

RESEARCH ARTICLES

EVIDENCE THAT G-CSF DOES NOT INHIBIT BACTERIAL TRANSLOCATION IN AN ANIMAL MODEL OF LPS 055: B5 ENDOTOXEMIA

Erol EROĞLU, M.D., Canan AGALAR*, M.D., Çağrı ERGİN*, M.D., Koray OKUR, M.D.,
Fatih AGALAR, M.D.

Süleyman Demirel University, School of Medicine, Departments of Surgery and Microbiology*
Isparta, Turkey

Gazi Medical Journal 2001; 12: 1-4

ABSTRACT

Purpose: The effect of G-CSF on bacterial translocation in an experimental endotoxemia model with LPS 055: B5 (*Escherichia coli* lipopolysaccharide) was evaluated. **Methods:** 68 adult male mice were used in the study. Seventeen mice were allocated to each group. The first group received 1 ml of physiologic saline intraperitoneally (ip). In group II G-CSF (50 µg/kg) was given ip, in group III LPS 055: B5 (20mg/kg⁻¹) was given ip, and in group IV, 24 hours following the G-CSF application, 20 µg/kg of endotoxin was applied ip. Twenty-four hours later, midline laparotomy was performed under aseptic conditions and blood samples were collected by sterile cardiac puncture. Mesenteric lymph nodes, spleen and liver specimens were also removed and homogenised. Homogenates were placed on EMB and blood agar. After 24 and 48 h of aerobic incubation the plates were examined and bacteria identified using standard microbiological techniques. **Results:** Leukocyte count of group II, III and IV were significantly higher than that of the control group ($p < 0.05$ for all comparisons). In group IV, this was also significantly higher than in the other groups ($p < 0.05$). No colony-forming bacteria could be isolated from any of the samples obtained from animals in the control and G-CSF applied groups, which suggested the absence of bacterial translocation in these mice. In the endotoxemia groups bacteria (*E.coli*) were isolated from the blood and tissue specimens in 8 animals. In group IV (endotoxin+G-CSF), there were colony forming bacteria (*E.coli*) in 5 animals, but this finding was limited to the mesenteric lymph nodes. **Conclusion:** LPS 055: B5 endotoxemia causes translocation of *E.coli* from gut, and although G-CSF could not prevent the bacterial translocation, it prevented the systemic expansion of bacteria and limited the spread of bacteria in the MNLs.

Key Words: Endotoxemia, Bacterial Translocation, Granulocyte Colony Stimulating Factor.

INTRODUCTION

Infections leading to multiple organ failure are the most important complications in the care of critically ill patients, and septic shock still remains a leading cause of death in non-coronary intensive care units (1). Some fungi (2), active synthetic oligonucleotides or bacterial DNA (3) can initiate septic shock and multiple organ

failure as well as gram positive microorganisms (4). Predominantly gram-negative bacteria (5) and their endotoxins (6) are widely accepted as the cause of sepsis. Endotoxin is probably the primary initiator of septic shock. Although a large proportion of patients have non-gram negative sepsis and endotoxemia (7), 40 percent of patients with septic shock lack any pathogenic

micro-organisms in the blood or tissue (8,9). It is well known that the gastrointestinal tract is the main source of endotoxin (10). Due to injuries from blunt multiple trauma, burns intestinal ischemia and re-perfusion, food deprivation or exogenously introduced endotoxin, an increase in intestinal mucosal permeability may result in the pathological migration of live bacteria into the blood-stream as well as to gut-associated lymphoid structures (11).

G-CSF (granulocyte colony-stimulating factor), a glycoprotein that stimulates granulocyte colonies, has been successfully used to treat critical infections in neutropenic patients (12) and in some non-neutropenic conditions (13). Therefore, we studied the effect of G-CSF on bacterial translocation in an experimental endotoxemia model with LPS 055: B5 (Escherichia coli Lipopolysaccharide).

MATERIALS AND METHODS

68 adult male mice from the Suleyman Demirel University, Laboratory Animal Breeding Unit were used in the study. Animals were housed under conventional environmental conditions, at ambient temperature with free access to pellet rodent chow and tap water ad libitum in the experiment. All of the guiding principles in the care and use of the animals were strictly adhered to throughout the entire study.

A preliminary study was performed to obtain the sublethal endotoxic dosage of endotoxin (Escherichia coli Lipopolysaccharide LPS 055: B5) (Sigma, St Louis Missouri, USA) with ten animals. Seventeen mice were allocated to each group. The first group received 1 ml physiologic saline intraperitoneally (ip). In group II G-CSF (Neupogen, Roche, Switzerland) (50mg/kg) was given ip, in group III LPS 055: B5 (20mg/kg-1) was given ip and in group IV 24 hours after G-CSF application endotoxin was applied ip (Table 1).

24 hours later, animals were anaesthetised with ketamine (100mg/kg ip) + xylazine (4mg/kg ip) intraperitoneally and midline laparotomy was performed under aseptic conditions and blood

Table 1: Study groups.

Group I	(control) 0.9% NaCl, 1 ml ip
Group II	G-CSF ip
Group III	Endotoxin ip
Group IV	G-CSF ip + Endotoxin (24 h later) ip

samples were collected by sterile cardiac puncture. Samples of blood (1 ml) from each animal were placed in a blood culture bottle containing brain heart infusion (BHI) broth for aerobic growth and the bottles were incubated for 7 days at 37°C. Subsequent subcultures were performed using blood agar base after 48 h and cosin-methylene blue agar after 7 days. An additional 0.5 ml of blood was drawn to evaluate the peripheral leukocyte count. Mesenteric lymph nodes, spleen and liver specimens were removed, weighed and homogenised in 2 ml BHI broth with the sterile-scalpel method of homogenising tissue. Homogenates were placed on EMB and blood agar. After 24 and 48 h of aerobic incubation at 37 °C, the plates were examined and bacteria identified using standard microbiological techniques.

Statistical evaluation was done using Independent Student's t test and differences were accepted as statistically significant when the p value was less than 0.05.

RESULTS

In the endotoxin applied groups (n=34) no death was seen in animals due to endotoxemia during the experiment. After applying endotoxin animals became calm in the first 2 hours and piloerection developed in 6 hours. After piloerection animals lost their normal activity.

Leukocyte counts of group II, III and IV were significantly higher than the control group (p<0.05). In-group IV it was also significantly higher than other groups (p<0.05) (Table 2).

No colony-forming bacteria could be isolated from any of the samples obtained from animals in the control and G-CSF applied group, which indicated the absence of bacterial translocation in these mice (Table 3). In the endotoxemia group bacteria (E.coli) were isolated from blood and tissue specimens in 8 animals. In group IV (endotoxin+G-CSF) there were colony forming bacteria (E.coli) in 5 animals, but it was limited to mesenteric lymph nodes. In blood, liver and spleen samples no

Table 2: Leukocyte counts.

Groups	Leukocyte counts/mm ³ (mean ± sd)
I	2170 ± 666
II	5222 ± 1117
III	5033 ± 1877
IV	8620 ± 1819

Table 3: Incidences of the presence of bacteria in tissue samples.

Groups	Lymph nodes	Liver	Spleen	Blood
I	-	-	-	-
II	-	-	-	-
III	3	2	3	3
IV	5	-	-	-

bacteria could be isolated (Table 3).

DISCUSSION

It is widely accepted that any injury (including exogenously applied endotoxin (14)) that disrupts the integrity of intestinal mucosa may facilitate the migration of bacteria out of the lumen which then triggers the complex interactions between immunological defence mechanisms and invading pathogens (15). The translocation of pathogen bacteria itself is regarded as an important etiologic factor for the development of multiple organ failure in injured patients (11). In the presented study it was found that after E.coli lipopolysaccharide application, bacterial growth was seen in mesenteric lymph nodes, liver, spleen and blood cultures. Prevention of infection after major trauma or hemorrhagic shock offers the added benefit of reduction in both morbidity and the cost and complications as hospital care. G-CSF was used as a preventive agent in this study design. G-CSF was found to be efficacious in a model of sepsis involving cecal ligation and puncture in mice by increasing the survival rate when administered either before or at the time of cecal ligation and puncture (16). In another study G-CSF was shown to be effective as an additional element to the concept of prophylaxis (17).

The effect of G-CSF on bacterial translocation may be related to several mechanisms. G-CSF has some qualitative effects on peritoneal defense cells (e.g. macrophages, polymorphonuclear leukocytes). In this model it was seen that G-CSF could not prevent bacterial translocation, but it prevented the spreading of microorganisms to other organs (liver, spleen, blood). The beneficial effects of G-CSF on chemotaxis, neutrophil phagocytosis, and bactericidal activities were demonstrated by several studies (18, 19). In hemorrhagic shock models G-CSF was found to be effective in preventing bacterial translocation, and is believed to increase the efficiency of the killing activity of first-line defense cells, which in turn results in

better annihilation of the translocated bacteria (20). G-CSF can also augment the recruitment of neutrophils into mesenteric lymph nodes. It is well known that bacterial products and inflammatory mediators stimulate the production of G-CSF (13). In a study of Gross-Weege et al. it was shown that serum levels of G-CSF increased during the acute infectious process (21). Higher G-CSF levels were more frequently observed in patients without infectious complications and in patients who survived (21). Although the fact that G-CSF has been found to modulate the expression of surface FC receptors for Ig A (22), in our study G-CSF could not prevent the translocation of bacteria, but the peripheral leukocyte numbers were significantly increased in the endotoxin + G-CSF applied group. Gorgen I et al has shown that G-CSF prevented lethality induced by LPS endotoxin by stimulating alveolar macrophages, bone marrow macrophages, Kupffer cells or peritoneal macrophages (23). Mononuclear phagocytes, which are the most likely candidate for a first line encounter with the invading microorganisms, could prevent the systemic bacterial spreading through liver, spleen, blood and limited the bacteria to mesenteric lymph nodes in this study design. The doses of endotoxin given to mice should be the endpoint of this result.

In conclusion, LPS 055: B5 endotoxemia causes translocation of E.coli from gut and G-CSF could not prevent the bacterial translocation, but it prevented a systemic expansion of bacteria and limited the spread of bacteria to mesenteric lymph nodes.

Correspondence to: Erol EROĞLU, M.D.
Eski Sümerbank Cad. 28/1
32040 ISPARTA -TÜRKİYE
Phone: 0 246 - 232 25 82
Fax : 0 246 - 232 25 82
E-mail: heroleroglu@hotmail.com

REFERENCES

1. Davies MG, Hagen P-O. Systemic inflammatory response syndrome. *Br J Surg* 1997; 84: 920-935.
2. Bone RC. Gram negative sepsis. Background clinical features, and intervention. *Chest* 1991; 100: 802-808.
3. Sparwasser T, Mielhke T, Lipford G, Borschert K, Hacker H, Heeg K. Bacterial DNA causes septic shock. *Nature* 1997; 386: 336-337.
4. Bone RC. Gram positive organisms and sepsis. *Arch Intern Med* 1994; 154: 26-34.
5. Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115: 457-469.
6. Raetz CRH, Ulevitch RJ, Wright SD, Sibley CH, Ding A, Nathan CF. Gram-negative endotoxin: an extra ordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J* 1991; 5: 2652-2660.
7. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parillo JE. Endotoxemia in human septic shock. *Chest* 1991; 99: 169-175.
8. Gutierrez-Ramos JC, Bluethman H. Molecules and mechanisms operating in septic shock: lessons from knockout mice. *Immunol Today* 1997; 18: 329-334.
9. Morrison DC, Ryan JL. Endotoxins and disease mechanisms. *Annu Revc Med* 1987; 38: 417-432.
10. van Deventer SJH, Cate JW, Tytgat GNJ. Intestinal endotoxemia. Clinical significance. *Gastroenterology* 1988; 94: 825-831.
11. Swank GM, Deitch EA. Role of gut in multiple organ failure in bacterial translocation and permeability changes. *World J Surgery* 1996; 20: 411-417.
12. Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Tabbera I, Kris M, Grous J, Picozzi V, Rousch G, Smith R, Gradhisar W, Yakanda A, Vincent M, Stewart and Glaspy J. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. *New Engl J Med* 1991; 325: 164-170.
13. Nelson S. Role of granulocyte colony-stimulating factor in the immune response to acute bacterial infection in the non-neutropenic host: An overview. *Clin Infect Dis* 1994; 2: 197-204.
14. Deitch EA, Berg R, Specian R. Endotoxin promotes the translocation of bacteria from the gut. *Arch Surg* 1987; 122: 185-190.
15. Kavuklu B, Agalar C, Guc MO, Sayek I. Evidence that aminoguanidine inhibits endotoxin-induced bacterial translocation. *Br J Surg* 1998; 85: 1103-1106.
16. Deitch EA, Morrison J, Berg R and Specian RD. Effects of hemorrhagic shock on bacterial translocation, intestinal morphology, and intestinal permeability in conventional and antibiotic-decontaminated rats. *Crit Care Med* 1990; 18: 529-536.
17. Lorenz W, Reimund PK, Weitzel P, Celik I, Kurnatowski M, Schneider C. Granulocyte colony stimulating factor prophylaxis before operation protects against lethal consequences of postoperative peritonitis. *Surgery* 1994; 116: 925-934.
18. Sartorelli KH, Silver GM, Gamelli RL. The effect of granulocyte colony-stimulating factor (G-CSF) upon burn induced defective neutrophil chemotaxis. *J Trauma* 1991; 31:529-530.
19. Roilides E, Walsh TJ, Pizzo PA, Rubin M. Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils. *J Infect Dis* 1991; 163:579-583.
20. Agalar F, Iskit A, Agalar C, Hamaloglu E, Guc O. The effects of G-CSF treatment and starvation on bacterial translocation in hemorrhagic shock. *J Surg Res* 1998; 78: 143-147.
21. Gross-Weege W, Dumon K, Dahmen A, Schneider EM, Roher HD. Granulocyte colony-stimulating factor (G-CSF) serum levels in surgical intensive care patients. *Infection* 1997; 25: 213-216.
22. Simms HH, D'Amico R. Granulocyte colony-stimulating factor reverses septic shock induced polymorphonuclear leukocyte dysfunction. *Surgery* 1994; 115: 85-93.
23. Gorgen I, Hartung T, Leist M, Niehoster M, Tiegs G, Uhlig S, Weitzel F, Wendel A. Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharide-induced toxicity via suppression of systemic tumor necrosis factor-alpha. *J Immunol* 1992; 149: 918-924.