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Title: Effect of vasopressors on the macro- and microcirculation during systemic inflammation in humans *in vivo*

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Abstract

Aim: Comparing the effects of different vasopressors in septic shock patients is hampered by high heterogeneity and the fact that current guidelines dictate the use of norepinephrine. Herein, we studied the effects of three vasopressor agents, norepinephrine, phenylephrine and vasopressin, on the macro- and microcirculation during experimental human endotoxemia, a standardized, controlled model of systemic inflammation in humans *in vivo*.

Methods: We performed a randomized controlled study in which 40 healthy male volunteers were assigned to a five-hour infusion of either 0.05 μg/kg/min norepinephrine (n=10), 0.5 μg/kg/min phenylephrine (n=10), 0.04 IU/min vasopressin (n=10), or saline (n=10), starting one hour before intravenous administration of two ng/kg lipopolysaccharide (LPS). The macrocirculation was monitored using arterial catheter-derived parameters with additional blood pressure waveform contour analysis (PCA) until 4.5 hours following LPS administration. Sublingual microcirculatory density and flow were assessed using a handheld video microscope until 6 hours post-LPS.

Results: LPS administration affected all macrocirculatory and microcirculatory parameters. The LPS-induced decrease in blood pressure and systemic vascular resistance (SVR) was refractory to low-dose norepinephrine and phenylephrine, and to a lesser extent, to vasopressin. Only vasopressin exerted effects on PCA parameters compared to placebo, by mitigating the LPS-induced decrease in diastolic blood pressure by stabilizing SVR and cardiac output. The endotoxemia-induced decreased indices of microvascular flow and density were not influenced by vasopressor therapy.

Conclusions: In a highly controlled model of systemic inflammation in humans *in vivo*, a five-hour infusion of various vasopressors revealed distinctive effects on macrohemodynamic variables without affecting the sublingual microcirculation.

Key Words: Sepsis, vasopressor, microcirculation, pulse contour analysis

Introduction

During septic shock, both systemic hemodynamics and the microcirculation are severely affected, and these alterations are associated with organ failure and impaired outcome (1). Norepinephrine is by far the most widely used vasopressor for septic shock. However, other vasopressors such as vasopressin or phenylephrine might hold an advantage when considering effects on both the microcirculation and systemic hemodynamics (2). Comparing the effects of different vasopressors in septic shock patients is hampered by the high heterogeneity of the disease and the fact that current guidelines dictate the use of norepinephrine as the first line vasopressor, and only advise the use of other compounds as 'add-on' treatment in catecholamine-resistant shock (3). Experimental human endotoxemia is a controlled, safe and reproducible model of systemic inflammation that mimics several of the microcirculatory and macrocirculatory changes observed in sepsis (4–6). In the present study, we aimed to study the effects of three vasopressor agents, norepinephrine, phenylephrine and vasopressin, on both the microcirculation and systemic hemodynamics during experimental human endotoxemia.

Materials and methods

2.1 Subjects, study design, and ethics

We performed a randomized controlled experimental endotoxemia study in forty healthy male volunteers (18-35 years) at the intensive care department of a tertiary care university hospital in the Netherlands (Clinicaltrials.gov NCT02675868). All subjects provided written informed consent and the study was approved by the local ethics committee (registration no. 2015-2079). Experiments were carried out in accordance with the Declaration of Helsinki, including recent revisions, and Good Clinical Practice guidelines.

2.2 Experimental human endotoxemia procedures

All subjects received an intravenous bolus injection with 2 ng/kg lipopolysaccharide (*E. coli*-derived LPS), and were randomized to receive either a five-hour infusion of 0.05 µg/kg/min norepinephrine (n=10), 0.5 µg/kg/min phenylephrine(n=10), 0.04 IU/min vasopressin (n=10) or placebo (NaCl 0,9%, n=10). Experimental procedures are detailed in our previous work (7). Infusion was started one hour before LPS administration. Furthermore, the study subjects received 1500 mL 2.5% glucose/0.45% saline during the hour prior to LPS administration, followed by 150 mL/h until six hours after LPS administration, and 75 mL/h for the remaining two hours. Both macro- and microcirculatory measurements were performed at baseline (T1), 30 minutes after initiation of vasopressor administration but before LPS administration (T2), 90 [macrocirculation] or 120 [microcirculation] minutes following LPS administration (T3, the height of the inflammatory response, characterized by peak levels of pro-inflammatory cytokines and flu-like symptoms(5)), 210 minutes post-LPS administration (T4, maximum hemodynamic effects(5), only macrocirculatory parameters were obtained at this timepoint), and 270 [macrocirculation] or 360 [microcirculation] minutes following LPS administration (T5, after cessation of vasopressor infusion).

2.3 Macrocirculation measurements

All macrocirculation parameters were blood-pressured derived. The radial artery was cannulated using a 20-gauge arterial catheter (Angiocath, Becton Dickinson Pty Ltd, Franklin Lake, NJ, USA) which was connected to an arterial pressure monitoring set (Edwards. Lifesciences LLC, Irvine, California, USA). The arterial blood pressure (ABP) signal was recorded on a laptop computer and stored on a hard disk with a sample rate of 200Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA) for off-line analysis. The ABP signal was analysed using custom-made MATLAB scripts (Matlab

R2017b, The MathWorks Inc. Massachusetts, USA). Mean arterial blood pressure (MAP) was acquired by taking a fourth order Butterworth low-pass filter with a cut-off frequency of 0.02 Hz from the raw ABP signal. Heart rate (HR) was acquired by automatic detection of R-peaks from the ECG-signal. The used pulse contour analysis (PCA) accounts for the dependence of arterial compliance on arterial pressure by scaling its cardiac output (CO) estimate to pulse pressure, with stroke volume (SV) equalling pulse pressure divided by the sum of systolic (SBP)- and diastolic blood pressure (DBP) as proposed by Liljestrand and Zander (8, 9). SV was subsequently multiplied by HR to calculate cardiac output (CO). Systemic vascular resistance (SVR) was approximated by dividing MAP by CO.

2.4 Microcirculation measurements

A minimum of five steady video clips of at least 10 seconds were obtained from the sublingual region using a video microscope (CytoCam-IDF, Braedius Medical, Huizen, The Netherlands). Video microscopy was performed by a trained investigator (LvL) after removal of saliva while avoiding pressure artefacts. Video scoring was performed according to Massey *et al*.(10). Vessel density was calculated as the number of vessels crossing arbitrary lines divided by the total length of these lines (i.e. Number of crossings). Quantification of flow (i.e. microvascular flow index (MFI) was categorized as 0:no flow, 1: intermittent flow, 2: sluggish flow, and 3: continuous flow, as described previously(4).

2.5 Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). Normality was assessed using Shapiro-Wilk tests. Effects of vasopressor agents before LPS administration were analysed using paired Student's T-tests on T1 and T2. LPS-induced changes over time were analysed using repeated measures one-way ANOVA on T2,

T3, T4, and T5 in the placebo group only. Differences between vasopressor and placebo-treated subjects over time during endotoxemia were tested using repeated measures two-way ANOVA (interaction term) on T2, T3 and, for macrocirculatory parameters only, T4. A two-sided p-value of <0.05 was considered statistically significant.

Results

3.1 Subjects and symptoms

There were no differences in baseline characteristics between the treatment groups, which are reported elsewhere (7). All subjects developed typical flu-like symptoms, peaking at 90 minutes following LPS administration, which were completely subsided 7-8 hours after the LPS challenge.

3.2 Effects of vasopressors prior to LPS administration

Administration of norepinephrine and phenylephrine caused an immediate increase in blood pressure, but did not affect other macrocirculatory parameters prior to LPS administration (Figure 1). Vasopressin did not affect any of the macrocirculatory parameters, and none of the vasopressors affected microcirculatory parameters before LPS administration (Figure 2).

3.3 Effects of vasopressors during endotoxemia

Except for SV, LPS administration resulted in significant changes of all macrocirculatory parameters (Figure 1). All blood pressure variables decreased, accompanied by a compensatory increase in HR, increased CO (at constant SV) and decreased SVR. MAP kinetics in the norepinephrine and phenylephrine groups were not significantly different from placebo. Vasopressin mitigated the LPS-induced decrease in DBP by stabilizing SVR and CO. The static blood pressures did not correlate to their corresponding PCA parameters (SVR, CO and SV) in any of the groups (Pearson correlation p-values >0.10). LPS

administration resulted in decreased microvascular density and flow, which were not changed by any of the vasopressors (Figure 2).

Discussion

Our study demonstrates that the decrease in blood pressure and SVR during experimental endotoxemia is refractory to low-dose norepinephrine and phenylephrine therapy, and to a lesser extent, to vasopressin administered at a dosage used in clinical practice for the treatment of septic shock. Vasopressin prevented the endotoxin-induced increase in CO and decrease in SVR. Furthermore, endotoxemia resulted in decreased indices of sublingual microvascular flow, which were not affected by any of the vasopressors.

Expectedly, both norepinephrine and phenylephrine caused an increase in blood pressure prior to LPS administration. While these elevated levels were maintained during the peak of the inflammatory response, the LPS-induced decrease in blood pressure was not prevented. Vasopressin did not increase blood pressure prior to LPS administration. Unlike patients with sepsis, this can be explained by the fact that vasoconstrictive effects of vasopressin infusion are antagonized by intrinsic activation of the baroreflex in healthy volunteers under non-inflammatory conditions (11). PCA allowed us to break down blood pressure into flow and resistance. Complementary to our previous findings, we anew showed that experimental human endotoxemia results in a loss of vascular resistance of the arterial bed (7). Interestingly, vasopressin mitigated the LPS-induced decrease in SVR, a hallmark of sepsis-induced hypotension (12).

Our study underscores that limiting hemodynamic monitoring in critically ill patients to solely blood pressure is insufficient, as it neglects the causative physiological processes (CO

and SVR) and its ultimate goal (improving microvascular perfusion). The lack of coherence between blood pressure and these other parameters is a well-known phenomenon in sepsis (13, 14). Accordingly, in our model, there were no correlations between blood pressure and PCA parameters. Furthermore, despite clear effects on the macrocirculation both prior to (norepinephrine and phenylephrine) and after LPS administration (vasopressin), the different vasopressors did not influence sublingual microcirculatory parameters. In accordance with earlier work (4), the sublingual microcirculation was profoundly altered during endotoxemia but remained intact (indicated by high >2 MFI-values). Previous work in a model of septic shock in pigs revealed that norepinephrine and phenylephrine improved macrocirculatory parameters (e.g. MAP and cardiac index) (2). However, both pressors only marginally affected microcirculatory flow measured in seven organs: norepinephrine decreased microcirculatory blood flow in the jejunal mucosa, whereas phenylephrine increased microcirculatory jejunal muscularis flow (2). As such, the sole measurement of blood pressure can be misleading, as it may suggest that vasopressor therapies or resuscitation manoeuvres are adequate, while perfusion at the tissue level is or remains markedly compromised (14).

Several study limitations deserve attention. First, knowing that the ideal model of sepsis does not exist, our model has proven to be highly controlled, reproducible and representative for several hallmarks of sepsis (5). Nevertheless, since healthy subjects were studied, only low dosages of norepinephrine and phenylephrine could be safely administered. Higher dosages of these agents may affect the microcirculation. Second, microcirculatory parameters were determined in the sublingual vascular bed. Although the sublingual area is the preferred site for noninvasive microcirculation measurements and this approach is widely accepted as a measure of the systemic microcirculation, we cannot exclude the possibility of heterogeneity

between different tissues. Third, because PCA converts pressure measurements into volume parameters using assumptions of the dynamic characteristics of the arterial vasculature, uncalibrated PCA may not yield accurate results upon changes in SVR. Furthermore, PCA remains arduous for implementation in everyday clinical practice, partly because of the use of inscrutable algorithms. We advocate for the use of well-documented, open source, and straightforward formulas, as employed in the present work.

In conclusion, various vasopressors exert distinctive effects on macrohemodynamic variables without affecting the sublingual microcirculation in a highly standardized controlled model of systemic inflammation in humans *in vivo*. Furthermore, our data indicate that blood pressure measurements do not adequately reflect physiological parameters that are of vital importance in the critical care setting, such as CO, SVR, and microvascular perfusion. Uncalibrated PCA could be a helpful, less-invasive tool in monitoring hemodynamic responses to interventions and in disease.

List of abbreviations

ABP	Arterial blood pressure
CO	Cardiac output
DBP	Diastolic blood pressure
HR	Heart rate
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
MFI	Microvascular flow index
PCA	Pulse contour analysis

SBP	Systolic blood pressure
SV	Stroke volume
SVR	Systemic vascular resistance

Declarations

Ethics approval and consent to participate

The human endotoxemia study was approved by the local Institutional Review Board (document number 2015-2079, NCT02675868) and written informed consent was obtained from all participants prior to the study.

Consent for publication

Not applicable

Availability of data and material

The materials described in this manuscript, including all relevant raw data, is freely available to any scientist wishing to use them for non-commercial purposes.

Competing interests

None declared.

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Figure 1. Macrocirculatory parameters before and after LPS administration.

A) Mean arterial pressure, B) Pulse Pressure, C) Cardiac output (pulse contour analysis), D) Systemic vascular resistance (pulse contour analysis), E) Heart rate (pulse contour analysis), F) Stroke volume (pulse contour analysis), G) Cardiac output (pulse contour analysis), and H) Systemic vascular resistance (pulse contour analysis). LPS was administered at timepoint 0, indicated by the vertical dashed line.

Data are expressed as mean and SEM. *: p<0.05, **: p<0.01 and ***: p<0.001 (paired Student's t-tests on -90 [T1] and -30 [T2] within treatment groups). ØØ: p<0.01 and ØØØ: p<0.001 over time (-90 – 270 [T1-T2]) within placebo group (repeated measures one-way ANOVA). ⊗: p<0.05 over time (-30 - 210 [T1-T4]) vs. placebo (repeated measures two-way ANOVA, time*treatment interaction term).

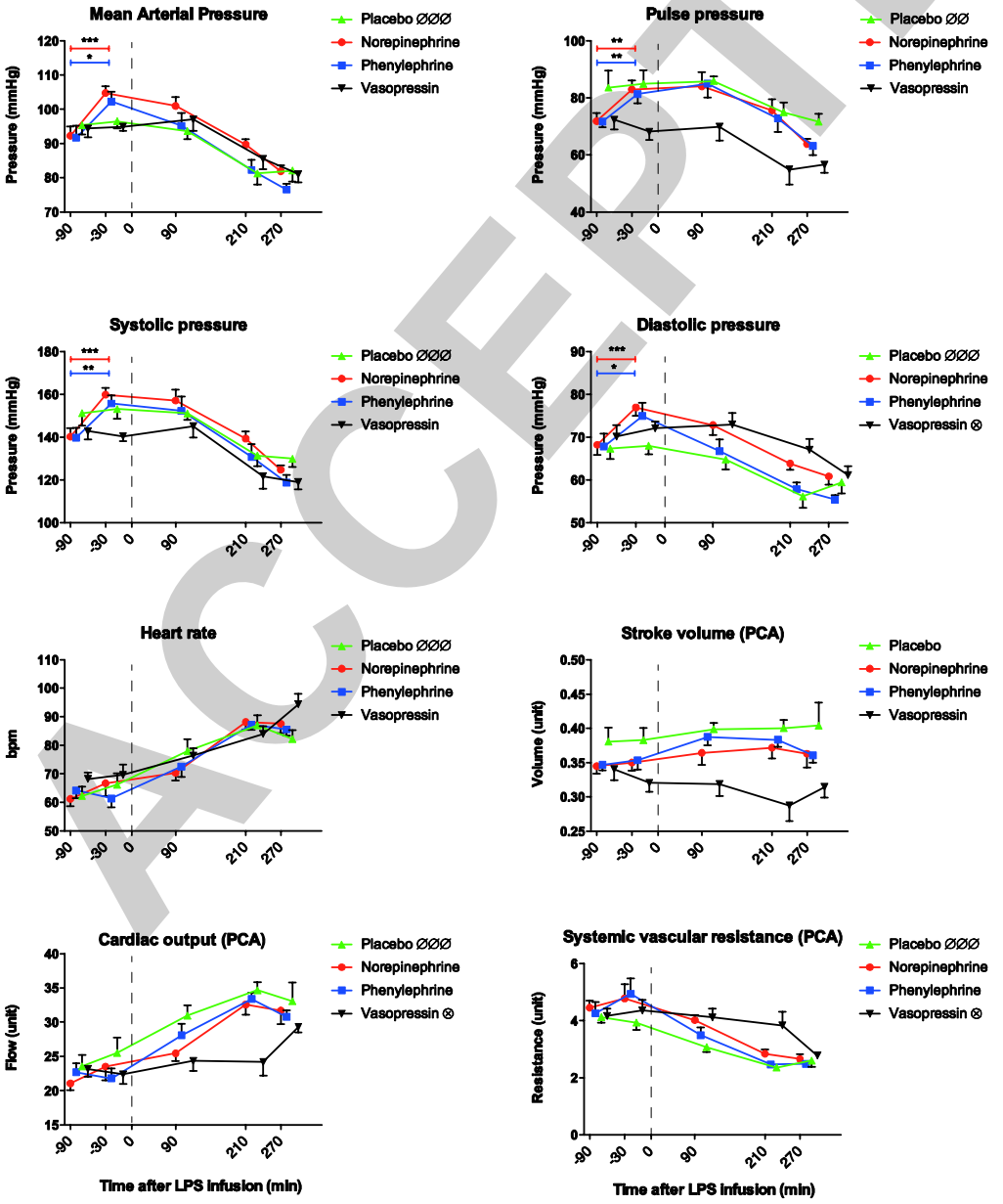

Figure 2. Sublingual microcirculatory parameters before and after LPS administration.

A) Number of crossings and B) Microvascular Flow index. LPS was administered at timepoint 0, indicated by the vertical dashed line. Data are expressed as mean and SEM. $^{\varnothing}$: $p<0.05$ over time (-90 - 270 [T1-T5]) within placebo group (repeated measures one-way ANOVA).