Cardiac effects of endothelin receptor antagonism in endotoxemic pigs

Konrad D¹, Haney M², Johansson G², Wanecek M¹, Weitzberg E¹ and Oldner A¹

¹Department of Physiology and Pharmacology
Section for Anaesthesiology and Intensive Care
Karolinska Institute
SE171 76 Stockholm, Sweden

and

²Perioperative and Surgical Sciences
Anesthesiology and Intensive Care Medicine
Umeå University
SE90185 Umeå, Sweden

Correspondence: David Konrad, MD
Dept Anaesthesiology and Intensive care
Karolinska Hospital
SE17176 Stockholm, Sweden
Phone: +46-73-966 18 53
Fax: +46-8-5177 5810
Mail to: david.konrad@ki.se
Abstract:

Myocardial depression in sepsis is frequently encountered clinically and contributes to morbidity and mortality. Increased plasma levels of endothelin-1 (ET-1) have been described in septic shock and previous reports have shown beneficial effects on cardiovascular performance and survival in septic models using ET receptor antagonists. The aim of the current study was to investigate specific cardiac effects of ET receptor antagonism in endotoxicosis. 16 domestic pigs were anesthetized and subjected to endotoxin for five hours. Eight of these pigs were given tezosentan (dual ET receptor antagonist) after three hours. Cardiac effects were evaluated using the left ventricular (LV) pressure-volume relationship. Endotoxin was not associated with any effects on parameters of LV contractile function (end-systolic elastance (Ees), preload recruitable stroke work (PRSW), powermax/end-diastolic volume (PWRmax/EDV) and dP/dTmax/end-diastolic volume (dP/dTmax/EDV)) but with impairments in isovolumic relaxation (time constant for pressure decay, tau) and mechanical efficiency. Tezosentan administration decreased Ees, PWRmax/EDV and dPdTmax/EDV while improving tau and LV stiffness. Thus, dual ET receptor antagonism was associated with a decline in contractile function but, in contrast, improved diastolic function. Positive hemodynamic effects from ET receptor antagonism in acute endotoxemia may be due to changes in cardiac load and enhanced diastolic function rather than improved contractile function.

Key words: sepsis, endotoxin, inotropy, end-systolic elastance, diastolic.
Introduction

Septic myocardial depression is a dire manifestation of sepsis, enhancing mortality in an already devastating disease (13). Diastolic as well as systolic dysfunction have been described (40, 45, 48) potentially causing deterioration of both right and left ventricular function (42, 44). The causative mechanisms are far from clear although a number of cytokines and nitric oxide have been proposed and investigated (31) (and references therein). The endothelins (ETs), also proposed in this context, are a family of peptides with powerful vasoactive properties first described in 1988 (64). Endothelin-1 (ET-1), which is probably the most important of the ETs as far as cardiovascular effects in humans are concerned, is mainly produced by the vascular endothelium and acts on two groups of receptors: ETA- and ETB-receptors, which are located on vascular smooth muscle cells mediating contraction. The ETB-receptor is also found on the endothelium mediating vasodilation by release of nitric oxide or prostacycline (15, 17).

In the heart, the predominant ET isopeptide is ET-1 (47), and both ETA and ETB receptors are found in the myocardium, endocardium, conducting system as well as in coronary vessels (4, 39). The ET system and ET-1 binding properties on cardiomyocytes are largely similar in pigs and humans (38).

The ET system is involved in the cardiovascular response to several disease processes. Increased plasma levels of ET-1 have been noted in association with acute myocardial infarction, congestive heart failure, pulmonary hypertension and septic shock (59). In human sepsis, ET-1 plasma levels are increased five-fold (61) and correlates to severity of illness as well as outcome (7). Previous reports, including those from our own group, have shown positive cardiovascular effects using ET-receptor antagonists in septic settings (10, 29, 43, 56). However, neither of these studies were designed to evaluate specific cardiac effects and the positive results seen may merely constitute alterations in cardiac pre- or afterload. Interestingly, other investigators have reported conflicting inotropic effects of ET-1 in normal versus pathological conditions (33, 53). In analogy, we
were recently able to demonstrate positive inotropic effects of intra-coronary administration of ET-1 in normal, anesthetized pigs (30).

The aim of the present study was to investigate the cardiac effects of ET-receptor antagonism in endotoxemic pigs. Left ventricular pressure-volume relations (LVPVR) were examined by means of conductance volumetry in an *in vivo* model of porcine endotoxemia. Based on our previous results, we postulated that dual ET-1 receptor antagonism by administration of tezosentan would improve myocardial contractile and diastolic function in a septic state.
Materials and methods

The Research Ethical Committee at Umeå University approved the experimental protocol for this study, which was conducted in conformity with the European Convention for the protection of vertebrate animals used for experimentation and other scientific purposes (Council of Europe No 123, Strasbourg 1985) and with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996, USA).

Sixteen female domestic land race pigs weighing between 38 and 55 kg were anaesthetized after fasting overnight with free access to water. After i.m injections of ketamine 10 mg·kg⁻¹, azaperone 4 mg·kg⁻¹, and atropine 50 μg·kg⁻¹, anaesthesia was induced with pentobarbital 12 mg·kg⁻¹ i.v. and maintained by a continuous infusion of pentobarbital 5 mg·kg⁻¹·h⁻¹, midazolam 0.3 mg·kg⁻¹·h⁻¹ and fentanyl 20 μg·kg⁻¹·h⁻¹. Intravenous fluids were administered as Ringer's Acetate at a rate of 20 ml·kg⁻¹·hour⁻¹ throughout the study period. After tracheotomy the animals were mechanically ventilated (Evita 4 ventilator, Draeger Medical, Lubeck, Germany) with an FiO₂ of 0.4, PEEP of 5 cm H₂O (Artema, Artema Medical, Stockholm, Sweden) with tidal volumes less than 10 ml·kg⁻¹. If arterial oxygen tension (PₐO₂) dropped below 6.5 kPa, FiO₂ was increased with increments of 0.10. Blood gas measurements were performed hourly (ABL 5, Radiometer, Copenhagen, Denmark). Body temperature was measured and maintained between 38° and 39° centigrade with the help of heating pads and a warming blanket.

All vascular catheters were placed through direct cutdowns onto the jugular or carotid vessels. A three-lumen central venous catheter (Arrow International, Pennsylvania, USA) and a thermistor-tipped pulmonary artery catheter (Optimetrix, Abbott, Illinois, USA) were placed. An arterial catheter was placed with the tip in the descending aorta. A 7.5 French (F) balloon occlusion catheter (Vascular Technologies, Solna, Sweden) was positioned in the inferior vena cava directly adjacent to the right atrium in order to provide a controlled transient restriction of venous return. Arterial, central venous, and
pulmonary artery pressures were measured using a fluid filled catheter system and
transducers (Gabarith PMSET, Becton Dickinson, New Jersey, USA). A 7 F left
ventricular (LV) pigtail combination tip manometer and conductance catheter (CA-
71083-PN, CD Leycom, Zoetermeer, Holland) was placed through an 8.5 F introducer in
the carotid artery system into the left ventricle using fluoroscopic guidance. A dual
thermistor-tipped coronary sinus catheter (Webster, California, USA) was placed in the
great cardiac vein. Catheter position was checked and rechecked using fluoroscopy and
minimal amounts of intravascular radiographic contrast (Visipaque, Amersham, Solna,
Sweden). An i.v. heparin infusion, 1000 IE per hour, was started when the cardiac
catheters were in place to minimize the risk of catheter-related thrombosis. At
termination of the experiment the pigs were euthanised using a combination of
pentobarbital bolus i.v. followed by a bolus of potassium i.v.

**Measurements and calculations**

The conductance volumetry technique is well described elsewhere (52) and we have
previously described this method in depth (8, 21). LV volume was measured using the 12-
electrode dual-field conductance catheter with 8 mm spacing between electrodes, and a
signal conditioning-amplifier (Leycom Sigma 5DF, Cardiodynamics, Zoetermeer,
Holland). The volume signal was calibrated using a stroke volume and flow reference
ratio derived from thermodilution cardiac output measurements obtained using the
pulmonary artery catheter and a thermodilution computer (WTI, Wetenskappelijk
Technische Instituut, Rotterdam, Holland). Parallel conductance for LV volume signal
was measured using the hypertonic saline method (51). Left ventricular pressure and
conductance data were recorded with a sampling rate of 250 Hz using a software package
(PC Conduct, Cardiodynamics, Zoetermeer, Holland). All circulatory measurements
were recorded and analyzed using a digital signal acquisition and analysis software
package (Acqknowledge, Biopac Systems, Santa Barbara, CA, USA).
Great cardiac vein flow ($Q_{GCV}$) was measured by thermodilution. Coronary oxygen ($O_2$) kinetics were calculated as follows: arterial $O_2$ content $= (($arterial partial pressure $O_2 \times 0.23) + \text{[hemoglobin concentration]}(1.39 \times$ arterial $O_2$ saturation)); great cardiac vein (GCV) $O_2$ content $= (($GCV$$ partial pressure $O_2 \times 0.23) + \text{[hemoglobin concentration]}(1.39 \times$ GCV $O_2$ saturation ($S_{GCVO2}$))); myocardial $O_2$ delivery (MDO$_2$) $= Q_{GCV} \times$ arterial $O_2$ content; myocardial $O_2$ consumption (MVO$_2$) $= (arterial$ $O_2$ content - GCV $O_2$ content) $\times Q_{GCV}$; and myocardial $O_2$ extraction ratio (MOE) $= 100 \times$ MVO$_2$/DO$_2$.

The units used for $O_2$ content is mL/L and for partial pressure kPa.

General hemodynamic parameters for each point in the protocol were measured: heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO), stroke volume (SV), central venous pressure (CVP), mean pulmonary artery pressure (MPAP), LV end-systolic volume, LV end diastolic volume (LVEDV), LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), LV maximal rate of change in pressure ($dP/dT_{max}$), and maximum negative rate of pressure change ($dP/dT_{min}$). End-diastole was identified as the maximum LV volume before isovolumic pressure increase, which was timed for the purpose of analysis of sequences with multiple heart cycles to 8-16 milliseconds before measured $dP/dt$ max or 8-16 milliseconds after the intracardiac ECG R wave. LV stroke work (SW) was measured from the integral of the pressure-volume area for each heart cycle. Power max ($Power_{max}$) was calculated for each beat as the maximal instantaneous pressure-flow product during systole (41). The analysis of contractile parameters was made from a selection of contiguous beats within physiological pressure ranges and also based on strong linearity in the end-systolic pressure-volume relation. The end-systolic points were initially estimated as maximal pressure/volume for each cycle, and these beats were used to establish an end-systolic pressure-volume relation (ESPVR) for all beats, with an x intercept. A tangent to this x intercept was then used to find a new end-systolic P/V point for all beats, and a final ESPVR (24). Total potential energy (PVA) was calculated for a single resting beat at the onset of a preload.
reduction sequence using the end-systolic pressure-volume relation (ESPVR) and then
(0.5)Pes(Ves-Vo), where Pes was LV end-systolic pressure, Ves was LV end-systolic
volume, and Vo was the LV volume at the x-intercept for the ESPVR. Stroke work (SW)
was calculated for the same beat, and myocardial efficiency was expressed as SW/PVA.
For diastolic parameters, tau is the time constant for pressure decay during the
isovolumic relaxation phase assuming a non-zero asymptote (9). Additionally, the half
time for pressure decay during isovolumic relaxation (t½) was measured (37). Also, for
each measurement point in the protocol a controlled preload alteration was performed
during a brief period of apnea using transient inflation of the balloon-tipped catheter to
occlude the inferior vena cava for a short period (6-8 seconds). A sequence of 6-12
contiguous heart cycles was later selected from this sequence for analysis, based on a
progressive beat-to-beat reduction in end-diastolic and end-systolic LV volumes. This
sequence was analysed for end-systolic elastance (Ees) (24) and preload recruitable stroke
work (PRSW) (18). All myocardial function parameters were calculated using custom
software.

Biochemical analyses

Plasma levels of ET-1-like immuno reactivity (ET-1 LI) were analysed with
radioimmunoassay as described by Hemsén (22). Troponin I in plasma was analyzed by a
two-position immunoenzymatic assay (Beckman Coulter Inc., Fullerton, CA, USA).

Experimental protocol

Upon completion of the preparation, a 45 minute stabilisation period was allowed.
After baseline measurements, an intravenous infusion of endotoxin (E. Coli B0111:B4,
Sigma, USA) was started in all animals beginning at 0.05 µg·kg⁻¹·h⁻¹ and gradually
increased to reach 0.25 µg·kg⁻¹·h⁻¹ within 45 minutes. After three hours, eight animals
received a short infusion of tezosentan (1 mg·kg⁻¹ in 10 minutes) followed by a continuous
infusion of tezosentan at 1 mg·kg\(^{-1}\)·h\(^{-1}\). General hemodynamics, blood gases and cardiac function were assessed every hour and plasma samples for analyses of ET-1 LI and troponin I were drawn at baseline, after three and five hours.
Statistical analysis

Data are presented as mean (±SEM). A univariate analysis for repeated measures of variance (ANOVA) was used for analysing changes over time from baseline until three hours for evaluating effects of endotoxin administration and for differences between groups prior to intervention. A repeated measures ANOVA using the time point three hours as a covariate was used for evaluating effects of tezosentan administration from four to five hours. Regarding ET-1 LI and troponin I, differences between groups post intervention were evaluated by ANOVA with analysis of the time-treatment interaction. Differences were considered significant at p < .05. A computer software program (STATISTICA 7.0 Stat Soft Inc., Tulsa, OK, USA) was utilised for statistical calculations.
**Results**

*Effects on general circulation and metabolic parameters (figure 1 and table 1)*

Endotoxemia evoked a hypodynamic response with a prominent pulmonary hypertension seen as decreases in CI, stroke volume, MAP and SvO₂, increases in HR, SVRI, CVP as well as MPAP and PVRI. Gas exchange deteriorated and a metabolic acidosis was noted. Tezosentan improved CI and stroke volume as well as reduced MAP, CVP and SVRI. Pulmonary hypertension was abolished without further effects on gas exchange. A tendency to an increase in base excess and SvO₂ was also seen in response to tezosentan.

*Effects on left ventricular systolic performance (figure 2 and table 2)*

The first three hours of endotoxemia were not associated with detectable changes in parameters of systolic function. A trend (p=0.052) for differences between groups prior to intervention were noticed regarding Ees. Tezosentan administration had negative effects on Ees, $\text{Power}_{\max}/\text{LVEDV}$, and $\text{dPdT}_{\max}/\text{LVEDV}$. A tendency towards decreased PRSW (p=0.067) and ejection fraction (p=0.060) was also seen in response to tezosentan. SW/PVA was slightly reduced by endotoxin and tezosentan had no effect on this parameter.

*Effects on diastole/isovolumic relaxation (figure 3)*

Endotoxin infusion had negative effects on the time constant for pressure decay, tau, whereas no significant effects regarding $t_{1/2}$ (p=0.08) or LV stiffness were seen. Upon tezosentan administration, tau and $t_{1/2}$ were improved and left ventricular stiffness decreased.
Coronary blood flow and oxygen utilization (table 2)

Neither $Q_{CV}$ nor $S_{CV}O_2$ were affected by either endotoxin or tezosentan whereas coronary perfusion pressure (CPP) was modestly increased by endotoxin and likewise modestly decreased by tezosentan. MDO$_2$ and MVO$_2$ were not affected by endotoxin but there were significant differences between groups prior to intervention. MDO$_2$ was slightly decreased in response to tezosentan. MOE was slightly increased in the tezosentan group compared with controls.

Biochemical parameters (figure 4)

Endotoxemia caused a two-fold increase in plasma ET-1 LI immunoreactivity. Tezosentan further increased ET-1 LI and resulted in a four-fold increase as compared with controls at five hours. Troponin levels were elevated in response to endotoxin by 57%. Tezosentan did not influence this parameter.
Discussion

In this study we have demonstrated opposite inotropic and lusitropic cardiac effects of dual endothelin-receptor antagonism in endotoxemic pigs. Firstly, tezosentan resulted in deterioration of left ventricular contractile performance. Secondly, tezosentan significantly improved diastolic performance. These findings are somewhat surprising and in contrast to previously published findings where heart function was assessed with less sensitive methods (29).

Endotoxemia per se was not associated with detectable changes in myocardial contractile function in the present study. Similar in vivo findings have been described by others (3, 46) whereas some authors have reported increased (12, 23) or decreased (1, 32, 62) contractile function in early endotoxemia. The abovementioned investigators have all used load-independent measures of contractility and endotoxin, although in different doses and serotypes and in various species. Therein lays possibly the explanation for the diverging reports. The manner by which endotoxin is infused is also important where a continuous infusion of endotoxin is preferable to bolus infusions as a model of human sepsis since it generally produces a more persistent pathophysiological response (20). Our current results suggest that endotoxin did not cause detectable LV contractile impairment, but sympathetic activation in response to endotoxin may very well have compensated for a negative inotropic effect of endotoxin as suggested by Smith et al (12, 50). The increase in HR seen following endotoxemia would support this concept but when blocking baroreceptor reflexes, Aghajani et al (2) still could not see evidence of impaired contractility in endotoxemic pigs. Intriguingly, Ishihara et al (23) reported biphasic, time-dependent changes in LV systolic performance in awake pigs receiving continuous infusion of endotoxin for 24 hours. They reported an initial increase in Ees in the first hours followed by a significant sustained decrease in Ees after seven hours and onward. Studies in humans of myocardial depression are invariably not done within the first few hours of sepsis debut but investigators often find depressed systolic function upon presentation in the ICU and days thereafter (48). Our findings, within the limited time
frame under which they are conducted, do not rule out the possibility that ET-1 may play a significant role in the clinical presentation of depressed systolic function at a later stage of sepsis as well. Interestingly, ET plasma levels have been shown to remain elevated in up to 28 days after onset of severe sepsis (55).

Although many studies performed in vitro in various species show conflicting results regarding myocardial effects of ET-1 (49, 65), there are several reports done in larger animals and humans that seem to indicate positive inotropic effects of ET-1 under non-disease conditions (33, 60). We recently reported that exogenous ET-1 administered into the coronary circulation had positive, dose-related effects on LV systolic performance in a non-septic setting (30).

Interestingly, in pathological states, such as congestive heart failure, ET receptor antagonism has shown positive effects in some clinical trials (54) but with increasing doses, the overall effect may be negative. Similarly, there are reports on ET-1 exerting negative inotropic effects during pathological conditions such as congestive heart failure (33, 53).

Despite an improved CI, tezosentan administration was associated with impairment of LV contractile status. In our previous studies, we have shown beneficial effects on CI, SV, SW and survival using dual ET receptor antagonists (29, 58). However, load-independent measures of LV contractile performance were not used in those studies. The positive effects seen may well have been due to reductions in afterload. In the present study we therefore utilized LVPVR to minimize loading confounders, a method previously validated by others (52). Sepsis and endotoxemia are associated with marked alterations in both pre- and afterload making the choice of method crucial for analyzing myocardial effects in vivo.

In the current paper Ees, PWR_max/LVEDV and dP/dT_max/LVEDV all decreased in response to tezosentan and there was a tendency for PRSW to move in the same direction. Since all these load-independent parameters show congruent results, the
conclusion that tezosentan had negative inotropic effects in this setting is fair. This is also in agreement with our recent study where intracoronary ET-1 administration was associated with increased myocardial contractile function in non-septic pigs (30), an effect likely mediated by ET\textsubscript{A} receptors. The inotropic effect of ET\textsubscript{A}-receptor activation has previously been described (25, 33) and this activation is thought to lead to increased sensitivity of the myofilaments for Ca\textsuperscript{2+} via the Na\textsuperscript{+}/H\textsuperscript{+} exchanger thus increasing cytoplasmic pH, increase in the inward Ca\textsuperscript{2+} current during depolarization and post-translational modification of myofibrillar proteins (63). Few investigators have proposed the ET\textsubscript{B} receptor as primarily responsible for the inotropic effects of ET-1 (5). Our current data implicates that the increase in ET-1 levels seen in endotoxemia may provide a response to uphold LV contractile function.

The effects of endotoxemia on general hemodynamics were primarily hypodynamic and pulmonary hypertension was prominent. Gas exchange was impaired seen as decrease in PaO\textsubscript{2} and increase in PaCO\textsubscript{2}. Tezosentan administration was associated with increases in CI and SVI as well as decreases in SVRI, MPAP, PVRI and CVP whereas MAP was further decreased and HR was unaffected. The beneficial findings on global hemodynamics are possibly related to the vasodilatory effects of dual ET-receptor antagonism, more pronounced in a state of sepsis where the ET system is markedly activated (four-fold increase in plasma ET-1 LI levels) than in a state of non-septic anesthetized pigs (35).

Mechanical efficiency (SW/PVA) was also studied and there was a modest decrease in response to endotoxin prior to intervention. In another pig model of endotoxemia, mechanical efficiency was also impaired (3) and similar findings has been shown in septic models in rats (26) and dogs (28). Contrarily, Constable \textit{et al} (12) demonstrated increased SW/PVA in endotoxemic neonatal calves. SW/PVA is most reliable as a measure of in vivo mechanical efficiency if ventricular load and heart rate are maintained relatively
constant during serial mechanical efficiency measures. These were not experimentally controlled in this model. In the literature there is some evidence of ET-1 improving contractile efficiency in vitro (63) (and references therein). This means that antagonizing the ET system would impair myocardial efficiency but, in the current study, tezosentan administration was not associated with further effects on SW/PVA.

Endotoxemia was associated with deterioration of isovolumic relaxation, seen as prolongation of tau, but had no evident effects on LV stiffness. Several investigators have reported similar findings (1, 62). In healthy volunteers, Kiely et al infused ET-1 intravenously and found impaired LV relaxation using echocardiographic parameters (27). In a cecal inoculation model in rats, Brahmbhatt and coworkers could show prolongation of tau at 12 and 24 hours post inoculation which was further prolonged by infusing bigET-1, a precursor of ET-1 (6). This suggests that sepsis per se as well as the ET system impairs LV isovolumic relaxation. Diastolic dysfunction is also seen in septic patients either as a sole manifestation of septic myocardial depression or in conjunction with systolic dysfunction (48).

Tezosentan improved isovolumic relaxation (tau and t½) and decreased LV stiffness. These findings are in line with our previous work (29) where high-volume resuscitated endotoxemic pigs improved measures of LV stiffness when treated with tezosentan in the same dosage. In a recent study from our group we administered either ET-1 or sarafotoxin 6c, a selective ETB-receptor agonist, into the coronary circulation (30). Both these peptides were associated with deteriorated isovolumic relaxation (tau and t½) which suggest that the ETB-receptor is strongly involved. ET receptor antagonism has also been beneficial in this regard in other models. Goldberg et al reported impairment in human myocyte relaxation upon ET-1 administration which was attenuated by an ET A-receptor antagonist (19) and Mebazaa et al, in papillary muscles from rabbits exposed to endotoxin in vivo, could show prolonged time to half relaxation.
which was counteracted by an ET$_A$-receptor antagonist (34). These authors found the ET$_A$-receptor responsible for the negative lusitropic effects whereas our previous results strongly suggest the ET$_B$-receptor (30). The present data do not discriminate which of the ET receptors are responsible for the effects seen. However, while being a dual ET receptor antagonist, tezosentan has a high ET$_A$/ET$_B$ antagonizing effect ratio (11). Therefore a high degree of ET$_A$-receptor antagonism would be expected in this model, suggesting that the results possibly were mainly due to ET$_A$-receptor antagonism. On the other hand, there is clear evidence of ET$_B$-receptor antagonizing effects by tezosentan seen as increased levels of plasma ET-1 LI. The elevation of plasma ET-1 upon ET receptor antagonism depends upon blocking pulmonary endothelial ET$_B$-receptors which are responsible for the clearing function of circulating ET-1 (16).

These results suggest that specific ET$_B$-receptor antagonism could be preferable, improving diastolic function without negatively affecting systole. However, in a previous study from our group, selective ET$_B$-receptor antagonism proved detrimental during endotoxemia, probably due to unopposed vasoconstriction mediated by ET$_A$ receptors and decreased ET clearance (57).

Endotoxemia was not associated with any effects on cardiac oxygen utilization parameters or $Q_{GCV}$. MOE decreased somewhat in response to endotoxin, an effect also seen in human sepsis (14). As previously reported in both human sepsis and animal endotoxemia (29) cardiac troponin I was increased in response to endotoxin. The mechanisms behind this phenomenon are yet unclear but mere ischemia is unlikely since neither in this model nor in human sepsis is myocardial hypoperfusion evident (14). Tezosentan administration was coupled to a modest decrease in MDO$_2$, possibly related to the concomitant decrease in hemoglobin, and a modest increase in MOE. These effects were quite small and are less likely to have had an impact on the results on cardiac function. Interestingly, a recent report from Merkus et al (36) suggests a local regulating
factor responsible for abolished ET-1 mediated constrictor effect on coronary resistance vessels. Cardiac troponin I was not affected by tezosentan.

In conclusion, in this porcine model of early endotoxemia dual ET receptor antagonism with tezosentan was associated with a reduction in contractile function despite improved global hemodynamic parameters. In contrast, ET receptor antagonism seemed to improve diastolic function. Positive hemodynamic effects from ET receptor antagonism in acute endotoxemia may be due to changes in cardiac load and enhanced diastolic function rather than improved contractile function.
Acknowledgements

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References


15. de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, and Vane JR. Pressor effects of circulating endothelin are limited by its removal in the


Figure legends

Figure 1.
Hemodynamic variables. General hemodynamics were studied following endotoxin administration (0.25 μg·kg⁻¹·h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg⁻¹·h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Effects of endotoxin prior to intervention are displayed as: ##=p <.01, ###=p < .001. Significant differences between groups post intervention are displayed as: ** =p< .01 and *** = p< .001.

Figure 2.
Myocardial contractile function parameters: end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), power_max / end-diastolic volume (Power_max/EDV) and dP/dT_max/EDV were studied following endotoxin administration (0.25 μg·kg⁻¹·h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg⁻¹·h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Differences between groups post intervention are displayed as: * = p< .05 and ** = p< .01.

Figure 3.
Diastolic parameters. Isovolumic relaxation parameters: tau and pressure half-time as well as left ventricular stiffness were studied following endotoxin administration (0.25 μg·kg⁻¹·h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg⁻¹·h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Effects of endotoxin prior to intervention are displayed as: #=p< .05. Differences between groups post intervention are displayed as: * = p< .05 and ** = p< .01.
Figure 4.

Endothelin-1 like immunoreactivity (ET-1 LI, a) and Troponin I (b) in arterial plasma were studied following endotoxin administration (0.25 µg·kg\(^{-1}·h^{-1}\)) for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg\(^{-1}·h^{-1}\)) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM). Effects of endotoxin are displayed as: ##=p<.01 and ###=p<.001. Differences between groups post intervention are displayed as: *** = p<.001.
Table 1. *Hemodynamic and blood gas derived parameters*./revised)

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<td>511 ± 52</td>
<td>473 ± 54</td>
<td>**</td>
</tr>
<tr>
<td>PVRI (mmHg kg min mL⁻¹)</td>
<td>ctrl</td>
<td>49 ± 9</td>
<td>228 ± 36</td>
<td>233 ± 14</td>
<td>306 ± 26</td>
<td>276 ± 29</td>
<td>273 ± 35</td>
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<td>36 ± 7</td>
<td>189 ± 18</td>
<td>204 ± 37</td>
<td>266 ± 35</td>
<td>111 ± 18</td>
<td>79 ± 11</td>
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<tr>
<td>SvO₂ (%)</td>
<td>5.1</td>
<td>ctrl</td>
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<td>67.7 ± 4.5</td>
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<td>48.9 ± 6.3</td>
<td>53.7 ±</td>
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<td>64.3 ± 5.0</td>
<td>65.8 ± 4.2</td>
<td>p=.051</td>
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<tr>
<td>Hemoglobin (g L⁻¹)</td>
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<td>85 ± 3</td>
<td>90 ± 3</td>
<td>98 ± 4</td>
<td>103 ± 4</td>
<td>101 ± 3</td>
<td>99 ± 4</td>
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<td>86 ± 1</td>
<td>92 ± 2</td>
<td>102 ± 2</td>
<td>107 ± 2</td>
<td>94 ± 1</td>
<td>90 ± 1</td>
<td>***</td>
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<tr>
<td>pH</td>
<td>ctrl</td>
<td>7.49 ± 0.2</td>
<td>7.47 ± 0.1</td>
<td>7.44 ± 0.3</td>
<td>7.41 ± 0.3</td>
<td>7.41 ± 0.3</td>
<td>7.42 ± 0.3</td>
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<td>7.50 ± 0.2</td>
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<td>7.45 ± 0.4</td>
<td>7.45 ± 0.3</td>
<td>7.49 ± 0.2</td>
<td>7.48 ± 0.2</td>
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<td>Base excess (mM)</td>
<td>ctrl</td>
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<td>3.3 ± 7</td>
<td>3.1 ± 9</td>
<td>2.1 ± 9</td>
<td>2.0 ± 1.3</td>
<td>2.1 ± 1.3</td>
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<td>4.9 ± 9</td>
<td>4.0 ± 8</td>
<td>3.7 ± 1.0</td>
<td>2.9 ± 9</td>
<td>5.3 ± 9</td>
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<td>Arterial pO₂ (kPa)</td>
<td>ctrl</td>
<td>25.3 ± 1.4</td>
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<td>13.5 ± 3.0</td>
<td>13.7 ± 3.1</td>
<td>17.9 ± 4.3</td>
<td>###</td>
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<tr>
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<td>tezo</td>
<td>27.4 ± 1.2</td>
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<td>19.7 ± 3.0</td>
<td>16.8 ± 3.5</td>
<td>18.5 ± 2.8</td>
<td>18.1 ± 2.5</td>
<td>NS</td>
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<tr>
<td>Arterial pCO₂ (kPa)</td>
<td>ctrl</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.3</td>
<td>5.7 ± 0.3</td>
<td>5.7 ± 0.3</td>
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<td>5.2 ± 0.3</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.2</td>
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Hemodynamics and blood gases were studied following endotoxin administration (0.25 µg·kg⁻¹·h⁻¹) for five hours.
After three hours of endotoxiaemia, tezosentan (1 mg·kg⁻¹·h⁻¹) was started (tezo, n=8) and compared with animals receiving endotoxin alone (ctrl, n=8). Data are presented as mean (SEM).
Effects of endotoxin prior to intervention (both groups) are displayed as: #=p<.05, ##=p<.01 and ###=p<.001.
Differences between groups post intervention are displayed as: *p<.05, **p<.01 and ***p<.001.
Table 2. *Cardiac effects* (revised)

Cardiac effects were studied using LVPVR following endotoxin administration (0.25 µg·kg⁻¹·h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg⁻¹·h⁻¹) was started (tezo, n=8) and compared with animals receiving endotoxin alone (ctrl, n=8). Data are presented as mean (SEM). Effects of endotoxin prior to intervention are displayed as: #=p< .05 and ###=p< .001. Differences between groups prior to intervention are displayed as: §=p< .05. Differences between groups post intervention are displayed as: * = p < .05 and ** = p < .01.

<table>
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<tr>
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<th>0h</th>
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<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>p-values</th>
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<tr>
<td>SW (mm Hg mL)</td>
<td>ctrl</td>
<td>6105 ±791</td>
<td>5061 ±789</td>
<td>5338 ±665</td>
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<td>6815 ±631</td>
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<td>6313 ±922</td>
<td>5230 ±665</td>
<td>4416 ±535</td>
<td>4631 ±554</td>
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<td>LVEDP (mmHg)</td>
<td>ctrl</td>
<td>14.4 ±1.5</td>
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<td>9.8 ±1.9</td>
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<tr>
<td>LVEDV (mL)</td>
<td>ctrl</td>
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<td>LVESP (mmHg)</td>
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<td>LVESV (mL)</td>
<td>ctrl</td>
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<td>40.0 ±4.8</td>
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<td>SW/PVA</td>
<td>ctrl</td>
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<td>EF (%)</td>
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<td>MDO2 (mL min⁻¹)</td>
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<td>16.9 ±4.4</td>
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<tr>
<td>MVO2 (mL min⁻¹)</td>
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</tr>
</tbody>
</table>
ET-1 LI (a)

Troponin I (b)

Copyright Information
Hemodynamic variables. General hemodynamics were studied following endotoxin administration (0.25 µg.kg⁻¹.h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg.kg⁻¹.h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Effects of endotoxin prior to intervention are displayed as: ##=p<.01, ###=p<.001. Significant differences between groups post intervention are displayed as: ** =p<.01 and *** = p<.001.
Myocardial contractile function parameters: end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), powermax / end-diastolic volume (Powermax/EDV) and dP/dTmax/EDV were studied following endotoxin administration (0.25 µg.kg⁻¹·h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg.kg⁻¹·h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Differences between groups post intervention are displayed as: * = p<.05 and ** = p<.01.
Diastolic parameters. Isovolumic relaxation parameters: tau and pressure half-time as well as left ventricular stiffness were studied following endotoxin administration (0.25 µg.kg⁻¹.h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg.kg⁻¹.h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM) with relative changes from baseline. Effects of endotoxin prior to intervention are displayed as: # = p < .05. Differences between groups post intervention are displayed as: * = p < .05 and ** = p < .01.
Endothelin-1 like immunoreactivity (ET-1 LI, a) and Troponin I (b) in arterial plasma were studied following endotoxin administration (0.25 µg.kg⁻¹.h⁻¹) for five hours. After three hours of endotoxemia, tezosentan (1 mg.kg⁻¹.h⁻¹) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM). Effects of endotoxin are displayed as: ##=p<.01 and ###=p<.001. Differences between groups post intervention are displayed as: *** = p<.001.