Original article

Effects of H_2 Receptor Blocking Agents on Bacterial Translocation in Burn Injury*

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Summary

We experimentally studied the effects of H_2 receptor blockers (ranitidine) on bacterial translocation (BT) in 42 male albino rats. Sham group (Group I, n = 12 rats) were exposed to 21 °C water while Burn group (Group II, n = 15 rats) and Ranitidine group (Group III, n = 15 rats) were exposed to 90 °C hot water for 10 seconds to produce a full thickness burn in 30 % of total body surface area. 300 mg/kg ranitidine was administered to Group III starting immediately after the burn injury. Rats were sacrificed on the fifth postburn day. Sham group gained weight while groups II and III had significant weight loss. Gastric pH increased with the administration of ranitidine. Both gram negative and total number of bacteria were found to be reduced in cecal stool cultures in Ranitidine group. Significant increase in BT was observed in Group III, and translocating bacteria were found to be different in Burn and Ranitidine groups with a final conclusion that administration of ranitidine changes intestinal ecological equilibrium and promotes BT.

Key words: Burn injury – Ranitidine – H_2 receptor blockers – Bacterial translocation

Résumé

Nous avons étudié les effets du blocage récepteur H_2 (ranitidine) sur la translocation bactérienne (BT) chez 42 rats mâles albinos. Un groupe témoin (Groupe 1, n = 12 rats) fut trempé dans de l'eau à 21° cependant que le groupe brûlé (Groupe 2, n = 15 rats) et le groupe ranitidine (Groupe 3, n = 15 rats) ont été exposés à de l'eau chauffée à 90° pendant 10 secondes pour produire une brûlure de pleine épaisseur sur 30 % de la surface totale. 300 mg/kg de ranitidine ont été administrés au groupe 3, en débutant immédiatement après la brûlure. Les rats ont été sacrifiés au 5ème jour postbrûlure. Le groupe témoin avait pris du poids cependant que les groupes 2 et 3 avaient une perte de poids significative. Le PH gastrique avait augmenté avec l'administration de ranitidine. L'existence de bactéries gram négatives et le nombre total de ces bactéries a été trouvé diminué dans les cultures de selles cœcales dans le groupe ranitidine. Une augmentation significative en BT a été observé dans le groupe 3 et les bactéries transloquées ont été trouvées différentes dans le groupe de brûlés et dans le groupe ranitidine avec la conclusion finale que l'administration de ranitidine modifie l'équilibre écologique intestinal et favorise la translocation bactérienne (BT).

Mots-clés: Brûlure, ranitidine – Bloqueur de la réception H_2 – Translocation bactérienne

Zusammenfassung

Experimentell wurde die Wirkung von H_2-Rezeptorenblockern (Ranitidin) auf die bakterielle Translokation bei 42 männlichen Albinoratten überprüft. Die Vergleichgruppe (Gruppe I, n = 12 Ratten) wurde einer Temperatur von 21 Grad im Wasserbad ausgesetzt, während die Tiere mit den Verbrennungen (Gruppe II, n = 15 Ratten) sowie die Ranitidin-Gruppe (Gruppe III, n = 15 Ratten) einer Temperatur von 90 Grad heißem Wasser für 10 Sekunden ausgesetzt wurden. Dies verursachte eine drittgradige Verbrennung von 30 % der Körperoberfläche. 300 mg/kg Körpergewicht Ranitidin wurden der Gruppe III sofort nach dem Verbrennungstrauma gegeben. Die Ratten wurden am 5. posttraumatischen Tag getötet. In Gruppe II und III fand sich ein signifikanter Gewichtsverlust im Gegensatz zur Kontrollgruppe. Der Magen-pH stieg mit der Gabe von Ranitidin an. Bei den Ratten der Ranitidin-Gruppe waren gramnegative Bakterien sowie die Gesamtzahl der Bakterien im zökalen Stuhl herabgesetzt. Es fand sich zudem eine signifikante Zunahme der bakteriellen Translokation in der Gruppe III und eine unterschiedliche Translokation der Bakterien bei den Ratten mit isolierter Verbrennung und der Ranitidin-Gruppe. Dies läßt die Schlußfolgerung zu, daß die Gabe von Ranitidin das intestinale, ökologische Gleichgewicht verändern kann und eine bakterielle Translokation fördert.

Schlüsselwörter: Verbrennungstrauma – Tiermodell – Ranitidingabe – H_2-Rezeptorenblocker – Bakterielle Translokation

Introduction

Sepsis still continues to be a major reason for morbidity and mortality despite progress in the management of burn injury (7, 9, 16, 20, 26). It has been clinically shown that, blood endotoxin levels rise in the early periods of burn trauma although the wound is sterile, and gram negative wound infection can be detected on the 4th – 7th postburn days (5). Studies have shown that the gastrointestinal mucosal barrier is damaged following burn injury, further leading to translocation of enteric bacteria which is responsible for the rise in blood endotoxin levels. Increase in

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cellular reabsorbtion of H^+ ions from the disturbed gastrointestinal mucosa is responsible for the development of gastric and duodenal so called Curling ulcers in burn patients (2, 21). H_2 receptor blockers used in the prophylaxis of those ulcers decrease the cellular reabsorbtion of H^+ ions. On the other hand, it is supposed that they are responsible for the change in microflora and abnormal bacterial colonization due to increase in gastric pH, leading to bacterial translocation (BT) (4, 14).

Material and methods

Forty-two male albino rats, weights ranging between 170–230 grams, were used in this study. All animals were maintained following the guidelines of the Surgical Research Committee of the Ege University, and the experimental studies were performed with the approval of the Ethical Committee of Surgical and Medical Researches of the same University. Rats were brought to our Surgical Research Laboratory four days prior to experiment in order to adapt to the environment and were fed with rat chow regularly. Animals were randomly assigned to three groups: Sham group (Group I, n = 12), Burn group (Group II, n = 15) and Ranitidine group (Group III, n = 15). Rats were anesthetized by intramuscular (i.m) injection of 50 mg/kg ketamine sulfate. Total body surface areas (TBSA) were calculated using the formula TBSA (cm^2) = 9.1 X $(weight)^{2/3}$ by *Horst* et al (11) to obtain a 30% burn of TBSA.

The method used to achieve burn trauma was adopted from the model used by *Walker* and *Mason* (12). The backs were shaved to allow direct skin contact between hot water. Group I were exposed to 21 °C water, while Group II and Group III were exposed to 95 °C water for 10 seconds to produce a full thickness burn injury in 30% of TBSA. All rats were resuscitated with intraperitoneal injection of 25 ml/kg saline following burn injury, and were allowed to feed with water and standard rat chow after recovering from anesthesia. Intramuscular injection of ranitidine (300 mg/kg/day) was started immediately in Group III in two split doses while the same volume of NaCl 0.9% was injected in the other two groups.

All three groups were sacrificed on the fifth postburn day. Laparotomy was performed under i.m. ketamine anesthesia and 0.5 ml blood sample was drawn from inferior vena cava for analysis. Mesenteric lymph nodes (MLN), spleen, liver and cecum were removed for quantitative cultures of translocating bacteria. A 5 cm. distal ileal segment from ileocecal junction was resected, the lumen was irrigated with sterile saline, and the wall of the segment was transected from the antimesenteric border for pathological analysis. Stomachs of all groups were opened from the lesser curvature and gastric pH was measured by pH meter (Sesa model 1400). Mesenteric lymph nodes (MLN), spleen, liver and cecum were homogenized. Blood and homogenized samples were prepared for aerobic and anaerobic cultures. The types and quantitative amounts of bacteria per gram/tissue were estimated 24 - 48 hours later. Presence of 100 or more bacteria/gram tissue - ml was accepted as the criteria for translocation. Ileal segments were analyzed pathologically and 5 different villus lengths were measured for each specimen after which average villus length was calculated.

Fisher's exact and chi square tests were used for statistical analysis of the incidence of bacterial translocation and the differences among translocating bacteria, while data about weights, gastric acid levels were analyzed by using *Kruskal-Wallis* variance analysis. Quantitative results of cultures, and villus lengths were analyzed by *Mann-Whitney* U and *Student*-t tests respectively.

Results

No wound infection was observed in any of the three groups. Mean weight loss and gain for the groups are plotted in Figure 1. Sham group (Group I) gained 12.91 ± 4.3 grams at the end of 4 days while burn group (Group II) lost 18.33 ± 4.87 grams. Mean weight loss for burn + ranitidine group (Group III) was 31.33 ± 4.34 grams. Group III had marked cachexia. The results were statistically significant for all groups ($p < 0.01$).

Figure 2 shows mean gastric pH for the three groups. Difference between Group I and Group II was statistically insignificant ($p > 0.05$) while gastric pH for Group III was significantly comparable to Groups I and II ($p < 0.01$).

Bacterial translocation was not observed in the Sham group. Incidence of BTwas 3/15 (20%) for Group II and 7/15 (48%) for

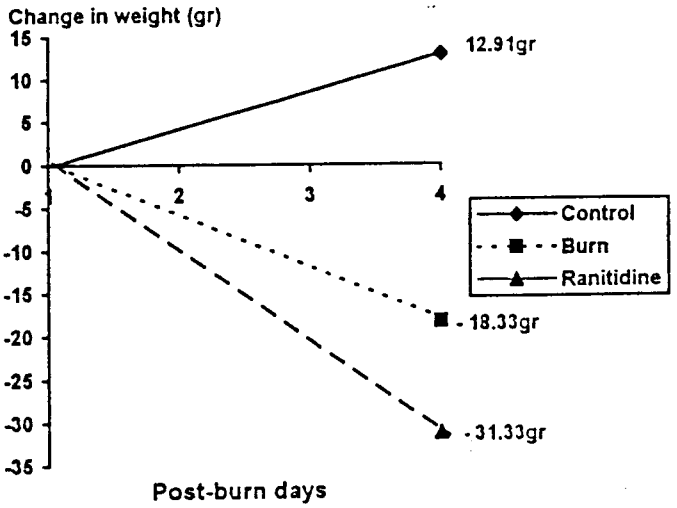

Fig. 1 Weight changes in groups.

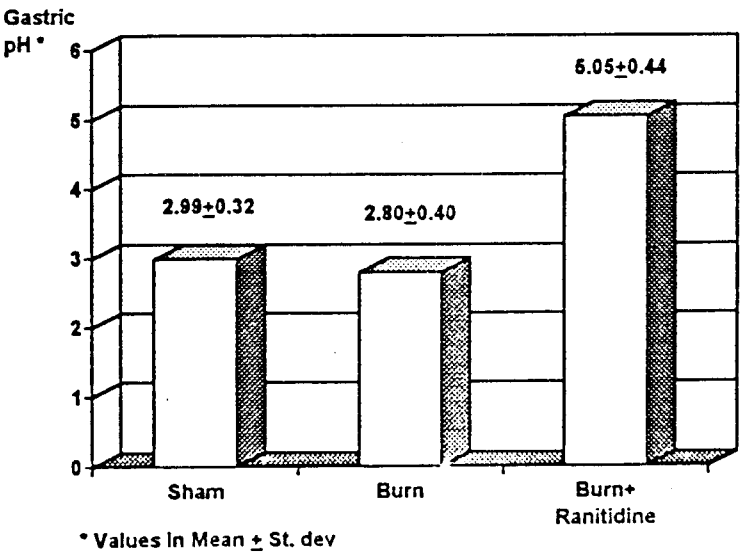

Fig. 2 Mean gastric pH for three groups.

Table 1 Incidence of bacterial translocation according to the sites of isolation.

	Incidence of BT	MLN	Spleen	Liver	Blood
Sham	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
Burn	3/15 (20%)	3/15 (20%)	1/15 (6.6%)	2/15 (13.3%)	0/15 (0%)
Burn + ranitidine	7/15 (48%)*	6/15 (40%)**	2/15 (13.3%)	2/15 (13.3%)	0/15 (0%)

* $p<0.01$ compared to Control Group
** $p<0.05$ compared to Control Group

Table 2 Quantitative results of cecal cultures.

	Total	Gram negative	Gram positive
Control	8215±3037	6551±3738	1663±1665
Burn	7672±5430		6480±5356
2214±1531			
Ranitidine	3937±3292*		2980±2217*
1104±1366**			

colony forming units (CFU)/gram cecal content ± St. dev.
* $p<0.01$ compared to Control and Burn Groups
** $p<0.05$ compared to Burn Group

Group III. The results for translocation were not significant between Group I and II ($p > 0.05$), while significant increase in BT was observed in Group III in comparison with Group I ($p < 0.01$). The incidence was comparable between Groups II and III ($p > 0.05$). Table 2 shows the incidence of translocation according to the sites of isolation. The main site of translocation was mesenteric lymph nodes. Significant increase in translocation to MLN was observed in Group III compared with Group I ($p < 0.01$), while it was statistically insignificant for Group II ($p > 0.05$). Incidence of translocation to spleen and liver was statistically insignificant for Groups II and III ($p > 0.05$). No microorganism could be isolated from blood samples.

Proteus, the major microorganism isolated in Group II, constitutes 72% of all translocating bacteria in the burn group. On the contrary, E. coli was isolated from all cultures in Group III. Bacteroides sp accompanied E. coli in only one rat in the ranitidine group. Bacteroides was the only anaerobic microorganism among the translocating bacteria. Significant increase in the translocation of E. coli was seen with the administration of ranitidine ($p < 0.05$).

There was no difference between Groups I and II in respect of cecal cultures ($p > 0.05$), but a significant decrease in total number of bacteria and total number of gram negative microorganisms was observed in cecal cultures with the administration of ranitidine ($p < 0.01$) (Table 2).

No significant change in villus lengths could be demonstrated in all three groups ($p > 0.05$).

Discussion

Experimental studies have shown that mesenteric blood flow falls by more than 50% in the first 8 hours, and rises to control levels after 20 hours following burn injury (17). The maximal incidence of BT occurs in the 24–48 hours post-burn. It is considered that damaging of the mucosal barrier due to ischemia reperfusion injury promotes bacterial translocation, and attempts to prevent intestinal ischemia by aggressive fluid resuscitation, inhibitors of xantine oxydase, and vasodilators reduce BT (6, 11, 19). Mucosal repair is completed within four days if burn injury is not complicated by any other factor, and translocated bacteria disappear from the MLN (10). Improper fluid resuscitation, infection, and protein malnutrition leads to continuation of mucosal damage and increase in BT. Ecological equilibrium of intestinal microflora changes with the administration of total parenteral nutrition, hyperosmolar diets, and broad spectrum antibiotics, facilitating BT from the damaged mucosa (3, 4). H_2 receptor blockers influence intestinal microflora by a similar mechanism and increase BT (4).

Wound infection leads to prolonged mucosal damage and increased BT in burn injury. No wound infecion was observed in the three groups in the series, and villus lengths were identical for all three groups. Therefore rats were sacrificed at the end of four days presuming that mucosal repair should be complete, and thus the incidence of BT should be expected to decrease by the fifth day. Indeed, significant increase in BT was observed with the administration of ranitidine although total number of bacteria were diminished in cecum. Translocation of different groups of bacteria in burn and ranitidine groups clearly shows that an ecological imbalance develops leading to colonization of more pathogenic microflora. H_1 antagonists are known to have an antimicrobial effect (13), and ranitidine may be acting in a similar way in reducing bacterial population while increasing endotoxins and other bacterial products. The term "bacterial translocation" includes the translocation of endotoxins and other bacterial deposits as well as translocation of live bacteria from the intestinal barrier, resulting in a wide spectrum of clinicopathological changes further leading to multiple organ failure (4, 14, 23). The increase in endotoxins in the intestinal system leads to activation of *Kupffer* cells and hepatic dysfunction (1). Production of tumor necrosis factor (TNF) increases with the activation of *Kupffer* cells. Bacterial translocation in burn injury elevate the production of m-RNA responsible for the synthesis of TNF α / cachexin (15). We could not measure blood endotoxin levels in our study, but we have noted a significant loss of weight in the ranitidine group which could be regarded as an indirect finding of the elevation of endotoxin levels in the portal system. Increase of endotoxin levels enhances the production of TNF, leading to cachexia become apparent.

Histological examination of intestinal mucosa revealed no change in villus lengths and morphologies. Therefore, we think ranitidine has no additive effect on mucosal injury after burn trauma, and the increase in BT with the administration of ranitidine is not due to the damage in the mucosal barrier. H_2 receptor blockers have an inhibiting effect on gastric secretions as well as some negative effects on pepsin and other exocrine secretions (12). Decrease in intestinal secretions reduces intestinal peristaltism which further leads to overgrowth of enteropathogens together with condensation of intraluminal endotoxins and translocation.

H_2 receptor blockers suppress T-suppresser cells which are responsible for immune deficiency in burn trauma (18, 25) hence, we do not think that BT with ranitidine is due to burn-induced immune deficiency.

We conclude that ranitidine enhances bacterial translocation by changing the ecological equilibrium in the intestinal microflora. Changing ecological balance promotes colonization of more pathogenic bacteria which can cross the intestinal barrier more

easily. These findings show that prophylactic use of ranitidine in burn trauma may be hazardous by causing BT. Mucosal protective agents, such as sucralfate, should be preferred if necessary.

References

[1] *Billiar JR, Maddaces MA, Wesil MA:* Intestinal gram negative bacterial overgrowth in vivo augments the in vitro response of Kupffer cells to endotoxin. Ann Surg 208 (1988) 532–540

[2] *Cheung L, Chang N:* The role of gastric mucosal blood floow and H^+ backdiffusion in the pathogenesis of acute gastric erosions. J Surg Res 22 (1977) 357–361

[3] *Deitch EA, Maejimo K, Berg R:* Effect of oral antiobiotics and bacterial overgrowth on the translocation of GI tract microflora in burned rats. J Trauma 25 (1985) 385–392

[4] *Deitch EA:* The role of the intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. Arch Surg 125 (1990) 403–404

[5] *Dobke MK, Simoni J, Ninnemann TJ,* et al: Endotoxemia after burn injury. Effect early excision on circulating endotoxin levels. J Burn Care Rehabil 10 (1989) 107–111

[6] *Fukushima R, Gianotti L, Alexander JW,* et al: The degree of bacterial translocation is a determinant factor for mortality after burn injury and is improved by prostoglandin analogs. Ann Surg 216 (1992) 438–445

[7] *Herndon DN, Zeigler ST:* Bacterial translocation after thermal injury. Critical Care Med 1993 50–54

[8] *Horst K, Mendel LB, Lafayette F,* et al: The metabolism of the albino rat during prolonged fasting at two different environmental temparatures. J Nutr 3 (1930) 177–200

[9] *Housinger TA, Brinkerhoff C, Warden GD:* The relationship between platelet count, sepsis and survival in pediatric burn patients. Arch Surg 128 (1993) 65–67

[10] *Jones II WG, Minei JP, Barber AE,* et al: Bacterial translocation and intestinal atrophy after thermal injury and burn wound sepsis. Ann Surg 211 (1990) 399–405

[11] *Jones II WG, Minei JP, Barber AE,* et al: Splanchnic vasoconstriction and bacterial translocation after thermal injury. Am J Physiol 261 (1991) H1190–H1196

[12] *Kayaalp O:* Histamin ve antihistaminikler. Tıbbi farmakoloji 4. baskı, cilt 3 (1989) 2717–2743

[13] *Kristiansen JE:* The antimicrobial activity of non-antiobiotics. APMIS Suppl 30 100 (1992) 7–14

[14] *Maejima, Deitch EA, Berg RD:* Bacterial translocation from the gastrointestinal tractus of rats receiving thermal injury. Infect Immun 43 (1984) 6–10

[15] *Marano MA, Moldawer LL, Fong Y,* et al: Cachectin / TNF production in experimental burns and pseudomonas infection. Arch Surg 123 (1988) 1383–1388

[16] *Mason AD, Mc Manus AT, Pruitt BA:* Association of burn mortality and bacteremia. Arch Surg 121 (1986) 1027–1031

[17] *Morris SE, Navaratnam N, Herndon DN:* A comparison of effects of thermal injury and smoke inhalation on bacterial translocation. J Trauma 30 (1990) 639–645

[18] *Nielsen HS, Nielsen H, Jensen S,* et al: Ranitidine improves postoperative monocyt and neutrophil function. Arch Surg 129 (1994) 309–315

[19] *O'Brien R, Merdoch J, Kuehn R,* et al: The effect of albumin or crystalloid resuscitation on bacterial translocation and endotoxin absorption following experimental burn injury. J Surg Res 52 (1992) 161–166

[20] *Sitting K, Deitch EA:* Effect of bacteremia on mortality after thermal injury. Arch Surg 123 (1988) 1367–1370

[21] *Skillman JJ, Silen W:* Stress ulcers. Lancet 16 (1972) 1303–1306

[22] *Souba WW, Herkowitz K, Klimberg US,* et al: The effects of sepsis and endotoxemia on gut glutamine metobolism. Ann Surg 214 (1990) 543–548

[23] *Sullivan BJ, Swallow CJ, Ginotti MJ,* et al: Bacterial translocation induces procoagulant activity in tissue macrophages. Arch Surg 126 (1991) 586–590

[24] *Walker HL, Mason AD:* A standart animal burn. J Trauma 8 (1968) 1049–1054

[25] *White WB, Ballow M:* Modulation of supressor-cell activity by cimetidine in patients with common variable hypogammaglobulinemia. N Eng J Med 312 (1985) 198–202

[26] *Winchurch RA, Thupari JN, Munster AM:* Endotoxemia in burn patients. Levels of circulating endotoxins are related to burn size. Surgery 102 (1987) 808–812

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YANIK İNCİNMESİNDE H_2 RESEPTÖR BLOKERLERİNİN BAKTERİYEL TRANSLOKASYON ÜZERİNDEKİ ETKİSİ

42 erkek albino sıçanda ranitidinin bakteriyel translokasyon üzerindeki etkisi deneysel olarak araştırılmıştır. Sham grubu (grup 1=12 sıçan) 21°C suya maruz bırakılırken yanık grubu (grup 2=15 sıçan) ve ranitidin grubu (grup 3=15 sıçan) total vücut yüzeyinin %30'u kadar tam kat bir yanık oluşturmak üzere 90°C suya 10 sn süresince maruz bırakılmıştır. Grup 3'e yanık incinmesinin hemen ardından başlamak üzere 300 mg/kg ranitidin uygulanmıştır. Sıçanlar 5. yanık gününde sakrifiye edilmiştir. Sham grubunda ağırlık artışı olurken grup 2 ve 3'te anlamlı ağırlık kaybı gözlenmiştir. Ranitidin uygulamasıyla gastrik pH anlamlı olarak artmıştır. Ranitidin grubunda çekal gayta kültürlerinde gram(-) ve total bakteri sayısında azalma saptanmıştır. Grup 3'te bakteriyel translokasyonda anlamlı artış saptanırken ranitidin ve yanık gruplarında transloke olan bakterilerin farklı olduğu gözlenmiş ve ranitidin uygulamasının intestinal ekolojik dengeyi değiştirerek bakteriyel translokasyonu arttırdığı kanaatine varılmıştır.

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Dear Doctor Erdener,

Cologne, 26 th of October 1996

Thank you very much for your interesting manuscript entitled:

Effects of H2 Receptor blocking agents on bacterial translocation in burn injury.

We are glad to be able to confirm that your manuscript has been accepted by the editorial board and our advisers for publication in the European Journal of Pediatric Surgery. We have sent your paper to the publishers and thank you very much again for having let us seen your paper.

With kind regards
yours sincerely

Prof Dr.A.M.Holschneider