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The role of poly(ADP-ribose) synthetase inhibition on the intestinal mucosal barrier after thermal injury

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Abstract

Oxidative and nitrosative stressor agents can trigger DNA strand breakage, which then activates the nuclear enzyme poly(ADP-ribose) synthetase (PARS). Activation of the enzyme depletes the intracellular concentration of energetic substrates such as nicotinamide adenine dinucleotide (NAD). This process can result in cell dysfunction and cell death. PARS inhibitors have been successfully used in ischemia–reperfusion injury, inflammation and sepsis in several experimental models. In our experimental study, we investigated the role of 3-aminobeanzamide (3-AB), a non-specific PARS inhibitor, on the intestinal mucosal barrier after burn injury. Twenty-four Wistar rats were randomly divided into three groups. The sham group ($n = 8$) was exposed to 21 °C water while the burn group ($n = 8$) and the burn + 3-AB group ($n = 9$) were exposed to boiling water for 12 s to produce a full thickness burn in 35–40% of total body surface area. In the burn + 3-AB group, 10 mg/kg of 3-AB was given intraperitoneally 10 min before thermal injury. Twenty-four hours later, tissue samples from mesenteric lymph nodes (MLN), spleen and liver were obtained under sterile conditions for microbiological analysis and ileum samples were obtained for biochemical and histopathological analysis. In burn group, the incidence of bacteria isolated from MLN and spleen was significantly higher than other groups ($P < 0.05$). 3-AB pre-treatment prevented burn induced bacterial translocation and it significantly reduced burn induced intestinal injury. Tissue malondialdehyde and 3-nitrotyrozine levels were found significantly lower than that of the burn group. These data suggest that the relationship between PARS pathway and lipid peroxidation in intestinal tissue and PARS has a role in intestinal injury caused by thermal injury.

Keywords: Poly(ADP-ribose) synthetase; Intestinal mucosal barrier; Thermal injury; Peroxynitrite; Bacterial translocation

1. Introduction

Despite the progress in the management of thermal injury, its complications such as systemic inflammatory response syndrome (SIRS), sepsis and multiple organ failure (MOF) still continue to be the leading causes of mortality and morbidity [1,2]. These septic events often occur in the absence of an identifiable focus of infection. However, gut hypoperfusion has been implicated as an initiating event in the development of these complications [3]. Normally, in the gut, there is a homeostasis between the intraluminal bacteria and their products and the intestinal mucosal barrier. Thermal cutaneous injury leads to a breakdown in the intestinal mucosal barrier and causes the disruption of mucosal integrity, which can induce bacterial translocation (BT) and thus septic complications and MOF in burn patients [4]. After thermal injury, there is a transient and selective splanchnic vasoconstriction, which is associated with decreased mesenteric blood flow and damage of the mucosal barrier due to

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ischemia–reperfusion (I–R) injury, which promotes BT from the gut [5,6]. The pathophysiological mechanisms that cause the damage of the mucosal barrier include the adhesion and activation polymorphonuclear neutrophils, the release of pro-inflammatory cytokines, as well as the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydroxyl radical, superoxide anion, hydrogen peroxide and peroxynitrite [7,8]. Recently, it has been reported that nitric oxide (NO) appears to play a paradoxical role in intestinal physiology. Small amounts of NO may have a cytoprotective effect by enhancing mucosal blood flow. However, there is an increasing body of evidence indicating that over production of NO may damage the mucosal barrier, leading to failure of the gut barrier function [9,10]. The toxicity of NO has been attributed to the peroxynitrite ($ONOO^-$) which is formed by reaction of NO with superoxide anion [11]. Peroxynitrite reacts with cellular lipid and protein components, consequently impairs their function. One pathway of cell death, triggered by peroxynitrite, leads to the loss of cellular energetics via the induction of DNA single-strand breaks, and subsequent activation of the nuclear enzyme poly(ADP-ribose) synthetase (PARS) [12]. PARS activation, which is most likely due to peroxynitrite production, depletes the intracellular concentration of energetic substrate, nicotinamide adenine dinucleotide (NAD), thus leading to cellular dysfunction and cell death [13]. Pharmacological inhibition of PARS has been shown to have beneficial effects against peroxynitrite-mediated cell injury [14]. Recently, beneficial effects of PARS inhibition were reported in many experimental models such as ischemia–reperfusion injury and sepsis. However, there is no published data regarding the effect of PARS enzyme inhibition in the small intestine after burn injury. For this reason, in this study, we aimed to investigate the role of 3-aminobenzamide (3-AB), a PARS inhibitor, on the intestinal mucosal barrier and bacterial translocation after thermal injury.

2. Materials and methods

The procedures used in this study and the handling of study animals were performed in adherence to National Institutes of Health guidelines on the use of experimental animals. The experimental protocol was approved by the Ethics Committee of University of Mersin. Wistar–Albino rats, weighing 200–250 g were housed at constant temperature with 12 h periods of light–dark exposure, respectively. Animals were allowed access to standard rat chow and water ad libitum acclimation period of at least 1 week before use in this study.

2.1. Experimental design

After stabilization period, 24 rats were randomly divided into three groups. The first group (sham group, $n = 8$) were exposed to 21 °C water and intraperitoneal saline injection (1 mL/100 g). The second group (burn group, $n = 8$) was exposed to thermal injury and given an intraperitoneal saline injection (1 mL/100 g). The third group (burn + 3-AB group, $n = 8$), received thermal injury 10 min after the 3-AB (A-0788; Sigma, St. Louis, MO, USA) and saline injection (10 mg/kg, intraperitoneally). All animals were sacrificed 24 h after thermal injury. A midline laparotomy was performed and mesenteric lymph nodes (MLN), spleen and liver specimens were obtained under sterile conditions. Then, samples of ileum were harvested for both histopathological and biochemical evaluation.

2.2. Thermal injury

Animals were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). The backs of animals were shaved to allow direct skin contact between skin and hot water. A marked area of the shaved dorsal skin exposed through a template and immersed in boiling water for 12 s. This procedure produced a full thickness burn of 30–35% of total body surface areas of rats [15]. The rats in sham group were exposed to room temperature water (21 °C) instead of boiling water. All animals in groups II and III were resuscitated by intraperitoneal injection of 1 mL/100 g. saline following burn injury and were allowed to feed with water and standard rat chow after recovering from anesthesia. No animals died within the first 24 h of post-burn period.

2.3. Microbiological analysis

Microbiological analysis was performed as described previously [16]. A 1 mL sample of blood from each animal was immediately placed into Bactec Peds Plus/F blood culture medium (Becton Dickinson Microbiolgical Systems, Cockeysville, MD, USA) and incubated at 37 °C for 7 days under aerobic conditions in a Bactec 9240 system. Broths were incubated at 35 °C until turbid, and the turbidity was adjusted to match that of a 0.5 McFarland standard (10^8 CFU/mL). By using normal saline, a 1/100 dilution of the suspension was made to give an adjusted concentration of 10^6 CFU/mL. From all culture materials, subsequent subcultures were performed on blood agar, eosin–methylene blue (EMB) agar and chocolate agar [16]. All samples were stained by acridine orange and gram technique. The liver, spleen and MLN specimens were placed into 2 mL of brain heart infusion (BHI) broth, weighed, and homogenized. These samples were placed on blood agar and EMB agar. All cultures were incubated under aerobic and anaerobic conditions and were examined at 24 and 48 h for presence of growth. The identification of bacterial species was performed by standard microbiological methods. Colonization was expressed as the number of CFU per gram of tissue homogenate (CFU/g).

2.4. Histopathological analysis

Sections of tissue were obtained from intestinal segments that were fixed in 10% formaldehyde and stained with hematoxylin–eosin (H&E). Mucosal injury, inflammation and hyperemia/hemorrhage were assessed and graded in a blinded manner by a pathologist using the histological injury scale previously defined by Chiu et al. [17]. Briefly, the mucosal damage was graded from 0 to 5 according to the following criteria: grade 0, normal mucosal villi; grade 1, development of subepithelial Gruenhagen's space at the apex of the villus, often with capillary congestion; grade 2, extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; grade 3, massive epithelial lifting down the sides of villi, possibly with a few denuded tips; grade 4, denuded villi with lamina propria and dilated capillaries exposed, possibly with increased cellularity of lamina propria; grade 5, digestion and disintegration of the lamina propria, hemorrhage and ulceration.

2.5. Detection of MDA levels

One part of the tissue was homogenized in 10 parts of 15 mmol/L KCL for malondialdehyde (MDA) assay MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to from a colored complex. Measurement of MDA content by TBA reactivity is the most widely used method for assessing lipid peroxidation [18]. The principle of the method is based on the measurement of absorbance of the pink color produced by interaction of TBA with MDA at 530 nm. Values were expressed as nanomoles per gram wet tissue weight.

2.6. Measurement of ileal tissue myeloperoxidase (MPO) activity

One part of the tissue was homogenized in 10 parts of HETAAB buffer for myeloperoxidase activity assay. The determination of tissue MPO activity depends on the fact that it reduces dimethoxybenzidine. Ileal segments were homogenized in 0.5% hexadecyltrimethylammonium bromide in 10 mM 3-(*N*-morpholino) propane sulfonic acid and centrifuged at 15,000 × *g* for 40 min. An aliquot of supernatant was mixed with a solution of 1% (w/v) dimethoxybenzidine and 1 nM hydrogen peroxide. After a 30 min of incubation, the reaction was stopped by adding 3 M HCI. Activity was measured spectrophotometrically as the change in absorbance at 410 nm at 37 °C by using a spectrophotometer [19]. Results are expressed as units MPO activity per gram tissue.

2.7. 3-Nitrotyrosine (3-NT) detection

3-NT and tyrosine were obtained from Sigma. H_2O_2, sodium acetate, citrate, NAOH, MnO_2, H_3PO_4, KH_2PO_4, K_2HPO_4 and were purchased from Merck (Deisenhofen, Germany). All organic solvents were HPLC grade. The tissues were homogenized in ice-cold phosphate-buffered saline (pH 7.4). Equivalent amounts of each samples were hydrolyzed in 6N HCl at 100 °C for 18–24 h, then samples were analyzed on an Agillent 1100® HPLC apparatus. The analytical column was a 5 μm pore size Spherisorb ODS-2 C_{18} reverse-phase column (4.6 mm × 250 mm; HiChrom, Reading, UK). The mobile phase was 50 mmol/L sodium acetate–50 mmol/L citrate/8% (v/v) methanol, pH 3.1. HPLC analysis was performed under isocratic conditions at a flow rate of 1 mL/min and UV detector set at 274 nm. 3-NT peaks were determined according to its retention time and the peaks were either confirmed by spiking with added exogenous 3-NT (10 μmol/L) or reducing to aminotyrosine [20]. 3-NT levels were expressed as nmol 3-NT/μmol tyrosine.

2.8. Statistical analysis

Statistical evaluation for proportional comparisons for quantitative cultures and biochemical values were analyzed using one-way ANOVA followed by the Tukey test. Logarithmic values of quantitative cultures values were used in analysis. Comparisons for intestinal injury score were analyzed by Kruskall–Wallis variance analysis followed by Dunn test. Data were presented as mean ± S.E.M. for microbiologic results and mean ± S.D. for biochemical analysis. *P*-values less than 0.05 were considered as statistically significant.

3. Results

All animals survived the experimental protocol. The incidence of BT within the groups is summarized in Table 1.

Table 1
Incidence of bacterial translocation and quantitative results in tissue specimens

Group	MLN		Spleen		Liver	
	Incidence	CFU/g	Incidence	CFU/g	Incidence	CFU/g
Sham	0/8 (0%)	≤0.17	0/8 (0%)	≤0.17	0/8 (0%)	≤0.17
Burn	7/8 (87.5%)*	6.08 ± 2.8	5/8 (62.5%)*	3.83 ± 3.5	3/8 (37.5%)	3.19 ± 3.8
Burn + 3-AB	3/8 (37.5%)	2.55 ± 3.96	2/8 (25%)	1.43 ± 3.25	1/8 (12.5%)	0.7 ± 2.82

Values expressed as logarithm of geometric mean (CFU/g tissue) ± S.E.M.
* $P < 0.05$, significantly different from the other groups.

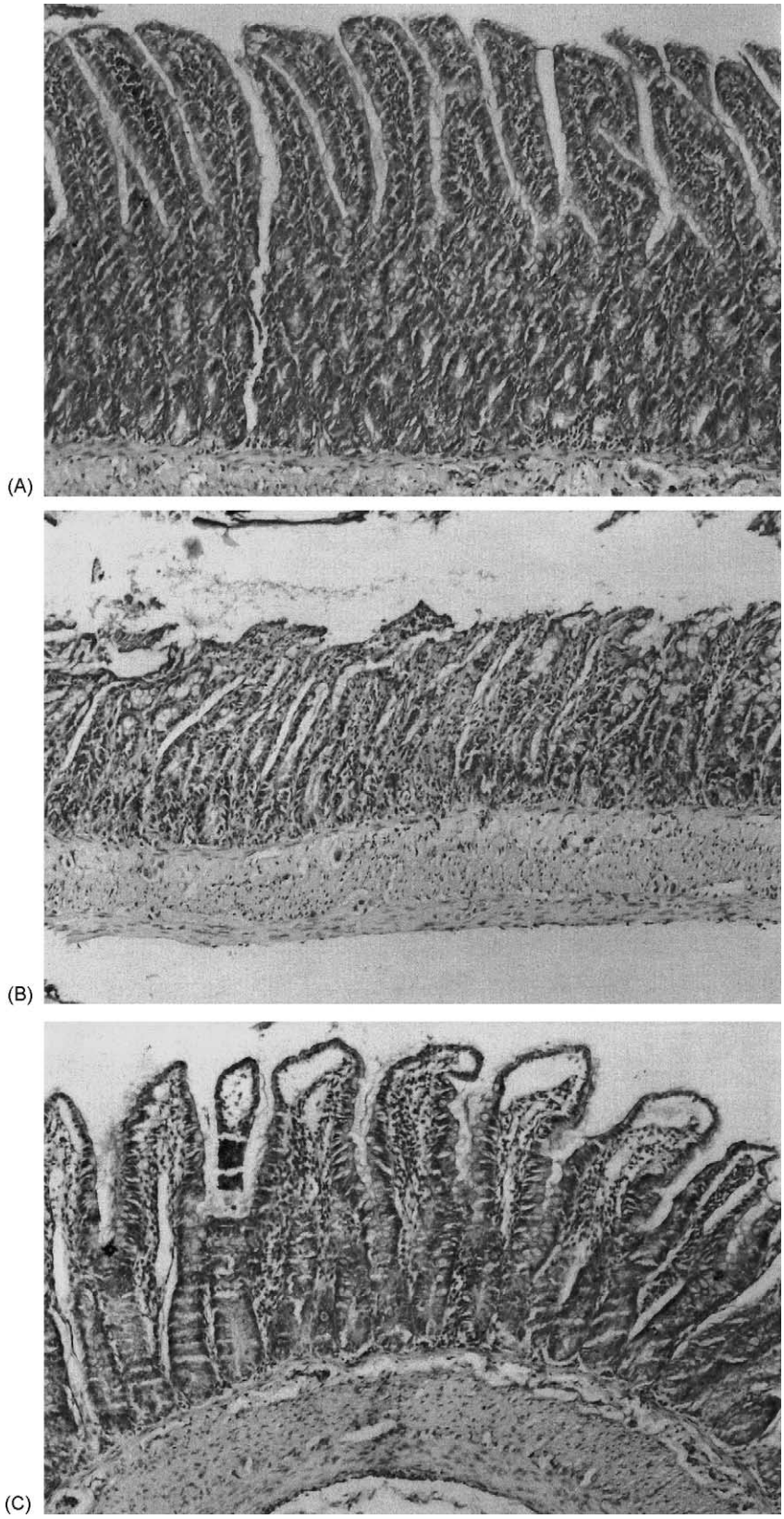

Fig. 1. Photomicrographs of small intestine segments. (A) Sham group showing normal histopathology. (B) Burn group showing denuded and massive epithelial lifting on the villi, and neutrophil infiltration in lamina propria. (C) Burn + 3-AB group showing development of subepithelial space at the apex of the villus. 3-AB pre-treatment before thermal injury in a decrease in epithelial injury (H&E, 100×).

Table 2
Descriptive statistics of histopathological scores of the ileal specimens obtained from the study groups

Group	*N*	Mean ± S.D.
Sham	8	0
Burn*	8	1.125 ± 1.126
Burn + 3-AB	8	0.25 ± 0.70

* $P < 0.05$, significantly different from the other groups.

BT did not occur in the sham control rats. Burn caused severe BT in rats in the second group, and the incidence of bacteria isolated from MLN and spleen was significantly higher than the other groups ($P < 0.05$). Also, burn caused BT in liver, but this finding was not statistically significant compared with the sham group. Although the incidence of BT in liver was reduced in the burn + 3-AB group compared to the burn group, this difference was not statistically significant (Table 1).

Samples of small intestine of the rats were assessed for tissue damage by histopathological examination. Photomicrographs of sections of the small intestine are presented in Fig. 1A–C. Thermal injury caused significant tissue damage (Table 2 and Fig. 2). These changes varied from denuded villi with exposed dilated capillaries to significant architectural distortion and lamina propria disintegration (Fig. 1B). The histopathological score of the burn group (1.125 ± 1.126)

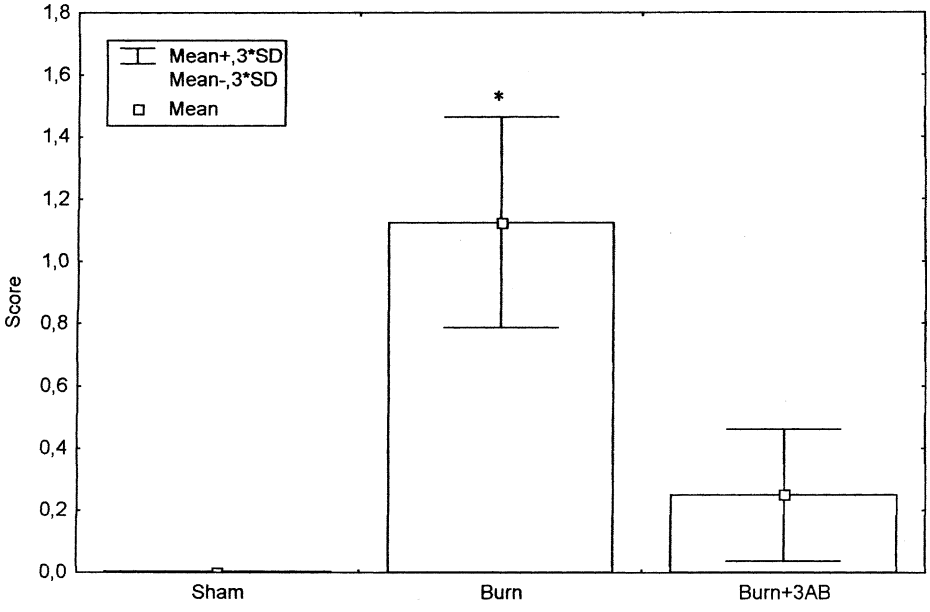

Fig. 2. Histopathological scores of the samples of small intestine. Results are presented as mean ± S.D. Burn group shows significantly higher injury scores when compared to sham control and burn + 3-AB groups. *$P < 0.05$, Kruskall–Wallis variance analysis followed by Dunn test.

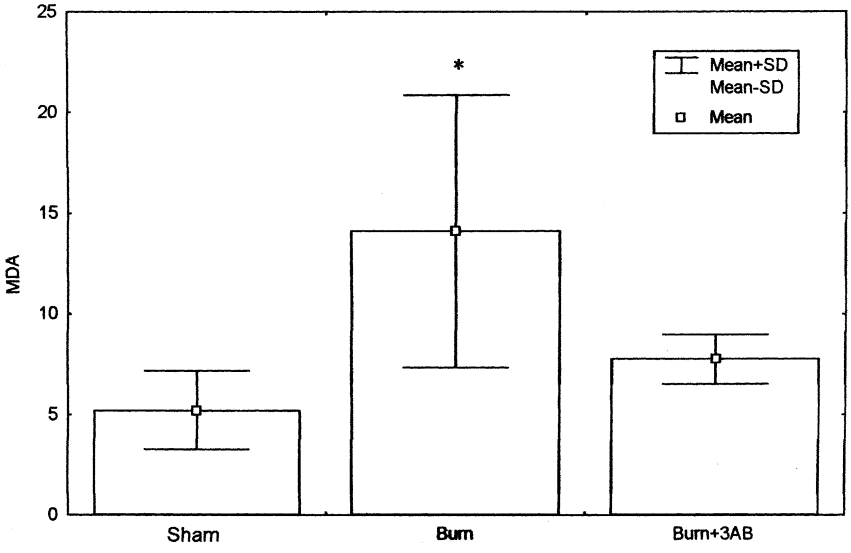

Fig. 3. Tissue MDA levels in all groups. Data expressed as mean ± S.D. *$P < 0.05$, significantly different from the others group.

Table 3
The results of biochemical analysis of tissue samples

Parameter	Sham	Burn	Burn + 3-AB	*P*-value
MDA (nmol/mL)	5.21 ± 1.94	14.1 ± 6.78*	7.75 ± 1.23	<0.05
3-NT (μmol/tyrosine/L)	21.88 ± 15.01	49.98 ± 7.13*	30.17 ± 19.07	<0.05

Values are expressed as mean ± S.D.
* $P < 0.05$, significantly different from the other group.

was significantly higher than the other groups (0 and 0.25 ± 0.70; $P < 0.05$) (Table 2). 3-AB pre-treatment significantly reduced the burn induced mucosal injury compared to the burn group ($P < 0.05$) (Figs. 1C and 2).

The results of biochemical analysis in the tissue are presented in Table 3. MDA levels were significantly increased in the burn group in comparison to sham and burn + 3-AB groups ($P < 0.01$). MDA levels were significantly reduced in the burn + 3-AB group with pre-treatment 3-AB (Fig. 3).

Thermal injury caused significant increase level of 3-NT in the burn group when compared to the sham group ($P < 0.01$). Increased level of 3-NT was suppressed by pre-treatment 3-AB (Table 3 and Fig. 4).

4. Discussion

In the present study, we demonstrated that PARS inhibition attenuated post-burn ileal injury and prevented burn induced BT. Thermal injury caused severe damage in intestinal barrier and BT accompanied it. The administration of 3-AB protected the intestinal barrier and prevented BT.

These results suggest a relationship between the BT and toxicity of nitric oxide on the intestinal mucosal barrier after burn. Experimental studies have shown that mesenteric blood flow decreases by more than 50% in the first 8 h after severe thermal injury, which rises back to control levels nearly 20 h after the thermal injury [5,6]. The main cause of decreased mesenteric blood flow is transient selective vasospasm in splanchnic area and this post-burn splanchnic vasospasm results in I–R injury to intestinal mucosa leading to increased BT from the gut. In addition, the maximal incidence of BT occurs in the first 24 h after burn [21,22]. The microbiological results of our study confirm these reports. In our study, thermal injury caused the BT on the post-burn 24 h.

The ischemia–reperfusion injury of small intestine has been shown to occur after a burn injury. Potential mechanisms causing the development and progression of I–R injury include the release of pro-inflammatory cytokines, as well as the formation of ROS and RNS [7]. The mechanism of the RNS mediated intestinal mucosal damage may involve the nitric oxide (NO)-derived $ONOO^-$, which activates a nuclear enzyme, PARS. Recent studies have shown that NO plays a very important and paradoxical role in intestinal physiology. Small amounts of NO may have a cytoprotective effect by enhancing mucosal blood flow. However, the excess production of NO can cause to impair the intestinal integrity by forming peroxynitrite with superoxide anion, and leading to failure of the gut barrier function [9,10]. Furthermore, Chen et al. showed that

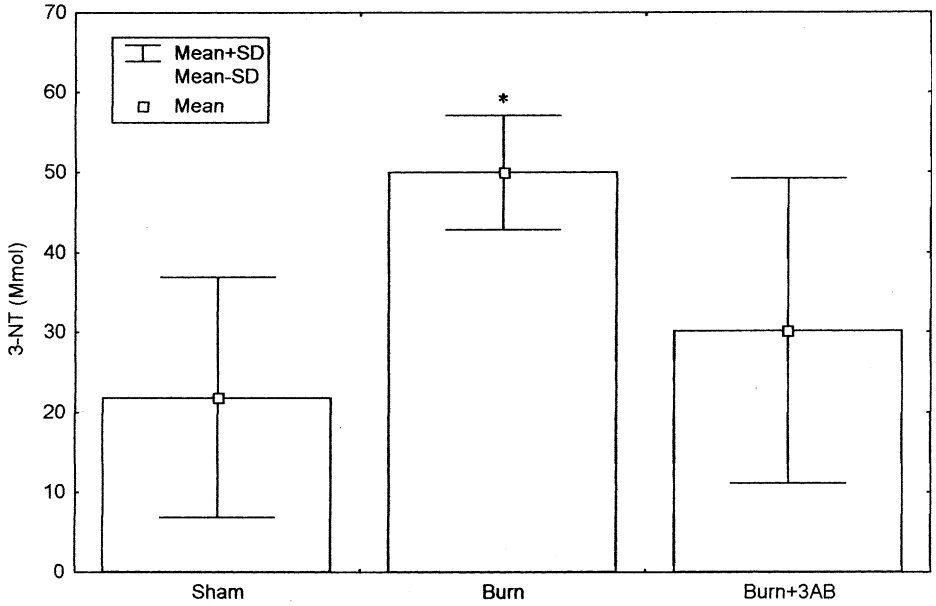

Fig. 4. Tissue 3-NT levels in all groups. All data is presented as mean ± S.D. *$P < 0.05$, compared to other groups.

inhibition of inducible nitric oxide synthase (iNOS) reduces the intestinal mucosal peroxynitrite levels and improves the barrier function of the intestinal mucosa after burn [23]. Our findings clearly showed that thermal injury attenuated the intestinal mucosal integrity. Histopathologic scores of the bowel specimens revealed that 3-AB pre-treatment protects the small intestine after burn (Table 2 and Fig. 2). These results suggested that peroxynitrite may play a role in intestinal injury.

Peroxynitrite is a strong oxidant product and as an oxidative and nitrosative stress mediator, it is mentioned as the ugly face of NO [24]. There are several pathways leading to cellular dysfunction which are initiated by $ONOO^-$. First, the great part of superoxide scavenging activity of superoxide dismutase can be inactivated by peroxynitrite [25]. Moreover, $ONOO^-$ causes lipid peroxidation on many cellular membranes, thereby impairing their function [26]. In our study, we observed that the level of tissue MDA, which is the product of lipid peroxidation, was significantly increased after thermal injury. This finding shows that $ONOO^-$ may lead to lipid peroxidation in the enterocyte membrane. Pre-treatment with 3-AB reduced the level of MDA in the small intestine after burn. This set of data showed that lipid peroxidation and oxidative stress were prevented with the protective effect of 3-AB. These results are parallel to the current literature which report the prevention of lipid peroxidation in lipopolysaccharide induced organ dysfunction by PARS inhibition [27,28].

Peroxynitrite can cause DNA strand breakage, which triggers the activation of PARS. PARS plays an important role in the repair of strand breaks in DNA and its activation results in depletion of cellular NAD^+ and ATP, and consequently in cell death [12,13]. Overall, this process has been termed the ''PARS Suicide Hypothesis'' [14]. Experimental studies have demonstrated that PARS inhibition has beneficial effects against organ dysfunction due to reperfusion injury and endotoxemia [7,14,27]. Shimoda et al. have clearly demonstrated that PARS inhibition reduces both the morphological and the physiological changes in the lung in model of burn and smoke inhalation [29]. The nitration of protein tyrosine residues by $ONOO^-$ results in the formation of 3-NT, the footprint of $ONOO^-$, and the appearance of this product in tissue samples is taken as a diagnostic tool for exposure to peroxynitrite [30]. However, 3-NT is not necessary for the formation of $ONOO^-$ since other nitrating agents, such as myeloperoxidase, lactoperoxidase, and lipogenase may also contribute to the 3-NT formation [31]. First, Chen et al. have reported that 3-NT has been detected in rat intestinal mucosa following thermal injury. In this study, they also have showed that inhibition of iNOS significantly decreased the 3-NT [23]. In our study, we observed that the level of 3-NT increased in burn group and it decreased by pre-treatment with 3-AB in burn + 3-AB group. Actually, administration of 3-AB did not completely prevent 3-NT formation in burn + 3-AB group (Fig. 4). The partial decrease in the 3-NT level in burn + 3-AB group was an indirect finding of the presence of possible nitrating mechanism other than $ONOO^-$ pathway. The rest of the 3-NT formation must be due to the contribution of other reactions, as mentioned before [24,32]. We did not exclude other nitrating mechanisms leading to the formation of 3-NT but we based this study on the idea that $ONOO^-$ is the most likely source in vivo.

In addition, there are several pathways of $ONOO^-$ cytotoxicity [33]. Beneficial effects of PARS inhibition have been documented by various tissue injury studies. Zingarelli et al. demonstrated that PARS inhibition reduced the expression of P-selectin and ICAM-1 in I–R model [34]. Furthermore, $ONOO^-$ causes a direct inhibition of mitochondrial respiration and membrane pumps [35]. Similarly, $ONOO^-$ may have caused damage on the mucosal barrier after burn as previously mentioned. Nuclear factor-kappa B (NF-κB), as a transcriptional regulatory protein, plays a central role in regulating cytokines and other mediators, which are involved in tissue injury associated with I–R [36]. Recently, Malata and Galinanes have demonstrated that $ONOO^-$ enhances NF-κB mediated pro-inflammatory signal transduction pathways [37]. PARS inhibition presenting anti-inflammatory and antioxidant effects may have a role in diminishing the activity of NF-κB in this experimental study.

In summary, thermal injury induces intestinal mucosal disintegrity; PARS inhibition ameliorates the intestinal mucosal injury and thus prevents BT after burn. The increase in BT incidence in post-burn injury may be due to the excess $ONOO^-$ production and its effects on the intestinal mucosal barrier. The results of this study support that PARS inhibition may provide a novel therapeutic approach in reducing gut barrier failure seen after thermal injury.

References

[1] Sitting K, Deitch EA. Effect of bacteremia on mortality after thermal injury. Arch Surg 1988;123:1367–70.
[2] Housenger TA, Brinkerhoff C, Warden GD. The relationship between platelet count, sepsis and survival in pediatric burn patients. Arch Surg 1993;128:65–7.
[3] Moore FA. The role of the gastrointestinal tract in postinjury multiple organ failure. Am J Surg 1999;178:449–53.
[4] Deitch EA, Berg R. Bacterial translocation from the gut: a mechanism of infection. J Burn Care Rehabil 1987;8:475–82.
[5] Morris SE, Navartam N, Hendro D. A comparison of effects of thermal injury and smoke inhalation on bacterial translocation. J Trauma 1990;30:639–45.
[6] Tokyay R, Zeigler ST, Traber DL, Stothert JC, Loick HM, Heggers JP, et al. Postburn gastrointestinal vasoconstriction increases bacterial and endotoxin translocation. J Appl Physiol 1993;74:1521–7.
[7] Cuzzocrea S, Zingarelli B, Costantino G, Szabo A, Salzman AL, Caputi AP, et al. Beneficial effects of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. Br J Pharmacol 1997;121:1065–74.
[8] Willy C, Dahouk S, Starek C, Kaffenberger W, Gerngrob H, Plappert UG. DNA damage in humane leucocytes after ischemia/reperfusion injury. Free Radic Biol Med 2000;28:1–12.

[9] Nadler EP, Ford HR. Regulation of bacterial translocation by nitric oxide. Pediatr Surg Int 2000;16:165–8.
[10] Salzman AL. Nitric oxide in the gut. New Horizon 1995;3:33–45.
[11] Pryor W, Sguadrito G. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol 1995;268:699–722.
[12] Szabo C, Zingarelli B, O'Conner M, Salzman AL. DNA strand breakage, activation of poly(ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci USA 1996;93:1753–8.
[13] Szabo C, Dawson WL. Role of poly(ADP-ribose) synthetase in inflammation and ischemia–reperfusion. TIPS 1998;19:287–98.
[14] Cuzzera S, Zingarelli B, Constantino G, Sottile A, Teti D, Caputi AP. Protective effect of poly(ADP-ribose) synthetase inhibition on multiple organ failure after zymosan-induced peritonitis in rat. Crit Care Med 1999;27:1517–23.
[15] Walker HL, Mason Jr AD. A standard animal burn. J Trauma 1968;8:1049–51.
[16] Isenberg HD. Clinical microbiology procedures handbook. 1st ed. Washington, DC: ASM; 1992, p. 15–7.
[17] Chiu CT, McArdle AH, Brown R, Scott H, Gurd F. Intestinal mucosal lesion in low-flow states. Arch Surg 1970;101:478–83.
[18] Yagi K. Lipid peroxides and related radicals in clinical medicine. In: Armstrong D, editor. Free radicals in diagnostic medicine. New York: Plenum Press; 1994. p. 1–15.
[19] Golowich SP, Kaplan SD. Methods in enzymology, vol. II. New York: Academic Press; 1955. p. 769.
[20] Ünlü A, Türközkan N, Cimen B, Karabicak U, Yaman H. The effect of *Escherichia coli*-derived lipopolysaccharides on plasma levels of malondialdehyde and 3-nitrotyrosine. Clin Chem Lab Med 2001;39:491–3.
[21] Hendron DN, Zeigler ST. Bacterial translocation after thermal injury. Crit Care Med 1993;21(2):S50–4.
[22] Fukushima R, Gianotti L, Alexander JW, Pyles T. The degree of bacterial translocation is a determinant factor for mortality after burn injury and is improved by prostaglandin analogs. Ann Surg 1992;216:438–45.
[23] Chen LW, Hsu CM, Wang JS, Chen JS, Chen SC. Specific inhibition of iNOS decreases the intestinal mucosal peroxynitrite level and improves the barrier function after thermal injury. Burns 1998;24:699–705.
[24] Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. Am J Physiol 1996;271: C1424–37.
[25] Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, et al. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch Biochem Biophys 1992;298:431–7.
[26] Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite induced membrane lipid-peroxidation: the cytotoxic potential of superoxide and nitric-oxide. Arch Biochem Biophys 1991;288:481–7.
[27] Özdülger A, Cinel I, Ünlü A, Cinel L, Marioglu I, Tamer L, et al. Poly(ADP-ribose) synthetase inhibition prevents lipopolysaccharide-induced peroxynitrite mediated damage in diaphragm. Pharm Res 2002;46:67–73.
[28] Dirlik M, Çağlıkülekçi M, Cinel I, Cinel L, Tamer L, Pata C, et al. The effect of PARS inhibition on ileal histopathology, apoptosis and lipid peroxidation in LPS-induced obstructive jaundice. Pharm Res 2003;48:139–49.
[29] Shimoda K, Marukami K, Enkhbaatar P, Traber LD, Cox RA, Hawkins HK, 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:L240–9.
[30] Ischiropoulos H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. Arch Biochem Biophys 1998;356:1–11.
[31] van der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. J Biol Chem 1997;272:7617–25.
[32] Beckman JS, Beckman TW, Chen J, Marshall BA, Freeman BA. Apparent OH radical production from peroxynitrite: implications for endothelial injury by nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620–4.
[33] Szabo C. Multiple pathways of peroxynitrite cytotoxicity. Toxicol Lett 2003;140–141:105–12.
[34] Zingarelli B, Salzman AL, Szabo C. Genetic distruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. Circ Res 1998;83:85–94.
[35] Szabo C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in a rat mode of endotoxic shock. Importance of activation of poly(ADP-ribose) synthetase by peroxynitrite. J Clin Invest 1997;100:723–35.
[36] Abraham E. NF-κB activation. Crit Care Med 2000;28:N100–4.
[37] Malata BM, Galinanes M. Peroxynitrite is an essential component of cytokine production mechanism in human monocytes through modulation of NF-{kappa}B DNA binding activity. J Biol Chem 2001;277:2330–5.