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Pars Inhibitor 3-Aminobenzamide Attenuates Lung Injury in Experimental Endotoxemia

A.Şükrü Taner*, Koray Topgül**, Fikri Küçükel***, Hilal Özer*, Selim Temel*

* Department of General Surgery, Ankara State Hospital, Turkey.
** Department of General Surgery, Ondokuz Mayıs University School of Medicine, Samsun, Turkey.
*** Department of General Surgery, Guven Hospital, Ankara, Turkey.

Background: The role of poly(ADP-ribose) synthetase (PARS) inhibitor 3-aminobenzamide on lung injury was investigated in endotoxin-induced sepsis in 21 male Wistar rats (250-300 g) allocated into three groups.
Methods: The control group, received intraperitoneal saline (1ml kg^{-1} : n=7); the second group, intraperitoneal endotoxin (*Escherichia coli* lipopolysaccharide 055:B5 10 mg kg^{-1} :n=7); the third group, intraperitoneal 3-aminobenzamide 10mg kg^{-1} and 1 ml kg^{-1} saline 10 min before endotoxin (n=7). Six hours later, rats were sacrified with overdose of anaesthetic. Bronchoalveolar lavage (BAL) of the right lung was performed and used for assey of protein concentration and neutrophil count. The wet/dry lung ratio of the left lower lobe was calculated. Intravascular neutrophils of the left lung were cleared out with saline and asseyed spectrophotometrically for myeloperoxidase activity. For statistical evaluation analysis of variance was used.
Results: When compared with controls, administration of endotoxin caused a significant increase in mean pulmonary myeloperoxidase activity which was associated with an increase in mean BAL neutrophil concentration and mean lung wet:dry weight ratios as an expression of pulmonary extravascular lung water and microvascular leakage of protein The increase in these values were not significant in the endotoxin+3-AB group.
Conclusion: The results of this study show that endotoxin challenge is associated with marked pulmonary injury and part of the anti-inflamatory effect of PARS in this injury may be related to the reduction of neutrophil recruitment into the inflamatory site.

Key Words: Sepsis, 3-Aminobenzamide, Pulmonary injury

Deneysel Endotoksemide Pars İnhibitörü 3-Aminobenzamid Akciğer Hasarini Azaltir

Amaç: Endotoksine bağlı endotoksemi geliştirilen üç gruba ayrılmış 21 erkek Wistar ratta (250-300g) PARS (poli ADP-riboz sentetaz) inhibitörü 3-aminobenzamidin akciğer hasarı üzerine etkisi araştırıldı.
Gereç ve Yöntem: Kontrol grubuna intraperitoneal olarak salin (1ml kg^{-1} n=7); ikinci gruba, intraperitoneal endotoksin (*Escherichia coli* lipopolysaccharide 055:B5 10 mg kg^{-1} :n=7); üçüncü gruba ise, endotoksinden 10 dakika önce olmak üzere intraperitoneal 3-aminobenzamid 10mg kg^{-1} ve 1 ml kg^{-1} salin uygulandı (n=7). Altı saat sonra fareler yüksek doz anestezik madde verilerek sakrifiye edildi. Protein konsantrasyonunu ve nötrofil sayımını yapmak için akciğer sağ lobuna bronkoalveolar lavaj (BAL) uygulandı. Sol lobun ise ıslak/kuru akciğer oranı hesaplandı. Sol akciğerin intravaskuler nötrofilleri salin ile yıkandı ve spektrofotometrik olarak myeloperoksidaz aktivitesi ölçüldü. İstatistiki değerlendirme için varyans analizi kullanıldı.
Bulgular: Kontrol grubu ile karşılaştırıldığında, endotoksin verilen grupta ortalama pulmoner myeloperoksidaz aktivitesinin, ortalama BAL nötrofil konsantrasyonlarının ve protein ve damar dışı akciğer sıvısının sızıntısının işareti olan ortalama ıslak/kuru akciğer oranı anlamlı şekilde artmış olduğu görüldü. Artmış olarak bulunan bu değerler açısından, endotoksin + 3-aminobenzamid grubunda anlamlı bir fark bulunamadı.
Sonuç: Bu çalışmanın sonuçları, endotokseminin belirgin bir şekilde akciğer hasarıyla gittiği ve inflamasyonun olduğu alanda PARS'ın etkisi ile nötrofillerin toplanmasında düşüşün ve bununda gelişen hasarla ilişkili olabileceğini göstermiştir.

Anahtar kelimeler: Sepsis, 3-aminobenzamid, Akciğer hasarı.

Endotoxic shock, where the host is under heavy oxidative stress, is known to impair critical cellular functions and is associated with the development of multiple organ failure (MOF).[1,2] Oxyradicals, nitric oxide and peroxinitrite play a crucial role in the inflamatory process.[3,4] A cytotoxic cycle triggered by oxidant induced DNA single strand breakage and subsequent activation of the nuclear enzyme poly (ADP-ribose) synthetase (PARS) have been shown to contribute to the cellular injury during various forms of oxidative stress in vivo, playing a role in the pathogenesis of MOF.[5,6] Pharmacological inhibition of PARS have been shown to have beneficial effects against oxidant and free radical mediated cell injury.[7] The most frequently used inhibitors of PARS are 3-aminobenzamide (3-AB) and nicotinamide. The purpose of this study was to determine the effect of in vivo PARS inhibition on endotoxin induced alveolar dysfunction.

MATERIALS AND METHODS

After fasting over night 21 male Wistar rats weighting 250-300 g were divided into three equal groups (seven per group). Controls received intraperitoneal (IP) saline only (1 mg kg^{-1}). The sepsis group received IP endotoxin (*E.coli* lipopolysaccaride –LPS- 0.55:B5 10 mg kg^{-1}, Sigma St Louis Missouri, USA). The sepsis plus 3-aminobenzamide group received IP 3-aminobenzamide (A-0788 Sigma) 10 mg kg^{-1} and saline 1 ml kg^{-1} 10 min before endotoxin.

Six hours later each animal was killed with an overdose of an anaesthetic. A sternotomy was performed and the left bronchus was clamped. Bronchoalveolar lavage (BAL) of the right lobe was performed three times with 1 ml saline containing etylenediamine tetra-acetic acid 0.07 mol/L. Total lavage fluid of 2 ml for each animal was centrifuged for 20 minutes at 40 ^{0}C at 1500 rpm, frozen at - 20 ^{0}C and used for assey of protein concentration.

Neutrophil count in BAL fluid was carried out by Diff-Quick staining of a representative sample of BAL and consecutive counting of neutrophils per high-power field.

Wet/dry lung ratio:

The wet/dry lung ratio of the left lower lobe was calculated after weighting the freshly harvested organ and heating at 90 ^{0}C in a gravity convection oven over a 72 h period during which time the weight of the residum became constant.

Myeloperoxidase assay:

The right ventricle was cannulated with a 22 gauge needle and the left pulmonary hilum clamped. Intravascular neutrophils of the left lung were cleared out with 30 ml saline. The remaining lung was weighted and homogenized on ice in 10 ml 0.5 per cent hexadecyltrimethyl ammonium bromide in potassium phosphate buffer 50 mmol/L at pH 6. The supernatant was assayed spectrophotometrically for myeloperoxidase activity. The change in absorbance with time at 460 nm was recorded. One unit of myeloperoxidase was defined as that degrading 1 μmol peroxide per min at 25 ^{0}C.

Results are expressed as mean ± SEM values. For statistical evaluation one way analysis of variance was used and $p<.05$ was concidered statistically significant.

RESULTS

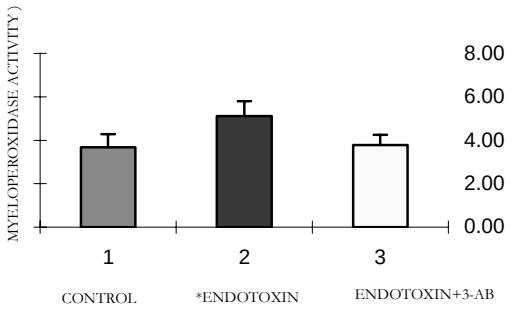
Administration of endotoxin caused a significant increase in mean pulmonary myeloperoxidase activity from 3.68 (±.60) in controls to 5.11 (±.16) units per gram wet lung weight which was preserved in the edotoxin + 3-AB group (3.78±.41 units/g) ($p< .05$) (Figure 1). This finding was parallel to an increase in mean BAL neutrophil concentration from 1.02 (±.4) in controls to 1.84 (±.12) in the group with endotoxin challenge ($p< .05$) which was found to be 1.09 (±.11) in the endotoxin + 3-AB group (Figure 2). Endotoxin caused a significant increase in mean lung wet:dry weight ratios as an expression of pulmonary extravascular lung water and microvascular leakage of protein (from 4.04±.65 and 390.2±55.8 mg/ml to 6.11±.15 and 785.6±104.2 mg/ml respectively) ($p<.05$). The increase in these values were not significant in the endotoxin+3-AB group (4.90±.46 and 412.1±35.6 mg/ml).

DISCUSSION

Endotoxemia results in significant organ dysfunction and failure of critical cellular functions. The functional abnormalities of the pulmonary microcirculation associated with endotoxemia manifested in an increased

endothelial and epithelial permability. There are many published studies demonstrating that PARS activation plays an important role in the pathogenesis of endotoxic shock, inflammation, and ischemia-reperfusion injury.[5,8-12] Also, many studies demonstrated that oxidants produced during endotoxemia the activation of the nuclear enzym PARS, resulting in intracellular energetic failure and dysfunction.[13] A characteristic feature of endotoxemia is the plasma leakage in various vascular beds including the lung. Which is rich source of resident macrophages abundant amounts of nitric oxid (NO) and peroxynitrite.[14, 15]

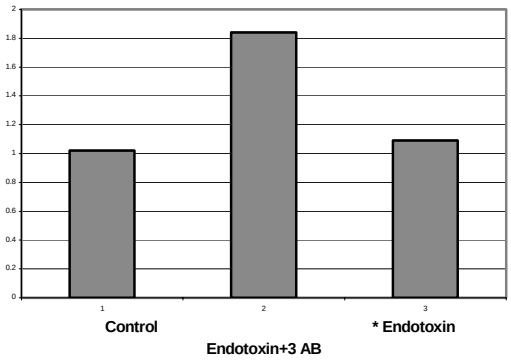
Figure 1. Mean alveolar myeloperoxidase activity of the study groups (*endotoxin challange caused a significant increase in mean myelopreoxidase activity, p<.05).



Peroxynitrite, and certain oxygen-derived oxidants, such as hydroxyl radical, mediate tissue injury by activating a variety of parallel cytotoxic pathways.

A cytotoxic cycle triggered by oxidant-induced DNA single strand breakage and subsequent activation of the nuclear enzyme poly(ADP-ribose) synthetase have been shown to contribute to the cellular injury during various forms of oxidant stress in vivo and in vitro [(5,6,16)] . Endotoxin causes excessive activation of the nuclear repair enxyme PARS which depletes cellular energy stores and leads to tissue dysfunction. The aim of this study was to determine the effect of in vivo PARS inhibition on endotoxin-induced alveolar dysfunction. The effect of 3-AB, an inhibitor of PARS activity was evaluated in a rat model of pulmonary injury under endotoxin challenge.

Figure 2. Mean Neutrophil concentration; (*****endotoxin challenge caused a significant increase in mean neutrophil concentration, p<.05)



It was shown that endotoxin challenge is associated with pulmonary injury as indicated by pulmonary edema and increased BAL protein concentrations. The reduced production of proinflammatory mediators and leukocyte infiltration in the lungs of PARS rat translated into a significant reduction of high permeability pulmonary edema, evidenced by a decreased protein content in the BAL. This finding may have clinical implications, as the formation of high permeability edema eventually results in alveolar collapse, reduced lung compliance, and severe gas exchange abnormalities.[17]

Myeloperoxidase activity, a haemo protein located in azurophil granules of neutrophils, has been used as a biochemical marker for neutrophil infiltration into tissue . Neutrophils might have a role in this injury as evidenced by increased neutrophil concentrations and myeloperoxidase activities found in the animals receiving LPS.

There are many studies about effects of the 3-aminobenzamide in endotoxemic or non endotoxemic models. Szabo et al.[18] found that PARS inhibition with 3-AB prevented both local and systemic inflammation. The beneficial effect of PARS inhibition on lung edema formation has already been confirmed by measuring the wet/dry ration, protein concentration in broncho-alveolar lavage, and pulmonary transvascular fluid flux.[19] In our study, the mean pulmonary myeloperoxidase activity was increased from 3,68 (±

60) in the control group to 5,11 (±16) in the endotoxin group because of administration of endotoxin. Also, the mean BAL neutrophil concentration was found in the endotoxin group higher than in the control group. Endotoxin caused a significant increase in mean lung wet/dry weight ratios. The ratio was found as 6.11±.15 in the endotoxin group. This result was higher than the others. Our results resembled the results of other studies in the literature.[20]

In conclusion the results of this study show that endotoxin challenge is associated with marked pulmonary injury and part of the anti-inflamatory effect of PARS in this injury may be related to the reduction of neutrophil recruitment into the inflamatory site.

REFERENCES

1- Altuntaş Y, Köksal G M, Sayılgan C, et al. Tavşanlarda oluşturulan intraabdominal SIRS modelinde erken dönemde oluşan değişikliklerin araştırılması. *Göğüs Kalp Damar Anestezi ve Yoğun Bakım Derneği Dergisi* 2003; 9: 4-10.

2- Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115: 457 – 469.

3- Wolfe TA, Dasta JF. Use of nitric oxide synthase inhibitors as a novel treatment for septic shock. *Ann Pharmacother* 1995; 29: 36 – 46.

4- Mercer DW, Smith GS, Cross JM, et al. Effect of Lipopolisaccharide on intestinal injury: Potential role of nitric oxide and lipid peroxidation. *J Surg Res* 1996; 63: 185 - 92.

5- Oliver FJ, Menissier-de Murcia J, Nacci C, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. *EMBO J* 1999; 18: 4446-4454

6- Szabo C, Zingarelli B, Salzman AL. Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ Res* 1996; 78: 1051 – 1063.

7- Szabo C. DNA strand breakage and activation of poly-ADP ribosyltransferase: A cytotoxic pathway triggered by peroxynitrite. *Free Rad Biol Med* 1996; 21: 855 –869.

8- Farivar AS, Woolley SM, Fraga CH,et al. Intratracheal poly (ADP) ribose synthetase inhibition ameliorates lung ischemia reperfusion injury. *Ann Thorac Surg* 2004 ;77(6):1938-1943.

9- Bowes J, McDonald MC, Piper J, et al. Inhibitors of poly (ADP-ribose) synthetase protect rat cardiomyocytes against oxidant stress. *Cardiovasc Res* 1999; 41: 126 – 134.

10- Cuzzocrea S, Zingarelli B, Costantino G, Sottile A, Teti D, Caputi AP. Protective effect of poly(ADP-ribose) synthetase inhibition on multiple organ failure after zymosan-induced peritonitis in the rat. *Crit Care Med* 1999; 27(8): 1517 – 1523.

11- Cuzzocrea S, Thiemermann C, Salvemini D, et al. Potential therapeutic effect of antioxidant therapy in shock and inflammation. *Curr Med Chem.* 2004 ;11(9):1147-1162.

12- 12-Virag L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev* 54: 375-429, 2002)

13- Szabo A, Salzman A L, Szabo C. Poly (ADP-Ribose) Synthetase activation mediates pulmonary microvascular and intestinal mucosal dysfunction in endotoxin shock. *Life Sciences* 1998; 63: 2133-2139.

14- Wizemann TM, Gardner CR, Laskin JD, et al. Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. *J Leukoc Biol.* 1994 ;56(6):759-768.

15- Arkovitz MS, Wispe JR, Garcia VF, et al. Selective inhibition of the inducible isoform of nitric oxide synthase prevents pulmonary transvascular flux during acute endotoxemia. *J Pediatr Surg.* 1996 ;31(8):1009-1015

16- Cuzzocrea S, Zingarelli B, Gilad E, et al: Protective effects of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase in a carrageenan-induced model of local inflammation. *Eur J Pharmacol.* 1998 .19;342(1):67-76.

17- Artigas A, Bernard GR, Carlet J, *et al* . The American-European Consensus Conference on ARDS. Part 2: Ventilatory, pharmacologic, supportive therapy, study design strategies, and issues related to recovery and remodeling. Acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1998; 157: 1332-1347.

18- Szabo, C., L. H. Lim, S. Cuzzocrea, S, et al. Inhibition of poly (ADP-ribose) synthetase attenuates neutrophil recruitment and exerts antiinflammatory effects. *J Exp Med* 1997; 186: 1041-1049

19- Shimado K, Murakami K, Enkhbaatar P, et al. Effect of poly(ADP ribose) synthetase inhibition on burn and smoke inhalation injury in sheep. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: 240-249.

20- Kiefmann R, Heckel K, Dörger M, et al. Role of poly(ADP-ribose) synthetase in pulmonary leukocyte recruitment. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: 996-1005.

Postal Adress:
Dr. Koray Topgül
OMÜ Tıp Fakültesi Genel Cerrahi AD
55139 SAMSUN/ TURKEY
Tel : 362 4576 000-3674
Fax : 362 4576 041
E-mail : korayt@omu.edu.tr