Effects of endothelin-1 on bacterial clearance in rabbits

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Abstract

As elevated endothelin-1 (ET-1) levels have been reported in systemic inflammatory diseases, the role of ET-1 as a promoter of inflammatory reactions is currently under investigation. The purpose of this study was to investigate the potential influence of ET-1 on systemic vascular pressure and immune function in terms of blood clearance and organ distribution of injected *Escherichia coli* in a rabbit model. To enable quantification of the clearance process, defined numbers of exogenous *E. coli* (10^8 cfu) were injected intravenously 60 min after starting the infusion of ET-1 (0.2 μg kg^{-1} min^{-1}; n=9) or after saline infusion (controls, n=9). Parameters monitored were arterial blood pressure, airway pressure, serum lactate concentrations and rates of bacterial elimination from the blood. At 180 min after *E. coli* injection, the animals were killed, and tissue samples of liver, kidney, spleen and lung were collected for bacterial counts. ET-1 infusion produced an increase in mean arterial pressure (83.9±3.9 mmHg vs. 50.1±4.1 mmHg at 120 min; P<0.01) associated with higher serum lactate concentrations (12.6±1.3 vs. 5.4±0.3 mg dL^{-1}; P<0.001) and a delayed bacterial elimination from the blood compared with controls. Furthermore, there was increased colonization of the lungs (3.6±0.5 × 10^3 cfu vs. 745±120 cfu; P<0.01), spleen (142.4±45.4 × 10^3 cfu vs. 22.7±5.2 × 10^3 cfu; P<0.05) and kidney (758±329 vs. 357±151 cfu; NS), reflecting a reduced bacterial killing function.

Introduction

Endothelin-1 (ET-1), a peptide with vasoactive properties, was first described by Yanagisawa *et al.* 1988 [1]. In the last decade, various actions were found to be induced by ET-1, such as bronchoconstriction [2], stimulation of leucocytes [3] and mitogenesis [4]. As elevated plasma levels were detected in patients suffering from septic complications [5,6] and in animal models of sepsis [7,8], the role of ET-1 as a mediator of septic complications is currently under investigation. ET-1 levels were elevated after administration of endotoxin [9, 10], indicating a role of ET-1 in inflammatory diseases. Furthermore, ET-1 plays an important role as a mediator of granulocyte-induced lung injury [11-13]. However, it is not known whether ET-1 only affects vascular reactions or

whether it also promotes the development of inflammatory diseases by influencing host defence mechanisms. The aim of this study was to examine the effects of ET-1 on systemic blood pressure and on bacterial killing capacity in anaesthetized rabbits in an attempt to clarify the pathogenic role of ET-1 in inflammatory disease. The elimination kinetics of exogenous *E. coli* from the blood and their tissue distribution in the liver, spleen, kidney and lung were studied to simulate bacterial invasion from various compartments, such as the gut, the urogenital tract, wounds or implanted catheters.

Materials and methods

The rabbit model

This study was approved by the Animal Subject Protection Committee of the District President at Karlsruhe. The care and handling of animals were in accordance with the National Institutes of Health guidelines.

Standard breed rabbits (n=18) of either sex weighing between 2500 and 3000 g were anaesthetized with ketamine (25 mg kg^{-1}) and xylazine (2 mg kg^{-1}) and anticoagulated with heparin-sodium (1000 U kg^{-1}) injected into an ear vein catheter. The animals were placed on a temperature-controlled preparation table. After aseptic placement of a tracheostomy tube, the rabbits were ventilated mechanically with room air (tidal volume 30 mL; frequency 30 min^{-1}) by means of an anaesthesia ventilation system (AV-1; Dräger, Lübeck, Germany) during the whole study period. A polyvinyl chloride catheter (inside diameter 1.4 mm) was inserted into the right carotid artery for arterial pressure measurements and blood sampling. Anaesthesia was maintained by the infusion of ketamine (10 mg kg^{-1} h^{-1}) and xylazine (2 mg kg^{-1} h^{-1}).

Monitoring

Arterial and airway pressures were monitored online via Statham strain gauge transducers connected to a Servomed recorder (Hellige, Freiburg, Germany). Intermittently, blood samples were drawn for measurements of pH, PaO_2, $PaCO_2$ and HCO_3^- (ABL 330, Radiometer, Copenhagen, Denmark), haemoglobin and O_2 saturation (OSM2, Radiometer), haematocrit (Adams Autokrit centrifuge; Clay-Adams, New York, USA) and lactate (test-combination lactate, fully enzymatic, Boehringer Mannheim, Mannheim, Germany).

Experimental protocol

After a 30-min steady-state period, during which the rabbits were haemodynamically stable, the rabbits were randomly assigned to one of two experimental groups. *E. coli* (10^8 colony-forming units; cfu) was injected intravenously at the beginning of the observation period (time 0). After 60 min, an infusion of 0.9% NaCl (5 mL h^{-1}) was started in the control group, which was maintained during the whole observation period (control group; n=9). In the other group, the infusion of ET-1 (0.2 μg kg^{-1} min^{-1}; 5 mL h^{-1}) (ET-1 group; n=9) was started 60 min before the application of *E. coli*. Arterial blood was extracted aseptically for culture just before and after bacterial injection at 1, 5, 15, 30, 45 and 60 min and thereafter at 30-min intervals. Three hours after bacterial injection, the animals were killed with an overdose of pentobarbitone and, subsequently, tissue samples of liver, spleen, kidney and lung were taken under aseptic conditions for quantitative bacterial determinations.

The dose of ET-1 to be used was estimated from dose-response studies in which a concentration of 0.2 μg kg^{-1} min^{-1} was able to induce a reproducible increase in arterial blood pressure, whereas higher concentrations of ET-1, which had previously been reported from experiments in rats [14],

killed the animals within 30 min. Human ET-1 levels during sepsis are increased to 19.9±2.2 pg mL^{-1} compared with 6.1±0.3 pg mL^{-1} in healthy volunteers [15]. In the present study, the relatively high concentrations of ET-1 were used in line with previous publications from Masaki and Yanagisawa [14] to demonstrate the pharmacological effects of ET-1 in an animal model.

Quantitative microbiology

Immediately after organ collection, cooled blood and tissue samples were prepared for bacterial culture. Blood samples were diluted by serial dilution into measured volumes of sterile saline. One hundred microlitres of each dilution was plated in duplicate onto cysteine-lactose, electrolyte-deficient agar plates, as described by Sandys [16]. Aseptically collected organs were weighed, and tissue samples (0.8-2 g) of each organ were homogenized in a mortar with 5 mL of sterile saline. Serial dilutions of tissue suspension (50 µL) were plated onto cysteine-lactose, electrolyte-deficient agar plates. The inoculated plates were incubated at 37°C for 24 h, and bacterial counts, appearing as cfu, were read. The final bacterial concentrations were calculated as the numbers of colonies per millilitre of blood and as colonies per gram of harvested tissue.

Histology

Representative tissue samples of liver, lung, spleen and kidney were frozen in liquid nitrogen and cut in slices of 10 µm for light microscopic examination.

Materials

ET-1 was purchased from Alexis (Läufelfingen, Switzerland) and used at a concentration of 0.2 µg kg^{-1} min^{-1} via infusion.

E. coli, an encapsulated, serum-resistant, non-haemolytic strain, freshly isolated from blood culture from a septicaemic patient, was cultivated on blood agar plates. The grown colonies were scraped from the plates, carefully homogenized by vortexing in tryptic soy broth, serially diluted, adjusted to a density of 10^8 cfu mL^{-1} and stored in a freezer until use. The amount of exogenous *E. coli* used was based on pilot experiments, investigating the clearance and organ distribution of different doses of *E. coli* (10^{10}, 10^8, 10^6) as used in previous studies [17,18]. For this study, a dose was chosen that showed reproducible elimination kinetics but did not induce severe haemodynamic changes.

Statistical analysis

Data are presented as means± standard error of the mean. Differences between the groups were tested by ANOVA with Bonferroni correction. Significance was accepted at $P<0.05$.

Results

Baseline values of mean arterial pressure (MAP) were similar in both groups (55.3±2.7 in the control group vs. 54.8±3.7 mmHg in the ET-1 group). A sustained increase in MAP was recorded beginning 30 min after the start of the ET-1 infusion (time −30 min). Compared with the control group, significant increases in MAP were reached 30 min before the injection of *E. coli* ($P<0.05$) and from time 0 until the end of the observation period ($P<0.01$ and $P<0.001$ respectively) (Fig. 1). *E. coli* injection (0 min) induced a transient pressure increase of approximately 10 mmHg in both groups, but MAP decreased progressively in the control group to 40.2±1.6 mmHg at the end of the observation period. In the ET-1 group, MAP reached the highest values from immediately before the injection of *E. coli* until 60 min thereafter (83.9±3.9 vs. 50.1±4.1 mmHg; $P<0.001$). During the subsequent experimental period, arterial blood pressure decreased to 50±2.9 mmHg (vs. 40.22±1.6

mmHg in the control group) at the end of the experiments ($P<0.05$). There was a continuous increase in serum lactate concentrations in both groups, which was most pronounced in the ET-1 group with a fourfold increase from 0 min (3.2±0.3 vs. 3.5±0.3 mg dL^{-1} in the control group) until the end of the experiments (12.6±1.3 vs. 5.4±0.3 mg dL^{-1} in the control group; $P<0.001$) (Fig. 2). Minimal changes in haemoglobin and haematocrit, which were similar in all groups, resulted from dilution effects caused by blood sampling and isovolaemic substitution with saline (Table 1). Furthermore, a decrease in endotoxin levels from immediately after *E. coli* injection until the end of the experiments was observed in both groups, with no significant differences between groups (Table 1).

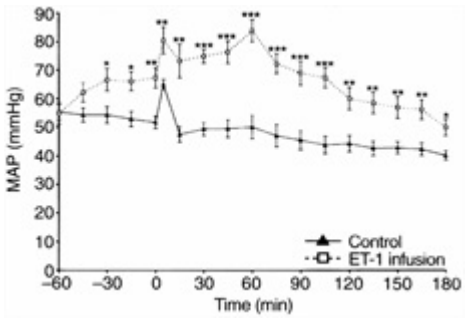

Fig. 1:
Mean arterial blood pressure (MAP) in rabbits treated with ET-1 and in NaCl-treated controls. Sixty minutes after starting the infusion, 10^8 cfu of *E. coli* were injected (time 0). Data are presented as means±standard error of the mean. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

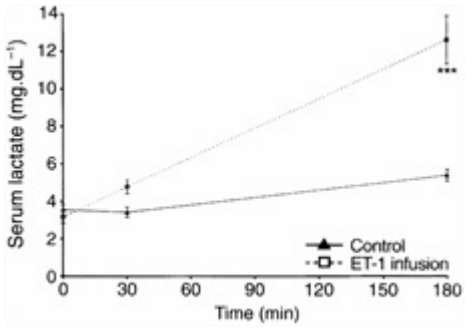

Fig. 2:
Serum lactate concentrations immediately before and after the injection of *E. coli* (10^8 cfu; time 0) in rabbits treated with ET-1 and in NaCl-treated controls. Data are presented as means±standard error of the mean. ***$P<0.001$.

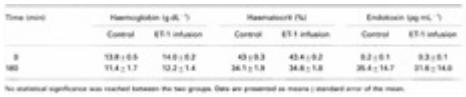

Table 1:
Haemoglobin, haematocrit and endotoxin levels in rabbits treated with ET-1 and in NaCl-treated controls before the injection of *E. coli* (10^8 cfu) and at the end of the observation period

Microbiological cultures from blood samples taken before *E. coli* injection were sterile in both groups. A delayed elimination of bacteria from the circulating blood was seen in the ET-1 group (Fig. 3), resulting in the detection of 27.8±17.2 cfu until the end of the experiments. In contrast to this, blood cultures from the control animals were almost sterile after 90 min. In comparison with the controls, the delayed clearance in the ET-1-treated animals was accompanied by higher counts of *E. coli* in liver, lung, kidney and spleen (Fig. 4). Tissue cultures from the control group showed the highest bacterial colonization in the liver (39.8±8.9 × 10^3 cfu) and the spleen (22.7±5.2 × 10^3 cfu). Lower counts were detected in the lung (745±120 cfu) and the kidney (357±151 cfu). In

contrast with this, ET-1 infusion resulted in significantly higher amounts of *E. coli* in spleen (142.4±45.4 × 10^3 cfu), lung (3.6±0.5 × 10^3 cfu), kidney (758±319 cfu) and liver (74.1±27.9 × 10^3 cfu), but statistically significant differences were only reached in the spleen (P<0.05) and lung (P<0.01).

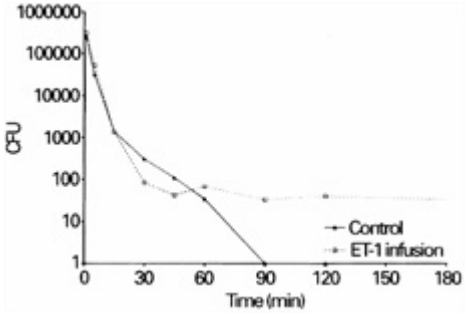

Fig. 3:
Time course of bacterial elimination from the blood after injection of *E. coli* (1.3 × 10^8 cfu) in groups pretreated with ET-1 or in NaCl-treated controls. Mean counts of cfu were plotted semi-logarithmically against time in minutes.

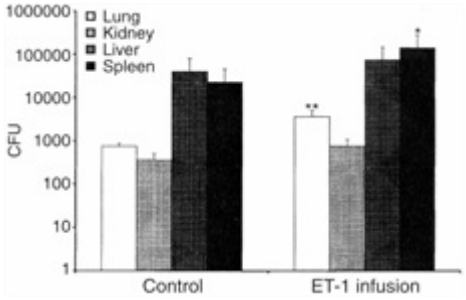

Fig. 4:
Comparative evaluation of organ distribution pattern of viable cfu of *E. coli* in tissue cultures. Data are presented logarithmically as means±standard error of the mean. *P<0.05; **P<0.01.

Signs of hypoperfusion in the liver, such as oedematous swelling of cells and the loss of nuclei, were observed with light microscopy of organ slices in the ET-1 group. Furthermore, a perisinusoidal infiltration has been seen. Regarding the other organs, no difference could be seen between the ET-1 and the control group.

Discussion

Nosocomial infections are the most common complications in intensive care units, leading to acute respiratory distress syndrome or multiple organ failure [19]. In view of therapeutic strategies, many studies have investigated the relation between critical illness and the enhanced predisposition of the organism for microbial invasion. A link between shock and the development of bacteraemia and endotoxaemia has often been reported [20,21]. A shock-induced immune suppression has been demonstrated in animal models [17,18]. The induction of haemorrhagic shock has resulted in an impaired inflammatory response [22] and a reduced peritoneal bacterial clearance [23].

The gut has been identified as a source of bacteria in many studies. The loss of intestinal barrier function leading to bacterial invasion into the blood and translocation into other organs has been related to intestinal hypoperfusion [24]. Increased ET-1 levels have often been reported after endotoxin exposure [9,10] or in sepsis [5,25]. The release of ET-1 may contribute to the vascular reactions associated with inflammation, such as pulmonary hypertension [26,27] and changes in systemic vascular resistance [14], but less is known about the effects of ET-1 on host defence mechanisms. The aim of the present study was to evaluate the potential influence of ET-1 on

bacterial clearance and to determine the role of ET-1 in mediating septic complications. The study was not designed to investigate possible enhanced translocation from the gut but, rather, whether systemic and organ clearance of intravenously applied *E. coli* is impaired after an infusion of ET-1. The injection of *E. coli* was chosen as a correlate of bacterial invasion from various compartments, e.g. the gut, the urogenital tract, wounds or catheters, into the circulation.

A significantly enhanced systemic blood pressure was registered after ET-1 infusion. This was in accordance with previous publications demonstrating systemic vasoconstriction after ET-1 application [28-30]. In contrast with this, systemic vasodilatation evoked by ET-1 has also been reported [31]. A delayed bacterial elimination from the blood was recorded in animals treated with ET-1 compared with NaCl-treated controls. The elimination kinetics (Fig. 2) of injected *E. coli* initially showed a rapid decrease in cfu in the blood within the first 15 min, followed by a delayed elimination of *E. coli* during the further observation period. Bacterial clearance was prolonged in the ET-1 group, leading to higher cfu counts in the blood. Furthermore, a significantly higher tissue colonization was found in lung and spleen in the ET-1 group. This hypothesis is supported by investigations in which enhanced vascular resistance has been found after ET-1 application in kidney [32], lung [33] and intestine [34]. One can only speculate on the underlying mechanisms leading to these findings, but it seems that ET-1 may impair host defence on the one hand by hypoperfusion and subsequent tissue hypoxia, which may be reflected by the increased lactate concentrations and the oedematous cell swelling in the liver. On the other hand, investigators have described a significant neutropenia after ET-1 infusion [35,36], which was not seen in the present study. ET-1 seems to play a proinflammatory role through its ability to activate neutrophils [27] and to stimulate elastase release by human neutrophils [3], thereby causing tissue injury. Supporting this hypothesis, ET-1-activated granulocytes have been shown to migrate into the vessel wall causing tissue damage in the human umbilical cord [3]. ET-1-induced release of tumour necrosis factor (TNF) has been found when monocytes were treated with ET-1 [37,38]. TNF is well known to impair bacterial clearance, leading to enhanced organ colonization [18]. The described actions of ET-1 may contribute to a reduced bacterial killing and an enhanced vascular permeability.

The current results indicate the crucial role of ET-1, not only as a regulator of vascular tone, but also as a central mediator affecting immune functions.

Acknowledgements

The authors thank B. Kaschta, S. Thuy (Department of Microbiology and Hygiene) and J. Christophel (Clinical Research Center Mannheim) for excellent technical assistance. This study was supported by grants from the Faculty of Clinical Medicine Mannheim, University of Heidelberg, and the Deutsche Forschungsgemeinschaft.

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