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Inhibition of poly(ADP-ribose) polymerase prevents vascular hyporesponsiveness induced by lipopolysaccharide in isolated rat aorta

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Abstract

Recent studies clearly show that there is a relationship between endotoxemia and impaired vascular responsiveness. The aim of this study was to investigate whether treatment with the new potent PARP inhibitor PJ34 could prevent the vascular hyporesponsiveness induced by lipopolysaccharide (LPS). Endotoxemia was induced in rats by LPS injection (20 mg kg^{-1}, i.p.). Administration of LPS caused a decrease in mean blood pressure and an increase in heart rate. In endothelium-denuded rings of thoracic aorta from untreated rats, contractile responses to KCl and phenylephrine decreased after LPS injection. Furthermore, there was a significant loss of endothelium-dependent vasodilatation in response to acetylcholine in LPS-treated rats. The animals pretreated with PJ34 (10 mg kg^{-1}, i.p., 30 min before LPS injection), the effect of LPS on vascular responsiveness was lower than the untreated ones. Pretreating the animals with PJ34 before the LPS challenge prevented the decline in mean blood pressure. However, this did not result in significant changes to the heart rate. The inhibitory effect of LPS treatment on both KCl- and phenylephrine-induced contraction responses was significantly antagonized by PJ34. Additionally, pretreatment of the rats with PJ34 attenuated the LPS-induced endothelial dysfunction in endothelium-intact aorta rings. This study demonstrates that PARP activation in the vascular system is an important contributory factor to the impaired vascular responsiveness associated with endotoxic shock. Hence, the pharmacological inhibition of PARP pathway might be an effective intervention to prevent endotoxin-induced vascular hyporesponsiveness.

Keywords: Lipopolysaccharide; Poly(ADP-ribose) polymerase; Vascular hyporesponsiveness; Aorta

1. Introduction

Despite the progress made in supportive care, septic shock continues to be a major cause of mortality. As a consequence of lipopolysaccharide (LPS) challenge, an animal model of sepsis, severe depression of the systolic and diastolic contractile function, tachycardia, and a reduction in mean arterial pressure were documented in both rats and mice [1]. Although severe hypotension and hyporesponsiveness to vasoconstrictors and endothelium-dependent vasodilator agents are hallmarks of sepsis that contribute to the associated high mortality rate [2–6], the mechanisms underlying LPS-induced vascular hyporesponsiveness could not be fully established.

Poly(ADP-ribose) polymerase (PARP), also known as poly(ADP ribose) synthetase (PARS), is an abundant nuclear enzyme that has been implicated in the cellular response to DNA injury [7,8]. Activation of PARP is triggered by single-strand breaks in DNA and subsequently results in a rapid depletion of intracellular NAD^+ and ATP. This slows the rate of glycolysis and mitochondrial respiration and eventually leading to cellular dysfunction and death [8,9]. Overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions associated with oxidant stress, including circulatory shock, reperfusion injury, stroke, diabetes, hyperhomocysteinemia, and aging [10–15]. Similarly, oxidative damage plays a key role in endotoxic shock, which is known to enhance the formation of reactive oxygen species (ROS) [16]. Therefore, activation of the PARP pathway within the vessel can be hypothesized to explain vascular hyporesponsiveness in endotoxic shock. LPS administration may lead to endothelial and/or muscle cell injury by acti-

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vating PARP pathway. In the previous studies, the PARP inhibitors were utilized to investigate the role of PARP pathway in LPS-induced vascular hyporeactivity [17–19]. Szabo et al. reported that PARP inhibitor 3-aminobenzamide (3-AB) improved vascular contractility in LPS-treated rats [17]. Similar findings were also reported with nicotinamide that has inhibitory effect on the PARP pathway [18]. In a different study, it was demonstrated that 3-AB improves endothelium-dependent relaxations in endotoxic shock [19]. The early studies referenced above utilized 3-AB or nicotinamide that is less specific, less potent, and exerts non-specific (scavenging) actions. As opposed to those, in the present study, we used PJ34, a more potent and specific inhibitor of PARP.

Herein, we tested whether the impaired vascular responsiveness in endotoxic shock is dependent upon the activation of the PARP pathway within the vessel. Furthermore, the effects of PJ34 treatment on the cardiovascular parameters of LPS-treated rats were also evaluated.

2. Materials and methods

2.1. Isolation of arteries

Male Wistar rats, 6–8 week of age, weighing 250–300 g were used in the present study. Rats were randomly divided into four groups. The first group that served as control (C group, $n = 6$) received only i.p. physiological saline of 1 ml kg^{-1}. The second group (LPS group, $n = 6$) received i.p. lipopolysaccharide (LPS group, 20 mg kg^{-1} diluted in physiological saline). The third group (C + PJ34 group, $n = 6$) was pretreated with PJ34 (10 mg kg^{-1}) 30 min before 1 ml kg^{-1} physiological saline injection. Finally, the fourth group (LPS + PJ34 group, $n = 6$) was pretreated with i.p. PJ34 30 min before LPS administration.

2.2. Measurement of heart rate and blood pressure

The animals were anaesthetized with i.p. injections of thiopentone sodium (60 mg kg^{-1}). Heart rate and blood pressure of rats were measured with a catheter that was inserted into their right carotid artery. All catheters were filled with heparinized saline. Body temperature was maintained at $37 \pm 1\,^{\circ}C$ using a heating lamp. After stabilization for 15 to 20 min, arterial blood pressure was recorded with a pressure transducer connected to an amplifier (MAY GTA0303, Ankara, Turkey) and a recorder (BIOPAC system MP 150, Ankara, Turkey). Heart rate was calculated from the blood pressure tracing. Hemodynamic measurements were recorded using a converter and displayed on a personal computer.

2.3. Organ bath technique

Eight hours later, rats were anesthetized by intraperitoneal injection of urethane (1.4 g kg^{-1}) and decapitated. The full length of thoracic aorta was removed and cleaned of the connective tissue. Thoracic aorta was cut into 3–4 mm width rings. Then, the rings were carefully suspended by two stainless steel clips passed through the vessel lumen in 20 ml organ baths filled with physiological salt solution (PSS) (mM: NaCl 118, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.2) maintained at $37\,^{\circ}C$ gassed with 95% O_2 and 5% CO_2 to obtain a pH of 7.4.

The rings were suspended under 1 g of tension, and the preparation was allowed to equilibrate for 60 min. Isometric tension was continuously measured with an isometric force transducer (FDT10-A, Commat Ltd., Ankara, Turkey), connected to a computer-based data acquisition system (TDA 97, Commat Ltd., Ankara, Turkey). After an equilibration period of 1 h, aortic rings were precontracted with phenylephrine (Phe, 10^{-6} M). This concentration was determined from the cumulative contraction–response curves to achieve 80% of the maximum contraction. In order to evaluate the contractility of aortic rings, Phe or KCl was used. In the first set of experiments, 20, 40 and 80 mM KCl responses were performed in rings obtained from all groups. After the washout, tissues were challenged with Phe (10^{-8} to 10^{-5} M) by addition of increasing concentrations of agonist to the baths in a cumulative manner and the isometric tension developed by the tissue recorded. The tissue response was allowed to reach a stable plateau (2–4 min) before each successive concentration of the agonist was added. In a separate experiment, relaxation responses were examined with, using acetylcholine (ACh, 10^{-9} to 10^{-5} M), an endothelium-specific vasodilator.

2.4. Drugs

Acetylcholine chloride, L-phenylephrine hydrochloride and PJ34 [*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide hydrochloride] were used. All drugs and the salts for the PSS were purchased from Sigma Chemical (St. Louis, MO). All drugs were prepared fresh daily during experiments, and were dissolved in distilled water before use.

2.5. Statistical analysis

All values are expresses as mean ± S.E.M. Responses to ACh are expressed as percentages of the reversal of the tension developed in response to phenylephrine. The logarithm of the concentration of agonists which elicited a 50% of maximal response (E_{max}) was designated as the EC_{50}. These values were determined by regression analysis of the linear portions of the log concentration–response curves. Sensitivity was expressed as pD_2 ($-\log EC_{50}$). Statistical analyses of the results were performed by ANOVA or Student's *t*-test where appropriate. A *P* value lower than 0.05 was considered significant.