Future drugs in atherosclerotic cardiovascular disease

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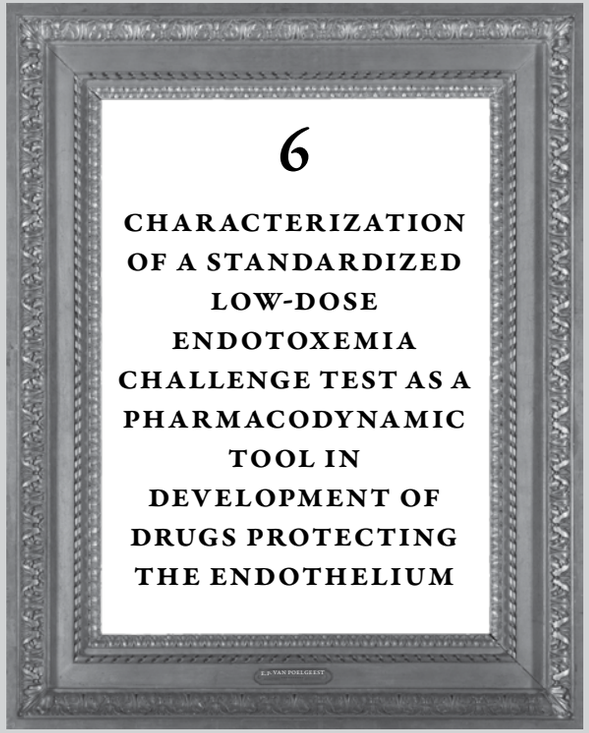

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CHARACTERIZATION OF A STANDARDIZED LOW-DOSE ENDOTOXEMIA CHALLENGE TEST AS A PHARMACODYNAMIC TOOL IN DEVELOPMENT OF DRUGS PROTECTING THE ENDOTHELIUM

EP van Poelgeest, MR Dillingh, KE Malone, EM Kemper, ESG Stroes, J Burggraaf, M Moerland. *Characterization of immune cell, endothelial, and renal responses upon human low-dose experimental endotoxemia.* Journal of Pharmacological and Toxicological Methods 2018; 89: 39-46.

ABSTRACT

BACKGROUND Although the effects of relatively high concentrations of endotoxin on endothelial activation/dysfunction and kidney markers has been described in literature, detailed insight in the LPS concentration-effect relationship, the magnitude, variability and timing of the response, and potential effects of endotoxemia on the kidneys is lacking. A study was performed to assess the effects of low- to moderate dose (0.5, 1 or 2 ng/kg) endotoxemia on the endothelium and kidneys as measured by a panel of novel highly sensitive kidney injury markers.

METHODS This was a randomized, double-blind, placebo-controlled study with single ascending doses of LPS (0.5, 1 or 2 ng/kg) administered to healthy male volunteers (3 cohorts of 8 subjects, LPS:placebo 6:2). Endothelial measures included selectins, cell adhesion molecules, and thrombomodulin. Renal measures included novel, sensitive and specific biomarkers of acute kidney injury.

RESULTS Endotoxin exposure resulted in consistent LPS dose-dependent responses in inflammatory markers, E- and P- Selectin, VCAM1, ICAM1, and thrombomodulin. The observed biological responses were transient, reaching a level of significance of at least <0.01 in the highest dose group and with an effect size which was dependent on the administered LPS dose. LPS-induced inflammatory and endothelial effects did not translate into a change in renal damage biomarkers, although at 2 ng/kg LPS, subtle and transient biomarker changes were observed that may relate to (subclinical) tubular damage.

DISCUSSION We demonstrated that administration of a single LPS dose of 2 ng/kg to healthy volunteers results in significant inflammatory and endothelial responses, without inducing clinically relevant signs of kidney injury. These findings support the application of the human endotoxemia model in future clinical pharmacology studies.

INTRODUCTION

The human endotoxemia model is a well-established model for investigation of the physiological mechanisms of systemic inflammation. In this experimental setting, purified lipopolysaccharide (LPS, also referred to as endotoxin) from the cell membrane of *Escherichia Coli* is administered intravenously to healthy volunteers resulting in flu-like signs and symptoms, and increased levels of inflammatory markers (cytokines and acute phase reactants). Recently, we published the results of a clinical study characterizing the inflammatory response at low to moderate LPS doses (0.5, 1, and 2 ng/kg) administered to healthy volunteers, focusing on clinical signs and symptoms and cytokine responses [1]. We demonstrated that this human endotoxemia model is a safe and robust methodological tool, feasible for use in clinical pharmacology studies to assess the anti-inflammatory effect of new investigational compounds.

Whereas the effect of *in vivo* endotoxin exposure on clinical signs and cytokine release in humans has been described extensively [2-4], limited information is available on the effect of lower LPS doses on the human endothelium and kidneys. This is in contrast with the detailed knowledge on the effects of endotoxin at a molecular/cellular level: LPS induces an inflammatory response via stimulation of Toll-like receptors (TLRs), innate immune receptors activated by tissue damage or by molecules associated with pathogen-associated molecular patterns (PAMPs) on invading microorganisms. Subsequent activation of multiple intracellular inflammatory pathways (e.g. MYD88-dependent and TRIF-dependent pathways) results in pro-inflammatory cytokine/chemokine release by leucocytes. LPS-induced cytokine/chemokine release leads to selectin-mediated rolling of leucocytes, firm adhesion of the leucocytes to the endothelium via cellular adhesion molecules and integrins, and transmigration of these cells through the endothelium to fight infection [5,6]. This protective inflammatory response, however, is accompanied by collateral tissue damage through inflammatory factors and reactive oxygen species secreted by cytokine-activated immune cells. The balance between protection and damage inflicting inflammation is delicate, and in various pathological conditions the beneficial effect of the inflammatory response may be outweighed by its harmful effects. In sepsis, for example, systemic inflammation and microvascular dysfunction ultimately leads to acute kidney injury (AKI) [7].

There are reports showing that LPS doses of 2-4 ng/kg activate the endothelium, reflected by an acute increase in circulating P and E-Selectin, vascular cell adhesion molecule (VCAM)1, intercellular adhesion molecule (ICAM)1, and thrombomodulin levels [7,8]. However, systematically collected and quantitative

data providing detailed insight in the relationship between LPS dose and vascular response are lacking. Moreover, data linking endotoxin-induced activation of the human vasculature and (subclinical) kidney injury are not readily available in the public domain. A human *in vivo* model with controlled and transient activation of the endothelium could qualify the human endotoxin challenge as a pharmacodynamic model for the early clinical development of new drugs, and non-pharmacological interventions such diet and life-style. This model would not only be relevant for new therapies targeting immune cells, but potentially also for compounds developed to improve or maintain integrity of the endothelium. Endothelial dysfunction is recognized as a key process in various pathological conditions including atherosclerosis [9-11], diabetes, insulin resistance [12] and sepsis-associated kidney injury [13], so pharmacological protection of the endothelial lining may be an important future target. Since LPS-induced systemic inflammation and endothelial activation may ultimately affect renal function, it is of importance that also the potential effects of the *in vivo* LPS challenge on the human kidney are quantified, to assure that this response is limited and transient.

We investigated the effect of an endotoxin challenge on endothelial activation, renal function and kidney injury markers. Healthy human volunteers were exposed to a single dose of LPS (0.5, 1 or 2 ng/kg, or placebo). The effects on the endothelium and the kidneys were quantified, in relation to the inflammatory response (cytokine release; presented earlier [1]). Endothelial activation measures included selectins, cell adhesion molecules, and TM. Renal measures included routine blood (e.g. creatinine, blood urea nitrogen) and urine (e.g. fractional excretion of sodium, sediments) markers of kidney injury, but also more sensitive and specific biomarkers for early detection of acute kidney injury (e.g. kidney injury molecule 1 (KIM1)) [14-16].

MATERIAL AND METHODS

Study setup The characteristics of our study have been published previously [1]. In short, 24 healthy male volunteers aged 18-28 years with normal kidney function (Modification of Diet in Renal Disease (MDRD) calculated creatinine clearance >60 mL/minute) and no abnormalities in urinary screen participated in this study, which was performed at the Centre For Human Drug Research in Leiden, The Netherlands. Participants were pre-hydrated with 1500 mL 2.5% glucose/saline (2.5% glucose/0.45% sodium chloride) for 2hrs. Subsequently, they received an intravenous single LPS dose (*Escherichia Coli* 113:H, 10:K negative, U.S. Standard Reference Endotoxin lot#3) or placebo (0.9% sodium chloride). The ratio LPS : placebo per dose group was 6 : 2, and doses comprised 0.5, 1 or 2 ng/kg bodyweight. Volunteers and investigators were blinded for LPS/placebo. Subjects were hydrated with 150 mL/hr glucose/saline by intravenous drip for a period of 6hrs after LPS/placebo administration. Blood samples were collected frequently over time at predefined time points. Urine samples were collected pre-dose, and during the 0-4hrs, 4-8hrs, 8-12hrs and 12-24hrs post-dose time intervals. All subjects provided written informed consent prior to study participation. The study was approved by the Medical Ethics Committee of the Amsterdam Medical Center, The Netherlands, and conducted in compliance with Dutch law on experiments in humans.

Bioanalysis Endothelial activation was measured using MesoScale Discovery's vascular injury panel 1 and V-PLEX™ vascular injury panel 2 (ICAM)1, ICAM3, (VCAM)1, TM, E-Selectin and P-Selectin). An enzymatic method was used to measure serum creatinine, and glomerular filtration rate was estimated using the 4-variable MDRD formula [17] and the chronic kidney disease epidemiology (CKD-EPI) equation (assumed to perform better than the MDRD formula in normal kidney function) [18]. Analysis of exploratory kidney injury biomarkers was performed for urinary beta2-microglobulin (B2MG; Immulite® 2000, a solid-phase two-side chemiluminescent immunometric assay), alpha-gluthatione S-transferase (αGST; Argutus Medical Alpha GST EIA enzyme immunoassay), N-acetyl-β-D-Glucosaminidase (NAG; N-acetyl-β-D-Glucosaminidase assay, Diazyme Europe GmbH), and KIM1 (Quantikine® human TIM-1/KIM-1/HAVCR immunoassay), and the MesoScale Discovery human Kidney Injury Panel V, containing Cystatin C, epidermal growth factor (EGF), neutrophil gelatinase-associated lipocalin (NGAL/Lipocalin-2), osteopontin (OPN), and uromodulin (UMOD; placebo and 2 ng/kg LPS groups only). For αGST measurement and the kidney injury panel, 100 µL of stabilizing buffer (BIO85STB, Argutus Medical) was added to 400 µL of urine.

Statistics All parameters are graphically presented as absolute readings per treatment group. Repeatedly measured parameters were analyzed after log-transformation, using a mixed model of variance with treatment, time, and treatment by time as fixed factors, subject as random factor and the baseline measurement as covariate. Treatment effects were calculated for each parameter for the time period from baseline up to 24hrs, except for the circulating cytokines for which the period was restricted to 6hrs post-dose. Contrasts (LPS versus placebo) were reported with the estimated difference, the 95% confidence interval (CI), and the p-value. All statistical analyses were performed using SAS for Windows Version 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Demographics, safety and circulating cytokines Demographic and baseline characteristics showed no significant differences between groups (Table 1). Administration of LPS resulted in a transient inflammatory response, as published by our group previously [1]. In short, LPS administration dose-dependently increased body temperature (+1.5°C for 2 ng/kg LPS) and heart rate (+28 bpm for 2 ng/kg LPS). LPS exposure resulted in elevated levels of C-reactive protein (CRP) and circulating cytokines, with clearly distinctive increases from placebo already at the lowest LPS dose level tested [0.5 ng/kg, contrast for timeframe 0-6hrs: tumor necrosis factor alpha (TNFα), +413%; interleukin 6 (IL6), +288%; and interleukin 8 (IL8), +254%; all p≤0.0001]. Maximal cytokine concentrations were measured in the 2 ng/kg group at 1.5-3hrs after LPS administration (TNFα: 222±68; IL6: 315±130; IL8: 329±84 pg/mL). CRP concentration was still increasing 24hrs after LPS dose administration.

Endothelial activation markers In the placebo-treated subjects (Figure 1A-E), all endothelial activation markers remained relatively stable over time. LPS administration dose-dependently increased endothelial activation markers (Figure 1, Table 2). An immediate increase after LPS dose administration was observed for circulating levels of VCAM1, TM, and P-Selectin. At an LPS dose of 2 ng/kg, for all these markers the responses reached a level of significance of at least <0.01 (Table 2). For ICAM1 and E-Selectin, a lag time of at least 2 hours was observed. The maximal responses in the 2 ng/kg LPS group for TM, E-Selectin and P-Selectin were reached at 6 hours after LPS administration, and amounted 4.1±0.5, 107±38 and 40±6 versus 3.3±0.7,15±3 and 24±4 ng/mL at baseline, respectively. For ICAM1 and VCAM1 the maximal response was only reached at 24 hours after LPS infusion and amounted 551±44 and 665±24, versus 320±57 and 432±29 ng/mL respectively at baseline. The response of ICAM3 to LPS administration was limited, and did not reach a level of statistical significance at any of the LPS dose levels tested.

Renal function and renal activation markers When expressed as urinary mass per hour (Figure 2A), effects of study-related hydration and diurnal effects were observed. In the placebo-treated group, baseline serum creatinine levels after prehydration amounted 78±4 μmol/L. Levels decreased in the course of the morning, to reach a minimum of 69 μmol/L observed at 6 hours after placebo administration (Figure 2B). In the subsequent hours, serum creatinine returned to baseline. In the LPS-treated subjects, serum creatinine levels followed a non-dose dependent pattern. No clear indications for an LPS-dependent effect on serum creatinine levels were observed (Table 2, Figure 2B). In the placebo-treated group, the estimated GFR (eGFR; calculated using the MDRD formula) after prehydration was 109±6 mL/min/1.73m^2 at baseline, increased in the course of the morning and reached a maximum increase to 126 μmol/L at 8 hours after placebo administration (Figure 2C). In the subsequent hours, eGFR returned to baseline. No clear indications for an LPS-dependent effect on eGFR were observed (Table 2, Figure 2C).

Baseline urinary B2MG concentration (Figure 3A, left panel) was 18±7 μg/L in the placebo-treated subjects, which is in line with previous published data (range in healthy volunteers 20 to 83 μg/L 19). Urinary B2MG concentration increased during the day in the placebo-treated subjects. Maximal concentration amounted 39±16 μg/L and was reached at 24 hours. This may be explained by relative hypovolemia after overnight fasting (see methods and discussion sections). Administration of 2 ng/kg LPS resulted in elevated urinary B2MG concentrations in the 8-24hrs time interval after LPS administration. This elevation amounted a maximum of 84±66 μg/L at 12hrs after LPS administration. The estimated difference over the 0-24hrs interval versus placebo was +88%, p=0.003 (Table 2). Correction for urinary creatinine excretion (Figure 3A, right panel) blunted this effect, although the LPS effect remained statistically significant (estimated difference over the 0-24hrs interval versus placebo +31%, p=0.03; Table 2).

Baseline urinary KIM1 and OPN concentrations (Figures 3B and 3C, left panel, respectively) were 0.18±0.07 and 389±288 μg/L, respectively in placebo-treated subjects. Urinary KIM1 and OPN concentrations increased over day in the placebo-treated subjects. Maximal concentrations were reached at 24 hours after placebo administration, amounting 0.58±0.2 and 883±413 μg/L for KIM1 and OPN, respectively. Administration of 2 ng/kg LPS resulted in elevated urinary KIM1 and OPN concentrations in the interval from 8 hours to 24 hours after LPS administration. This elevation amounted a maximum of 1.27±0.8 μg/L at 24 hours for KIM1, and of 1520±941 μg/L for OPN, respectively. The estimated differences over the 0-24hrs interval versus placebo were 121%, p=0.003 for KIM1, and 85%, p=0.04 for OPN (Table 2). Correction for urinary creatinine excretion (Figures 3B and C, right panel) blunted this effect. For KIM1 this corrected LPS effect remained statistically significant (estimated difference over the 0-24hrs interval versus placebo 44%, p=0.006, whereas for OPN, there was no statistically significant effect anymore (estimated difference over the 0-24hrs interval versus placebo 23%, p=0.5; Table 2).

Also for other renal activation markers (NAG, Cystatin C, UMOD, EGF, NGAL; Figures 3D to 3H), an increase in urinary concentration over day was observed

in the placebo-treated subjects. For these markers, no significant effects of LPS treatment could be observed (Table 2). However, for NAG, UMOD and EGF, the estimated contrasts between LPS and placebo (Table 2) and the graphical time courses (Figures 3D, 3F and 3G, respectively) did suggest the possibility of LPS-dependent effects: the estimated differences between 2 ng/kg LPS and placebo amounted 51% (p=0.1), 36% (p=0.08), and 44% (p=0.07) for NAG, UMOD and EGF, respectively (Table 2). For a considerable number of urinary NGAL and αGST measurements (33% and 89% of total, respectively), readings were below the limit of quantification (6 and 6.25 µg/L, respectively). Therefore, statistical analyses are not presented for these parameters. When evaluated on an individual basis, though, we observed that in the placebo and 0.5 and 1 ng/kg LPS dose groups there were almost no measurable αGST concentrations, whereas in the 2 ng/kg LPS group 3 out of 6 individuals showed measurable levels of αGST excretion at 12hrs after LPS administration, ranging from 10.6 to 84.3 ng/mL (range in healthy volunteers 1.1 to 64 ng/mL) [20].

DISCUSSION

LPS administration dose-dependently increased endothelial activation markers. Although it has been described previously that administration of 2 and 4 ng/kg LPS increases the circulating levels of P- and E-Selectin, VCAM1, ICAM1, and TM [7,8], the quantitative and temporal effects of lower LPS doses on these markers are unclear. We provided these insights, and demonstrated that VCAM1, TM, P-Selectin, ICAM1 and E-Selectin are suitable measures for monitoring LPS-induced endothelial activation. P-selectins promote endothelial activation. E-selectins are expressed on activated endothelial cells and mediate the adhesion of immune cells at sites of inflammation. ICAM1 and VCAM1 interact with leucocytes and are involved in subsequent transmigration into the tissues. TM is a cell surface-expressed glycoprotein present on vascular endothelial cells with an important role in coagulation and complement regulation. The explored markers all demonstrated a very low variability over day, with changes from baseline not exceeding 5% in the placebo group. For most parameters the maximal LPS response was observed at 6hrs post-dose, with the exception of VCAM1 which required a longer period (24hrs) to reach its maximal response upon LPS exposure. For ICAM1 and E-Selectin a lag time in LPS response was observed which most likely relates to cytokine-induced gene transcription [20,21]. The response of ICAM3 to LPS administration was limited, which probably relates to the fact that ICAM3 is constitutionally expressed on resting leucocytes but not on endothelium [22], and its expression is not increased by inflammation [23]. Power calculations were performed which showed that for E-Selectin in a parallel study design, at an LPS dose level of 2 ng/kg, a sample size of 3 per treatment group will have a power of 0.82 to detect a 70% inhibition; a sample size of 15 per group will have a power of 0.82 to detect a 30% inhibition using a two-sample t-test with a 2-sided significance level of 0.05. Under the same conditions, it would be possible to demonstrate a 70% and 30% inhibition with a sample size of 6 and 28 per treatment group, respectively, with a power of 84% and 81%, respectively for VCAM1. These power calculations support the application of low to moderate LPS dose-induced endothelial response as methodological tool for monitoring of drug effects in future pharmacology studies, even though our study was based on a relatively small sample size of 6 subjects per treatment arm. This is valuable information, since the pharmacological inhibition of adhesion molecules has been demonstrated so far predominantly in preclinical studies[24-28], and sporadically in human studies with generally high LPS doses (4 ng/kg) [29].

In addition to the effect on endothelial activation markers, we also explored whether exposure to a single low to moderate dose of LPS would translate into an effect on the kidneys, as endothelial alterations are important in the etiology and progression of renal damage [30]. Since experimental endotoxemia may occasionally result in vasovagal collapse [31], we decided to administer glucose/saline to all study participants from 2hrs pre-dose up to 6hrs post-dose. In the placebo-treated group, urinary volume doubled and eGFR increased by 17% within the first 4-8hrs and had normalized within 12hrs post-dose; serum creatinine levels decreased by a maximum of 12% 6hrs post-dose and had also normalized within 12hrs post-dose. Obviously, the glucose/saline infusion comprising of a total volume of 2.4L over 8hrs may have played an important role in these responses. In the LPS treated subjects, no clear indications were observed for LPS-related changes in kidney function, underlining the renal safety when considering the LPS challenge a methodological tool for future pharmacology studies. However, parameters like urinary volume, serum creatinine and GFR are relatively crude measures when assessing potential effects of an intervention on the kidneys. For example, even administration of 4 ng/kg LPS did not change traditional kidney function measurements creatinine and urea clearance, fractional sodium excretion and total 24hr urinary volume in healthy volunteers [32,33]. These traditional kidney function measurements lack sensitivity and fail to detect early subtle signs of kidney injury [34], while the extent of injury and poor outcomes associated with acute kidney injury worsen with delayed recognition of impending damage. We therefore selected an extensive panel of sensitive and early kidney injury markers

to provide insight into the functionality of different anatomical locations of the nephron. Important issues to consider regarding our renal injury marker data are the effects of overnight fasting and (pre-) hydration that may have influenced urinary concentrations of these markers. After overnight fasting there is relative hypovolemia resulting in elevated concentrations of serum creatinine and solutes in the urine due to decreased urinary volume. (Pre-) hydration restores intravascular volume, leading to an increase in glomerular filtration and increased urinary output, and therefore decreased serum creatinine and solutes in the urine. These diurnal and hydration effects were indeed observed for urinary mass (volume), serum creatinine, eGFR, and urinary concentration of most kidney biomarkers in both the placebo and LPS treated groups. However, there appears to be an untoward effect of the 2 ng/kg LPS dose as significant increases in urinary concentrations of B2MG, KIM1 and OPN were observed in the time interval 8-24hrs after LPS administration. B2MG, which is shed from lymphocytes, is filtered freely across the glomerulus and completely reabsorbed by proximal tubular cells [35]. KIM1 is a transmembrane protein expressed by proximal tubular epithelial cells with phagocytic capacity [35], and a sensitive marker for renal injury [36]. OPN is a secreted phosphoprotein with renal expression that is induced in the distal tubule upon inflammation [37,38]. Because of these characteristics, the observed changes in B2MG, KIM1 and OPN may be indicative of subtle tubular damage. Importantly, however, we observed that correction for urinary volume blunted this effect (absolute B2MG, KIM1 and OPN excretion did not differ between treatment groups). For NAG, Cystatin C, UMOD, EGF, no significant effects of LPS treatment could be observed, although for some of these measures the estimated contrasts between LPS and placebo and the graphical time courses did suggest the possibility of LPS-dependent effects. In summary, an LPS dose of 2 ng/kg does not result in transient kidney dysfunction or overt expression of kidney injury markers, but it does induce some injury responses with borderline significance. Possibly, a further increase in LPS exposure may result in more distinct kidney effects. These data are in line with the observation that administration of 4 ng/kg LPS to healthy volunteers did result in significant increases in B2MG excretion over time [33]. In the same study, NAG excretion was enhanced. This is indicative of tubular dysfunction, and clinical data indicate that NAG can be considered an early marker of mild tubular injury [39]. It may also be useful to compare the observed responses in kidney injury markers with the responses reported in literature in case of overt kidney injury. For example, reported urinary B2MG and KIM1 concentrations in drug-induced and septic AKI were ~3 to 4 fold higher than in our study [40,41]. Moreover, urinary KIM1 levels corrected for urinary creatinine levels in patients with AKI from various etiologies (including, but not limited to nephrotoxicity and sepsis) amounted 3-10 ng/mg creatinine [42-44]. In our study, the highest LPS dose increased KIM1 excretion to a maximum of 0.8 ng/mg creatinine (data not shown). These comparisons indicate that the observed responses in our study are only moderate. For αGST it was difficult to draw conclusions on LPS effects due to a considerable number of measurements below the limit of quantification (6.25 μg/L). It is known that in healthy volunteers urinary αGST concentrations are very low, since αGST is a general detoxification enzyme that is produced in situations of cellular stress/inflammation. An increased αGST excretion may reflect toxicity-induced tubular dysfunction/injury [45,46]. This is in line with our findings: αGST was undetectable except for 3 out of 6 individuals in the 2 ng/kg LPS group at 12hrs after LPS administration. An enhanced αGST excretion upon LPS administration (2 ng/kg) has been described before in a group of healthy volunteers [33].

In summary, we demonstrated that administration of a single low to moderate dose of LPS to healthy volunteers is well-tolerated and induces a sufficiently robust inflammatory [1] and metabolic [47] response that is apparently devoid of untoward renal effects. Also, we showed a dose-dependent transient endothelial response (E- and P-Selectin, VCAM1, ICAM1, and TM), reaching a level of significance at a dose level of 1-2 ng/kg. These findings support the application of the human endotoxemia model in future clinical pharmacology studies.

Table 1. Demographic characteristics.

	LPS			Placebo
	0.5 ng/kg (N=6)	1 ng/kg (N=6)	2 ng/kg (N=6)	(N=6)
Age (yrs)	23 (4)	22 (2)	24 (3)	22 (2)
Weight (kg)	72.6 (6.8)	67.1 (10.1)	73.0 (8.5)	74.3 (9.3)
BMI (kg/m2)	22.1 (1.3)	20.7 (1.5)	22.2 (2.2)	21.4 (2.4)

BMI = body mass index; SD = standard deviation; N=number of subjects in treatment group;
*Placebo subjects were pooled for the three different cohorts.

Table 2. Group contrasts. Data are presented as estimated treatment effect (with 95% confidence interval) for 0.5, 1 and 2 ng/kg LPS versus placebo (pooled for the three different cohorts) from baseline up to 24 hours post-dose.

	0.5 ng/kg LPS vs placebo	1 ng/kg LPS vs placebo	2 ng/kg LPS vs placebo
ENDOTHELIAL ACTIVATION MARKERS			
Thrombomodulin	-6 (-15 - 5)% p=0.3	8 (-4 – 21)% p=0.2	16 (5 - 29)% p=0.008
ICAM1	13 (3 – 23)% p=0.01	30 (19 – 42)% p<.0001	42 (30 - 55)% p=<.0001
ICAM3	-10 (-23 - 4)% p=0.1	6 (-9 - 23)% p=0.4	10 (-8 - 32)% p=0.3
VCAM1	8 (-3 – 20)% p=0.1	20 (9 – 32)% p=0.001	25 (13 - 38)% p=0.0001
E-Selectin	35 (2 – 79)% p=0.03	110 (56 – 182)% p<.0001	280 (186 - 404)% p=<.0001
P-Selectin	-5 (-26 – 21)% p=0.7	17 (-10 - 51)% p=0.2	42 (11 - 82)% p=0.009
RENAL FUNCTION			
Urinary mass	-28 (-64 - 47)% p=0.3	-10 (-53 - 72)% p=0.7	-47 (-73 - 3)% p=0.06
Serum creatinine	7 (-0.2 - 16)% p=0.06	-3 (-9 – 4)% p=0.4	6 (-1 -13)% p=0.1
eGFR (MDRD)	-9 (-18 - -0.5) p=0.04	3 (-6 - 11) p=0.5	-7 (-15 - 1.6) p=0.1
KIDNEY INJURY MARKERS			
Urinary B2MG	30 (-12 - 91)% p=0.2	19 (-19 – 74)% p=0.4	88 (27 - 178)% p=0.003
B2MG/Creat	-4.6 (-26 – 23)% p=0.7	20 (-6 – 53)% p=0.12	31 (3-67)% p=0.03
Urinary KIM1	32 (-25 - 132)% p=0.3	9 (-34 – 79)% p=0.7	121 (38 - 256)% P=0.003
KIM1/Creat	12 (-15 - 47)% p=0.4	15 (-10 - 48)% p=0.2	44 (13 – 83)% p=0.006
Urinary NAG	-18 (-54 - 47)% p=0.5	69 (-8 - 210)% p=0.08	51 (-14 - 167)% p=0.1
NAG/Creat	-19 (-49 - 28)% p=0.3	65 (0.9 - 171)% p=0.05	11 (-31 - 80)% p=0.7
Urinary Cystatin C	--	--	20 (-57 – 231)% p=0.6
Cystatin/Creat			0 (-44 - 79)% p=0.99
Urinary OPN	--	--	85 (3 - 235)% p=0.04
OPN/Creat			23 (-39 - 145)% p=0.5
Urinary UMOD	--	--	36 (-4 - 94)% p=0.08
UMOD/Creat			-9.8 (-42 - 40)% p=0.6
Urinary EGF	--	--	44 (-4 – 116)% p=0.07
EGF/Creat			-4 (-37 - 47)% p=0.83

ICAM1 indicates intercellular adhesion molecule 1; ICAM3, intercellular adhesion molecule 3; VCAM1, vascular cell adhesion molecule 1; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; B2MG, beta2-microglobulin; KIM1, kidney injury molecule 1; NAG, N-acetyl-β-D-glucosaminidase; OPN, osteopontin; UMOD, uromodulin and EGF, epidermal growth factor.

Figure 1. Serum concentrations ± SD (error bars) of endothelial activation markers: ICAM1 (A), VCAM1 (B), E-Selectin (C), P-Selectin (D), and TM (E). LPS administration was at T=0.
For readability, only the standard deviations of the two extremes are shown.

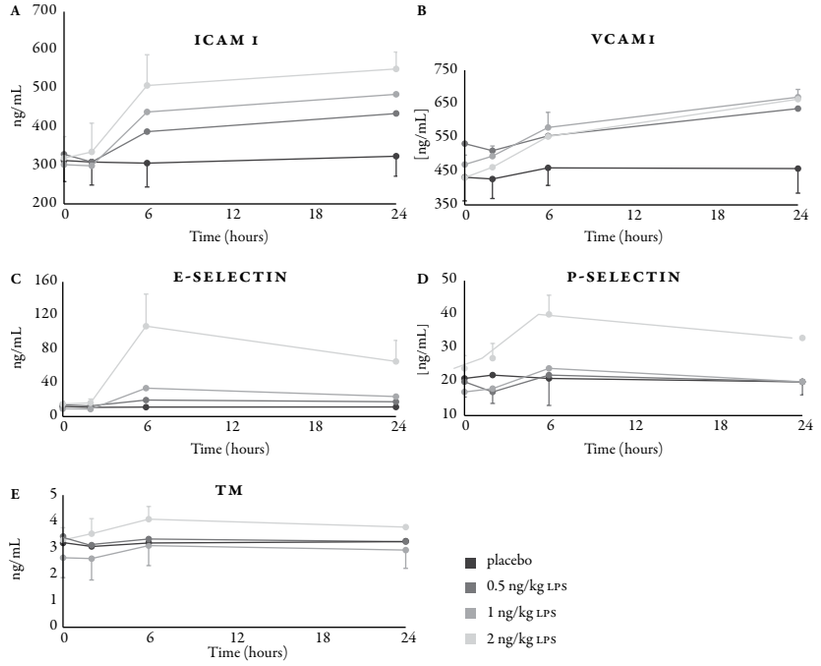


Figure 2. Absolute urinary mass ± SD (error bars) in grams of collected urine excreted per hour (A), and serum concentrations of renal function measurements serum creatinine (µmol/L) (B) and estimated glomerular filtration rate ± SD (error bars) according to the MDRD formula (mL/min/1.73m²) (C). LPS administration was at T=0. For readability, only the standard deviations of the placebo and 2 ng/kg LPS group are shown in panels B and C.

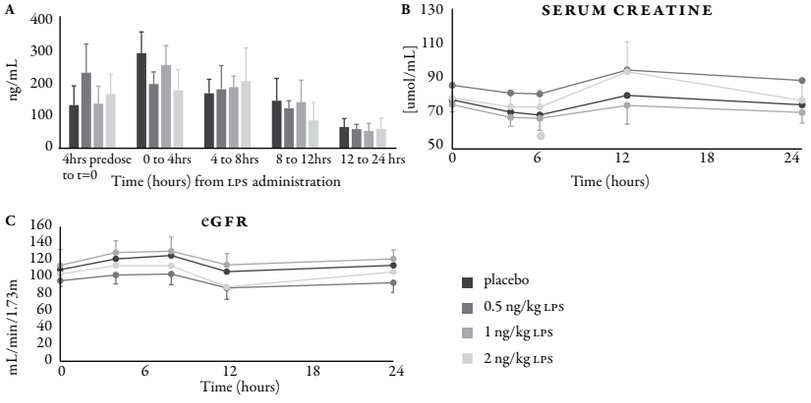
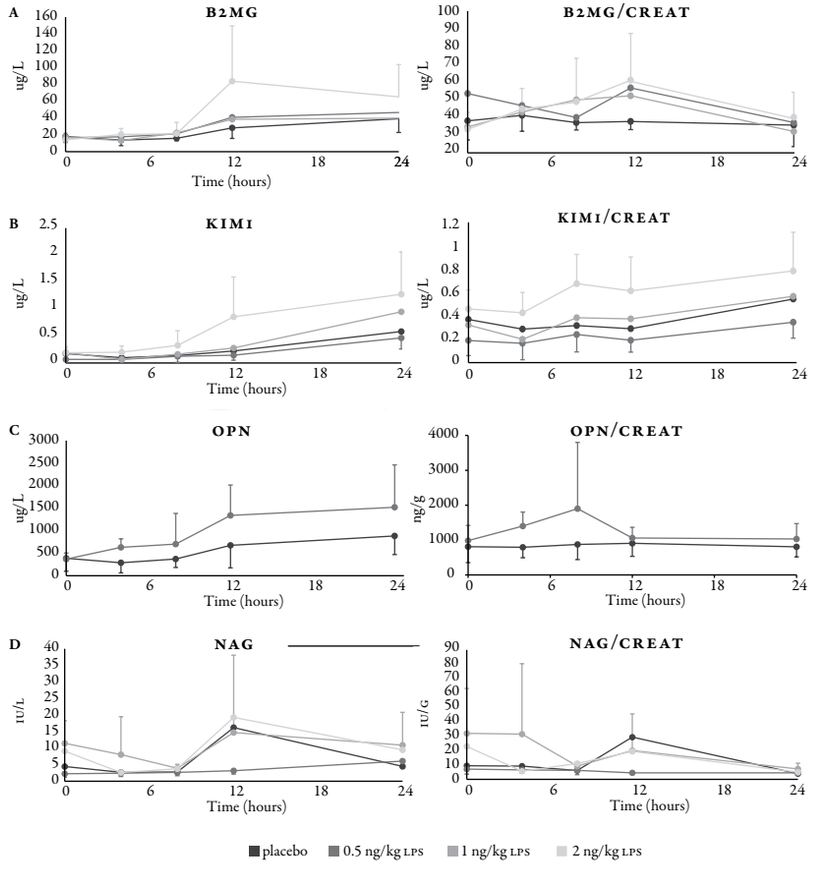
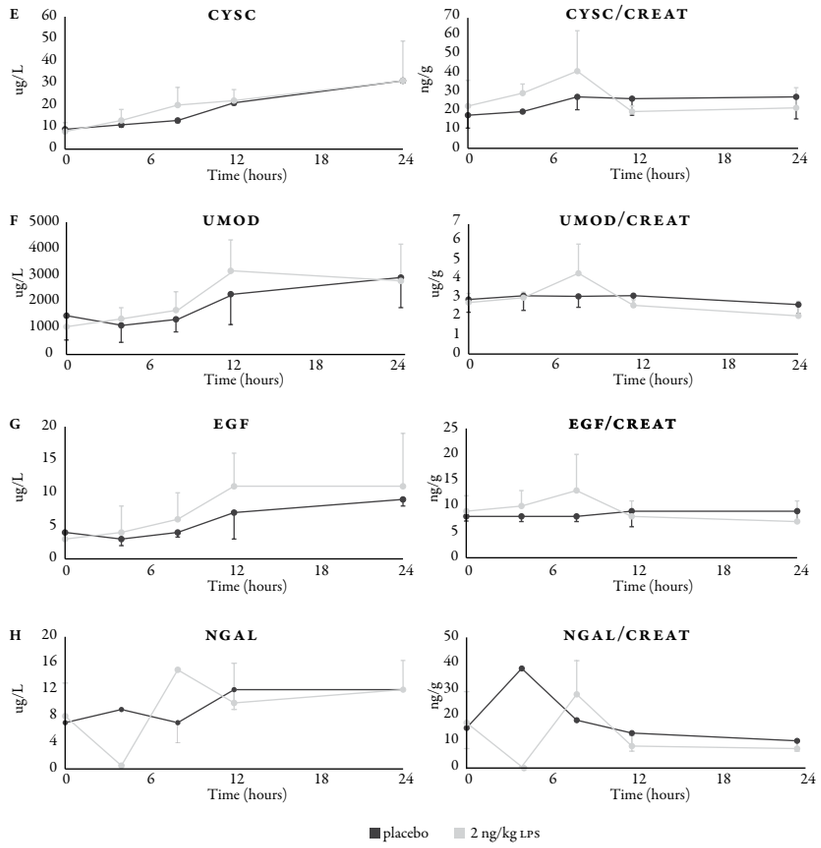


Figure 3. Urinary concentration of renal injury markers B2MG (A), KIM1 (B), OPN (C), NAG (D), CysC (E), UMOD (F), EGF (G) and NGAL (H), in absolute values (left panels), and corrected for urinary creatinine concentration (right panels) ± SD (error bars).



LPS administration was at T=0. For readability, only the standard deviations of the two extremes are shown.

Fig. 3. (continued)



LPS administration was at T=0. For readability, only the standard deviations of the two extremes are shown.

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