

SYSTEMIC AND HEPATOSPLANCHNIC HEMODYNAMIC AND METABOLIC EFFECTS OF THE PARP INHIBITOR PJ34 DURING HYPERDYNAMIC PORCINE ENDOTOXEMIA

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ABSTRACT—Activation of the poly(ADP-ribose)polymerase (PARP), a highly energy-consuming DNA-repairing enzyme, plays a crucial role in the pathogenesis of multiorgan failure. Most results, however, were derived from experiments with hypodynamic shock states characterized by a markedly decreased cardiac output (CO) and/or using a pretreatment approach. Therefore, we investigated the effects of the novel potent and selective PARP-1 inhibitor PJ34 in a posttreatment model of long-term, volume-resuscitated porcine endotoxemia. Anesthetized, mechanically ventilated and instrumented pigs received continuous intravenous (i.v.) lipopolysaccharide (LPS) over 24 h. Hydroxyethyl starch was administered to maintain a mean arterial pressure > 65 mmHg. After 12 h of LPS infusion, the animals were randomized to receive either vehicle (Control, n = 9) or i.v. PJ34 (n = 6; 10 mg/kg over 1 h followed by 2 mg/kg/h until the end of the experiment). Measurements were performed before as well as at 12, 18, and 24 h of LPS infusion. In all animals CO increased because of reduced systemic vascular resistance (SVR) and fluid resuscitation. PJ34 further raised CO ($P < 0.05$ vs. control group) as the result of a higher stroke volume indicating its positive inotropic effect. In addition, it diminished the rise in the ileal mucosal-arterial PCO_2 gap, which returned to baseline levels at 24 h of LPS, and improved the gut lactate balance ($P = 0.093$ PJ34 vs. control) together with significantly lower portal venous lactate/pyruvate ratios. By contrast, it failed to influence the LPS-induced derangements of liver metabolism. Incomplete PARP inhibition because of dilutional effects and/or an only partial efficacy when used in post-treatment approaches may account for this finding.

KEYWORDS—Blood flow, oxygen, gut, ileal mucosal-arterial PCO_2 gap, liver, lactate/pyruvate ratio, metabolism

INTRODUCTION

There is emerging evidence that the activation of type 1 poly(ADP-ribose) polymerase (PARP-1) (1), a highly energy-consuming DNA-repairing enzyme, plays a crucial role in the pathogenesis of multiorgan failure and cellular dysfunction (2, 3). The most important effects of PARP-1 activation are vascular hyporeactivity, myocardial failure, gut epithelial dysfunction, and inhibition of cell metabolism (2, 3).

PARP-1 is activated in the presence of DNA single-strand breaks produced by free radicals such as peroxynitrite, which are generated in a great quantity in inflammatory processes like sepsis. The activated enzyme cleaves the substrate NAD^+ into ADP-ribose and nicotinamide, then attaches the ADP-ribose to various proteins to build poly(ADP)ribose units. This energy-consuming reaction results in a marked depletion of cellular energy-rich phosphates, ultimately leading to mitochondrial damage and cell necrosis (3).

Strategies attempting to inhibit PARP-1 activity have proven

to be beneficial in various experimental models, such as myocardial (4–6) or splanchnic ischemia-reperfusion injury (7, 8), endotoxin-induced damage to the lung (9), and hemorrhagic (10–12), or polymicrobial septic shock (13, 14). Most of these studies, however, were performed on rodents with hypodynamic shock states associated with a profound depression of cardiac output. Moreover, experimental series in larger species (swine) used a pretreatment approach (6, 14, 15). Neither of the designs, however, mimics the clinical situation of human sepsis or septic shock, which are characterized by a sustained increase in cardiac output that results from decreased systemic vascular resistance along with excessive fluid resuscitation (16).

Recently, our group investigated the effects of nicotinamide as a PARP inhibitor in a “post-treatment” porcine model of long-term, well-resuscitated, hyperdynamic, and hypermetabolic endotoxemia. In fact, nicotinamide prevented the fall of blood pressure while maintaining the cardiac output but failed to improve any LPS-related derangements of systemic and hepatosplanchnic metabolism (17). The low potency and the deficiency in selectivity probably have to be blamed for this lacking beneficial effect. Nevertheless, other authors reported that more specific PARP inhibitors also failed to prevent endotoxin-induced organ dysfunction either completely (18) or when used in a post-treatment approach (19).

Just now, PJ34, a novel (20) and highly potent (the *in vitro* IC_{50} is 10,000 times lower than that of the prototypical compound 3-aminobenzamide) PARP-1 inhibitor, was shown to be of benefit to various experimental settings like stroke (21)

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and diabetic endothelial dysfunction (22). Furthermore, PJ34 treatment improved the myocardial function in pigs during both an ischemia-reperfusion injury (15) and a hypodynamic septic shock induced by polymicrobial peritonitis which ultimately resulted in an improved survival rate (14).

To evaluate the efficacy of this substance under clinically relevant conditions, we investigated its effects on a porcine model of long-term, volume-resuscitated, hyperdynamic endotoxemia (17, 23) in a post-treatment approach. Because of the critical role of the hepatosplanchnic region in the pathogenesis of septic shock and multiple organ dysfunction and failure (24), we focused our specific attention on the intestinal and liver perfusion, O_2 kinetics, and energy metabolism.

MATERIALS AND METHODS

Animals and preparation

The experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. The study protocol was approved by the University Animal Care Committee as well as the federal authorities for animal research (Regierungspräsidium Tübingen, Baden-Württemberg, Germany). Fifteen domestic pigs of either sex with a median bodyweight of 51 (range 40–54) kg were investigated. The anesthesia and the surgical preparation of animals have been described in detail previously (17, 23). Briefly, animals were fasted but had free access to water for 18 h before the experiment. They were pre-anesthetized with 5 mg/kg azaperone (Stresnil®, Janssen, Neuss, Germany) and 2.5 mg atropine (Atropinsulfat®, Braun, Melsungen, Germany®). After induction of anesthesia and endotracheal intubation, anesthesia was maintained until the end of the experiment by continuous intravenous pentobarbital (Nembutal®, Sanofi Wintrop, Munich, Germany) infusion (6–10 mg kg⁻¹ min⁻¹). The depth of anesthesia was controlled by EEG. Intravenous buprenorphin (0.3 mg; Temgesic®, Boehringer, Mannheim, Germany) was added every 4 h and before any surgical stimulus to prevent a rise in heart rate and arterial pressure as a result of inadequate anesthesia. Muscle paralysis was achieved with alcuronium (14 mg h⁻¹; Alloferin®, Hoffmann-La Roche, Basel, Switzerland). The pigs were mechanically ventilated (FiO₂ 0.35; PEEP 5 cm H₂O; Siemens Servo 900B, Erlangen, Germany) with a tidal volume of 15 mL kg⁻¹ at a respiratory rate of 10–12 breaths/min adjusted to maintain arterial PCO₂ between 35–45 mmHg. A central venous catheter for drugs and fluid administration was introduced through the left jugular vein. A balloon-tipped thermodilution catheter was placed via the right jugular vein for the measurement of central venous, mean pulmonary artery (MPAP) and pulmonary artery

occlusion pressure (PAOP) as well as cardiac output. In one femoral artery, a catheter was introduced for continuous blood pressure recordings and blood sampling, in the other one a thermistor-tipped fiberoptic catheter was placed for thermal-dye double indicator dilution. Ringer's solution (10 mg kg⁻¹ h⁻¹) was infused intravenously (i.v.) as baseline fluid. A midline laparotomy was performed, and precalibrated ultrasound transit time flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and the common hepatic artery, distal to the gastroduodenal branch. Flows were continuously recorded. A catheter was then introduced into the portal vein, and an angiography catheter was placed into one hepatic vein under ultrasound guidance via the right jugular vein. The correct position of the catheter was confirmed during postmortem inspection. A loop-ileostomy was performed, and a fiberoptic PCO₂ sensor (Paratrend 7, Diametrics Medical, High Wycombe, UK) was introduced about 15 cm into the lumen. Then, the abdominal wall was closed. The temperature of the ileostoma was maintained with sponges moistened with warm physiologic saline and by covering the ileostoma with a colostomy sack. A cystostomy catheter for urine collection was placed percutaneously. Body temperature was kept within 0.5°C of baseline values using a heating mattress or external cooling as needed. A postsurgical stabilization period of 8 h was allowed before baseline measurements were obtained.

Measurements and calculations

Cardiac output (CO) was determined as the mean of four injections of 10-mL ice-cold physiologic saline randomly spread over the respiratory cycle. The intrathoracic blood volume (ITBV) was measured by arterial thermal-green dye double indicator dilution after injection of 10 mL of ice-cold indocyanine green (2.5 mg mL⁻¹; 17, 23). The total hepatic blood flow (Q_{Liv}) was obtained as the sum of the continuously recorded portal venous (Q_{Pv}) and hepatic arterial (Q_{HA}) blood flow. Arterial, mixed venous, hepatic venous, and portal venous blood samples were analyzed for PO₂, PCO₂, and pH (Stat Profile Ultra, NOVA Biomedical, Waltham, MA), respectively as well as total hemoglobin content and hemoglobin O₂ saturation (IL 682 CO-Oximeter, Instrumentation Laboratories, Lexington, MA; calibrated for pig blood). Systemic O₂ delivery (DO_{2sys}) and uptake (VO_{2sys}) were calculated from standard formulas. The intestinal O₂ extraction was calculated as the quotient of arterial–portal venous O₂ content difference divided by the arterial oxygen content. Liver DO₂ (DO_{2liver}) and VO₂ (VO_{2liver}) were calculated as the product of Q_{Pv} and Q_{HA} times the portal venous and hepatic arterial O₂ content, respectively, and the portal–hepatic venous and the arterial–hepatic O₂ content differences where appropriate. To calculate the arterial, portal, and hepatic venous lactate/pyruvate (L/P) ratios the lactate and pyruvate concentrations were spectrophotometrically determined as described previously (17, 23). Intestinal and hepatic lactate fluxes were subsequently calculated as the product of portal venous and hepatic arterial blood flow times the arterial–portal venous, portal–hepatic venous, and arterial–hepatic venous concentration differences, respectively, as appropriate.

Ileal mucosal PCO₂ was measured continuously by a precalibrated fiberoptic PCO₂ sensor (17, 23, 25). Subsequently the ileal mucosal–arterial PCO₂ gap was

Study Protocol

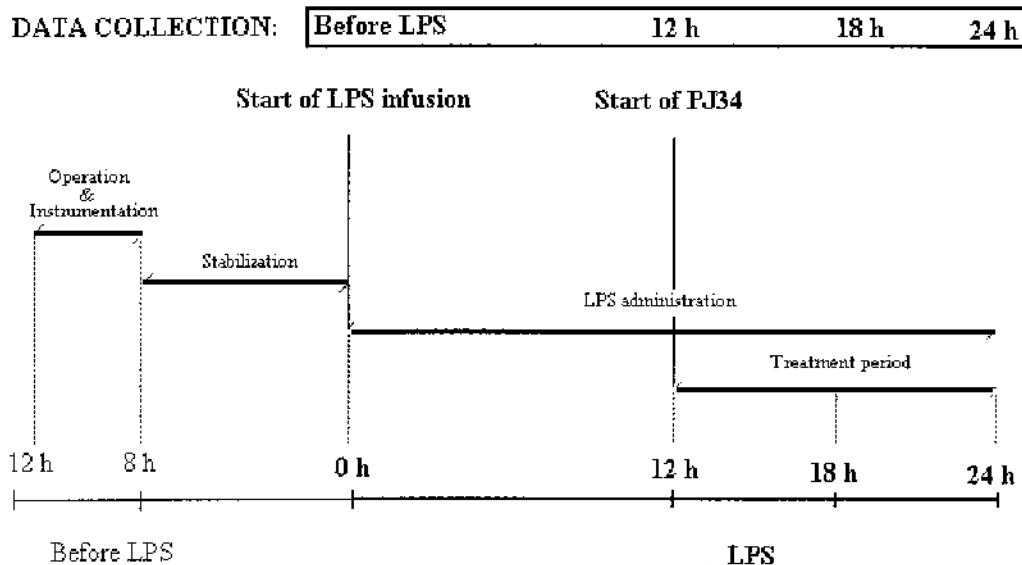


FIG. 1. Schematic presentation of the experimental protocol. PJ34 was administered with a loading dose of 10 mg kg⁻¹ over 1 h and continued at a rate of 2 mg kg⁻¹ h⁻¹ for the following 11 h.

TABLE 1. Endotoxin infusion rate, urine output, and cumulative colloid (hydroxyethyl starch, HES) infusion in the control (n = 9) and PJ34 (n = 6) groups during the second half of the experiment, i.e., from 12–24 h of LPS infusion

		12–24 h LPS
Endotoxin infusion rate ng kg ⁻¹ min ⁻¹	Control	25 (19–43)
	PJ 34	26 (19–52)
Urine output ml kg ⁻¹ h ⁻¹	Control	5.3 (3.0–10.3)
	PJ 34	10.7 (3.9–14.2)
HES infusion ml kg ⁻¹	Control	412 (333–627)
	PJ 34	421 (317–554)

All data are median (range).

calculated as the difference between the mucosal PCO₂ and the simultaneously measured arterial PCO₂.

Protocol

Figure 1 shows the time course of the experiments. The animals were assigned to two groups: endotoxin group without treatment except for volume resuscitation (control; n = 9) and PJ34 group (PJ34; n = 6). After baseline data collection a central venous LPS infusion (*Escherichia coli* 0111:B4, SIGMA Chemical, Lot: 110K4060; 20 mg L⁻¹ in 5% dextrose) was started (0.08 mg h⁻¹) until MPAP reached 50 mmHg. The infusion rate was subsequently adjusted to result in moderate pulmonary hypertension with MPAP = 35–45 mmHg. The dosage required to obtain this goal after 10 h of infusion, i.e., 2 h before the first data collection during LPS infusion, was maintained until the end of the experiment. Hydroxyethyl starch 6% (HAES-Steril® 200.000/0.5 Fresenius Kabi, Erlangen, Germany) was administered as necessary to maintain MAP > 60 mmHg. To keep the blood glucose level between 5–7 mmol L⁻¹, a mixture of 10% glucose and xylitol each (GX 20%, Pharmacia, Erlangen, Germany) was infused if necessary. After 12 h of LPS infusion, in the treatment group a continuous i.v. PJ34 infusion was initiated and continued until the end of the experiment. The PARP inhibitor was administered with a loading dose of 10 mg kg⁻¹ over 1 h and continued at a rate of 2 mg kg⁻¹h⁻¹ for the rest of the experiment. Recently, this dose of PJ34 has been shown (by immunohistochemistry) to effectively block the PARP-1 enzyme in septic pigs (14).

In addition to the baseline measurements further data acquisition was performed at 12, 18, and 24 h of the LPS infusion (corresponding to before, 6, and 12 h, respectively, after the start of PJ34 administration). When the last set of data had been obtained, the animals were killed by KCl injection under deep anesthesia.

Statistical analysis

All values shown are median and range. Differences within each group, i.e., between values before and either during endotoxin or endotoxin plus PJ34 infusion, respectively, were tested using Friedman analysis of variance on ranks and subsequent Dunnett's test for multiple comparisons. Differences between groups were analyzed by the Mann-Whitney Rank Sum Test. *P* < 0.05 was considered significant.

RESULTS

Table 1 shows that the animals of both experimental groups received virtually identical amounts of colloid volume resuscitation as well as endotoxin infusion rates whereas urine output tended to be higher after the PJ34 infusion had been started (*P* = 0.108 vs. control). While three of the control animals died of refractory hypotension before the scheduled termination of the experiment all animals in the PJ34 group survived until the end of the experiment. Systemic hemodynamic and O₂ exchange data are summarized in Table 2 and Figure 2. All animals developed normotensive and hyperdynamic circulation characterized by sustained increase in CO because of reduced SVR together with excessive volume resuscitation as documented by the increased cardiac filling pressures and ITBV. PJ34 infusion significantly increased CO beyond the values of the control group (Fig. 1A) due to a significantly higher stroke volume (Fig. 2B) whereas neither heart rate nor filling pressures or ITBV revealed intergroup

TABLE 2. Systemic hemodynamic, gas exchange, and acid-base data in the control (n = 9) and PJ34 (n = 6) groups

		Before LPS	12 h LPS	18 h LPS	24 h LPS
HR (beats/min)	Control	80 (71–92)	108 (94–133)#	112 (100–131)#	119 (81–128)#
	PJ34	81 (72–98)	109 (85–132)#	111 (105–135)#	121 (113–133)#
MAP (mmHg)	Control	79 (70–87)	74 (59–95)	75 (60–98)	87 (52–98)
	PJ34	81 (67–96)	76 (67–90)	78 (60–95)	78 (72–83)
MPAP (mmHg)	Control	22 (18–24)	36 (31–45)#	36 (30–47)#	37 (30–41)#
	PJ34	23 (18–25)	40 (36–44)#	37 (33–45)#	38 (31–47)#
CVP (mmHg)	Control	9 (5–12)	17 (14–19)#	19 (12–26)#	17 (12–19)#
	PJ34	7 (5–10)	18 (11–19)#	17 (15–19)#	18 (16–20)#
PAOP (mmHg)	Control	10 (5–12)	18 (13–19)#	18 (12–27)#	14 (12–23)#
	PJ34	8 (5–14)	18 (16–20)#	19 (15–20)#	20 (16–21)#
CO (mL kg ⁻¹ min ⁻¹)	Control	74 (58–103)	142 (92–173)#	160 (147–213)#	149 (85–183)#
	PJ34	84 (69–100)	140 (92–175)#	179 (132–218)#	209 (153–228)#§
SV (mL/kg)	Control	0.99 (0.73–1.29)	1.25 (0.91–1.58)	1.46 (1.19–2.13)#	1.25 (1.04–1.55)#
	PJ34	0.96 (0.90–1.28)	1.28 (1.09–1.51)#	1.60 (1.25–1.63)#	1.65 (1.36–1.90)#§
SVR (dynes s ⁻¹ cm ⁻⁵)	Control	1509 (1040–1793)	657 (535–972)#	554 (319–774)#	656 (369–834)#
	PJ34	1345 (1109–1811)	671 (605–971)#	517 (426–764)#	454 (430–576)#
ITBV (mL kg ⁻¹)	Control	21 (19–29)	31 (21–37)#	34 (29–41)#	33 (24–55)#
	PJ34	21 (18–22)	30 (24–36)#	35 (28–47)#	35 (31–38)#
DO _{2sys} (mL kg ⁻¹ min ⁻¹)	Control	8.9 (7.5–11.7)	14.7 (9.8–20.1)#	16.7 (11.0–18.6)#	16.0 (9.1–21.0)#
	PJ34	10 (7.7–11.5)	14.9 (10.1–20.6)#	17.2 (10.8–20.0)#	17.9 (15.4–20.3)#
VO _{2sys} (mL kg ⁻¹ min ⁻¹)	Control	4.2 (3.6–5.0)	4.4 (3.5–6.0)	4.2 (3.2–5.4)	4.2 (3.7–4.4)
	PJ34	4.2 (3.1–4.8)	4.3 (3.0–5.7)	4.2 (2.6–5.0)	4.4 (2.4–5.2)
L/P ratio arterial	Control	12 (6–21)	15 (10–21)	14 (11–30)	16 (11–25)
	PJ34	10 (9–20)	13 (8–17)	11 (9–16)	13 (11–17)
PaCO ₂ (mmHg)	Control	36 (33–41)	37 (32–52)	36 (30–47)	35 (22–40)
	PJ34	36 (35–51)	37 (32–61)	37 (32–44)	40 (38–47)
Arterial pH	Control	7.49 (7.46–7.52)	7.35 (7.23–7.43)#	7.34 (7.14–7.44)#	7.37 (7.21–7.40)#
	PJ34	7.50 (7.47–7.56)	7.39 (7.29–7.46)#	7.39 (7.24–7.45)#	7.34 (7.17–7.45)#

All data are median (range), #*P* < 0.05 vs. before LPS, §*P* < 0.05 vs. control.

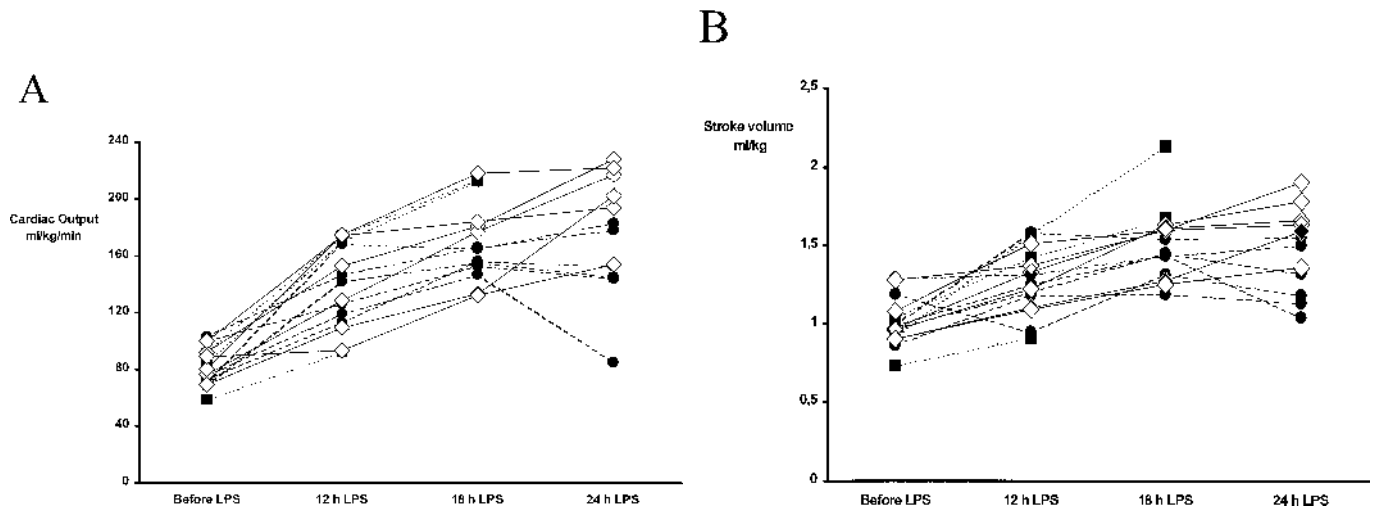


FIG. 2. Individual response of cardiac output (A) and stroke volume (B) in the control (black symbols) and PJ34 treated (open rhombus, solid line) animals. Animals in the control group which did not survive until the end of the experiment ($n = 3$) are presented as black squares and dotted lines, those which survived until 24 h of LPS infusion as black circles and broken lines.

differences (Table 2). The increased CO resulted in a significant rise of DO_{2sys} without intergroup difference, while VO_{2sys} remained unchanged in both groups. Nevertheless, progressive metabolic acidosis developed, again there were no intergroup differences (Table 2).

Table 3 presents the data on intestinal and liver perfusion and metabolism. Because of the enhanced cardiac output, Q_{PV} markedly increased in both groups 12 h after starting the LPS infusion. The intestinal O_2 extraction progressively decreased in either group. Despite the well-preserved macrocirculatory O_2 availability, an ongoing regional acidosis developed in response to LPS in both groups, but again there were no intergroup differences. By contrast, PJ34 improved the gut lactate

balance ($P = 0.093$ vs. control), which was associated with significantly lower portal venous L/P ratios at 24 h of endotoxin. PJ34 group also blunted the otherwise progressive rise in the ileal mucosal-arterial PCO_2 gap, which had returned to baseline levels until the end of the experiment (Fig. 3). Although the hepatic arterial blood flow (Q_{HA}) was not actually affected in either group, the increased CO led to a rise in Q_{PV} and thereby Q_{Liv} . There were, however, no intergroup differences. PJ34 significantly increased DO_{2liver} whereas no effect was present in the control animals. Despite this elevated DO_{2liver} and unchanged VO_{2liver} , the LPS infusion caused a drop in hepatic lactate uptake rate and, likewise, a progressive hepatic venous metabolic acidosis in both groups. The hepatic

TABLE 3. Hepatosplanchnic hemodynamic, gas exchange, and acid-base data in the control ($n = 9$) and PJ34 ($n = 6$) groups

		Before LPS	12 h LPS	18 h LPS	24 h LPS
Q_{PV} ($mL\ kg^{-1}\ min^{-1}$)	Control	19 (13–26)	28 (15–50)#	33 (15–45)#	31 (16–40)#
	PJ34	18 (12–25)	21 (18–33)	28 (19–36)#	32 (25–42)#
Q_{HA} ($mL\ kg^{-1}\ min^{-1}$)	Control	2.0 (1.1–7.3)	2.0 (1.3–8.3)	3.3 (2.0–10.4)	3.4 (1.3–10.1)
	PJ34	3.0 (2.0–4.0)	2.6 (1.3–5.7)	3.0 (2.0–10.2)	3.6 (2.2–11.9)
Q_{Liv} ($mL\ kg^{-1}\ min^{-1}$)	Control	21 (17–31)	31 (23–52)#	35 (25–49)#	34 (17–43)#
	PJ34	21 (14–29)	25 (19–38)#	35 (22–40)#	39 (30–45)#
Intestinal O_2 extraction (%)	Control	38 (24–46)	25 (17–34)#	21 (18–35)#	20 (15–33)#
	PJ34	39 (27–57)	33 (23–54)	25 (18–35)#	22 (17–27)#
DO_{2liver} ($mL\ kg^{-1}\ min^{-1}$)	Control	1.7 (1.5–2.6)	2.7 (1.7–4.8)#	2.8 (1.7–3.4)#	2.9 (1.3–4.2)
	PJ34	1.8 (0.9–2.4)	2.0 (0.9–3.6)	2.8 (1.3–3.4)#	3.0 (1.8–3.3)#
VO_{2liver} ($mL\ kg^{-1}\ min^{-1}$)	Control	0.9 (0.3–1.4)	0.9 (0.3–1.6)	0.9 (0.2–1.4)	0.8 (0.2–1.3)
	PJ34	0.7 (0.3–1.0)	0.9 (0.6–1.5)	0.7 (0.3–1.4)	0.8 (0.2–1.5)
L/P ratio pv	Control	12 (6–17)	15 (10–18)#	14 (11–23)#	18 (14–22)#
	PJ34	10 (9–19)	13 (8–17)	13 (9–15)	13 (11–14)§
Gut lactate balance ($\mu mol\ kg^{-1}\ min^{-1}$)	Control	-5.0 (-7.5--1.8)	-1.5 (-5.9-2.9)	0.2 (-5.0-1.9)	-2.2 (-8.6--0.6)
	PJ34	-2.2 (-7.2--0.6)	-1.0 (-2.9-0.8)	-1.9 (-5.0-0.3)	-0.5 (-1.8-1.7)
pH pv	Control	7.45 (7.41–7.46)	7.31 (7.20–7.38)#	7.29 (7.12–7.39)#	7.30 (7.18–7.38)#
	PJ34	7.46 (7.45–7.53)	7.33 (7.25–7.40)#	7.37 (7.23–7.41)#	7.30 (7.14–7.40)#
L/P ratio hv	Control	15 (5–22)	14 (8–26)	14 (9–22)	16 (11–25)
	PJ34	14 (8–29)	13 (8–15)	11 (9–15)	13 (11–15)
Liver lactate balance ($\mu mol\ kg^{-1}\ min^{-1}$)	Control	10.8 (-1.0–22.4)	4.9 (-6.2–19.2)#	0.1 (-14.2–16.0)#	0.2 (-9.7–7.7)#
	PJ34	11.1 (5.6–14.9)	7.7 (-1.9–12.1)#	6.1 (0.8–16.0)#	-1.0 (-4.1–12.2)#
pH hv	Control	7.45 (7.42–7.47)	7.26 (7.16–7.36)#	7.30 (7.12–7.38)#	7.29 (7.15–7.37)#
	PJ34	7.46 (7.44–7.51)	7.31 (7.26–7.40)#	7.35 (7.20–7.40)#	7.30 (7.13–7.41)#

All data are median (range), # $P < 0.05$ vs. before LPS, § $P < 0.05$ vs. control.

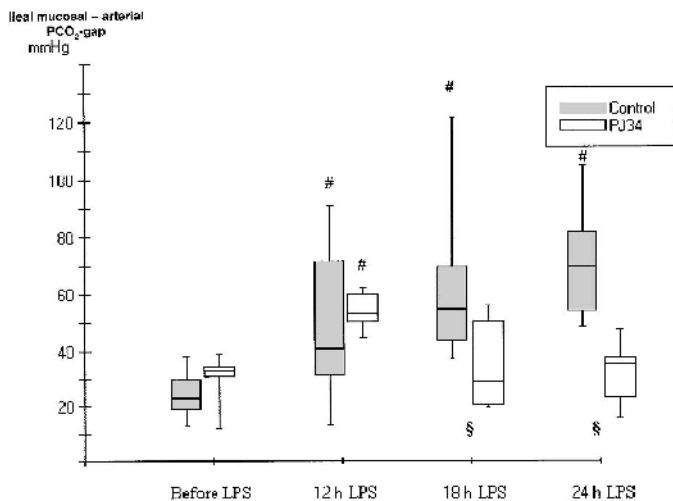


FIG. 3. Ileal mucosal-arterial PCO₂ gap in the control (grey box plots) and PJ34 treated (open box plots) animals. Data are median, quartiles and range. #Designates a significant difference ($P < 0.05$) vs. before LPS. §Designates significant difference ($P < 0.05$) between the two groups.

venous L/P ratio revealed neither inter- nor within-group differences over the experiment.

DISCUSSION

There is ample evidence in the literature demonstrating the beneficial effects of PARP inhibition on shock-induced organ failure (2, 3, 18), especially on the hepatosplanchnic region (19, 26–28). The only data in larger species, however, had been obtained from pre-treatment experiments (6, 14, 15). Because there is relevant information that the benefit of PARP inhibition is not necessarily transferable to a post-treatment approach (29), the aim of the present study was to investigate the effects of the novel potent PARP-1 inhibitor PJ34 in a post-treatment model of long-term, normotensive and hyperdynamic porcine endotoxemia. The key findings were that PJ34 1) improved CO as the result of an increased stroke volume and 2) attenuated the otherwise progressive LPS-induced deterioration of intestinal energy balance as assessed by the ileal mucosal-arterial PCO₂ gap.

LPS infusion with aggressive colloid resuscitation resulted in a hyperdynamic circulatory state characterized by a sustained increase in CO associated with a fall in SVR in both experimental groups. PJ34 further increased CO beyond the values of the control animals, which was associated with a significantly higher stroke volume. Although neither heart rate nor the cardiac filling pressures revealed any significant differences between the two experimental groups, there was a tendency towards higher PAOP values in the PJ34-treated animals. It must be underscored, however, that the ITBV values were identical. The intrathoracic blood volume has been shown to far better reflect cardiac preload than PAOP, in particular during mechanical ventilation (30). Therefore, given the uninfluenced blood pressure, PJ34 probably increased the stroke volume to a direct positive inotropic effect and/or by preventing the impairment of cardiac function. This finding corroborates previous reports demonstrating a beneficial effect of PARP inhibition on myocardial contractility in porcine

models of myocardial reperfusion injury (6) or hemorrhagic shock (10). Furthermore, Faro et al. recently reported a positive inotropic effect of PJ34 that was documented by improved dp/dt_{max} values after regional myocardial ischemia in pigs because of a reduced infarct size (15). Moreover, Pacher et al. proved this compound successful to ameliorate diabetes-induced myocardial dysfunction (31), and Goldfarb et al. finally showed such a positive inotropic effect in a porcine model of polymicrobial peritonitis using ultrasound measurements of cardiac muscle shortening (14).

Despite the well-preserved intestinal macrocirculatory O₂ availability and O₂ extraction, the LPS infusion markedly deteriorated the gut energy balance, i.e., the ileal mucosal-arterial PCO₂ gap and the portal venous L/P ratios. However, PJ34 completely blunted this progressive rise of both the portal venous L/P ratios and the PCO₂ gap. In addition, it is noteworthy that PJ34 also tended to ameliorate the intestinal lactate balance which, however, did not reach statistical significance when compared with the control group ($P = 0.093$). Improved systemic lactate levels and, in particular, gut luminal lactate release have also been reported by Lobo et al. during short-term endotoxic shock in rabbits (32). Our observations are in good agreement with previous reports that PARP inhibition is associated with an enhanced gut mucosal integrity in shock states. Salzman et al. showed that mucosal acidosis, regardless of its origin, was able to initiate derangements of the mucosal barrier function (33). PARP activation demonstrated in hemorrhagic shock (19) led to a markedly increased energy consumption in the tips of the gut villi (3). PARP inhibition, however, prevented this development of mucosal dysfunction in both endotoxic (26) and hemorrhagic (19) shock. What is more, mice deficient in the functional PARP enzyme were protected against disruption of the intestinal barrier function after mesenteric ischemia-reperfusion injury (8). Although we could not directly quantify the metabolic activity of the gut mucosal layer it is tempting to speculate that PJ34 exerted its beneficial effects through a direct effect on the cellular energy balance. This is based on the fact that PJ34 considerably lowered the portal venous L/P ratio, a marker of the cytosolic redox state of the portal venous-drained viscera.

We can only speculate whether a beneficial effect on the gut microcirculation also contributed to the restoration of the ileal mucosal-arterial PCO₂ gap during PJ34 infusion. In fact, impaired villous perfusion (25) resulting in microvascular O₂ “shunting” (34) has been shown in endotoxic pigs. However, PJ34 has been reported to ameliorate endotoxin-induced endothelial dysfunction in such models not only by normalization of mean arterial pressure but also by reversion of the LPS-induced depression of the vasodilator response to acetylcholine (3). As a consequence we suggest that the beneficial effect of this compound in our experiments might have been mediated at least in part by a restored vasoreactivity affiliated with improved vasoregulation as recently reported in experimental diabetes (31).

The LPS infusion impaired the hepatic metabolic capacity despite the well-preserved hepatic blood flow and O₂ exchange as documented by the progressive fall in hepatic lactate clearance and the development of hepatic venous acidosis. In

contrast to the documented role of PARP activation in oxidative stress-induced hepatocyte damage (35) in the present study PJ34 failed to attenuate any of the LPS-driven derangements in liver metabolism. The latter finding reproduces our previous results when using nicotinamide as a PARP inhibitor (17). This missing benefit of PJ34 on liver metabolism is also supported by recent findings in other models of acute inflammation showing that a post-treatment approach could only partly attenuate or restore organ dysfunction (29). Moreover, in severe hemorrhagic shock and resuscitation PARP inhibition also failed to influence organ damage and metabolic acidosis when administered as a treatment strategy and not prophylactically (19). It should be noted that we were unable to perform PARP staining in the liver, and, therefore, we can only speculate whether PJ34 completely blocked PARP activity. The high potency of the compound (21) as well as the fact that we used the same infusion rate that had previously been shown to efficiently block PARP in septic pigs (14), however, makes this assumption less likely. Nevertheless, it must be emphasized that almost complete PARP inhibition was only demonstrated in heart and lung tissue in that study (14) whereas no data were reported on hepatic PARP activity. Moreover, in our study an insufficient drug concentration at the target organ (liver) resulting from dilutional effects due to large volume resuscitation and/or drug loss into third spaces such as ascites—both circumstances are common in the hyperdynamic model used—may have curtailed the efficacy of the compound.

Although our study was not designed to evaluate outcome a clear tendency to improved survival was present in the PJ34 group: all animals in the treatment group survived until the scheduled end of the observation period whereas three of nine died prematurely in the control group. This finding corroborates recently published results in septic shock models on polymicrobial peritonitis both in pigs (14) and PARP-deficient mice (13). Moreover, our finding probably gains in importance in the context of lacking differences between the two experimental groups at the end of the experiments: potentially beneficial effects of PJ34 allegedly were missed because at the end of the experiment comparison was only possible to the small number of surviving controls.

In summary, during normotensive, hyperdynamic porcine endotoxemia the novel highly selective PARP-1 inhibitor PJ34 improved cardiac output and stroke volume, probably due to a positive inotropic effect. In addition, PJ34 nearly completely blunted the otherwise progressive impairment of intestinal wall energy balance. By contrast, it failed to influence the LPS-induced derangements of liver metabolism. Incomplete PARP inhibition due to dilutional effects and/or an only partial efficacy when used in post-treatment approaches may account for this finding. Taken together, the current study further supports the hypothesis that potent PARP inhibitors may be of significant beneficial and therapeutic effects in circulatory shock of various etiologies.

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