Inhibition of PARS attenuates endotoxin-induced dysfunction of pulmonary vasorelaxation

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Inhibition of PARS attenuates endotoxin-induced dysfunction of pulmonary vasorelaxation. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L769–L776, 1999.—Endotoxin (Etx) causes excessive activation of the nuclear repair enzyme poly(ADP-ribose) synthase (PARS), which depletes cellular energy stores and leads to vascular dysfunction. We hypothesized that PARS inhibition would attenuate injury to mechanisms of pulmonary vasorelaxation in acute lung injury. The purpose of this study was to determine the effect of in vivo PARS inhibition on Etx-induced dysfunction of pulmonary vasorelaxation. Rats received intraperitoneal saline or Etx (Salmonella typhimurium; 20 mg/kg) and one of the PARS inhibitors, 3-aminobenzamide (3-AB; 10 mg/kg) or nicotinamide (Nic; 200 mg/kg), 90 min later. After 6 h, concentration-response curves were determined in isolated pulmonary arterial rings. Etx impaired endothelium-dependent (response to ACh and calcium ionophore) and -independent (sodium nitroprusside) cGMP-mediated vasorelaxation. 3-AB and Nic attenuated Etx-induced impairment of endothelium-dependent and -independent pulmonary vasorelaxation. 3-AB and Nic had no effect on Etx-induced increases in lung myeloperoxidase activity and edema. Lung ATP decreased after Etx but was maintained by 3-AB and Nic. Pulmonary arterial PARS activity increased fivefold after Etx, which 3-AB and Nic prevented. The beneficial effects were not observed with benzocid acid, a structural analog of 3-AB that does not inhibit PARS. Our results suggest that PARS inhibition with 3-AB or Nic improves pulmonary vasorelaxation and preserves lung ATP levels in acute lung injury.

poly(ADP-ribose) synthase; pulmonary artery; 3-aminobenzamide; nicotinamide; myeloperoxidase

ENDOTOXEMIA RESULTS in the increased production of both nitric oxide (13, 34) and superoxide anion (35). When both of these species are present in large amounts, the formation of peroxynitrite (ONOO−), a potent oxidant and nitrating reagent, is favored (4, 12, 34). Nitric oxide is the only known endogenous molecule produced in high enough concentrations in pathological conditions that can effectively compete with superoxide dismutase for superoxide (5). Peroxynitrite is a strong activator of DNA single-strand breaks, resulting in excessive stimulation of poly(ADP-ribose) synthase (PARS) (31). This process depletes NAD+ (23), leading to inhibition of glycolysis and decreased ATP formation (21). This mechanism of cellular injury has been proposed as a major pathway involved in the vascular dysfunction observed in endotoxic shock (32, 39, 40). Peroxynitrite formation in acute lung injury, as evidenced by 3-nitrotyrosine formation, has been found in endotoxin-treated rats (34) and in human autopsy specimens with sepsis-induced diffuse alveolar damage (15). In nonsepsis models of pulmonary inflammation, PARS inhibition prevented edema from excitatory amino acid (N-methyl-D-aspartate) toxicity in the isolated perfused rat lung (22) and improved cellular energetics in macrophages harvested from rats subjected to carrageenan-induced pleurisy (8) as well as in a human pulmonary epithelial cell line exposed to peroxynitrite (30).

Endotoxin-induced acute lung injury is characterized by lung edema, neutrophil sequestration, and increased pulmonary vascular resistance (7, 9). We and others (9, 16, 18, 25) have found that endotoxin causes dysfunction of pulmonary vasorelaxation in response to agonists that require the generation of cGMP. This endotoxin-induced dysfunction of pulmonary vasorelaxation is mediated, in part, by polymorphonuclear leukocytes; neutrophil depletion attenuates this acute lung injury in a rat model (24).

Activation of PARS in response to peroxynitrite-mediated DNA single-strand breaks may be responsible for the cellular energy depletion and vascular dysfunction associated with endotoxemia. In vivo administration of a PARS inhibitor attenuated the impairment of contractility in thoracic aortic rings (32) and improved mean arterial pressure (41) in endotoxin-treated rats. The dysfunction in endothelium-dependent relaxant responses in rat thoracic aortic rings caused by endotoxin was also ameliorated by the PARS inhibitor 3-aminobenzamide (3-AB) (27). PARS inhibition with 3-AB also reduced neutrophil recruitment and tissue edema in zymosan- and carrageenan-triggered models of local inflammation (29).

We hypothesized that in vivo inhibition of PARS would attenuate endotoxin-induced impairment of pulmonary vasorelaxation. The purpose of this study was to determine the effect of the PARS inhibitors 3-AB and nicotinamide (Nic) on endotoxin-induced, cGMP-mediated pulmonary vasomotor dysfunction. Endothelium-dependent relaxation was studied with the receptor-dependent agonist ACh and the receptor-independent agonist calcium ionophore A-23187. Endothelium-independent relaxation was examined with direct stimu-
lation of vascular smooth muscle with the use of the nitric oxide donor sodium nitroprusside (SNP). We also examined the effect of PARS inhibition with 3-AB and Nic in endotoxemia on lung ATP levels and pulmonary arterial PARS activity. A secondary purpose of this study was to determine the effect of 3-AB and Nic on endotoxin-induced lung edema and myeloperoxidase activity as a measure of neutrophil accumulation. The results of this study demonstrate that PARS inhibition with 3-AB and Nic in endotoxemia 1) attenuates dysfunctions of endothelium-dependent and -independent mechanisms of pulmonary vasorelaxation, 2) has no effect on lung myeloperoxidase activity and edema, and 3) maintains lung ATP levels.

MATERIALS AND METHODS

Animal care and housing. All animals received humane care in compliance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing 250–300 g were quarantined in quiet, humidified, light-cycled rooms for 2–3 wk before use. Rats were allowed ad libitum access to food and water throughout quarantine.

Experimental protocol. Rats were administered normal saline (NS; 1 ml ip), endotoxin [Et; 20 mg/kg ip Salmonella typhimurium lipopolysaccharide (LPS) in 1 ml of NS], a PARS inhibitor alone (3-AB, 10 mg/kg ip, or Nic, 200 mg/kg ip, in 1 ml of NS), or EtX followed by 3-AB or Nic. Rats received 3-AB or Nic 90 min after EtX to avoid any potential interference due to the presence of nitric oxide synthase (NOS) induction. The doses of 3-AB and Nic were chosen on the basis of multiple previous in vivo studies employing them in rats (8, 32, 33, 41). A similar set of experiments were performed with the use of a structural analog of 3-AB that does not inhibit PARS [benzoic acid (BA); 10 mg/kg ip]. Rats were provided chow and water ad libitum during the 6-h period after initial injection. Norats died during the 6-h experimental time course. A previous experiment using the same dose of EtX resulted in 15% mortality at 72 h (unpublished data).

Isolated pulmonary arterial ring preparation. Isolated pulmonary arterial rings were harvested and prepared as previously described (9, 24). Five rats (10 rings) were studied in each group. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). Median sternotomy was performed, and heparin sulfate (500 USP units) was injected into the right ventricular outflow tract. Lungs were then surgically removed, externally rinsed with saline, and blotted dry. Five lungs from five rats were studied in each group.

Lung myeloperoxidase assay. Segments of lung weighing 400–500 g were snap frozen in liquid nitrogen for subsequent determination of myeloperoxidase (MPO) activity. Lung tissue was homogenized for 30 s in 4 ml of 20 mM potassium phosphate buffer, pH 7.4. Lung protein was quantified with the use of the Coomassie plus protein assay (Pierce, Rockford, IL). The samples were then centrifuged for 30 min at 40,000 g at 4°C (Beckman L-80 Ultracentrifuge; Beckman Instruments, Palo Alto, CA). The pellet was resuspended in 4 ml of 50 mM potassium phosphate buffer, pH 6.0, containing 0.5 g/dl cetrimonium bromide. The samples were sonicated for 90 s at full power (ultrasonic homogenizer; Cole-Parmer Instruments, Chicago, IL), incubated in a 60°C water bath for 2 h, and centrifuged for 10 min at maximum speed (Eppendorf 5415C; Baxter, San Diego, CA). The supernatant (25 µl) was added to 725 µl of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml o-dianisidine and 5 × 10−4% hydrogen peroxide. Absorbance of 460-nm visible light was measured between 1 and 3 min (Beckman DU7 spectrophotometer; Beckman Instruments, Irvine, CA). MPO activity (units/mg lung protein) was then calculated as

\[ \text{MPO activity} = \frac{(A_{460})(13.5)}{\text{l lung protein}} \]

where A460 is the change in absorbance of 460-nm light from 1 to 3 min after the initiation of the reaction. The coefficient 13.5 was empirically determined such that 1 unit of MPO activity is the amount of enzyme that will reduce 1 µmol peroxide/min (3). Lung protein is measured in milligrams.

Lung wet-to-dry weight. Harvested left or right lungs were weighed for determination of wet weight. In separate specimen containers, they were dried over a desiccant (Drierite, Xenia, OH) at 4°C for 5 days to a constant weight and then reweighed.

Lung ATP assay. Lung ATP levels were determined with the use of a quantitative, enzymatic assay (Sigma Diagnostics, St. Louis, MO). Lungs were harvested as described in
Lung harvest for myeloperoxidase assay, wet-to-dry weight determination, and ATP assay, and were immediately frozen with the use of liquid nitrogen. Segments of frozen lung weighing 100–200 mg were ground to a fine powder with the use of mortar and pestle and then sonicated (ultrasonic homogenizer) at full power on ice for 30 s in 1 ml of 12% TCA. Segments of frozen lung weighing 100–200 mg were ground to a fine powder with the use of liquid nitrogen. Segments of frozen lung were immediately frozen on ice. Homogenization of the samples was performed by sonication (ultrasonic homogenizer) at full power for 30 s in 1 ml of 12% TCA. Supernatant (the volume containing 20 µg of protein), PARS buffer (10 µl), 1 mM NAD (10 µl), 2 µCi 32P-labeled poly(ADP) ribosylation of proteins. The labeled ADP present in the supernatant was then measured by scintillation counting after TCA precipitation onto a filter. Calculation of PARS activity proceeded as follows:

PARS activity = \[ \frac{\text{total cpm/reaction}}{\text{reaction time} \times \text{vol sample} \times \text{NAD sp act}} \]

where units are as follows: PARS activity, nmol·min\(^{-1}\)·µl\(^{-1}\); reaction time, min; vol (volume of) sample, µl; and NAD specific activity (sp act), counts·min\(^{-1}\)·(cpm)·nmol\(^{-1}\). The specific activity of NAD was determined by calculating the total counts in a background control reaction containing 10 nmol of NAD. Results are expressed as picomoles per minute per microliter.

Reagents. Standard reagents as well as the S. typhimurium strain used were obtained from Sigma Chemical (St. Louis, MO), with the exception of A-23187 (Calbiochem, La Jolla, CA). Fresh solutions were prepared daily with either deionized water or NS as the diluent. Concentrations are expressed as final molar concentrations in the organ chambers.

Statistical analysis. Statistical analyses were performed on a Macintosh Quadra computer with StatView software (Brain Power, Calabasas, CA). Data are presented as means ± SE of the number of rings or lungs studied at each point of data collection. In ring experiments, comparisons between groups were made at the same concentrations. Statistical evaluation utilized standard one-way ANOVA with post hoc Bonferroni-Dunn correction. P < 0.05 was accepted as statistically significant.

RESULTS

Effects of PARS inhibition with 3-AB and Nic on Etx-induced impairment of cGMP-mediated pulmonary vasorelaxation. The vasoconstriction response to the α-adrenergic agonist PE is unchanged after Etx (Fig. 1). Etx administration significantly impaired endothelium-dependent, receptor-dependent pulmonary arterial vasorelaxation (response to ACh), and PARS inhibition with 3-AB or Nic attenuated this injury (Fig. 2A). Rings from saline-treated rats were preconstricted with PE to 285 ± 16 mg tension and relaxed to 16 ± 4 mg tension at 10\(^{-6}\) M ACh, and rings from Etx-treated rats were preconstricted to 283 ± 16 mg tension and relaxed to 168 ± 12 mg tension. In Etx+3-AB and Etx+Nic rats, 106 ± 13 and 66 ± 7 mg PE-induced ring tension remained at 10\(^{-6}\) M ACh, respectively (P < 0.05 vs. Etx alone, P < 0.05 vs. control).

Endothelium-dependent, receptor-independent cGMP-mediated pulmonary vasorelaxation (response to calcium ionophore A-23187) was significantly impaired after Etx, and both PARS inhibitors were beneficial (Fig. 2B). Control rings were preconstricted to 299 ± 13 mg tension and relaxed to 19 ± 4 mg tension at 10\(^{-6}\) M A-23187. Rings from Etx-treated rats were preconstricted to 280 ± 8 mg tension, and those from Etx+3-AB and Etx+Nic rats were preconstricted to 283 ± 6 and 288 ± 10 mg tension, respectively. In the Etx-treated rat group, 117 ± 9 mg tension remained in response to 10\(^{-6}\) M A-23187 compared with 60 ± 9 (Etx+3-AB) and 45 ± 8 (Etx+Nic) mg tension in the
PARS inhibitor groups (P < 0.05 vs. Etx alone, P < 0.05 vs. control).

Endothelium-independent cGMP-mediated vasorelaxation (response to SNP) by direct stimulation of guanylate cyclase was also impaired after Etx treatment, and, again, PARS inhibition with 3-AB and Nic attenuated this dysfunction (Fig. 2C). Control rings were preconstricted to 292 ± 8 mg tension, with 4 ± 2 mg tension remaining at 10^{-6} M SNP, and Etx rings were preconstricted to 292 ± 8 mg tension and relaxed to 80 ± 10 mg tension. PARS inhibition resulted in 22 ± 6 (Etx + 3-AB) and 21 ± 4 (Etx + Nic) mg tension remaining at 10^{-6} M SNP (P < 0.05 vs. Etx alone, P < 0.05 vs. control).

Administration of the PARS inhibitor 3-AB or Nic alone did not affect cGMP-mediated vasorelaxation (data not shown). Neither endothelium-dependent (either receptor-dependent ACh or receptor-independent A-23187) nor endothelium-independent (SNP) vasorelaxation responses were different in comparison with saline-treated control rats.

Administration of BA, a compound that is similar to 3-AB but does not inhibit PARS, had no effect on cGMP-mediated pulmonary vasorelaxation in saline-treated control rats (data not shown). Responses in rings from Etx + BA rats were not different compared with those from Etx-treated rats (P > 0.05 vs. Etx) for both endothelium-dependent and endothelium-independent cGMP-mediated pulmonary vasorelaxation (Fig. 3).

Effects of PARS inhibition with 3-AB and Nic on lung MPO activity in endotoxemia. Etx increased lung MPO activity more than threefold in comparison with saline-treated control rats. Treatment with 3-AB or Nic did not affect cGMP-mediated pulmonary vasorelaxation in Etx-treated rats (P > 0.05 vs. Etx). Treatment with BA did not affect cGMP-mediated pulmonary vasorelaxation in Etx-treated rats (P > 0.05 vs. Etx).

*P < 0.05 vs. control at same concentration of agonist.
attenuate this effect (Fig. 4). Lung MPO in controls was 0.21 ± 0.09 units/mg protein. Endotoxia in 6 h resulted in 0.75 ± 0.07 units MPO/mg protein (P < 0.05 vs. control). Lung MPO activity after Etx and either 3-AB (0.84 ± 0.05 units MPO/mg protein) or Nic (0.65 ± 0.10 units MPO/mg protein) was not different from that in Etx-treated rats (P > 0.05 vs. Etx).

Effect of PARS inhibition with 3-AB and Nic on lung edema after Etx. Lung wet-to-dry weight ratios (W/D) were significantly greater after Etx treatment, and the PARS inhibitors did not demonstrate any beneficial effects (Table 1). Although endotoxia resulted in an increase in W/D to 4.76 ± 0.03 from the control value of 4.43 ± 0.03 (P < 0.05), neither 3-AB (W/D 4.79 ± 0.03) nor Nic (W/D 4.80 ± 0.05) attenuated this injury (P > 0.05 vs. Etx).

Effect of PARS inhibition with 3-AB and Nic on lung ATP levels in endotoxia. The Etx-induced decrease in lung ATP levels at 6 h was prevented by PARS inhibition with 3-AB and Nic (Fig. 5). Etx reduced lung ATP from the control value of 13.39 ± 0.50 nmol/mg protein (P < 0.05). Etx+3-AB-treated rats had lung ATP levels similar to those of control rats (12.47 ± 0.50 nmol/mg protein, P = 0.20) that were also different from those of rats treated with Etx alone (P < 0.05). The lung ATP levels of Etx+Nic-treated rats were also similar to those of saliné-treated rats (13.65 ± 0.69 nmol/mg protein, P = 0.74 vs. control) and different from those of rats treated with Etx alone (P < 0.05). 3-AB or Nic alone had no effect on lung ATP levels in saline-treated rats (data not shown). The lung ATP levels of Etx+BA-treated rats (8.02 ± 0.33 nmol/mg protein) were not different from those of Etx-treated rats (P = 0.37).

Effect of PARS inhibition with 3-AB and Nic after Etx on pulmonary arterial PARS activity. Pulmonary PARS activity increased more than fivefold after Etx treatment, and the PARS inhibitors 3-AB and Nic prevented this increase (Fig. 6). Etx increased pulmonary arterial PARS activity from the control value of 0.188 ± 0.010 pmol·min⁻¹·µl⁻¹ to 1.050 ± 0.047 pmol·min⁻¹·µl⁻¹ (P < 0.05). The pulmonary arteries from Etx+3-AB-treated rats had PARS activity similar to those of control rats (0.176 ± 0.015 pmol·min⁻¹·µl⁻¹, P = 0.83) and different from rats treated with Etx alone (P < 0.05). Similarly, the pulmonary arterial PARS activity of Etx+Nic-treated rats (0.236 ± 0.27 pmol·min⁻¹·µl⁻¹) was not different from control (P = 0.48) but was different from Etx alone (P < 0.05). Neither 3-AB nor Nic alone affected pulmonary arterial PARS activity in saline-treated rats (data not shown). The pulmonary arterial PARS activity of Etx+BA-treated rats (0.951 ± 0.148 pmol·min⁻¹·µl⁻¹) was not different from that of Etx-treated rats (P = 0.32).

**DISCUSSION**

We found that PARS inhibition with 3-AB and Nic in endotoxia attenuates the dysfunction of NO-mediated pulmonary vasorelaxation and does not affect lung MPO activity and edema but maintains lung ATP levels. Although other studies have demonstrated beneficial effects of PARS inhibitors on the systemic vascular dysfunction in endotoxic shock (27, 32, 39, 41), this is the first study to our knowledge that examines the effect of in vivo PARS inhibition on the Etx-induced impairment of pulmonary arterial vasorelaxation. Two distinct PARS inhibitors, 3-AB and Nic, ameliorated the Etx-induced impairment of both endothelium-dependent and -independent mechanisms of pulmonary vasorelaxation. The prototypical, competitive PARS inhibitor 3-AB does not directly scavenge peroxynitrite (32) and does not prevent the development of DNA strand breakage (40). The beneficial effects of 3-AB appear to be related to PARS inhibition as opposed to other possible pharmacological properties of this drug. Its inactive analog, benzoic acid, did not affect the Etx-induced dysfunction of pulmonary vasorelaxation. PARS generates Nic in its enzymatic catalysis of the transfer of ADP-ribose to various proteins.

![Graph](http://ajplung.physiology.org/content/199/3/L773/F4)

**Fig. 4. Lung myeloperoxidase (MPO) activity in control, Etx-, Etx+3-AB-, 3-AB (alone), Etx+Nic-, and Nic (alone)-treated rats. Etx caused a significant increase in lung MPO activity. Values are means ± SE; n = 5 rats/group. *P < 0.05 vs. control and P > 0.05 vs. Etx alone.**

![Table 1](http://ajplung.physiology.org/content/199/3/L773/T1)

**Table 1. Lung wet-to-dry weight ratios**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Lung W/D</th>
<th>P Value vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.43 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Etx</td>
<td>4.76 ± 0.03*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Etx + 3-AB</td>
<td>4.79 ± 0.03*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3-AB</td>
<td>4.46 ± 0.03</td>
<td>0.52</td>
</tr>
<tr>
<td>Etx + Nic</td>
<td>4.80 ± 0.05*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nic</td>
<td>4.42 ± 0.05</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rats/group. Values are lung wet-to-dry weight ratios (W/D) in control, endotoxin (Etx), Etx+3-AB, and Etx+Nicina for each group. Two rats died before the end of the experiment, and their data were excluded from the analysis. The mean values of the control group were significantly different from those of the Etx-treated group. The mean values of the Etx+3-AB and Etx+Nic groups were not significantly different from those of the control group. The mean values of the 3-AB, Etx+3-AB, and Etx+Nic groups were significantly different from those of the Etx-treated group. The mean values of the Etx+Nic group were significantly different from those of the Etx+3-AB group. The mean values of the Etx+Nic group were significantly different from those of the control group. The mean values of the Etx+3-AB and Etx+Nic groups were significantly different from those of the control group. The mean values of the Etx+Nic group were significantly different from those of the control group.

*P < 0.05 vs. control.
Therefore, Nic can decrease PARS activity through negative feedback. Its beneficial effect may also stem from conversion back to NAD\(^+\), thus directly restoring cellular energy levels (41). Although Nic is somewhat less specific than 3-AB, both compounds are potent inhibitors of PARS activity (2). In vivo administration of PARS inhibitors 3-AB and Nic 90 min after Etx prevented decrease in lung ATP (\(\#P < 0.05\) vs. Etx alone and \(P > 0.05\) vs. control). BA, a structural analog of 3-AB that does not inhibit PARS, did not affect a decrease in lung ATP after Etx.

![Fig. 5. Lung ATP levels in control, Etx-, Etx+3-AB-, Etx+Nic-, and Etx+BA-treated rats. Values are means \(\pm\) SE; \(n = 5\) rats/group. Etx caused a significant decrease in lung ATP levels (\(*P < 0.05\) vs. control). In vivo administration of PARS inhibitors 3-AB and Nic 90 min after Etx prevented decrease in lung ATP (\(\#P < 0.05\) vs. Etx alone and \(P > 0.05\) vs. control). BA, a structural analog of 3-AB that does not inhibit PARS, did not affect a decrease in lung ATP after Etx.](http://ajplung.physiology.org/)

In the current study, we found that PARS inhibition with 3-AB and Nic had no effect on Etx-induced lung MPO activity. Previous work in our laboratory (24) has demonstrated the importance of the neutrophil in this model of Etx-induced dysfunction of pulmonary vasorelaxation. Neutrophil depletion with the use of vinblastine or rabbit anti-rat neutrophil antiserum before Etx attenuated the impairment of the response to cGMP-mediated pulmonary vasorelaxation. However, neutrophil depletion did not totally eliminate the dysfunction of pulmonary arterial vasorelaxation. Thus there exist neutrophil-independent mechanisms of vasomotor dysfunction in Etx-induced acute lung injury, and increased PARS activity may be one of these mechanisms contributing to vascular dysfunction. Our results agree with a recent study that also found no effect of 3-AB on Etx-induced increases in rat lung and ileum MPO activity (26). In contrast to our findings, Szabo et al. (26) found a reduction in pulmonary microvascular leakage in 3-AB-treated rats. These results may be explained by the fact that a pretreatment as well as a posttreatment dose of 3-AB was utilized. Perhaps the Etx-induced increase in the lung wet-to-dry ratio is mediated by an early, PARS-dependent mechanism.

In contrast, PARS inhibition decreased neutrophil recruitment in other models of non-Etx-mediated inflammation. The PARS inhibitor 3-AB reduced myocardial neutrophil accumulation after ischemiareperfusion injury (38), and studies in PARS\(^{-/-}\) mice demonstrated a role for this enzyme in the regulation of the adhesion molecules P-selectin and intercellular adhesion molecule-1 (42). Szabo et al. (29) recently found that PARS inhibition with 3-AB prevented both local and systemic inflammation after carrageenan or zymosan challenge. The protective effects appeared to be more pronounced in the severe forms and delayed phase of inflammation, and inhibition of PARS increased the rate of adherent neutrophil detachment.

![Fig. 6. Pulmonary arterial PARS activity in control, Etx-, Etx+3-AB-, Etx+Nic-, and Etx+BA-treated rats. Values are means \(\pm\) SE; \(n = 5\) rats/group. Etx caused a marked increase in pulmonary arterial PARS activity (\(*P < 0.05\) vs. control). In vivo administration of PARS inhibitors 3-AB and Nic 90 min after Etx prevented increase in PARS activity (\(\#P < 0.05\) vs. Etx alone and \(P > 0.05\) vs. control). BA, a structural analog of 3-AB that does not inhibit PARS, did not affect increase in pulmonary arterial PARS activity after Etx.](http://ajplung.physiology.org/)

from the endothelium. In these nonendotoxia models, PARS inhibitors were administered before the inflammatory stimulus, suggesting that an early PARS-dependent mechanism is involved in neutrophil accumulation and edema. The lack of effect by 3-AB and Nic on the Etx-induced increase in MPO activity in the current study and in the study by Szabo et al. (26) suggests that lung neutrophil recruitment in severe endotoxia may be mediated by a PARS-independent step. Further investigation is needed to establish the effect of PARS inhibition on neutrophil accumulation and function in endotoxia.

We further examined the role of PARS in Etx-induced acute lung injury by measuring lung ATP levels and pulmonary arterial PARS activity. The maintenance of lung tissue ATP after 3-AB treatment in endotoxia suggests that PARS activation may be an important pathway in the reduction of cellular energy levels in this model of lung injury. However, lung ATP levels may not accurately represent pulmonary vascular energy status, but previous studies suggest that endotoxia depletes cellular energy levels and that this energy deficit may be linked to a dysfunction of pulmonary arterial vasorelaxation. In rat aortic smooth muscle cells, the PARS inhibitors 3-AB, Nic, and PD-128763 inhibited the reduction in cellular NAD+ and ATP as well as the suppression of mitochondrial respiration in response to LPS and interferon-γ stimulation (32). Rodman et al. (20) found that inhibitors of oxidative phosphorylation reduced receptor-dependent relaxation in both aortic and pulmonary arterial rings. In vivo administration of both 3-AB and Nic prevented the Etx-induced increase in pulmonary arterial PARS activity, confirming the action of the inhibitors in the tissue with which we performed our vasorelaxation experiments. Furthermore, benzoic acid, a compound that is structurally similar to 3-AB but does not inhibit PARS, had no effect on lung ATP levels, pulmonary arterial PARS activity, or the dysfunction of pulmonary vasorelaxation in endotoxia.

Peroxynitrite formation is the proposed initial stimulus leading to PARS activation, with subsequent impairment of cellular energetics and vascular function in endotoxia (32, 39, 40). Although the presence of peroxynitrite in the lung has been demonstrated experimentally in Escherichia coli Etx-treated rats (34) and clinically in autopsy specimens from patients with sepsis-induced pulmonary injury (15), its role in the pulmonary circulation remains unclear. Other investigators have found that peroxynitrite itself is a direct pulmonary arterial vasodilator in the dose range of 10–100 µM (6, 37). However, this observed effect of peroxynitrite occurs at concentrations that are not physiologically relevant because peroxynitrite would remain in the nanomolar range even in disease states (17). Although PARS activity was not measured directly, Chabot et al. (6) found an inhibitory effect of 3-AB on this peroxynitrite-induced vasodilation. However, the effective dose of 3-AB was 10 mM, and a dose of 1 mM 3-AB did not affect the vasodilation to peroxynitrite. The high concentration of 3-AB needed to observe inhibition calls into question the specificity of this dose on PARS activity. The published IC50 of 3-AB is 33 µM, with 88% inhibition of PARS occurring at 1 mM (2). Although peroxynitrite may not be the stimulus for pulmonary arterial PARS activation, hydroxyl radical, a species also present in endotoxia, can cause PARS activation and subsequent endothelial damage (1, 14). Further work is needed to define the relative roles of peroxynitrite and hydroxyl radical in PARS activation and vascular injury.

Although both pulmonary arterial PARS activity and lung ATP levels returned to control values with 3-AB administration, the Etx-induced dysfunction in cGMP-mediated vasorelaxation was only partially attenuated. PARS-independent mechanisms of vascular dysfunction may also contribute to the injury, as has been observed in other models of inflammation (11, 32). Cellular damage in oxidative stress occurs by parallel and/or synergistic pathways, which may or may not involve peroxynitrite generation and PARS activation. Indeed, inhibition of PARS represents just one potential strategy to reduce nitric oxide- or peroxynitrite-mediated cellular injury. Other agents such as specific inhibitors of iNOS, superoxide dismutase mimetics, and scavengers of peroxynitrite also offer promise in the therapy of shock and other inflammatory diseases.

In summary, we found that the PARS inhibitors 3-AB and Nic in Etx-induced acute lung injury attenuate the dysfunction of pulmonary vasorelaxation and maintain lung ATP levels but do not affect lung MPO activity and edema. The data presented here suggest that PARS activation in endotoxia contributes to the development of pulmonary vascular dysfunction. PARS inhibition may provide a novel therapeutic approach in ameliorating the vascular dysfunction seen in sepsis and acute lung injury.

This work was supported in part by National Institute of General Medical Sciences Grant GM-49222, National Institute of Child Health and Human Development Grant HD-36256-01 (to D. D. Bensard), and an American College of Surgery Faculty Research Grant (to R. C. McIntyre, Jr.). E. J. Pulido is the Kiwanis Trauma Research Fellow. Address for reprint requests and other correspondence: R. C. McIntyre, Jr., Dept. of Surgery, Campus Box C-313, Univ. of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262 (E-mail: robert.mcintyre@uchsc.edu).

Received 15 December 1998; accepted in final form 10 June 1999.

REFERENCES


