Consequences of lipopolysaccharide and n-3 polyunsaturated fatty acid administration on aortic function of spontaneously hypertensive rats

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Abstract. The aim of the work was to study the delayed effect of lipopolysaccharide (LPS) administration on endothelial function of the aorta of rats with genetic hypertension. Further, the possibility to ameliorate LPS-induced changes by n-3 polyunsaturated fatty acids (n-3 PUFA) was tested. Rats received a bolus of 1 mg/kg LPS i.p.; n-3 PUFA were administered in the dose of 30 mg/kg daily for 10 days p.o.. Ten days after receiving of LPS, the body weight gain of rats was statistically lower compared to control rats (p < 0.05). n-3 PUFA administration to LPS rats had no effect on this parameter. The TBARS and NAGA concentrations in plasma were significantly increased in the LPS group (p < 0.05) and n-3 PUFA administration returned them to control values. In functional studies, phenylephrine (PE, 1 µmol/l) evoked contraction of aortas which was not statistically different among experimental groups. However, endothelium-dependent relaxation was depressed in the LPS group (p < 0.05) and n-3 PUFA slightly recovered it to control values. In conclusion, oxidative stress seems to be responsible for aortic endothelial dysfunction detected 10 days after administration of LPS to rats. n-3 PUFA slightly improved the function of the endothelium injured by LPS, probably thanks to their antioxidant properties. Prolonged administration of higher doses of n-3 PUFA should defend the vascular endothelium against detrimental effect of bacterial inflammation.

Key words: Lipopolysaccharide — Omega-3 fatty acids — Spontaneously hypertensive rats — Aorta

Abbreviations: CRP, C-reactive protein; DHA, docohexaenoic acid; eNOS, endothelial NO-synthase 2; EPA, eicosapentaenoic acid; HTG, hypertriglyceridemic; IL1β, interleukin 1β; iNOS, inducible NO-synthase 2; LPS, lipopolysaccharide; n-3 PUFA, omega-3 polyunsaturated fatty acids; NAGA, N-acetyl-β-D-glucosaminidase; PE, phenylephrine; PSS, physiological salt solution; SHR, spontaneously hypertensive rat; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor α.
doses are associated with low-grade and chronic non-
resolving inflammation. Elevated circulating endotoxin
may program host leukocytes into a low-grade “memory”
state and contribute to the pathogenesis of diverse diseases
including atherosclerosis, diabetes, Parkinson’s disease, etc.
(Manco et al. 2010; Mehta et al. 2010; Wiesner et al. 2010;
Morris et al. 2015).

Recent evidence indicates that the endothelium does not
play a passive role in systemic inflammatory states. Endothelial
cells produce an abundance of inflammatory mediators and
elements of the immune and coagulation systems in the host
response to inflammatory stimulation. The barrier function
of the endothelium is impaired by endotoxins and is likely to
contribute to adverse outcomes. A number of authors have
recently developed a concept of the aberrant and dysfunctional
endothelial barrier as the central pathophysiological process
in LPS-induced inflammation and septic shock (Schouten et
al. 2008; Opal and van der Poll 2015).

Endothelial dysfunction is now well established as a pi-
voval early event in the development of major cardiovascular
diseases including hypertension, atherosclerosis and diabe-
tes. Pre-clinical studies have indicated that polyphe-nol-rich
food and food-derived products, e.g. grape-derived products
and omega-3 fatty acids, can trigger pathways in endothe-
"ials promoting an increased formation of NO and
endothelium-dependent hyperpolarization. Moreover, in-
take of such food-derived products has been associated with
the prevention and/or the improvement of an established
endothelial dysfunction in several experimental models of
cardiovascular diseases and in humans with cardiovascular
diseases (Auger et al. 2016).

The lipid A component of LPS in picomolar concen-
trations is sufficient to cause endothelial cell injury by
promoting the expression of tissue factor and proinflam-
matory cytokines like tumor necrosis factor a (TNF-α) and
interleukin 1β (IL1β) (Miller et al. 2005), leading to apop-
tosis of endothelial cells (Bannerman et al. 2002). Different
bacterial infections accompany us from birth to old age.
Presumably, each inflammation might affect the function of
the endothelial membrane and consequently lead to injury
of vessels and organs they supply. Regular consumption of
compounds with the protective membrane effects could be
beneficial for normal function of vessels.

Polyunsaturated fatty acids are natural constituents of the
diet, with a wide spectrum of physiological effects (Komatsu
et al. 2003; Calder 2012). Their anti-inflammatory properties
were documented experimentally and confirmed by clinical
trials. In cultured macrophages polyunsaturated fatty acids
decreased the expression of 10 genes related to inflammation
(Allam-Ndoul et al. 2016). Clinical trials on fish oil in pa-
"ients with rheumatoid arthritis (Kremer et al. 1985) showed
a significant anti-inflammatory activity of the combination of
eicosapentaenoic (EPA) and docosahexaenoic acid (DHA).

Various studies confirmed that chronic consumption of fish oil
or omega-3 polyunsaturated fatty acids (n-3 PUFA) reduced
atherosclerosis (Shim et al. 2016) and ameliorated heart failure
(Chrysohoou et al. 2016). n-3 PUFA protect the function and
integrity of the endothelium against injury, preventing struc-
tural remodeling of the vascular wall. Our previous studies
showed n-3 PUFA diet-induced modulation of Cx43 expres-
sion in the aorta and heart of hypertriglyceremic (HTG)
and hypertensive rats (Mitasikova et al. 2008; Dlugosova et
al. 2009a, 2009b), as well as modulation of endothelial Cx40
expression in the aorta of Wistar rats injected with LPS (Frim-
mel et al. 2014), supporting the involvement of intercellular
communication in protective effects of n-3 PUFA.

In essential hypertension, in animals as well as in humans,
chronic vascular and immune dysfunctions are closely as-
associated. In spontaneously hypertensive rats (SHR), vari-
ous vascular alterations have been reported, e.g. increased
activity of the sympathetic system, endothelial dysfunction,
arterial compliance decrease, and medial hypertrophy
(Folkow 1982; Chobanian 1990). SHR also exhibit immune
abnormalities with defective leukocyte-endothelial cell
interactions, depressed T lymphocyte functions, decreased
delayed-type hypersensitivity (Dzielak 1990, 1992), and
chronic inflammatory process in the cardiovascular system
(Hinglais et al. 1994). All these observations suggest direct
interaction of the cardiovascular and immune systems in
SHR. Thus, the response of the rat organism to LPS admin-
istration can be influenced by hypertension.

The aim of the work was to study the effect of LPS on
endothelial function of the aorta of rats with genetic hyper-
tension (SHR) 10 days after LPS administration. Further,
the possibility to ameliorate LPS-induced changes by n-3
PUFA was tested.

Materials and Methods

The investigation conformed to the Guide for the Care and
Use of Laboratory Animals. The experiments were approved
by the State Veterinary and Food Administration of the
Slovak Republic.

Experiments were done on 3-month-old male SHR
(Breeding station Dobrá Voda, Slovakia), weighing 220.4
± 5.4 g. They were housed in a room with air temperature
of 22–24°C, 45–60% humidity and regular light control – 12
hours light and 12 hours dark. They were given standard
rodent chow and water ad libitum. All procedures were done
at the same time during the light phase of the L/D cycle. The
acclimatization period lasted 10 days.

The rats were randomly assigned to three experimental
groups (each of 8 animals): C, control group without any
treatment; LPS, rats received bolus of 1 mg/kg LPS i.p.;
LPS+n-3 PUFA, LPS rats treated with n-3 PUFA in the dose
of 30 mg/kg daily for 10 days p.o. by gavage. The chosen dose was based on our previous studies (Dlugosova et al. 2009b). Administration of n-3 PUFA (57% EPA and 43% DHA – commercial nutritional supplement MaxiCor, SVUS Pharma, Czech Republic) was started on the same day as that of LPS. Treatment of healthy animals with n-3 PUFA had no effect on the parameters measured and are thus not presented in this work. LPS (Escherichia coli serotype 055:BS, Sigma Chemical, Germany) was dissolved in sterile 0.9% NaCl solution. The rats from the control group were injected with the same volume of sterile 0.9% NaCl solution.

The animals were weighed at the beginning and end of the experiment, systolic blood pressure was measured at the same period by non-invasive plethysmographic method on the rat tails. After 10 days of the experiment, the rats were anesthetized with thiopental (50 mg/kg i.p.) and killed by heart excision. Blood was used for evaluation of markers of oxidative stress and the thoracic aorta was removed. Markers of oxidative stress – concentrations of thiobarbituric acid reactive substances (TBARS) – were determined in plasma according to Esterbauer (1993). The plasma specific activity of lysosomal N-acetyl-β-D-glucosaminidase (NAGA), a marker of cellular injury, was assayed according to standard methods as described previously in Navarova and Nosalova (1994).

**Isolated rat aorta**

The thoracic aorta was excised and transferred into oxygenated physiological salt solution (PSS). The arteries were cleaned of adherent tissue and cut into rings, approx. 2–3 mm long. Special care was taken not to damage the endothelium. The rings were mounted between two hooks in water-jacketed (37°C) chambers containing PSS bubbled with a mixture of 95% O₂ and 5% CO₂ at pH 7.4. The composition of PSS was (in mmol/l): NaCl (118.0), KCl (4.7), KH₂PO₄ (1.2), MgSO₄ (1.2), CaCl₂ (2.5), NaHCO₃ (25.0), and glucose (11.0). The preparations were connected to an isometric force transducer (Experimetria Hungary), stretched passively to 10 mN and equilibrated for 60 minutes.

**Experimental protocol:** After the equilibration period, contraction was induced by submaximal concentration of phenylephrine (PE, 10⁻⁶ mol/l). At the plateau of the contraction, the effect of acetylcholine in the cumulative concentrations of 10⁻⁸ – 10⁻⁵ mol/l was tested as a measure of endothelial function. After washing with PSS and reaching the initial tension value, concentration-response curves of sodium nitroprusside (10⁻¹⁰ – 10⁻⁷ mol/l) were performed in PE-precontracted preparations.

**Statistical analysis**

Data are expressed as mean ± SEM. The mechanical responses are expressed as percentages of the PE-induced contraction. Statistical analysis was performed by using ANOVA with Bonferroni posttest. Statistical significance was indicated at p < 0.05. The pD₂ values (–log of concentration inducing 50% of maximal response) were calculated by Graph Pad.

**Results**

Ten days after a non-lethal dose of LPS (1 mg/kg b.w.), we evaluated the physiological state of the rats. As seen in Fig. 1, body weight gain of LPS rats was statistically lower compared to controls. n-3 PUFA administration to LPS rats had no effect on this parameter. Blood pressure changes were not found among the experimental groups. The final systolic blood pressure of each group did not increase significantly in comparison to starting values (Fig. 2). The TBARS concentration in plasma was significantly (p < 0.05) increased in the LPS group (4.06 ± 0.36 µg/mg prot.) and n-3 PUFA administration returned it to the control values (LPS+n-3 PUFA: 3.49 ± 0.18 µg/mg prot., C: 3.18 ± 0.07 µg/mg prot.). Similarly, plasma specific NAGA activity was augmented in the LPS group (from 0.14 ± 0.01 to 0.18 ± 0.02 µg 4-NP/min/mg prot., p < 0.05) and ameliorated in the LPS+n-3 PUFA group (0.13 ± 0.02 µg 4-NP/min/mg prot.) (Fig. 3).

The LPS dose used increases levels of C-reactive protein (CRP) which justify the presence of inflammation (Frimmel et al. 2014).

In functional studies, PE in the concentration of 1 µmol/l evoked contraction which was not statistically different among the experimental groups (not shown). In the control group, acetylcholine induced endothelium-dependent relaxation with the pD₂ value of 7.78 ± 0.11 and the maximal relaxation of 25.61 ± 4.78% of PE-induced contraction. However, responses of LPS aortas were attenuated (pD₂ 7.39 ± 0.25, maximal response 49.33 ± 8.43% of PE-induced contraction) in the LPS+n-3 PUFA group vs. control (LPS vs. C). Data are means ± SEM (standard error of the mean) from 8 animals. * p < 0.05 vs. C.
contraction). n-3 PUFA administered to LPS animals slightly shifted endothelium-dependent relaxation to that of controls – pD2 value 7.72 ± 0.20, maximal relaxation 41.97 ± 7.31 (Fig. 4). No differences between groups were found in the response to sodium nitroprusside (not shown).

Discussion

Single LPS administration caused loss of body weight in SHR rats. Likewise, reduced body weight gain was found in HTG rats using the same LPS experimental model (Frimmel et al. 2016). Our data correspond with the study of Valles et al. (2000) who demonstrated that even a single exposure to stressors, including LPS, can cause long-lasting reduced food intake and affect consequently the body weight, which is mediated by different metabolic or neural mechanisms (Liu et al. 2016). On the other hand, we did not find changes in systolic blood pressure after LPS administration to SHR. We speculate that this might be due to dose- and route-dependent application of LPS, duration of its influence, as well as higher resistance of SHR to LPS (Bernard et al. 1998).

It is known that administration of LPS or cytokines (tumor necrosis factor or interleukin-1) to animals produces a shock-like syndrome, characterized by low blood pressure and hyporeactivity to vasoconstrictor agents (Fink and Heard 1990; Bone 1991). This response is rapid and it was confirmed also in experiment by Vo et al. (2005). These authors showed in Wistar rats that acute application of LPS to precontracted aortic rings induced endothelium-dependent relaxation which reached maximum in up to 5 hours. Moreover, isolated blood vessels of Wistar rats exposed to LPS or removed from LPS-treated animals had diminished responses to vasoconstrictor agents (Joly et al. 1994; Wu et al. 1995). Effects of LPS on the vascular system, however, seem to be dose-, and time-dependent. In our experiments, we administered a nonlethal dose (bolus of 1 mg/kg i.p.) of LPS to SHR rats and observed its effect after 10 days. As expected, the results are impacted by this protocol. Contractions induced by phenylephrine were not influenced probably as a consequence of subsided acute response. The hypotensive effect induced by the toxin seems to be dependent also on the rat strain. The SHR should be more resistant to the hypotensive effect of toxins. However, controversial results were obtained with this model. While Bernard et al. (1998) found that SHR had a greater ability to resist endotoxin shock than normotensive controls, on the other hand, Yen et al. (1997) observed a shorter survival time in SHR after LPS injection in comparison with normotensive rats.

Whereas LPS-induced vascular hyporesponsiveness to constrictor agents may be due to excessive generation of NO by inducible NO-synthase2 (iNOS) (Vo et al. 2005), the mechanism underlying LPS-induced endothelial dysfun-
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...is still not clear and seems to be dependent also on animal species and vessels used. The endothelium-dependent vasodilatation of the thoracic aorta of Wistar rats was found to be significantly inhibited six hours after 10 mg/kg LPS administration (Uğurel et al. 2016). The endothelial injury was accompanied by decreased eNOS and phospho-eNOS expression. Thus changes of the ratio in eNOS/iNOS production seem to be accountable for vascular response in the acute phase of LPS administration. The most important result of our experiments is that even 10 days after LPS administration changes in endothelial function were demonstrated by reduced endothelium-dependent relaxation. At the same time, we also observed enhanced activity of the lysosomal enzyme NAGA in the LPS group compared to controls. Increased plasma and organ activity of the lysosomal enzyme NAGA indicates cell injury ongoing in the organism during pathological processes. This was found in different pathological experimental models (Nosáľová et al. 2010) as well as in patients with hypertension (Lisowska-Myjak et al. 2011) and with type 1 diabetes mellitus (Mandic and Filipovic 1998). Therefore, augmented NAGA activity in our study indicates cell injury remaining 10 days after LPS administration. Moreover, increased plasma levels of TBARS in animals injected with LPS suggest the presence of oxidative stress in these rats. Oxidative stress is involved in atherogenesis, which is initiated by endothelial injury in cases with cardiovascular risk factors, including diabetes mellitus, hypertension, cigarette smoking, dyslipidemia, obesity, and metabolic syndrome (Husain et al. 2015; Salmanoglu et al. 2016). Participation of oxidative stress in induction of endothelial dysfunction was confirmed by the beneficial action of antioxidants which were able to suppress the detrimental effect of reactive oxygen species (Sotníková et al. 2008, 2011). Results of Requintina and Oxengruk (2003) indicated that the effect of LPS on lipid peroxidation is dose-, time-, and species-dependent. Using the identical experimental model, we recently demonstrated in Wistar rats LPS-induced impairment of endothelium-dependent relaxation of the aorta (Frimmel et al. 2014) accompanied with increased values of NAGA activity and levels of TBARS. On the other hand, Wu et al. (1995) observed significantly impaired acetylcholine-induced relaxation in thoracic aortic rings obtained from Wistar Kyoto rats treated with LPS, but not in those from SHR. As these authors studied effects of 5 mg/kg i.v. LPS during 5 hours, we suppose that this discrepancy may be explained by different experimental protocols used.

In order to confirm endothelial damage, we studied the effect of sodium nitroprusside on the aorta to identify the effect of LPS on endothelium-independent relaxation. No differences among experimental groups were found. Our previous studies, using the same experimental LPS model, also demonstrated that LPS did not affect endothelium-independent relaxation of the aorta in Wistar and hHTG rats (Frimmel et al. 2016). The data indicate changes in endothelial function 10 days after LPS injection.

The importance of n-3 PUFA for the cardiovascular system has come under the spotlight during the last decades. Data from clinical (Colussi et al. 2016) and experimental studies (Mitiková et al. 2008) support the hypothesis that consumption of n-3 PUFA lowers the risk of cardiovascular diseases. The n-3 index – a percentage of EPA + DHA of total fatty acids in red blood cells (von Schacky and Harris 2007) – may be a marker of increased cardiovascular risk. Our results showed that n-3 PUFA (30 mg/kg b.w.) administered for 10 days were able to suppress oxidative stress and tissue injury in the LPS group of rats. The influence of n-3 PUFA on the endothelium was not so evident, although endothelium-dependent relaxation, injured by LPS, was slightly improved. Shim et al. (2016) evoked endothelial injury in the atherosclerosis-induced erectile dysfunction rat model. They found that omega-3 fatty acids in high doses and administered over 4 weeks were able to significantly improve intracavernosal pressure and had a beneficial role against pathophysiological consequences, such as fibrosis or hypoxic damage, in this experimental model. Further, supplementation of 4 g/day omega-3 fatty acids to healthy volunteers for 4 weeks significantly decreased postprandial triglyceride elevation and postprandial endothelial dysfunction (Miyoshi et al. 2014). We thus suggest that n-3 PUFA could have a protective effect on endothelial dysfunction induced by LPS when administered over a longer time in higher doses.

In conclusion, oxidative stress seems to be responsible for aortic endothelial dysfunction detected 10 days after LPS administration.
administration of LPS to rats. In the dose of 30 mg/kg daily, n-3 PUFA slightly improved the function of the endothelium injured by LPS, probably thanks to their antioxidant properties. Prolonged administration of higher doses of n-3 PUFA should defend the vascular endothelium against detrimental effect of bacterial inflammation.

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