, micro-centrifugal tubes and routine microbiological procedures; and to obtain a pair of cecal LNs, a pair of spleen tissues (0.5x0.5x0.5 cm) with partial splenectomy, and a pair of liver biopsies (1x1x1 cm) from the caudate lobe.

The common bile duct (CBD) was found and held by a plane pick-up in the animals in the SHAM groups (Groups I, II and IIA). After being tied twice from its upper and lower parts with 5/0 polypropylene, the CBD was cut in the CBDL group animals (Groups III, IV, IVA and V). After controlling bleeding, abdominal closure of two layers was performed by atraumatic 4/0 polypropylene and then awakened rats were taken to a separated and warm house.

Activity, wound healing, color of the skin, urine and feces (daily), and body weights of rats (weekly) were monitored after the first operation. None of the medical drugs was given in Group 0. In Groups I and III, 0.5 ml/d of saline was administered subcutaneously. Six µg/kg/d of GM-CSF (Leucomax® 150 µg/ml, Novartis/Schering-Plough; Novartis Pharma AG; Basel, Switzerland) was given subcutaneously to Groups II and IV postoperatively and Group V preoperatively; 1µg/kg/d of GM-CSF was given subcutaneously to Groups IIA and IVA postoperatively for 7 days. Groups IIA and IVA were included in the study in order to identify dose-response relation.

Seven days later, after 12-hour fast, in the second operations (except Group 0), a pair of cecal LNs, a pair of spleen tissues (0.5x0.5x0.5 cm) with partial splenectomy, and a pair of liver biopsies (1x1x1 cm) from the caudate lobe were taken under the same anesthesia as with laparotomy. The first series of biopsies were taken into the micro-centrifugal tubes, containing measured volume and weight of brain-heart infusion broth (BHI®, Difco Laboratories; Detroit, MI). The second series of biopsies were taken for histopathological examination. At the end of second operations, the abdomen was checked for bleeding and abdominal closure of two layers was performed by atraumatic 4/0 polypropylene, and then awakened rats were taken to a separated/warm house.

All rats were monitored for 24 hours and examined for activity, wound healing, and color of the skin, urine and feces. No medical treatment was administered after the 2nd operation until the end of study (the 20th day). Dead rats with previous jaundice, hypoactivity, less appetite, malnourishment, poor/infected wound healing, ascites and systemic infection as pneumonia diagnosed by autopsy were recorded as mortality of sepsis and excluded from the house. There were 2 deaths in Group 0 by attack through incision line and damage of viscera. Survived rats were sacrificed by decapitation after the 21st day according to "Recommendations for Euthanasia of Experimental Animals of the Commission of the European Communities" and autopsied. The experimental period and monitoring were limited to 20 days since significant difference in survival was determined among the groups.

Microbiologic evaluations were performed under double-blind condition at the Clinical Pathology Laboratory of Hacettepe University. In the first series, all biopsy specimens were disintegrated, homogenized in sterile conditions and incubated in blood agar for 24-48 hours. Positive specimens were identified according to routine procedures using Gram's staining, coagulase, catalase and the Sceptor System (Becton Dickinson Diagnostic Instrument Systems®; Towson, MD, USA) when indicated. Isolated translocated bacteria were *E. coli, Klebsiella spp., P. aeruginosa, Enterococcus sp., S. aureus, Yersinia enterocolitica, and Acinetobacter spp.* Contamination was observed in 14 (6.2%) out of 225 samples while disintegrating the biopsy specimens. These contaminated microorganisms were found to be Micrococcus, Bacillus and Diphtheroids and half of them were identified in the same specimen with other translocated enteric bactericeae.

Histopathologic evaluation was assessed at the Pathology Laboratory of Türkiye Yüksek İhtisas Hospital under a double-blind condition for the second series of specimens. Tissue samples were fixed in 10% formalin for paraffin sections. Four µm thick paraffin sections were stained by hematoxylin and eosin (HE). In liver specimens, proliferation of biliary ducts, enlargement of central vein, and lymphocyte infiltration of portal space were evaluated, and Kupffer cells were counted in x1000 magnification. Kupffer cells were re-counted by immunohistochemically with CD-68. In spleen specimens, macrophages, lymphoid follicles and congestion; and, in LN specimens, pattern of

reaction were assessed, and findings were compared with Group 0 or SHAM controls.

Independent variables of the study were group, GM-CSF dose, and obstructive jaundice. Dependent variables were the incidences of BT in LN, liver and spleen tissues, the incidence of Kupffer cells in liver tissue and survival.

Statistical analyses

Data was coded and recorded using the SPSS for Windows 13.0 version. In epidemiologic analysis, relative risks (RR) of operation and jaundice and the relative efficacy (RE) and dose-response relation of GM-CSF were calculated. Formulations of RR and RE were included in the text and are also shown in the Tables. In statistical analysis, Pearson chi-square test was used for groups in cross-tables of translocations of LN, liver and spleen tissues. Wilcoxon signed-ranks test was used to compare body weights before the 1st and 2nd operations within the groups. Body weights, survival periods and Kupffer cell incidence between the groups were compared by Kruskal Wallis test. After Kruskal Wallis test, post hoc multiple comparison test was used to identify statistically significant pairs. Survivals in different groups were also compared by Kaplan-Meier log-rank test. P values less than 0.05 were accepted as statistically significant in each test.

RESULTS

Postoperative activity in Groups 0, V and the SHAM groups was similar to that observed preoperatively, except for the jaundiced groups. Animals in the CBDL groups showed decreased activity, weight loss, poor wound healing and wound infections. According to Table 1, animals in CBDL only group (Group III) showed decreased body weight with no significant difference. There was a significant difference in body weights of rats before the 1st and 2nd operations in the preoperative GM-CSF-administered group (Group V) ($p<0.05$).

Objective evaluation was performed for BT, histopathological examination and survival issues.

Bacterial translocation

There were significant differences between groups with respect to BT of LN, liver tissues and total

Table 1. Body weights in groups before the operations

Groups	Body weight(g)		
	Before 1st operation	Before 2nd operation	P value *(Wilcoxon)*
0-Group 0	193.0±24.9	-*	-
I-SHAM	178.7±18.8	180.5±13.6	*0.683*
II-SHAM + 6 µg/kg GM-CSF	167.7±21.3	178.6±18.3	*0.066*
IIA-SHAM + 1 µg/kg GM-CSF	177.3±20.3	184.5±18.9	*0.035*
III-CBDL**	170.5±14.0	163.4±4.0	*0.172*
IV-CBDL + 6 µg/kg GM-CSF	173.0±12.4	177.9±6.3	*0.407*
IVA-CBDL + 1 µg/kg GM-CSF	186.6±16.5	178.7±7.5	*0.125*
V-PREOP 6 µg/kg GM-CSF + CBDL	174.4±16.1	180.9±12.4	*0.025*
Total	176.6±18.2	177.8±13.7	*0.151*
P Value *(Kruskal Wallis Test)*	*0.131*	*0.005*	-

There was no significant difference between the groups with respect to body weights before the 1st operation; however, statistically significant differences were determined before the 2nd operation. Body weights were decreased in CBDL- and increased in GM-CSF-applied groups.
*: Animals in Group 0 were operated once. **CBDL: Common bile duct ligation.

Table 2. The incidence of bacterial translocation in specimens according to groups

Groups	Translocations							
	LN		Liver		Spleen		Total	
	+/-	%	+/-	%	+/-	%	+/-	%
0-Group 0	0/10	0.0	0/10	0.0	0/10	0.0	0/15	0.0
I-SHAM	6/4	60.0	3/7	30.0	3/7	30.0	12/18	40.0
II-SHAM + 6 µg/kg GM-CSF	2/8	20.0	2/8	20.0	2/8	20.0	6/24	20.0
IIA-SHAM + 1 µg/kg GM-CSF	3/7	30.0	3/7	30.0	3/7	30.0	9/21	30.0
III-CBDL*	7/3	70.0	7/3	70.0	3/7	30.0	17/13	56.7
IV-CBDL+ 6 µg/kg GM-CSF	3/7	30.0	3/7	30.0	3/7	30.0	9/21	30.0
IVA-CBDL+ 1 µg/kg GM-CSF	2/8	20.0	2/8	20.0	2/8	20.0	6/24	20.0
V-PREOP 6 µg/kg GM-CSF+CBDL	1/9	10.0	0/10	0.0	0/10	0.0	1/30	3.3
Total	24/51	32.0	20/55	26.7	16/59	21.3	60/165	26.7
Pearson X^2 test **P**	**0.024**		**0.028**		**0.554**		**0.000**	

The incidence of translocation in GM-CSF-given groups was lower than in controls with significant differences in LN and liver specimens. Bacterial translocation was prevented by GM-CSF administration before CBD ligation.
*CBDL: Common bile duct ligation. **LN: Lymph node.

Table 3. Kupffer cell incidence according to groups

Groups	Kupffer cells / (x1000)	
	Means ± SD	Range
0-Group 0	3.6 ± 0.5	3-4
I-SHAM	3.4 ± 0.5	3-4
II-SHAM + GM-CSF 6 µg/kg	7.6 ± 0.5	7-8
IIA-SHAM + GM-CSF 1 µg/kg	5.6 ± 0.5	5-6
III-CBDL	0.3 ± 0.5	0-1
IV-CBDL + GM-CSF 6 µg/kg	3.4 ± 0.5	3-4
IVA-CBDL + GM-CSF 1 µg/kg	1.5 ± 0.5	1-2
V-PREOP GM-CSF 6 µg/kg + CBDL	1.7 ± 0.5	1-2
Total	3.4 ± 2.3	0-8

Kruskal Wallis Test x^2:70.060; p: 0.000 p < 0.001

Pairwise significant differences according to post hoc test: 0-II, 0-IIA, 0-III, 0-IVA, 0-V, I-II, I-IIA, I-III, I-IVA, I-V, II-IIA, II-III, II-IV, II-IVA, II-V, IIA-III, IIA-IV, IIA-IVA, IIA-V, III-IV, III-IVA, III-V, IV-IVA, IV-V

The incidence of Kupffer cells was increased with GM-CSF administration in both SHAM and CBDL groups.
*CBDL: Common bile duct ligation.

(p<0.05, Table 2). The highest translocation incidence was seen in LNs of Group III (CBDL only), at 70.0%. There was no translocation in Group 0 (naive) and Group V according to liver and spleen cultures.

All of the animals in the SHAM groups showed translocation. Surgical trauma was a cause of BT in the LN, liver and spleen tissues. Biliary obstruction was also a cause of translocation in the LN and liver (RR of jaundice=1.2-2.3 fold) *(RR = RR in Group III / RR in Group I=70/60=1.2 for LN and RR=70/30=2.3 for liver).*

GM-CSF prevented translocation in LNs caused by operation (RE of GM-CSF in surgical trauma=3-fold) *(RE of GM-CSF = [(RR in Group II)$^{-1}$ / (RR in Group I)$^{-1}$] = (1/20) / (1/60) = RE=3.0).*

GM-CSF administration decreased BT ratios caused by obstructive jaundice in LN and liver (RE of GM-CSF in CBDL group=2.3-fold) *(RE of GM-CSF = [(RR in Group IV)$^{-1}$ / (RR in Group III)$^{-1}$] = (1/30)/(1/70) = RE=2.3).*

When administered preoperatively, GM-CSF prevented translocation in the liver completely (RE of preoperatively administered GM-CSF in CBDL group=7-fold - Completely) *(RE of preoperatively administered GM-CSF for liver = [(RR in Group V)$^{-1}$ / (RR in Group III)$^{-1}$] = (1/0)/(1/70)=RE=Completely)* and decreased translocation in LN tissue *[RE for LN (RR in Group V)$^{-1}$ / (RR in Group III)$^{-1}$] =(1/10)/(1/70)=RE=7.0).*

Histopathologic examinations

There were no specific histopathologic findings in spleen or LN specimens but findings were present in the liver (Figures 2, 3). In the liver specimens, the incidence of Kupffer cells was increased in SHAM groups with GM-CSF administration with 7-8 cells/x1000 area, and decreased in CBDL groups with 0-1 cell/x1000 area (Table 3, Figure 2) with significant differences (p<0.001). The decrease in incidence of Kupffer cells was prevented by GM-CSF in CBDL groups (Table 3, Figure 3).

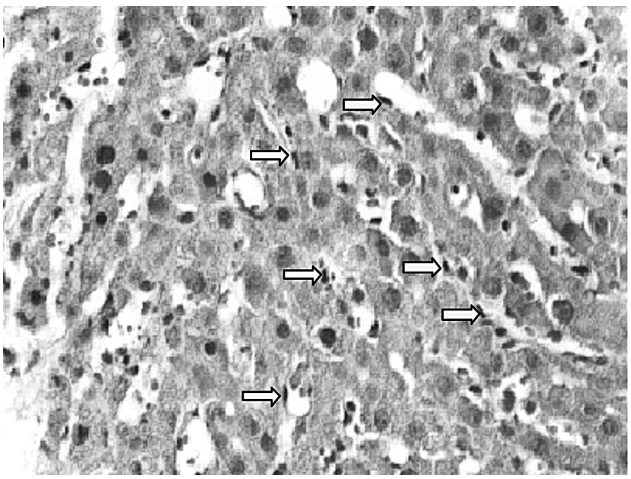

Figure 2. A liver specimen of SHAM + 6 µg/kg/d GM-CSF Group, with increased incidence of Kupffer cells (arrows) (hematoxylin and eosin, x 200).

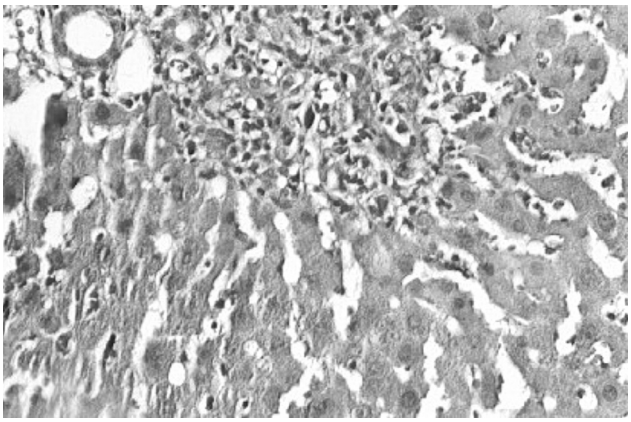

Figure 3. Ductular proliferation in a liver specimen of CBDL + 6 µg/kg/d x 7 days of GM-CSF Group (hematoxylin and eosin, x200).

Survival

There were statistically significant differences in survival among the groups according to both Kruskal Wallis and Kaplan-Meier tests ($p<0.001$ for each). The best (median: 20.0 days) survival was found in Groups 0 and V, at the end of experiment, while the worst survival was observed in the CBDL only group (Group III), with a median 11.5 days (Table 4, Figure 4). According to Table 4, a dose–response relation can be considered with respect to median survival.

DISCUSSION

The gastrointestinal tract is not only the organ responsible for nutrient absorption, it is also a metabolic and immunologic system, functioning as an effective barrier against endotoxin and bacteria in its lumen (46). There have been many disturbances in the homeostasis between the intraluminal toxins (bacteria, bacterial endo- and exotoxins) and the mucosal barrier, which consists of mucus secretion, proliferative epithelial cell replacement, intercellular tight junctions, membrane impermeability, active transport mechanisms, perfusion and integrity of mucosal barrier. These disturbances can cause systemic infections as clinical sepsis or sepsis syndrome, endotoxemia, hypermetabolic responses, and multiple organ dysfunction syndrome in patients with surgical trauma and jaundice (47, 48).

The limitations of the study were its relatively small sample size, contaminations during disintegration of specimens for routine microbiological procedures, the idea of "administration of human GM-CSF in rats", and lack of evaluation of Kupffer cell activity and functions, hepatic blood flow and diagnosis of the cause of mortality. In our study, fasting, general anesthesia, surgical trauma and obstructive jaundice were the possible causes of BT. We did not prefer intravenous or intraperitoneal anesthesia, because both may have caused other translocations. Obviously, sepsis and its complications were the major cause of mortality in CBDL rats. However, just before death, the cause of BT cannot be easily addressed in these groups and a series of confounding factors should be considered. To prevent the effects of confounding factors, we evaluated 7th day tissue translocations after SHAM or CBDL operations.

According to subjective evaluation of groups, postoperative activity in Group 0 and SHAM groups was similar to preoperative status except in CBDL groups. Animals in the CBDL group (Group III) showed decreased activity and weight and poor wound healing because of the aforementioned abnormalities. However, in Groups IV and V, no decreased activity, weight loss or impaired wound healing was observed. These subjective findings are similar to the literature.

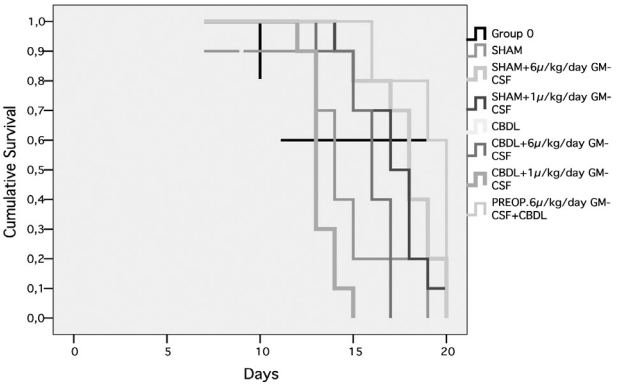

Figure 4. Survival of rats according to groups.
The best survival was determined in Group 0 and Group V, whereas the worst was in Group III. Kaplan-Meier logrank: 69.874; P=0.000<0.001.
CBDL: Common bile duct ligation.

Table 4. Survival of rats according to groups (days)

Groups	Survival mean ± SD	Median	Min - Max
0-Group 0	16.2 ± 5.2	20	10-20
I- SHAM	14.3 ± 3.4	14	7-19
II-SHAM + GM-CSF 6 µg/kg	17.8 ± 2.0	18	14-20
IIA-SHAM + GM-CSF 1 µg/kg	17.1 ± 1.9	17.5	14-20
III-CBDL	11.7 ± 1.9	11.5	9-15
IV-CBDL + GM-CSF 6 µg/kg	15.9 ± 1.3	16	13-17
IVA-CBDL + GM-CSF 1 µg/kg	13.3 ± 0.8	13	12-15
V-PREOP GM-CSF 6 µg/kg + CBDL	19.0 ± 1.6	20	16-20
TOTAL	15.6 ± 3.2	15	7-20
Kruskal Wallis Test P 0.000 < ***0.001***			
Pairwise differences according to post hoc test	*0-III, I-II, I-V, II-III, II-IVA, IIA-III, IIA-IV, III-IV, III-V, IVA-V*		

Not only surgical trauma but also obstructive jaundice decreased the survival of rats and GM-CSF increased the survival both in SHAM and obstructive jaundice groups with a parallelism of dose-response relation. The longest survival was seen in Group V, which was preoperatively administered GM-CSF, whereas the worst survival was in the CBDL group.
*CBDL: Common bile duct ligation.

Translocation ratios with surgical trauma and obstructive jaundice show a wide spectrum in the literature, principally resulting from the technical method of experiments, standardization problems and methods of evaluating translocation. The translocation ratio itself was evaluated in some studies, while in others colony forming units were evaluated. In one endotoxin study, translocation in mesenteric LN was 7.1% in SHAM, 64% in CBDL, and 28% in rhGH administered+CBDL (49), while in another, bacteria translocated in extraintestinal organs was observed in 70% of mice (50). In a non-lethal hemorrhagic shock study, translocation in mesenteric LN was 70% in fasted, 31.6% in fed, and 11.1% in fed+G-CSF-administered groups (4, 5). According to a study comparing enteral (EN) and parenteral (TPN) nutrition, the translocation ratios of mesenteric LN were reported as two-thirds in TPN, 33.3% in EN and 0.0% in control groups (51). In another experimental study, oral 4-day penicillin, clindamycin and metronidazole treatments promoted 100%, 97% and 62% of translocations to mesenteric LN, respectively (5). In CBDL rats, translocation ratios to mesenteric LN were reported as 62.5% and 0% in the control or SHAM-operated groups, respectively (52). According to another study, translocation through the thoracic duct in rats with E. coli infused to the CBD was reported as 40% with 300-1.7 x 10^7 colony forming units (53).

In our study, translocation ratio was found to be as much as 70.0% to mesenteric LN and liver in the jaundiced group (Group III), but in other groups, ratios were lower than those of Group III. There was no spontaneous translocation in Group 0. Both surgical trauma (in SHAM group, Group I) and CBDL (in Group III) caused translocations with different RRs, with statistically significant differences among the groups.

GM-CSF administration prevented translocations in SHAM groups (Group II, IIA) and also in obstructive jaundice groups (Groups IV, IVA). GM-CSF administration before CBDL also decreased the translocation ratios, and the lowest ratios were calculated in this group. There is no published study in the literature on this subject; we believe this observation shows the prophylactic effect of GM-CSF and the issue warrants further evaluation in new studies.

At first, our translocation ratios may be thought to be higher than those reported in the literature. However, these rats were operated twice in one week, meaning two fasting periods, two administrations of anesthesia and two surgical trauma episodes; thus, the high translocation ratios may be a result of the cumulative effects of these risk factors.

There is some information in the literature stating that G-CSF prevented translocation (54) and increased blood interferon (IFN)-α levels (55) in the cases with obstructive jaundice. However, there was no information on GM-CSF preventing translocation. We found an increase in the incidence of translocation in the SHAM group compared to Group 0, and possible causes of this increase are the same as mentioned above. “Surgical trauma causes high incidence of BT” (RR: 1.0-2.5).

In the patients with CBDL, incidences of sepsis, delayed wound healing and weight loss were increased. The negative effect of surgical trauma increased with obstructive jaundice. In CBDL animals, we observed weight loss and delayed wound healing in subjective evaluation, and high incidence of translocation in the jaundiced rats compared to the SHAM groups.

GM-CSF prevents translocation by enhancing immunologic functions, the stimulation of an enhanced rate of phagocytosis, induction of bactericidal or bacteriostatic mechanisms, or stimulation of an increased production of neutrophils and macrophages, and also by increasing the Kupffer cell count. Kupffer cell functions are endotoxin, cytokine, and bacteria scavenging, cytokine production, and O_2-derived radical production. In experimental models of sepsis, cytokine (TNF-α, IL-1, IL-6)- and O_2-derived radical production from Kupffer cells are increased and endotoxin, cytokine, and bacteria scavenging is overhelmed (14). According to one study, after CBD ligation, 33% of rats died within two weeks (56). Not only the number of Kupffer cells, but also activity (bacterial clearance, trapping, phagocytosis, killing ability, etc.) and function of the Kupffer cells and hepatic blood flow are important for host defense in individuals with jaundice (57-59). We showed an increase in the number of Kupffer cells in liver tissue of GM-CSF-administered rats and the survival of these animals was increased with a dose-response relation, probably by the combination of all the aforementioned mechanisms in our study. We did not hypothesize regarding the activity and function of the Kupffer cells or hepatic blood flow, since these issues have already been studied and will be studied further by researchers.

Based on the evidence of this study, GM-CSF may be used in selected individuals for the treatment and prophylaxis of BT caused by surgical trauma and obstructive jaundice, given the positive effects regarding increased physical activity, less weight loss or increase in body weight, better wound healing according to subjective evaluations, lower or no translocation, higher Kupffer cell incidence in liver, and better survival observed in the GM-CSF-administered jaundiced groups compared with controls. Further, adequate host defense can be provided by using GM-CSF before the surgical trauma or CBDL. However, it is suggested that GM-CSF should be used in patients with surgical trauma and obstructive jaundice after well-designed, Phase-II and III studies, clinically.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Experimental Laboratory of Ankara University, TIVAK, and the Laboratory of Clinical Pathology of Hacettepe University Hospital for their assistance in this study.

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