

DENEYSSEL TIKANMA İKTERİNDE NÖTROFİL FAGOSİTOZUNA GRANÜLOSİT KOLONİ SİTUMULAN FAKTÖRÜN (G-CSF) ETKİLERİ

EFFECTS OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) ON NEUTROPHIL PHAGOCYTOSIS DURING EXPERIMENTAL OBSTRUCTIVE JAUNDICE

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ÖZET

Amaç: Ekstrahepatik safra yollarının tıkanması immün sistem elemanlarının çoğunun deprese olmasına yol açar. G-CSF farklı durumlarda bozulan nötrofil fonksiyonlarını düzeltmektedir. Bu çalışmada, tıkanma ikterinde nötrofil fagositoz fonksiyonlarındaki değişiklikleri ve G-CSF uygulamasının bu fonksiyonlara etkisinin araştırılması planlandı.

Gereç ve yöntem: Sıçanlar 5 gruba ayrıldı: Sham grup (Grup 1), ve koledokun iki defa bağlanıp kesildiği diğer dört grup. Bu dört grubun 2'sine (Grup 3 ve 5) deney sırasında G-CSF verildi. Nötrofil fagositoz indeksi grup 2 ve 3'de 15. günde, grup 1, 4 ve 5'de 21. günde belirlendi.

Bulgular: Nötrofil fagositoz indeksi safra kanalı bağlanmasını takiben (Grup 2) 15. günde artarken 21. günde azaldı (Grup 4). G-CSF verilen gruplarda nötrofil fagositoz indeksinde 15. ve 21. günlerin sonunda artış saptandı (Grup 3 ve 5).

Sonuçlar: Sonuç olarak, nötrofil fagositoz indeksi, uzamış tıkanma sarılığı durumunda ileriki günler ve haftalarda G-CSF uygulamasıyla düzelmektedir.

Anahtar kelimeler: Tıkanma ikteri, Nötrofil fagositozu, G-CSF

SUMMARY

Background: Obstruction of the extrahepatic biliary tree produces profound depression of many components of the immune system. G-CSF improves diseased function of neutrophils in various conditions. In this study, we planned to investigate the changes on neutrophil phagocytosis in obstructive jaundice and the effect of G-CSF administration on this function.

Methods: Rats were divided into 5 groups as follows: the sham group and four other groups that underwent double ligation and division of common bile duct. Two of these four groups (Group 3 and 5) received G-CSF during experiment. Neutrophil phagocytosis index was determined for group 2 and 3 at the end of the 15 days and for group 1, 4 and 5 at the end of the 21 days.

Results: Neutrophil phagocytosis index significantly increased at the end of the 15th day after the bile duct ligation (Group 2) and significantly decreased at the end of the 21st day after the bile duct ligation (Group 4). Neutrophil phagocytosis index in G-CSF-treated groups was significantly increased at the end of the 15th days (Group 3) and increased at the end of the 21st day (Group 5).

Conclusions: As a result, neutrophil phagocytosis index is improved if G-CSF is administered later in the course of prolonged jaundice.

Key Words: Obstructive jaundice, Neutrophil phagocytosis, G-CSF

INTRODUCTION

Surgical intervention in patients with obstructive jaundice is associated with significant morbidity and mortality from sepsis, bleeding disorders and renal failure (1,2). The pathophysiological events causing these complications in obstructive jaundice are not well understood, though septic complications in jaundiced patients may well be caused by profound depression of many components of the immune system (3-5). In cholestatic patients, decreased reticuloendothelial system

functions, depressed nonspecific immunity, reduced T cell responses, portal and systemic endotoxemia, reduced bacterial clearance and elevated bacterial translocation might be observed besides structural hepatic injury, increased intestinal mucosal permeability and differentiation of intestinal and hepatic blood flow (3,5-11). Neutrophil chemotaxis and phagocytic function, intracellular bacterial killing function and superoxide generation of neutrophils are also thought to be impaired by obstructive jaundice (3,12,13). Improvement of liver

function by internal and external biliary drainage may have a therapeutic role in improving neutrophils and tissue macrophage phagocytic capacity (6,9,10). On the other hand, many human and animal studies have shown beneficial effects of G-CSF on neutrophil functions (14,15). This study was designed to investigate the changes in neutrophil phagocytosis and effects of G-CSF on this function during experimental biliary obstruction.

MATERIAL -METHOD

Experiment

This study was approved by the ethical committee of Kocaeli University and performed following standard guidelines for the care and use of laboratory animals. Albino rats of Wistar strain, handled in compliance with the Division of Experimental Medicine in Kocaeli University, Turkey, (175-225 g) were kept in stainless steel cages, given food and water ad libitum, and quarantined seven days before surgery. Food intake was stopped 10 hours prior to surgery, but free access to water was allowed. The animals were anesthetized with ketamine HCl (20 mg/kg, intraperitoneal) and xyalazine (15 mg/kg, intramuscular).

Group 1 (n=5) had sham operation and saline was intraperitoneally administered. Following midline laparotomy under sterile conditions, common bile duct was demonstrated and twenty-seven rats underwent double ligation and division of the common bile duct (CBDL) according to Lee's description (16). Abdominal layers were closed with appropriate suture materials. All animals were maintained under the same conditions after surgery. A semi-liquid diet and tap water were provided at the end of the postoperative 6th h. CBDL-groups were randomly divided into four groups. Group 2 and 4 were respectively observed until 15th and 21st day. One group of these animals (n=7) received one dose subcutaneous

injection of G-CSF (10 mg/ kg/ day) (Roche Co., İstanbul) on 11th, 12th, 13th, and 14th days (Group 3). The other eight CBDL animals received one close subcutaneous injection of G-CSF (10 mg/ kg/ day) on 17th, 18th, 19th and 20th days (Group 5). At the end of the 15th and 21st days of CBDL, blood samples of rats in CBDL and CBDL- G-CSF treated groups were taken by cardiac puncture, and animals were sacrificed. Same procedures without G-CSF administration were respectively made for other two groups which contain six rats in each. Cholestatic changes of the liver were evaluated by gross inspection and by microscopic examination of liver.

Studies on Neutrophil Function

White Blood Cell (WBC) Counts and Neutrophil Phagocytosis Index: Blood for blood smears and quantification of the absolute numbers of circulating leukocytes were obtained by intracardiac puncture. The total circulating white blood cell count/ mm³ was determined. Neutrophil phagocytosis index was determined by modified method of Penny et al (17).

Biochemical Evaluation

Blood samples were centrifuged (5000 rpm, 10 minutes) and serum samples were stored in -20°C until used. Bilirubin, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in blood were determined within two days of autopsy with CIBA Corning Extra Plus autoanalyser.

Statistical Analysis

The statistical analysis of biochemical parameters and WBC and neutrophil phagocytosis index among the groups were carried out using non-parametric ANOVA and Newman-Keuls Multiple Comparison Test. A p value <0.05 was considered to be statistically significant.

Table. ALP, AST and total bilirubin levels of the groups, and their differentiation.

| | GROUP 1 (n=5) | GROUP 2 (n=6) | GROUP 3 (n=7) | GROUP 4 (n=6) | GROUP 5 (n=8) |
|-----------------------|------------------|------------------|------------------|------------------|------------------|
| AST U/L | 11±3 | 561±147 | 593±130 | 1107±213 | 1247±168 |
| ALP U/L | 20±1.5 | 482±107 | 543±178 | 643±168 | 638±169 |
| Total Bilirubin mg/dl | 0.3±0.1 | 11±3 | 10±1 | 14±2 | 13±2 |

AST: p0.05 (Group 1-2, 1-3, 2-4, 3-4); p0.001 (Group 1-4, 1-5);

ALP: p0.05 (Group 1-2, 1-3, 1-5); p0.001 (Group 1-4);

Total bilirubin: p0.01 (Group 1-2, 1-3); p0.001 (Group 1-4, 1-5).

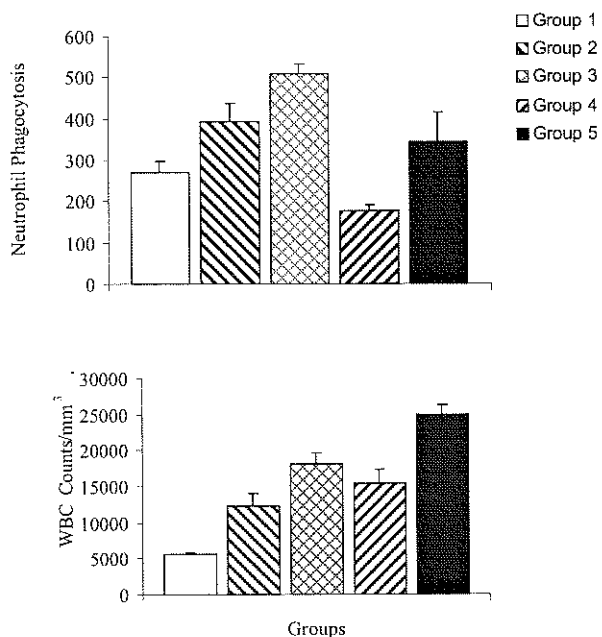


Fig. Comparisons of white blood cell count and neutrophil phagocytosis index of each group. Neutrophil Phagocytosis: $p < 0.05$ (Group 1-2, 1-4); $p < 0.001$ (Group 1-3, 2-4, 3-4); $p < 0.01$ (Group 1-5, 2-3, 3-5, 4-5) White Blood Cell Counts: $p < 0.05$ (Group 1-2, 1-3, 2-3, 3-4); $p < 0.001$ (Group 1-4, 1-5, 4-5, 2-5);

RESULT

The hair and the tails of the rats in group 2, 3, 4, and 5 turned to yellow and urine of these rats was darkened two days after bile duct ligation. Biochemical parameters of liver damage are summarized in table. Serum total bilirubin and AST levels in four CBDL groups were significantly increased when compared with the sham group ($p < 0.05$). AST levels were significantly different in group 2 compared to group 4 and group 3 compared to group 4 ($p < 0.05$). AST, ALP and bilirubin levels were not significantly different in group 2 compared to group 3 and group 4 compared to group 5 ($p > 0.05$).

WBC counts in group 2,3, ($p < 0.05$) 4, and 5 ($p < 0.001$) were significantly increased when compared with group 1. There was significant differentiation in group 2 and 4 when compared with group 5 ($p < 0.001$) (Fig).

When the neutrophil phagocytosis index values were compared, there was a significant difference between the sham group and CBDL-groups ($p < 0,05$). While neutrophil phagocytosis index in CBDL-groups increased until 15th day, neutrophil phagocytosis index increased by administration of G-CSF. When the common bile duct obstruction has continued, it decreased at the 21st day. There was significant difference between group 4 and 5 at the end of 21st days after bile duct ligation ($p < 0,001$). Although neutrophil phagocytosis index in group 4 decreased, it increased in group 5 by administration of G-

CSF until 21 days.

DISCUSSION

Obstructive jaundice is frequently associated with septic complications and renal disorders (18,19). Mortality following operations on the biliary tract varies from 9-27% in patients with obstructive jaundice, among whom wound infection, septicemia, and formation of abdominal abscesses are leading causes of death (4,20). The basis for this predisposition to infective complications is not understood, but may be due to alterations in host defense mechanisms (4,21).

Previous studies have demonstrated impaired reticuloendothelial functions in jaundiced-patients and animals (5,8). Reticuloendothelial system as a part of the host defense system is responsible for removing circulating particulate matter, such as bacteria, endotoxin, dead or damaged cells, and tissue debris (5). The exact pathophysiological mechanisms that cause these complications are, however, yet not fully understood, though septic complications in jaundiced patients may well be caused by reticuloendothelial dysfunction (22).

Septic complications has been considered to be the major causative factor for the morbidity and mortality noted following biliary tract surgery in jaundiced patients (12,21,23). Endotoxemia and bacterial translocation in obstructive jaundice are due to lack of gastro-intestinal bile flow (11). Studies demonstrated that cholestasis affects the systemic neutrophil functions by impeding chemotaxis, phagocytosis, and superoxide release, which are all critical in eliciting an adequate immune response (21). Phagocytosis is an energy-dependent process, which would be depressed by the antimetabolic effects of increased blood bilirubin levels which inhibit cellular respiratory enzymes (5,24). Serum inhibitory factors for neutrophils cannot be demonstrated in vitro (21).

It has been demonstrated that oral administration of bile salts decrease endotoxin absorption and inhibit bacterial translocation by prevention of bacterial growth (7,11). Improvement of liver function by therapeutic decompressive biliary drainage may have a therapeutic role in improving such impairments in neutrophils and tissue macrophage phagocytic capacity. Subsequent improvements of host defense may accordingly diminish the susceptibility of the cholestatic host to infective complications (21,25,26).

In literature, there is not any study which demonstrates the direct effects of G-CSF on neutrophil functions of jaundiced patients. We hypothesized that G-CSF may have beneficial effects on neutrophil functions in cholestatic rats and investigated its effect on neutrophil phagocytosis in common bile duct ligated rats. As demonstrated in this study, WBC counts increase together with elevated alkaline phosphatase, bilirubin and AST levels which indicate cholestasis and liver cell necrosis and these findings are

similar to previous studies (5,27).

In a previous study, enhanced neutrophil activity was observed within 12 hours of bile duct ligation and it remained increased during the 15 days (12). On the other hand, Rougheen et al (4) reported that neutrophil phagocytosis was impaired 21 days after the onset of obstructive jaundice. In the present study, we determined an increase in neutrophil phagocytosis index at the end of the 15 days and a decrease at the end of the 21 days. These data are similar to previous report of Analay et al (27). Differentiation of neutrophil phagocytosis at 15 and 21 days of obstructive jaundice may be due to changes of superoxide release of neutrophils. This hypothesis has to be studied.

Granulocyte-colony stimulating factor is a hematopoietic growth factor that supports the proliferation and differentiation of neutrophilic granulocytes in vitro and in vivo (28,29). This improvement may be obtained by an increase in the number of mitoses, size, basophilia, and cytoplasmic granulation of myeloblasts and promyelocytes, acting as a mediator of an early peripheral neutropenia and striking nuclear hypersegmentation (28). Beneficial effects of G-CSF were reported in various diseases (30-33). In two different studies, Canturk et al (14,15) previously reported that G-CSF improved neutrophil counts and phagocytosis in the experimental diabetic models. In the present study, we detected an increased neutrophil phagocytosis index in G-CSF administrated groups when compared with that of other groups. These data suggest that G-CSF increases neutrophil phagocytosis in all phase of obstructive jaundice and administration of G-CSF may improve immune resistance by normalization neutrophil functions from beginning of obstructive jaundice to further phase.

Finally, as stated above, the beneficial effects of G-CSF on neutrophil counts and phagocytosis were determined 15 and 21 days after performing CBDL. In the light of this suggestion, we recommend the use of G-CSF itself or together with replacement of bile salts or definitive surgical drainage from the beginning to further days or weeks of prolonged obstructive jaundice or chronic cholestasis. This approach may improve neutrophil functions and decrease postsurgical morbidity and mortality. Lack of any significant toxicity for G-CSF is also an important advantage for administration in malignant and even benign obstructive jaundice. Further studies measuring G-CSF levels are required for investigating host immune status either clinically or experimentally.

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