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ÖZGÜN ARAŞTIRMA/ORIGINAL ARTICLE

Yanık Sonrası Gelişen Bakteriyel Translokasyon Üzerine Amifostin'in Etkisi: Deneysel Çalışma

Effect of Amifostine on Bacterial Translocation After Burn Injury: An Experimental Study

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ÖZ

Amaç: Bağırsak epitel bariyerinin bozulmasının yanık yaralanmasını takiben meydana geldiği gösterilmiştir. Bu süreç, patojenlerin bağırsak lümeninden sistemik dolaşıma ve uzak organlara yer değiştirmesine yol açarak sepsis riskini artırır. Bu çalışmanın amacı, sıçan yanığı yaralanma modelinde amifostinin bakteriyel translokasyon üzerindeki etkisini incelemektir.

Gereç ve Yöntem: Toplam 27 erkek Wistar albino sıçanı dokuzlu üç gruba ayrıldı. Grup I bir kontrol grubuydu. Grup II ve grup III, toplam vücut yüzey alanının %30'u üzerinde üçüncü derece yanıklara maruz bırakıldı ve grup III'e intraperitoneal olarak 200 ml/kg amifostin uygulandı, ardından üçüncü derece yanıklardan sonra 10 ml/kg/gün idame dozu uygulandı. 48 saat sonra karaciğer, dalak, mezenterik lenf düğümleri ve çekumdan doku ve kan örnekleri alındı ve kültür ekimi yapıldı.

Bulgular: Kan kültürleri tüm gruplarda negatifti. Kontrol grubunda kolonizasyon sadece çekumda görülürken, grup II ve III'te karaciğer, dalak, mezenterik lenf nodları ve çekumda kolonizasyon tespit edildi. Bakteriyel kolonizasyon en sık çekum ve mezenterik lenf düğümlerinde bulunurken, grup II ve III arasında çekum (p = 0,298) ve mezenterik lenf düğümlerinde (p = 0,418) bakteri sayıları önemli ölçüde farklılık göstermedi.

Sonuç: Amifostin tek başına yanık yaralanmaları ile ilişkili bakteriyel translokasyonu kontrol etmede etkili değildir. Bakteriyel translokasyonu etkileyen bir dizi faktör olduğu için bu sonuçlar dikkatle yorumlanmalıdır.

Key Words:
Amifostine
Bacterial Translocation
Burns

ABSTRACT

Objective: Disruption of the intestinal epithelial barrier has been shown to occur following burn injury. This process can lead to translocation of pathogens from the gut lumen to the systemic circulation and distant organs thereby increasing the risk for sepsis. The aim of this study was to examine the effect of amifostine on bacterial translocation in a rat burn injury model.

Material and Method: A total of 27 male Wistar albino rats were divided into three groups of nine. Group I was a control group. Group II and Group III were subjected to third-degree burns over 30% of the total body surface area, and group III was administered amifostine 200 ml/kg intraperitoneally, followed by a 10 ml/kg/day maintenance dose after undergoing third-degree burns. After 48 hours, tissue and blood samples were obtained and cultured from the liver, spleen, mesenteric lymph nodes, and cecum.

Results: Blood cultures were negative in all groups. In the control group, colonization appeared only in the cecum, but in groups II and III, colonization was found in the liver, spleen, mesenteric lymph nodes, and cecum. While bacterial colonization was most frequently found in the cecum and mesenteric lymph nodes, bacterial counts did not significantly differ in the cecum (p = 0.298) and mesenteric lymph nodes (p = 0.418) between groups II and III.

Conclusion: Amifostine alone is not effective in controlling bacterial translocation associated with burn injuries. These results should be interpreted with caution as there are a number of factors that affect bacterial translocation.

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Objective

Burns, which are among the most common types of trauma, may result in death due to sepsis and infectious complications depending upon the severity of the injury (1). A systemic inflammatory response affecting distant organs may develop early after a burn (2). In addition to skin inflammation, irritation has also been reported in the lungs, liver, and intestines (3). Mesenteric vasoconstriction has reportedly developed in the intestines due to a burn, and that vasoconstriction resulted in a hypoxic environment around the intestines (4). Hypoxia causes oxidative stress, cell death, and an impaired epithelial barrier. As intestinal permeability increases, bacterial translocation develops in the mesenteric lymph nodes playing an important role in the pathogenesis of sepsis, which is the main cause of mortality in burn patients (5,6).

Amifostine, with an organic triphosphate structure, is thought to act as a free radical scavenger (7). Due to its cell protective effects, amifostine has been developed as a shielding agent against radiation and chemotherapy damage. Its polyamine-like structure and sulfhydryl group enable it to affect cellular processes and protect cells from the harmful effects of chemotherapeutics and ionizing radiation (8). It is thought that amifostine may also have anti-oxidant and cell protective effects on the blood-gut barrier. The effect of amifostine on bacterial translocation has been evaluated in a limited number of studies including one involving a radiation enteritis model (9).

In the present experimental study we aimed to evaluate the effect of amifostine on bacterial translocation induced by a burn injury. If this study demonstrates that amifostine prevents bacterial translocation, prophylactic use of amifostine in burn patients may reduce patients' comorbidity and reduce the secondary harmful effects of burn.

Material and Method

The study protocol was approved by the Baskent University School of Medicine Ethics Committee for Animal Experiments on 06/08/2013 (no. DA 13/05), which is in line with the National Institutes of Health Guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978). Animals were obtained from the Baskent University Production and Research Center. The experiments were performed at Baskent University School of Medicine Research Unit Laboratories. The study included 27 male Wistar albino rats weighing 180 to 350 g (mean: 286 g). All animals were cared for under optimal standard conditions.

Study Design

The rats were divided into three groups of nine rats as follows: group I (control group), group II (burn injury group) and group III (burn injury + amifostine treatment group).

After all rats in groups were anesthetized via intraperitoneal injection, the burn process was initiated by exposing the skin for ten seconds to a brass plate which had been heated for two minutes (Figure 1). The method reported by Gilpin et al. was used to calculate 30% of the body area (10). The third-degree burn was confirmed by histopathologic methods. Following the burn initiation in group III, a 200 mg/kg amifostine (Ethyol®, Er-Kim, Turkey) loading dose (intraperitoneal) followed by a 10 mg/kg/day (subcutaneous) maintenance dose was administered.

Figure 1: Third degree burn formation on rats

After 48 hours, following a sterile laparotomy, blood samples were taken from the portal vein and tissue samples were taken from the liver middle lobe, spleen, mesenteric lymph nodes, and cecum. Microbiological assessment was performed on the tissues and blood samples. When bacterial growth was observed, the type and number of colonies were recorded.

Microbiological Assesment

In order to prevent contamination in the microbiological examination, first blood and finally cecum samples were taken. Samples were placed in sterile 5 mL tubes containing thioglycollate broth (BD, USA). Samples other than blood were homogenized with tissue dissociator (gentleMACS Dissociator, Germany).

Blood samples, which taken from the portal vein of the rat were inoculated on blood agar, MacConkey Agara, two Scheadler Agara and Sabouraud Dextrose Agara using single spore method. Dilutions of tissue samples were obtained using a homogenizer. 100 microliter samples

from each dilution, were inoculated on blood agar, MacConkey Agara, two Scheadler Agara and Sabouraud Dextrose Agara using single spore method. One of the Schaedler Agar pairs was incubated under anaerobic conditions and the other under aerobic conditions for at least 48-72 hours at 37 °C. All other media were incubated at 37 °C for 24-72 hours under aerobic conditions.

Colonies growing on the plates were counted and typed using standard microbiological methods. The number of colony forming units (colony forming units-CFU) per gram of tissue was calculated according to the formula given below.

Number of bacteria (CFU/gram) = Number of bacteria in 1cc (CFU/ml) / Tissue weight (g)

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 9.0) program. The logarithmic transformation (log10) was performed because bacterial numbers varied widely among the groups. The data are expressed as the mean and standard deviation. A one-way ANOVA and *t* test were used to compare groups. A *P* value < 0.05 was considered significant.

Results

In the control group, there were no bacteria found in any area except the cecum. In the burn group, one rat had bacteria recurrence in the liver, one in the spleen, eight in the mesenteric lymph nodes, and nine in the cecum. Likewise, in the burn + amifostine group, one rat had bacteria in the liver, one in the spleen, eight in the mesenteric lymph nodes, and nine in the cecum (Table 1). The most frequently observed bacteria were *Escherichia coli* and *Enterococcus faecalis* (Table 2).

Table 1. Pathogen occurrence according to areas and groups.

	Control	Burn	Burn + Amifostine
Blood	-	-	-
Liver	-	1/9 (11%)	1/9 (11%)
Spleen	-	1/9 (11%)	1/9 (11%)
MLN †	-	8/9 (89%)	8/9 (89%)
Cecum	9/9 (100%)	9/9 (100%)	9/9 (100%)

†*MLN: Mesenteric lymph nodes*

In all groups the most frequent occurrence of bacteria was observed in the cecum; however, there was no difference between groups in terms of bacterial count (p = 0.298) (Table 3). It was observed that the most frequent occurrence of bacteria other than in the cecum was found in the mesenteric lymph nodes; however, no significant difference was observed between the groups (p = 0.418).

Discussion

It has been shown that after burns injuries, intestinal permeability leads to increases in bacterial translocation to mesenteric lymph nodes or distant organs and bacterial translocation is in turn associated with sepsis and mortality (11). In our study for the first time, the effect on bacterial translocation in a burn model treated with amifostine was evaluated. Although amifostine has cell-protective and anti-oxidant properties, it has not been found to be effective in bacterial translocation.

Bacterial translocation has been described in association with ileus, colorectal cancer, cirrhosis, obstructive hepatitis, acute pancreatitis, abdominal surgeries, bowel transplantation, hemorrhagic shock, and heart diseases (5,12-14). Despite the fact that the presence of bacterial translocation has been confirmed in a number of studies, only a few studies have identified factors that have an impact on bacterial translocation. Among these identified factors, immunodeficiency is considered to be most important. Vaishnavi stated that bacterial translocation is constantly seen in healthy individuals but only becomes clinically important when immunity is inadequate (15). In addition to insufficient immunity, factors such as changes in the normal ecological balance of the gut, barrier permeability, trauma, and oxidative stress have also been implicated in bacterial translocation. It was hypothesized that amifostine would have an effect on bacterial translocation due to its anti-oxidant properties; however, a positive result was not obtained from our tissue samples. The most likely reason for these results is that bacterial translocation has a multi-dimensional etiology. Since this was a pilot study, our findings should be interpreted cautiously.

The effect of amifostine on bacterial translocation has only been assessed in one study thus far to the best of our knowledge. Recently, the study by Taş et al. evaluated the effect of amifostine on bacterial translocation created by radiation enteritis (9). In their study, amifostine did not have an effect on bacterial translocation when administered at the same dosage and in a similar manner as in our study. However, unlike in our study, amifostine reportedly reduced bacterial overgrowth. Amifostine has the capacity to repair DNA fragmentation after radiation damage, which can explain this difference (16). Taş et al. concluded that amifostine alone is not sufficient to prevent bacterial translocation (9).

Table 2. Pathogen analysis of tissue cultures.

	Test subject	1	2	3	4	5	6	7	8	9
LIVER	Control	-	-	-	-	-	-	-	-	-
	Burn	-	-	-	-	-	*E. faecalis* 11	-	-	-
		-	-	-	-	-	-	-	-	-
	Burn	-	-	*E. coli* 6	-	-	-	-	-	-
	+Amifostine	-	-	*E. faecalis* 6	-	-	-	-	-	-
SPLEEN	Control	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-
	Burn	-	-	-	-	-	*E. faecalis* 28	-	-	-
		-	-	-	-	-	-	-	-	-
	Burn	-	-	-	-	-	-	-	*E. coli* 4	-
	+Amifostine	-	-	-	-	-	-	-	-	-
MLN†	Control	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-
	Burn	*E. coli* 157	*E. coli* 6	*E. coli* 24	*E. faecalis* 4	*E. faecalis* 3		*E. faecalis* 21	*E. coli* 31	*E. faecalis* 10
		E. faecalis 19	*E. faecalis* 56	*E. faecalis* 73						
	Burn	*E. coli* 40	*E. coli* 17	*E. coli* 16	*E. faecalis* 13		*E. faecalis* 4	*E. faecalis* 4	*E. coli* 3	*E. coli* 13
	+Amifostine			*E. faecalis* 80					*E. faecalis* 3	
CECUM	Control	*E. coli* 5.952	*E. coli* 95	*E. coli* 41.026	*E. coli* 9.615	*E. coli* 1.238	*E. coli* 114	*E. coli* 534	*E. coli* 64.815	*coli* 6.250
		E. faecalis 20.833	*E. faecalis* 269	*E. faecalis* 15.385	*KNS* 19.231		*E. faecalis* 170	*E. faecalis* 76	*E. faecalis*	*E. faecalis* 2.500
	Burn	*E. coli* 2.121	*E. coli* 123	*E. coli* 839	*E. coli* 1.200	*E. coli* 1.600	*E. coli* 3.000	*E. coli* 625	*E. coli* 833	*E. coli* 250
		E. faecalis 1.818	*E. faecalis* 123	*E. faecalis* 140		*E. faecalis* 240	*E. faecalis* 5.000	*E. faecalis* 15.625	*E. faecalis* 4.167	
	Burn	*E. coli* 160.000	*E. coli* 240.000	*E. coli* 30.000	*E. coli* 15.000	*E. coli* 33.333	*E. coli* 50	*E. coli* 50	*E. coli* 200	*E. coli* 85.714
	+Amifostine	*E. faecalis* 600.000		*E. faecalis* 10.000		*E. faecalis* 33.333	*E. faecalis* 50	*E. faecalis* 50	*E. faecalis* 467	*E. faecalis* 57.143

†*MLN: Mesenteric lymph nodes.*

Table 3. Comparison of groups in terms of pathogens recurrence in the cecum.

Group	Average number of bacteria	
	MLN†	Cecum
Control		3.70 ± 0.97
Burn	1.36 ± 0.64	3.30 ± 0.63
Burn + Amifostine	1.13 ± 0.48	4.10 ± 1.46
P-value	0.418	0.298

†*MLN: Mesenteric lymph nodes*

The limitation of our study is the lack of pathological assessment of the tissue samples taken. In future studies, the antioxidant effect of amifostine can be demonstrated with pathological examination and its preventive effect on bacterial translocation can be evaluated.

As a conclusion in the prevention of bacterial translocation produced by a burn model, amifostine has limited activity. We found that despite its cell-protective and antioxidant properties, amifostine is not effective in reducing bacterial translocation associated with burn injury in a rat model.

Yazarlık Katkısı: Fikir/Hipotez: ErK Tasarım: NA Veri toplama/Veri işleme: EmK Veri analizi: EmK Makalenin hazırlanması: NA, EmK

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References

1. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006;19:403-434.
2. Stoecklein VM, Osuka A, Lederer JA. Trauma equals danger–damage control by the immune system. J Leukoc Biol 2012; 92:539-551.
3. Shankar R, Melstrom KA, Gamelli RL. Inflammation and sepsis: past, present, and the future. J Burn Care Res 2007;28:566-571.
4. Magnotti LJ, Deitch EA. Burns, bacterial translocation, gut barrier function, and failure. J Burn Care Rehabil 2005;26:383-391.
5. MacFie J, O'Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P. Gut origin of sepsis: a prospective study investigating associations between bacterial translocation, gastric microflora, and septic morbidity. Gut 1999;45:223-228.
6. Choudhry MA, Rana SN, Kavanaugh MJ, Kovacs EJ, Gamelli RL, Sayeed MM. Impaired intestinal immunity and barrier function: a cause for enhanced bacterial translocation in alcohol intoxication and burn injury. Alcohol 2004;33:199-208.
7. Nicolatou-Galitis O, Sarri T, Bowen J et al. Systematic review of amifostine for the management of oral mucositis in cancer patients. Support Care Cancer 2013;21:357-364.
8. Grdina DJ, Kataoka Y, Murley JS. Amifostine: mechanisms of action underlying cytoprotection and chemoprevention. Drug Metabol Drug Interact 2000;16:237-279.
9. Tas S, Ozkul F, Arik MK, Kiraz A, Vural A. The effect of amifostine on bacterial translocation after radiation induced acute enteritis. Acta Cir Bras 2016;31:156-160.
10. Gilpin DA. Calculation of a new Meeh constant and experimental determination of burn size. Burns 1996;22:607-611.
11. Earley ZM, Akhtar S, Green SJ et al. Burn Injury Alters the Intestinal Microbiome and Increases Gut Permeability and Bacterial Translocation. PLoS One 2015;10:e0129996.
12. Kompan L, Kremzar B, Gadzijev E, Prosek M. Effects of early enteral nutrition on intestinal permeability and the development of multiple organ failure after multiple injury. Intensive Care Med 1999;25:157-161.
13. Wilmore DW, Smith RJ, O'Dwyer ST, Jacobs DO, Ziegler TR, Wang XD. The gut: a central organ after surgical stress. Surgery 1988;104:917-923.
14. Carrico CJ, Meakins JL, Marshall JC, Fry D, Maier RV. Multiple-organ-failure syndrome. Arch Surg 1986;121:196-208.
15. Vaishnavi C. Translocation of gut flora and its role in sepsis. Indian J Med Microbiol 2013;31:334-342.
16. Santini V. Amifostine: chemotherapeutic and radiotherapeutic protective effects. Expert Opin Pharmacother 2001;2:479-489.