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The role of nitric oxide on neutrophil chemostaxis and bacterial translocation in a sepsis model: An experimental study

Nitrik oksitin sepsis modelinde nötrofil kemotaksisi ve bakteriyel translokasyon üzerindeki rolü: Deneysel Çalışma

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Background/aims: *This experimental study investigated the possible effects of aminoguanidine, L-arginine and superoxide dismutase on bacterial translocation and neutrophil chemotaxis in endotoxemic rats.* ***Methods:*** *Six groups of male Wistar rats were used for the study. Normal saline solution was given intraperitoneally to the control group while lipopolysaccharide content of E.coli 0127:B8 was given intraperitoneally to the other five groups. Normal saline, aminoguanidine, aminoguanidine+L-arginine, L-arginine+superoxide dismutase and L-arginine alone was administered intravenously to the endotoxemic groups. Peritoneal neutrophil chemotaxis, quantitative cultures of the mesenteric lymph nodes, spleen, liver and cecum and histopathological examinations of tissue samples from the liver, spleen, mesenteric lymph nodes and ileum were then evaluated.* ***Results:*** *In endotoxemic rats, chemotaxis was found to be lower compared with the control group. Infusion of L-arginine increased peritoneal neutrophil chemotaxis significantly. Intraperitoneally administered lipopolysaccharide resulted in mesenteric lymph node bacterial translocation. In the groups given L-arginine+superoxide dismutase or L-arginine alone, no mesenteric lymph node bacterial translocation occurred. Histopathological examination of ileum revealed marked improvements in the endotoxemic groups treated with aminoguanidine+L-arginine and superoxide dismutase+L-arginine.* ***Conclusions:*** *Endotoxemia inhibits peritoneal neutrophil chemotaxis, abrogates gastrointestinal mucosal integrity and increases bacterial translocation. Aminoguanidine, L-arginine or superoxide dismutase improves these pathological changes and decreases bacterial translocation.*

Key words: Nitric oxide, chemotaxis, bacterial translocation.

Amaç: *Bu deneysel çalışma aninoguanidin, L-arginin ve süperoksit dismutazın endotoksemik ratlarda bakteriel translokasyon ve nötrofil kemotaksisi üzerine olan etkilerini araştırmak amacıyla planlanmıştır.* ***Yöntem:*** *Çalışmada altı grup erkek Wistar ratlar kullanılmıştır. Kontrol grubuna intraperitoneal olarak serum fizyolojik verilmiştir. Diğer beş gruba ise intraperitoneal olarak E.coli 0127:B8 suşunun lipopolisakkarid içeriği verilmiştir. Endotoksemik gruplara serum fizyolojik, aminoguanidin, aminoguanidin+L-arginin, L-arginin+superoksit dismutaz ve sadece L-arginin intravenöz olarak verilmiştir. Peritoneal nötrofil kemotaksisi, mezenterik lenf bezleri, dalak, karaciğer ve çekumun kantitatif kültürleri ve karaciğer, dalak, mezenterik lenf bezleri ile ileumun histopatolojik incelemeleri değerlendirilmiştir.* ***Bulgular:*** *Endotoksemik ratlarda kontrol grubuyla karşılaştırıldığında nötrofil kemotaksisi düşük bulunmuştur. L-arginin infüzyonunun kemotaksisi anlamlı oranda arttırdığı tespit edilmiştir. İntraperitoneal olarak verilen lipopolisakkarid, mezenterik lenf bezlerinde bakteriel translokasyona sebep olmuştur. L-arginin+superoksit dismutaz ve sadece L-arginin verilen gruplarda mezenterik lenf bezine translokasyon görülmemiştir. İleumun histopatolojik incelenmesinde ise aminoguanidin+L-arginin ve superoksit dismutaz+L-arginin verilen endotoksemik ratlarda belirgin düzelme saptanmıştır.* ***Sonuç:*** *Endotoksemi peritoneal nötrofil kemotaksisini inhibe etmekte, gastro-intestinal mukozal bütünlüğü bozmakta ve bakteriel translokasyonu arttırmaktadır. Aminoguanidin, L-arginin ve superoksit dismutaz bu patolojik değişiklikleri düzeltmekte ve bakteriel translokasyonu azaltmaktadır.*

Anahtar kelimeler: Nitrik oksit, kemotaksi, bakteriel translokasyon.

INTRODUCTION

Endotoxemia complicated by multiple organ dysfunction syndrome is a major cause of death in intensive care units, with a mortality rate exceeding 50%. The outcome is determined not only by the infection but also by the intensity of the immuno-inflammatory response (1,2).

The inflammatory response is largely mediated by cytokines, which are released into the systemic circulation. A complex interaction of cytokines

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and cytokine-neutralizing molecules probably determines the clinical presentation and cause of endotoxemia. Intervening in this sequence of events to modify the host inflammatory response may prove to be a beneficial treatment strategy for endotoxemia, but currently tested anticytokine therapies have been largely unsuccessful (3).

A current focus of investigation is the microcirculatory dysfunction associated with the systemic inflammatory response found in endotoxemia. Recently, endothelial-derived relaxation factor has been shown to play an important role in the pathogenesis of endotoxemic shock. Endothelial-derived relaxation factor has been demonstrated to be nitric oxide (NO) (4). In gut-derived endotoxemia, arginine supplementation in the diet has been shown to improve survival. The effect of arginine may be mediated by the generation of NO, since inhibition of NO synthase (NOS), namely, N-methyl-L-arginine, abrogates the beneficial effects of arginine (5). NO may promote bacterial killing either directly through its cytotoxic effect or indirectly by increasing local blood flow through its vasodilatory effects (6). However sustained release of NO may lead to cellular injury and gut barrier failure, resulting in enhanced bacterial translocation (7,8).

This experimental study investigated the effects of aminoguanidine as an inhibitor of inducible nitric oxide synthase, L-arginine as a precursor of nitric oxide and superoxide dismutase on bacterial translocation and neutrophil chemotaxis in endotoxemic rats.

MATERIALS AND METHODS

The study design was approved by the ethics committee of Ankara Numune Educational and Research Hospital. Sixty-five male Wistar rats (250-300 g) obtained from Hacettepe University Medical School Animal Laboratory were used in the study. They had been deprived of food but not water overnight (12 h) and were anesthetized with 60 mg/kg ketamine hydrocloride (Ketalar,) and 10 mg/kg xylasine hydrocloride (Rompun,) which was administered intramuscularly. The right internal jugular vein was catheterized with a 24 gauge catheter (Neoflon,). The rats were divided into two main groups, A and B. Group A was also divided into five groups with eight rats in each group: 1A, 2A, 3A, 4A and 5A. A 1 ml (=10 mg)/kg dose of lipopolysaccharide (LPS) (Eschericia coli 0127:B8) was given intraperitoneally to these groups.

Table 1. Characteristics of study groups.

Group	*n*	*Treatment*
1A	9	LPS (E.coli) (IP)
1B	8	NSS (IP)
2A	8	LPS (E.coli) (IP) +Aminoguanidine (IV)
2B	4	NSS (IP) +Aminoguanidine (IV)
3A	8	LPS (E.coli) (IP) +Aminoguanidin+L-arginine (IV)
3B	4	NSS (IP) +Aminoguanidine+L-arginine (IV)
4A	8	LPS (E.coli) (IP) +L-arginine+SOD (IV)
4B	4	NSS (IP) +L-arginine+SOD (IV)
5A	8	LPS (E.coli) (IP) + L-arginine (IV)
5B	4	NSS (IP) + L-arginine (IV)

NSS: Normal saline solution
LPS : Lipopolysaccharide
IP: Intraperitoneally
IV : Intravenously

Group B was divided five groups, as 1B, 2B, 3B, 4B and 5B and they constituted control groups. Group 1B included eight rats and each of the other control groups included four rats. Two hours before anesthesia, 1 ml/kg of normal saline solution (NSS) was given intraperitoneally to the main control group (Group 1 B) and the secondary control groups (group 2B, 3B, 4B and 5B). During the study period, 0.6 ml/kg/h NSS was administered with an LC 5000 infusion pump (Abbott Lab.). For the evaluation of chemotaxis, 15 ml sodium kazeinate was given intraperitoneally to all rats 16 hours prior to laparotomy.

In group 2 (A and B), 5 mg/kg/h of aminoguanidine was infused for four hours. In group 3 (A and B), 5mg/kg/h of aminoguanidine and 100 mg/kg/h of L-arginine was infused for four hours. In group 4 (A and B), L-arginine (100 mg/kg/h) and superoxide dismutase (3600 U/kg/h) were infused for four hours. In group 5 (A and B), only L-arginine was infused. The characteristics of the study groups are summarized in Table 1. Laparotomy was performed at the 6th hour of the experiment. In the secondary control groups (group 2B, 3B, 4B and 5B), only chemotaxis and in groups 1A, 1B, 2A, 3A, 4A and 5A, chemotaxis, bacterial translocation and histopathological analyses were performed.

Peritoneal neutrophil chemotaxis was measured as described by Boyum (9). Quantitative cultures of the mesenteric lymph nodes, spleen, liver and cecum were obtained and these cultures were processed as previously described (10). Tissues of liver, spleen, mesenteric lymph nodes and ileum

Table 2. Significant differences between control and LPS-treated groups.

Chemotaxis (µm)	*Groups*	*Groups* 1A	2A	1B	3A	5A	4A
32.48±3.59	1A						
45.42±5.33	2A	*					
50.10±2.96	1B	*	*				
50.67±2.41	3A	*	*				
59.47±3.86	5A	*	*	*	*		
85.79±4.67	4A	*	*	*	*	*	

*: p<0.05

were obtained after laparotomy, fixed in 10% formalin, and embedded in paraffin. The paraffin sections (5 m thick) were stained with hematoxylin and eosin. A pathologist evaluated the slides in a blinded fashion. Chemotaxis and bacterial translocation were evaluated with variant analysis and Duncan multiple range test statistically. Histopathological findings were evaluated with the Fridmen test, and chi-square test was used to determine differences between groups.

Table 3. Mesenteric lymph node bacterial translocation results

Groups	*n*	*positivity*
Control (1B)	8	0/8
E.coli (1A)	9	8/9
E.coli+Aminoguanidin (2A)	8	3/8
E.coli+Aminoguanidin+L-arginine (3A)	8	2/8
E.coli+L-arginine+SOD (4A)	8	0/8
E.coli+L-arginine (5A)	8	0/8

RESULTS

Peritoneal neutrophil chemotaxis

In group 1A, which was given LPS alone, chemotaxis was found to be lower, compared with the other groups. In groups 2A, 3A, 4A and 5A, which were treated with aminoguanidine, L-arginine and SOD, chemotaxis was significantly increased (p<0.05). But in groups, which L-arginine was infused (3A, 4A and 5A), chemotaxis was increased significantly higher than the group in which only aminoguanidine was infused (group 2A). The significant differences between the control group and LPS-treated groups are shown in Table 2.

Bacterial translocation

Intraperitoneally administered LPS resulted in mesenteric lymph node bacterial translocation. No bacterial growth was noted in the liver and spleen in these groups. The mesenteric lymph node bacterial translocation results of the groups are shown in Table 3. In groups 4A and 5A, no mesenteric lymph node bacterial translocation occurred and this result is similar to that of the control group. The significant diffrences between the LPS-treated group and other groups is shown in Table 4.

Table 4. Significance between LPS-treated group and other groups according to mesenteric lymph node bacterial translocation

Bacteria Concentration (x106/gr tissue)	*Groups*	*Groups* 1A	2A	3A	1B	4A	5A
18.80±13.32	1A						
1.06±1.82	2A	*					
0.12±0.24	3A	*					
0.00	1B	*	*	*			
0.00	4A	*	*	*			
0.00	5A	*	*	*			

*: p<0.05

Table 5. Significance between groups according to histopathological results of ileum.

		Groups					
Total score#	*Groups*	*1B*	*1A*	*2A*	*3A*	*4A*	*5A*
9	1B						
21	1A	*			*	*	*
16	2A						
13	3A						
12	4A						
11	5A						

*: $p<0.05$

#: Sum of pathological scores of rats in the same group.

Histopathological results

Congestion of the central vein was observed in the liver of the LPS-treated and all groups other than the control group. Also, dilatation of the sinusoids and neutrophil infiltration was prominent, with no difference between the LPS-treated group and the other groups. It was only in the control group that no major histopathological changes were observed. There was also no difference between the groups in mesenteric lymph nodes.

In the LPS-treated group, marked leukocyte infiltration of lamina propria, edema of submucosa and ulceration were seen on histopathological examination of the ileum. But in groups treated with aminoguanidine+L-arginine (group 3A), L-arginine+SOD (4A) or L-arginine alone (5A), histopathological examination of ileum revealed marked improvement in these findings. The significance between the groups is shown in Table 5.

DISCUSSION

Endotoxic shock is characterized by hypotension, intravascular coagulation, and increases in vascular permeability, hemoconcentration and gastrointestinal damage. These effects of endotoxin may be the result of a direct action of this lipopolysaccharide component of bacterial cell walls on the vascular endothelium or as a consequence of the release of secondary mediators (11-13).

Studies have recently focused on nitric oxide (NO), the labile vasodilator formed from L-arginine by endothelial cells and which was originally characterized as endothelium-derived relaxing factor (14-16). In addition to endothelial cells, NO can also be formed by other cells, including macrophages and neutrophils, which may be involved in endotoxic shock (17,18). The synthesis of NO by these cells with inducible NO synthase (iNOS) can be selectively inhibited by the L-arginine analogue, NG-monomethyl-L-arginine (NMA) (16,17). Inhibition of endothelial-derived NO by NMA in anesthetized animals produces an increase in systemic arterial blood pressure and inhibits the hypotensive action of acetylcholine and other endothelium-dependent vasodilators, suggesting that NO may have an important regulatory role on the vasculature in vivo (19,20).

In endotoxic and hemorrhagic shock, an enhanced formation of NO by the iNOS contributes to hypotension and vascular hyporeactivity to vasoconstrictor agents (21). As endotoxemia is also associated with tissue hypoperfusion as well as activation of platelets and neutrophils, it is conceivable that the inhibition of the release of NO by endothelial NO synthase aggravates the endotoxin-induced tissue ischemia, resulting in an increased incidence of multiple organ dysfunction syndrome (22).

Neutrophils play an important role in the host immune system against microbial infections. Decreasing numbers of neutrophils and/or function abnormalities leads to host susceptibility to infection. Christou et al. (23) found 66% sepsis and 37% mortality rates among 254 surgical patients with impaired neutrophil chemotaxis. The metabolism of L-arginine to NO has been shown to be important for the effector functions of many cell types, including neutrophils. Kaplan et al. (24) demonstrated that chemotaxis of neutrophils was markedly inhibited in NMA-treated cells and this inhibition could be overcome if L-arginine or cGMP were added to the NMA. These findings supported the hypothesis that L-arginine metabolism to NO and its effect in the cGMP level may be important for the dynamic changes required for neutrophil chemotaxis.

Both beneficial and detrimental effects of NO directed investigators to research the effects of selective NOS inhibitions. Wu et al. (22) demonstrated that aminoguanidine, a selective inhibitor of iNOS activity in vivo, attenuates the circulatory failure as well as the multiple dysfunction syndrome associated with endotoxic shock in the rat. Inhibition of endothelial NO synthase activity attenuated the circulatory failure, but not the multiple organ dysfunction syndrome, caused by endotoxemia.

In the present study, it was found that sepsis inhibits peritoneal neutrophil chemotaxis. This inhibition was overcome with aminoguanidine or L-arginine. In sepsis, the activation of iNOS begins at the 2nd hour and we infused aminoguanidine to septic rats at the same time as activation of iNOS was thought to begin. We hypothesized that inhibition of iNOS with aminoguanidine at the time of its activation and stimulation of endothelial NOS with L-arginine might improve neutrophil chemotaxis. This improvement was higher if SOD was added because it inhibits the reaction of NO with superoxide radicals. This finding also supported the hypothesis of the beneficial effect of endothelial NO on neutrophil chemotaxis. The effects of aminoguanidine, L-arginine and SOD on neutrophil chemotaxis in both LPS-treated and NSS-treated groups were also analyzed to understand the mechanism of their effects. In the NSS-treated group, no changes on neutrophil chemotaxis were observed but in the LPS-treated group, a significant increase of neutrophil chemotaxis was found. This suggested that the effects of these agents were NO-dependent.

In the second part of this study it was found that both L-arginine and L-arginine+SOD blocked bacterial translocation to mesenteric lymph nodes. It is known that NO protects mucosal integrity in the gastro-intestinal system and that L-arginine improves mesenteric blood flow in endotoxemia and these mechanisms may explain our results. However, it was also found that in the aminoguanidine-treated group, mesenteric lymph node bacterial translocation was significantly lower than in the only LPS-treated group and this significance was more prominent if L-arginine was added. This finding suggested the beneficial effect of constitutive NOS and the detrimental effect of iNOS on bacterial translocation.

The histopathological examination of ileum revealed submucosal edema, inflammation in lamina propria and ulceration in the LPS-treated group. These findings were similar to the results of Deitch and Berg's study (25). We found that aminoguanidine, L-arginine and SOD improved this pathological changes in LPS-treated rats. Hutcheson et al. (26) also reported that NMA increased mucosal damage and L-arginine protected the detrimental effect of NMA. It is known that SOD decreases mucosal damage in ischemia-reperfusion injury, but in endotoxemia its effect may be explained as its inhibitory effect on the reaction between NO and superoxide radicals. This hypothesis may explain why L-arginine+SOD had a greater effect in decreasing endotoxemic mucosal injury.

In conclusion, endotoxemia inhibits peritoneal neutrophil chemotaxis, abrogates gastro-intestinal mucosal integrity and increases bacterial translocation. Aminoguanidine, L-arginine or SOD improves these pathological changes and decreases bacterial translocation. The effects of these agents result in the inhibition of iNOS and protection of constitutive NOS. L-arginine and SOD could be used as therapeutic agents in sepsis, and aminoguanidine could be used at the time in which iNOS is activated.

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