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Pentoxifylline improves bacterial clearance during hemorrhage and endotoxemia

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Abstract

Objective

The aim of this study was to investigate whether the methylxanthine-derivative pentoxifylline (PTX) affects bacterial clearance of the organism in states of hemorrhage and endotoxemia.

Design

Prospective, randomized, controlled trial.

Setting

Experimental laboratory in a university hospital.

Subjects

Fifty-four female chinchilla rabbits.

Interventions

To quantify the clearance process, defined numbers (10^7 CFO) of *Escherichia coli* bacteria were injected intravenously into anesthetized rabbits, 60 mins after induction of hemorrhage ($n = 9 + 3$) or infusion of endotoxin (lipopolysaccharide [LPS]; 40 [micro sign]g/kg/hr; $n = 9 + 3$) and after saline infusion (control; $n = 9$), respectively. Hemorrhage was induced by bleeding, standardized by defined reduction of mean arterial pressure (30% of baseline value). To evaluate the potential effects of PTX on bacterial elimination and killing, in states of hemorrhage and endotoxemia, blood clearance of *E. coli* and colonization of different organs were investigated after pretreatment with PTX (30 mg/kg) as a bolus injection followed by continuous infusion of PTX (50 mg/kg/hr) in hemorrhagic ($n = 9$) and endotoxemic rabbits ($n = 9$). Three additional experiments were performed to evaluate the effects attributable to PTX itself.

Measurements and Main Results

Parameters monitored were rates of bacterial and LPS elimination from the blood, arterial blood pressure, blood gases, and serum lactate concentrations. Additionally, flow cytometric analysis of respiratory burst activity was performed. Three hours after bacterial injection, the animals were killed, and tissue samples of liver, kidney, spleen, and lung were collected for bacteriologic examinations. Compared with the controls, hemorrhage and endotoxemia resulted in a significantly prolonged elimination of injected *E. coli* from the blood. The delayed blood clearance was associated with a significantly ($p < .01$) higher bacterial colonization of all organs, which was most pronounced in the lung. Pretreatment with PTX slightly enhanced blood clearance of *E. coli* as well as of LPS, and significantly reduced ($p < .05$) the colonization of lung and kidney after hemorrhage and endotoxemia. Furthermore, PTX suppressed polymorphonuclear neutrophil respiratory burst activity.

Conclusions

Hemorrhage and endotoxemia induce impaired bacterial clearance from blood and tissue. Treatment with PTX may reduce the risk of bacterial infections by attenuating bacterial colonization of organs in states of hemorrhage and endotoxemia. (Crit Care Med 1999; 27:756-763)

Key Words: bacterial clearance; organ colonization; *Escherichia coli*; bacteremia; infection; pentoxifylline; hemorrhage; endotoxemia; flow cytometry; respiratory burst

Despite intensive research efforts and improved therapeutical strategies, the frequency and mortality of sepsis in trauma patients remains high. During inflammatory reactions of the organism, activation and stimulation of various cascade systems lead to extensive tissue injury, resulting in severe organ dysfunction such as adult respiratory distress syndrome or multiple organ failure.

The high frequency of septic complications after trauma, shock, or burn injury points toward a reduced resistance against invasion of bacteria and their toxins into the circulation and colonization of different organs. In previous experiments, the impairment of defense mechanisms, particularly of the bacterial clearance from the blood, was described after the experimental activation of the coagulation system and the complement cascade ^[1], or after induction of hypoxia or hemorrhagic shock and during endotoxemia ^[2]. Since the importance of overwhelming granulocyte activation for the development of sepsis-induced tissue damage became evident, various therapeutic strategies have been investigated that attempted to specifically influence this state of uncontrolled hyperinflammatory reactions. One of the substances that evoked major scientific interest during the last decades is the methylxanthine derivative, pentoxifylline (PTX).

Because of its positive effects on blood flow by mitigating platelet aggregation, improving erythrocyte plasticity, and reducing blood viscosity, PTX has been used clinically for several years in the treatment of peripheral obliterative vascular diseases [3,4]. Silomon et al. [5] recently demonstrated the protective effects of PTX during hemorrhagic shock/resuscitation caused by the modulation of hepatocyte calcium regulation and reported that PTX inhibits the uncontrolled activation of granulocytes, particularly in the lung [6]. PTX has been reported to suppress the release of proinflammatory cytokines, such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-6 [7,8], and the recent findings of our group indicated delayed bacterial clearance because of TNF [9]. Moreover, it was demonstrated that PTX inhibits the production of oxygen radicals [10], the release of lysosomal enzymes [11], and the formation of thromboxane A₂ [12]. By preventing the expression of adhesion molecules on the surface of polymorphonuclear granulocytes, reduced adherence on endothelial cells was shown after PTX [13]. Physiologic defense mechanisms coincide with alterations of the immune competence of the host, which may increase the frequency of septic complications. Thus, this study investigates the extent of the effect of PTX on the defense mechanisms of granulocytes, such as phagocytosis and burst activity, during hemorrhage and endotoxemia, and whether this affects the clearance rate of circulating bacteria and endotoxin (lipopolysaccharide [LPS]).

MATERIALS AND METHODS

Animal Model

Fifty-four female chinchilla rabbits weighing between 2 and 3 kg were anesthetized with ketamine (50 mg/kg body weight) Ketanest[trade mark sign], Parke Davis, Berlin, Germany) and xylazine (4 mg/kg body weight) (Rompun[trade mark sign], Bayer, Leverkusen, Germany) and were anti-coagulated with heparin-sodium (1000 immunizing units/kg body weight) injected into an ear vein catheter. The animals were placed in a supine position on a temperature-controlled (35[degree sign]C [95[degree sign]F]) operation table. After tracheotomy and intubation, the rabbits were mechanically ventilated with room air (tidal volume, 30 mL; frequency, 30/min) via a respirator (Servo ventilator 900D, Siemens, Elema, Sweden) during the whole observation period. A polyvinyl chloride catheter (inner diameter, 1.4 mm) was inserted into the right carotid artery for measurements of arterial blood pressure and for collection of blood samples. While monitoring the hemodynamic condition, anesthesia was maintained by injection of ketamine (5-10 mg/kg/hr) and xylazine (0.5-1.5 mg/kg/hr). In addition to the basal fluid requirement of 3 to 4 mL/kg of body weight, blood loss from sampling was replaced by isovolemic injection of saline.

Monitoring

After instrumentation of the rabbits, arterial and airway pressures were continuously monitored via Statham strain gauge transducers connected to a Sirecust 404A recorder (Siemens, Munich, Germany). Blood samples were drawn intermittently for measurements of pH, PaO₂, PaCO₂, HCO₃, hemoglobin, oxygen saturation, and hematocrit (288 Blood Gas System, Ciba-Corning, Fernwald, Germany), as well as of leukocyte and differential blood counts and polymorphonuclear neutrophil burst activity.

Bacterial Inoculum

An encapsulated, serum-resistant, nonhemolytic strain of *Escherichia coli*, freshly isolated from the blood of a septicemic patient, was cultured on blood agar plates. After 10 hrs of incubation at 37[degree sign]C (98.6[degree sign]F), the colonies were harvested and homogenized by vortexing in tryptic soy broth, adjusted to a density of 10⁷ colony-forming units (CFU)/mL and frozen in aliquots at -70[degree sign]C (-94[degree sign]F) until use. The used amount of 10⁷ CFU of *E. coli*

in the current study was based on pilot experiments that provided a reproducible clearance rate and organ distribution without inducing severe hemodynamic changes that might influence clearance function by tissue hypoperfusion. In control animals, the injection of 10^7 CFU of *E. coli* was completely cleared from the circulation during the time period of 60 to 90 mins, thus allowing registration of acceleration or impairment of the clearance kinetics in the different experimental groups.

Lipopolysaccharide

A trichloroacid preparation of LPS from *E. coli* O111, extracted according to the Boivin method, was provided by one of our investigators (R.U.).

Experimental Protocol

After a 30-min period of stable hemodynamics, the animals were randomly assigned to the respective groups, and the first blood samples for baseline measurements were collected.

Control Groups (n = 9 + 3)

After a 60-min period during which saline was infused (0.1 mL/min), a standardized amount of *E. coli* (10^7 CFU suspended in 1 mL of tryptic soy broth) was injected into the ear vein catheter. For bacterial analysis, arterial blood was aseptically drawn at 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, and 180 mins after bacterial injection. Blood gases, leukocyte counts, hematocrit, and hemoglobin concentrations were determined at 30-min intervals. Polymorphonuclear neutrophil oxidative burst activity was determined before and at 60, 90, and 180 mins after *E. coli* injection. In addition, blood samples were collected for the detection of endotoxin immediately before bacterial injection and at 1, 30, 120, and 180 mins. At the end of the experiment, the animals were killed with ketamine and xylazine. Subsequently, tissue samples of liver, spleen, kidney, and lung were removed under aseptic conditions for bacterial cultures. For the determination of effects caused by PTX itself, three animals received PTX (30 mg/kg body weight) (Trental[trade mark sign], Hoechst Marion Roussel, Germany) without induction of hemorrhage or endotoxemia.

Hemorrhage (n = 9 + 3)

During continuous hemodynamic monitoring, a standardized reduction of the mean arterial blood pressure from 60% to 75% of the baseline value was induced by controlled bleeding from the carotid artery. For 1 hr before bacterial injection, the mean arterial blood pressure was maintained at this level by withdrawal ([similar]30% of the total volume) or retransfusion of autologous blood, respectively. Sixty minutes after the induction of hemorrhage, the bacterial suspension was injected. Three additional experiments were performed according to this procedure, but without injection of bacteria, to determine bacteremia that potentially occurs because of translocation from the gut.

Pentoxifylline Treatment in Hemorrhage (n = 9)

Immediately before the induction of hemorrhage, PTX (30 mg/kg body weight) was injected, followed by a continuous infusion of PTX (50 mg/kg/hr) until the end of the experiment.

Endotoxemia (n = 9 + 3)

E. coli LPS (40 [micro sign]g/kg/hr) of a non-lethal dose, chosen from pilot studies with several LPS concentrations, was infused during the entire period of the experiment. Sixty minutes after the start of the LPS infusion, *E. coli* (10^7 CFU) was injected. To investigate the possibility of LPS-

induced bacterial or endotoxin translocation from the gut, three additional animals were included without injection of bacteria.

Pentoxifylline Treatment in Endotoxemia (n = 9)

After the steady-state period, PTX (30 mg/kg body weight) was injected. In addition, a continuous infusion of PTX (50 mg/kg/hr) followed until the end of the experiment.

Quantitative Microbiology

Blood and tissue samples were chilled and assayed in duplicates after the end of the experiment. After incubation of the cultures at 37[degree sign]C (98.6[degree sign]F) for 24 hrs, the bacterial numbers (CFU) were counted. The final bacterial concentration was calculated as the numbers of colonies/mL of blood or as colonies/g of tissue, respectively.

Blood Cultures. Blood samples were serially diluted in sterile saline. An aliquot of 100 [micro sign]L of whole blood and of each dilution step was plated onto cysteine-lactose electrolyte-deficient agar plates, according to the method of Sandys ^[14].

Organ Cultures. Aseptically collected organs (liver, spleen, lung, and kidney) were weighed, and 0.8 to 2 g of each organ was homogenized (Ultra-Turrax) in 3 mL of sterile saline. Serial dilutions of tissue suspension (100 [micro sign]L) were plated onto cysteine-lactose electrolyte-deficient agar plates.

Polymorphonuclear Neutrophil Burst Activity

Treatment of Samples. The amount of intracellular oxygen radical production of leukocytes was measured in freshly drawn heparinized whole blood with a commercially available test kit (Bursttest, Orpegen Pharma, Heidelberg, Germany). Two aliquots (100 [micro sign]L) of each sample were incubated for 10 mins at 37[degree sign]C (98.6[degree sign]F) with phosphate buffered saline (Dulbecco's) as negative control or with E. coli that was opsonized with antibodies from pooled serum ^[15,16]. During oxidation, the nonfluorescent substrate, dihydrorhodamine 123, was taken up by phagocytes and converted during the respiratory burst to a green fluorescent compound (rhodamine 123). This oxidation is highly specific for respiratory burst activity.

Flow Cytometry. For the determination of burst activity, 15,000 cells from each sample were measured by a laser flow cytometer (FACScan, Becton Dickinson, Heidelberg, Germany). The principle of flow cytometry is the simultaneous measurement of different physical cell characteristics stimulated by an argon ion laser (488 nm) and has previously been described in detail ^[17]. Polymorphonuclear neutrophils were identified as a leukocyte subpopulation by gate selection (multiple document interface for Windows 3.1, WinMDI 2.0, by J. Trotter) and the respective respiratory burst activity was determined by the amount of rhodamine 123, expressed as mean channel fluorescence (FL-1) per cell.

Detection of Endotoxin

Taking into account plasma-related factors that interfere with the limulus amebocyte lysate-endotoxin reaction and considering that each plasma sample has a different slope of the standard curve when spiked with endotoxin, an automated, kinetic, turbidimetric limulus amebocyte lysate microtiter test with individual internal standardization was used. The principle is to establish an endotoxin reference curve in each sample by spiking the samples (diluted 1:5 and heated 10 mins at 80[degree sign]C [176[degree sign]F]) with different LPS (NP3, Novo Pyrexal, Weidner, Waldorf, Germany) concentrations. After the addition of lysate (Pyrotell, Associates of Cape Cod, Weidner, Waldorf, Germany) the increase in optical density was measured at 37[degree sign]C (98.6[degree

sign]F) at 30-sec intervals for 100 mins at 405 nm (Thermomax Photometer, Heidelberg, Germany). The deviation from the linear slope in the lower range of the spiked LPS represents the endogenous unknown endotoxin content of the sample. The sensitivity of this assay is 0.1 pg/mL.

Statistical Analysis

Data are presented as the arithmetic mean \pm SEM. A logarithm of bacterial counts was used for statistical comparison. Differences between groups were tested by one-way analysis of variance and subsequently verified by a multiple range test (Student-Newman-Keul's). Significance was accepted at $p < .05$.

The experiments were performed in agreement with the commission for animal protection of the local government. The care and handling of animals were in accordance with the National Institutes of Health guidelines.

RESULTS

Microbiological Results. In all groups, the blood cultures were found to be sterile before the injection of bacteria. In the control group, the number of bacteria decreased rapidly within 20 mins after bacterial injection and, after 90 mins, bacteria were no longer detectable in the blood. The elimination of bacteria was markedly prolonged in both the hemorrhage and the LPS groups in which circulating bacteria were detectable until the end of the experiment. After hemorrhage, bacterial concentrations were significantly higher ($p < .05$) during the entire time of the experiment ([Figure 1](#), top) and after infusion with LPS at 1, 5, 15, and 90 mins ($p < .05$). PTX infusion without induction of hemorrhage or endotoxemia did not result in significant differences in bacterial clearance or colonization of the different organs compared with the controls. However, the additional infusion of PTX in the hemorrhage group resulted in lower bacterial counts ($p < .01$) at 5 and 120 mins ([Figure 1](#), bottom). PTX treatment in endotoxemia ([Figure 1](#), bottom) reduced bacterial counts at 1 min ($p < .01$) and 5 mins ($p < .05$). After injection of *E. coli*, the delayed bacterial clearance from the circulation in hemorrhage and endotoxemia was accompanied by a significantly higher colonization of organs. Compared with the control group, significantly higher colony counts were determined in the examined organs during endotoxemia ($p < .001$; [Figure 2](#), top) and hemorrhage ($p < .01$; [Figure 2](#), bottom). Compared with the hemorrhage group, pretreatment with PTX resulted in significantly lower bacterial counts in the kidney ($p < .01$) as well as in the lung ($p < .05$). This difference was even more distinct in the LPS group. During endotoxemia, PTX treatment resulted in a marked reduction in bacterial counts in the lung from $141,000 \pm 120,000$ CFU/g without PTX to 1040 ± 332 CFU/g ($p < .001$). Significantly decreased bacterial numbers were also isolated from the kidney ($p < .001$) and liver ($p < .01$) in the PTX group.

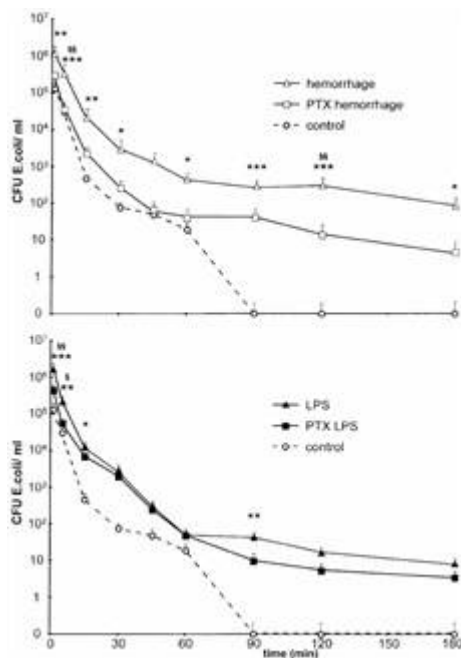


Figure 1:

Top: Elimination of *Escherichia coli* from blood of controls and after the induction of hemorrhage with and without pentoxifylline (PTX). * $p < .05$, ** $p < .01$, *** $p < .001$ vs. controls; sup [section sign][section sign] $p < .01$ vs. hemorrhage with PTX treatment. Bottom: Elimination of *E. coli* from blood of controls and after lipopolysaccharide (LPS) infusion with and without PTX. * $p < .05$, ** $p < .01$, *** $p < .001$ vs. control; sup [section sign] $p < .05$, sup [section sign][section sign] $p < .01$ vs. endotoxemia (LPS) with PTX treatment.

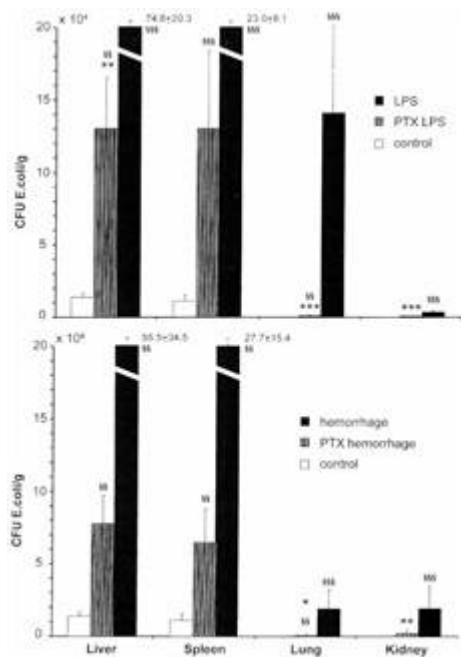


Figure 2:

Top: Bacterial counts in different organs at 3 hrs after injection of 10⁷ colony-forming units of *Escherichia coli* in controls and after infusion of lipopolysaccharide (LPS) with and without pentoxifylline (PTX). ** $p < .01$, *** $p < .001$ vs. endotoxemia (LPS) with PTX treatment; sup [section sign][section sign] $p < .01$, sup [section sign][section sign] $p < .001$ vs. controls. Bottom: Bacterial counts in different organs at 3 hrs after injection of 10⁷ colony-forming units of *E. coli* in controls and after induction of hemorrhage with and without PTX. ** $p < .01$, *** $p < .001$ vs. hemorrhage with PTX treatment; sup [section sign][section sign] $p < .01$, sup [section sign][section sign] $p < .001$ vs. controls.

Additional experiments tested whether hemorrhage and endotoxemia itself lead to invasion of *E. coli*, e.g., from the gut, into the blood and other organs during the observation period. Sterile blood and organ cultures were found at any time in hemorrhage ($n = 3$) and endotoxemia ($n = 3$). Thus, we conclude that bacterial translocation does not occur during the experimental procedure, and consequently, the decreasing numbers of circulating *E. coli* reflect the clearance function of the macroorganism.

Lipopolysaccharide Clearance. To determine the concentration of endotoxin injected with the *E. coli* suspension and to study the clearance rate during the infusion of LPS, its levels were measured before the injection of *E. coli* and at 30, 60, 120, and 180 mins. No endotoxin was detectable before injection of bacteria or infusion with LPS. Endotoxin levels were 98.7 ± 11.4 ng/mL 1 hr after infusion of $40 \text{ [micro sign]g/kg/hr}$ of LPS. In the control and the PTX groups, endotoxin levels continuously decreased after bacterial injection until the end of the experiment to $3.9 \pm 1.9\%$ (control group) and $7.4 \pm 2.1\%$ (PTX group) of the 1-min values. During the entire period of the experiment after hemorrhage, a slower clearance rate of endotoxin was observed ([Figure 3](#)); the endotoxin values at 180 mins were significantly higher ($p < .01$) than in the control group. Treatment with PTX resulted in a more rapid clearance of endotoxin, with significantly lower endotoxin values at 120 mins ($p < .05$) and 180 mins ($p < .01$) compared with the hemorrhagic animals without PTX.

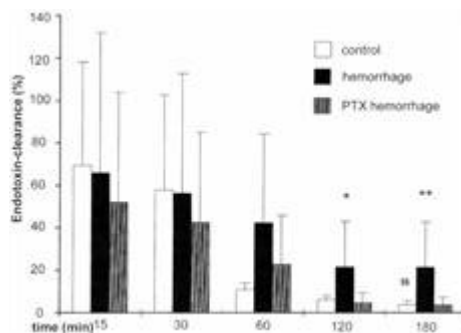


Figure 3:

Elimination of *Escherichia coli* endotoxin from the blood after induction of hemorrhage with and without pentoxifylline (PTX) related to the 1-min values after injection of *E. coli*. * $p > .05$, ** $p > .01$ vs. hemorrhage with PTX treatment; sup [section sign][section sign] $p < .01$ controls vs. hemorrhage.

Burst Activity of Polymorphonuclear Neutrophils. As expected, no spontaneous burst activity was measurable in polymorphonuclear granulocytes in the groups without LPS infusion before *E. coli* was injected, whereas markedly increased fluorescence occurred during LPS infusion ([Figure 4](#)). After *E. coli* injection, respiratory burst activity (FL-1) increased from 4 ± 2 to 43 ± 9 in the control group ($p < .05$) and from 2 ± 1 to 37 ± 2 ($p < .001$) in the PTX group at 5 mins. After the induction of hemorrhage, FL-1 values also increased at 5 mins from 2 ± 1 to 27 ± 2 ($p < .001$) and, after PTX treatment, from 1 ± 1 to 26 ± 3 ($p < .005$). Until the end of the experiment, a continuous decrease in polymorphonuclear neutrophil stimulation was registered in all groups. In those groups that were treated with PTX, the burst activity was reduced at all times of the experiment and was most expressed in the group after LPS infusion. No significant differences occurred among the different groups.

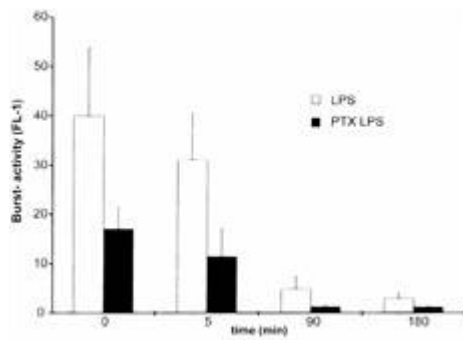


Figure 4:

Spontaneous respiratory burst activity of rabbit granulocytes after lipopolysaccharide (LPS) infusion with and without pentoxifylline (PTX) treatment.

Hemodynamic and Metabolic Parameters. In the control group, mean arterial blood pressure, hemoglobin, hematocrit, and blood gases (PaO_2 , PaCO_2 , SaO_2 , pH) remained within 10% of zero values during the observation period. After infusion of PTX, no significant differences in the serum parameters measured or in mean arterial blood pressure were observed as compared with the controls. To determine the bacterial clearance rate under standardized hemodynamic conditions, the mean arterial blood pressure was decreased by bleeding from 60% to 75% of baseline values. This decrease from 42 ± 1 mm Hg to 29 ± 1 mm Hg was reached within 20 mins and was maintained at this level during the first 60 mins of the experiment by autologous transfusion or further bleeding, respectively. After bleeding, hemoglobin decreased from 11.2 ± 0.6 g/dL to 9.4 ± 0.4 g/dL within 30 mins. The lowest levels of hemoglobin (8.3 ± 0.4 g/dL) were measured at 180 mins after injection of bacteria, correlating with the lowest values in mean arterial blood pressure (23 ± 3 mm Hg) at this time. In the LPS group, the mean arterial blood pressure decreased from 48 ± 2 mm Hg to 39 ± 2 mm Hg during this time period. Both after hemorrhage or infusion of LPS, the course of the mean arterial blood pressure correlated with a decrease in blood pH. At 180 mins, the pH values in the hemorrhage group reached a minimum of 7.2 ± 0.1 and of 7.1 ± 0.1 in the LPS group. Concomitantly, in both groups, a continuous rise in serum lactate was registered. After hemorrhage, the lactate concentration increased from 2.2 ± 0.3 mmol/L to 16.0 ± 2.0 mmol/L and, after LPS infusion, from 2.5 ± 0.2 mmol/L to 11.7 ± 1.5 mmol/L.

DISCUSSION

The activation of immunocompetent cells, and, in particular, of mononuclear phagocytes and polymorphonuclear neutrophils, plays a key role curing inflammatory reactions. Under physiologic conditions, these reactions serve to eliminate invading microorganisms via phagocytosis and killing by toxic oxygen radicals and proteases. Under pathologic conditions during sepsis in which hyperinflammatory processes are induced, polymorphonuclear neutrophils are activated to such an extent that host defense mechanisms induce severe tissue injury. Thus, for successful therapeutical approaches, substances are required that are capable of inhibiting these hyperinflammatory reactions. A variety of strategies attempting to reduce polymorphonuclear neutrophil-mediated tissue injury have been investigated. Agents used in this context include neutralizing antibodies against endotoxins and cytokines^[18,19], such as TNF and IL-1, or monoclonal antibodies against adhesion molecules^[20,21], antioxidants^[22,23], or adenosine^[24-26]. The use of the xanthine derivative, PTX, is another promising therapeutical approach in the treatment of polymorphonuclear neutrophil-mediated cytotoxic tissue reactions. Besides the well-known positive effects of PTX on blood flow, PTX is also capable of affecting uncontrolled inflammatory reactions by augmenting the regulatory defense mechanisms of the host. Increased resistance against sepsis or endotoxemia was observed in mice and rats, and, in recent studies, in human patients^[27,28]. PTX inhibits the production of oxygen radicals of polymorphonuclear neutrophils^[11,29,30] and their adherence to endothelial cells by inhibiting the expression of adhesion molecules

on the cell surface of polymorphonuclear neutrophils [13,31,32]; it also reduces the production of inflammatory cytokines, in particular of TNF [7,33,34], and prevents platelet aggregation [35,36]. Moreover, PTX improves perfusion of the microvascular bed and tissue oxygenation [37,38]. The inhibition of phosphodiesterase, as the underlying mechanism, is implied in these effects that result in the inhibition of hydrolysis of cAMP, which, in turn, increases intracellular cAMP [39,40]. In view of the beneficial activities of PTX, it was the aim of this study to investigate the effects of PTX on granulocyte function and bacterial clearance in blood and bacterial spread into organs. The pathophysiologic event states of endotoxemia and hemorrhage that were selected as clinically relevant in our experiments were those that were accompanied by a high frequency of septic complications. To enable quantitative bacterial analysis in this animal model, a defined number of *E. coli* bacteria was injected intravenously that was representative for the invasion of bacteria from various compartments, such as from the gut, the urogenital tract, implanted catheters, or infected wounds. Subsequently, the clearance of *E. coli* from the circulation and bacterial seeding in organs were examined under control conditions and after treatment with PTX in hemorrhage or endotoxemia. The amount of PTX infusion chosen corresponded to the treatment with PTX reported in recent animal experiments [8,41]. To answer the question whether bacterial isolates originating from potential intestinal translocation falsify the results of the clearance rate of injected *E. coli*, experiments were performed with hemorrhage or endotoxemia without the injection of *E. coli*. The possibility of translocating bacteria may be excluded, because no bacteria were isolated from blood or from the different organs throughout these experiments. Corresponding to previous results in the same animal model [2,9] after induction of hemorrhage or endotoxemia, a delayed bacterial clearance from blood with significantly higher counts of circulating bacteria was observed. Treatment with PTX resulted in an accelerated clearance of bacteria from the blood in hemorrhage as well as in endotoxemia. Interestingly, PTX-induced differences were more distinct in the bacterial organ distribution. The predominant seeding of injected *E. coli* in liver and spleen (more than 99% of bacterial load) favors the crucial contribution of tissue cells of the mononuclear phagocyte system to the elimination of bacteria from the blood. In hemorrhage and endotoxemia, an altered filter function of liver and spleen was observed, with significantly higher bacterial seeding in lung and kidney amounting to 6% in hemorrhage and to 11% in endotoxemia. Moreover, significantly higher numbers of bacteria were counted in all organs studied. Treatment with PTX resulted in significantly lower bacterial seeding in liver, lung, and kidney.

Because development of endotoxemia is a frequent event in the initial phase of sepsis and endotoxin levels are thought to be of prognostic value regarding the survival rate, this study includes measurement of endotoxin clearance under different pathophysiologic conditions. Endotoxin clearance was accelerated under PTX treatment during hemorrhage (120 mins, $p < .05$; 180 mins, $p < .01$) and endotoxemia.

Oxidative burst capacity of polymorphonuclear neutrophils and mononuclear phagocytes plays a central role in the bactericidal defense mechanisms against invading bacteria. Therefore, granulocyte burst activity was of special interest in these experiments. We were able to demonstrate that PTX suppresses respiratory burst activity of granulocytes in hemorrhage as well as in endotoxemia.

In summary, the findings of this study demonstrate that PTX has a beneficial effect on *E. coli* clearance from the blood and on the seeding of bacteria in lung, kidney, liver, and spleen during hemorrhage or endotoxemia. These results are in agreement with experimental studies demonstrating that PTX increased the survival rate in endotoxemia [8] and hemorrhage [42] as well as the elimination rate of bacteria [43]. An important underlying mechanism resides in the PTX-induced modulation of the potentially cytotoxic function of polymorphonuclear neutrophils. Thus, after treatment of sepsis with PTX, significantly reduced levels of TNF- α [44] and IL-6 were observed in animal experiments as well as in clinical studies [41,45]. The reduced release of proinflammatory mediators may be the reason for the reduced burst activity of poly-

morphonuclear neutrophils by PTX treatment observed in our experiments. The failure of PTX, however, to improve bacterial elimination from the blood after 60 mins despite decreasing the colonization of liver and spleen and reducing polymorphonuclear neutrophil burst activity emphasizes the impact of organ-related mononuclear phagocyte system cells on bacterial clearance. An important pathomechanism in the progression of sepsis in context with endotoxemia or hemorrhage is an impairment of the microcirculation resulting in hypoxic tissue injury. Wang et al. [46] described some beneficial effects of PTX on microcirculation and tissue oxygenation of the liver in hemorrhage that may play an important role in addition to the modulation of the cytokine pattern for the PTX-induced reduction of bacterial seeding in tissues observed in this study. Whether the accelerated endotoxin clearance by the treatment of hemorrhage with PTX shown in our experiments is a correlate of an improved liver perfusion needs further investigation. In this study, treatment with PTX led to significant improvement of hemorrhage- or endotoxemia-induced impairment of bacterial clearance from blood and tissue. Further clinical studies may provide evidence for a reduced risk of infections of critically ill patients with hemorrhage or endotoxemia by treatment with PTX.

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