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LPS-induced endotoxic shock does not cause early brain edema formation – An MRI study in rats

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Abstract. *Objective and design:* Early microcirculatory failure is assumed as a key factor in the development of a septic encephalopathy. However, brain edema is also a common finding in sepsis syndromes possibly interfering with the vasoregulative mechanisms of the brain. We assessed the occurrence of brain edema in a rat model of endotoxic shock.
Material and subjects: Eleven mechanically ventilated male CD-rats.
Treatment: Intravenous application of 5 mg/kg LPS (n = 8) or vehicle (n = 3).
Methods: Apparent diffusion coefficient (ADC) and T2-relaxation time (T2RT) were quantified on cerebral MRI at baseline and repeatedly for up to 3.5 h after LPS-injection. Change in blood pressure was compensated with norepinephrine. Brain water content was quantified using the wet/dry method.
Results: All LPS-treated rats developed endotoxic shock. No significant difference in T2RT or ADC was detectable before and after LPS-injection (T2RT: baseline 60.33 ± 1.21; after 3.5 h 60.15 ± 0.59; ADC: baseline 6.86 ± 0.51; after 3.5 h 6.75 ± 0.33). Post-mortem analysis did not indicate a difference in brain water content between septic and non-septic animals.
Conclusions: Reports of early microcirculatory failure seem not to be related to the occurrence of early (≤3.5 h) brain edema.

Key words: Shock – Sepsis – Brain edema – MRI – Rat – Lipopolysaccharide

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Introduction

Systemic sepsis, endotoxic shock and severe inflammatory response syndrome (SIRS) are the commonest causes of death in intensive care units [1–3]. Modern concepts assume microcirculatory failure as an important factor for the occurrence of early organ dysfunction [4, 5]. In a rat model of LPS-mediated shock early microcirculatory failure was demonstrated for the neurovascular coupling mechanism. Microcirculatory failure preceded changes in evoked potential responses indicating inadequate perfusion of active neurons [7, 8]. Microcirculatory failure occurred 60 min after application of 5 mg/kg b.w. LPS intravenously, whereas evoked potentials significantly decreased 180 min after LPS application. Cerebral edema is a frequent finding in sepsis syndromes. Vasogenic edema is caused by a blood brain barrier breakdown, whereas cytotoxic edema is caused by energy depletion of neurons due to microcirculatory failure [9–13]. One of the first signs are increased pinocytosis of endothelial cells, perimicrovascular edema with swelling and rupturing of the endfeet of surrounding astrocytes [14, 15]. Besides neurons the endothelial cells and astrocytes are relevant structures of the vasoregulative principles of the brain [8, 16].

The MRI-technique is a powerful and sensitive in-vivo technique, which allows sequential investigation of changes in the water content of the brain. The apparent diffusion coefficient (ADC) of water is sensitive to the cytotoxic edema [17], whereas elevated values of T2, the nuclear MR transverse relaxation time of water, detect vasogenic edema [18–29]. To assess the occurrence and time course of the different forms of brain edema and its relation to the previously reported early microcirculatory failure we used the MRT in the same rat model of endotoxic shock. The data were compared to the traditional method of brain edema determination by the wet/dry weight relation from brains at the end of experiments.

Materials and methods

Animal preparation

All procedures performed on the animals were in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and aproved by the local Animal Care and Use Committee.

Eleven male CD-rats weighing 298 to 307 g were initially anesthetized with 5 % isoflurane delivered in a 7:3 N_2O/O_2 mixture of gases. Anaesthesia was maintained with 2–3 % isoflurane. PE-50 catheters were inserted into the right and left femoral artery for monitoring of blood pressure and for obtaining blood samples to measure pH, $PaCO_2$ and PaO_2 (blood gas analyzer Rapidlab 348; Bayer Vital GmbH, Fernwald, Germany). Another PE-50 polyethylene tubing was inserted intravenously for LPS application. The animals were tracheotomized, paralyzed with pancuronium bromide ($0.2\,mg*kg^{-1}*h^{-1}$) and artificially ventilated (Harvard Rodent Ventilator; Harvard, South Natick, Mass., USA). The inhalative anaesthesia was switched to α-Chloralose (80 mg/kg; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) to exclude a possible interference of isoflurane with the endothelial nitric oxide system. Supplementary doses of chloralose (30 mg/kg) were given every hour. During surgical procedure, body temperature was continuously monitored with a rectal probe and maintained at 37.0 °C with a thermostatically regulated water flow system.

MR-Imaging

The head of the animals was fixed in a body restrainer with tooth-bar and a cone shaped head holder and rats were placed in a MRI spectrometer. Ventilation was undertaken with a two-way airway system (inspiration air flow separated from exspiration airways) with the valve being close to the trachea. Temperature was monitored using a rectal probe and maintained 37 °C by a thermostatically regulated water flow system during the entire imaging protocol. Mean arterial blood pressure was controlled and norepinephrinhydrochloride (Arterenol, Aventis Pharma GmbH, Germany) was injected intravenously if necessary to keep mean arterial blood pressure levels between 80 and 100 mmHg.

The restrainer was then placed into a custom-designed linear polarized volume resonater (diameter 60 mm) and tuned and matched manually.

MRI imaging was performed using a Bruker PharmaScan spectrometer (70/16, 7.0 T, 16 cm; Bruker BioSpin, Ettlingen, Germany) that operates at 300,51 MHz for the ^1H and is equipped with a 300 mT/m self shielding gradient system. Localizer images were acquired by a spin echo sequence (TR= 150 ms, TE= 12 ms, slice thickness = 4 mm) with three orthogonal slices – axial, coronal and sagittal - for positioning. Fast RARE sequences (20 contiguous slices, 1 mm thickness, TR = 2500 ms, TE = 41.8 ms) were used to verify strictly symmetric positioning of the animals and were repeated after correction of the animals position, if necessary.

To map the apparent diffusion coefficient of water (ADC), diffusion weighted images were acquired with the use of a fat suppressing EPI-sequence. Six contiguous, coronal slices with a thickness of 2 mm were collected with a field of view of 32 × 32 mm and a matrix size of 128 × 128 (TR = 3003 ms, TE = 38,6 ms, number of averages = 4). A 4-fold segmentation was used to reduce image artifacts caused by local field inhomogeneity. Five sets of coronal images were recorded for quantitative determination of ADC, with equidistant diffusion gradient values of 10, 40, 70, 100 and 130 mT/m. With a diffusion gradient duration (δ) of 9 ms and a gradient separation time (Δ) of 15 ms, this results in five b-Values of 6.96, 111.3, 340.8, 695.6 and 1175.5 s/mm^2. The acquisition time for each EPI sequence was 4.5 minutes.

To optimize image quality of the diffusion-weighted EPI images, a volume shim with a volume selective double spin echo sequence (TR = 1 s, TE = 30 ms, voxel-size 10 × 8 × 15 mm^3) was performed before the acquisition of the first diffusion series. The achieved full width at half maximum (FWHM) of the water line was about 25 to 35Hz.

The ADC-maps were calculated by a least squares fit with use of the Image Processing Tool of the Paravision 3.02 software (Bruker, Ettlingen, Germany)

T2-relaxation-time was mapped using a CPMG (Carr Purcell Meiboom Gill) spin echo sequence. 16 slices with a thickness of 2 mm were acquired with a field of view of 37 × 37 mm and a matrix size of 512 × 256 (TR = 3833.5 ms, 90° excitation and 180° rephasing pulses, NEX = 1). Twelve echoes were collected, starting with TE = 18 ms (step size ΔTE = 18 ms), resulting in a range from 18 to 216 ms. Acquisition time for each CPMG sequence was 16 minutes and 21 seconds.

The T2-maps were calculated by a least squares fit with use of the Image Processing Tool of the Paravision 3.02 software (Bruker, Ettlingen, Germany)

Study protocol

After baseline ADC- (two times) and T2-imaging, n = 8 rats received 5 mg/kg b.w. LPS (LPS from Escherichia coli, O111:B4; Sigma-Aldrich Chemie GmbH) or n = 3 vehicle (1 ml 0.9 % NaCl) intravenously within 3 minutes. At regular intervals pH, $PaCO_2$ and PaO_2 were measured. After sepsis induction the first images were performed approximately 18 min later when blood pressure levels were at a minimum. Diffusion weighted imaging followed by a T2-imaging was performed every 30 minutes up to 228 min after LPS administration. Then the animals were removed from the scanner and the last blood samples were obtained for measuring lactate. Finally, the animals were deeply anaesthetized using 5 % isoflurane (delivered in air at 5l/h for 5 minutes) and euthanized by decapitation.

Quantification of ADC and T2-relaxation-time

T2-relaxation-time and ADC were calculated from regions of interest (ROI) that were placed within the cortex (2 ROI´s) and the basal ganglia (1 ROI) on both hemispheres using Image Processing Tool of the Paravision 3.02 software (Bruker, Ettlingen, Germany) (figure 1). Mean T2-time- and ADC-Values were measured on all slices and averaged.

Post-mortem analysis

To analyze absolute brain water content (%H_2O), the wet weight of each hemishpere was measured separately. The tissue was then dried to a constant weight and weighed again. %H_2O was calculated using following equation:

$$\%\ H2O = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \cdot 100$$

Statistical procedures

Data are presented as mean ± standard deviation and were compared using a repeated measure analysis of variance (RMANOVA) with 2 inter-subject-factors (LPS vs. Placebo) and 9 within-subject levels according to the time-course of T2RT and ADC. A p-level <0.05 was considered statistically significant.

Results

Physiological parameters

Two animals died during the experiment, one immediately and the other one 2 hours after LPS injection.

In the LPS group mean arterial blood pressure dropped statistically significant from 120 ± 15 mmHg to 69 ± 24 mmHg

after LPS administration ($p < 0.001$) and was then stabilized within several minutes on levels around 100 mmHg using norepinephrine-infusion (range: 97 ± 23 to 112 ± 6 mmHg; p = n.s. as compared to baseline). The pH levels declined in the sepsis group from 7.40 ± 0.07 progressively to 7.29 ± 0.14 ($p < 0.001$) at the end of experiments. In the placebo group blood pressure (baseline: 120 ± 12 mmHg; end: 115 ± 16 mmHg) and pH (baseline: 7.36 ± 0.12; end: 7.37 ± 0.14) maintained stable over time.

At the end of the experiment lactate level was 2.04 ± 0.65 mmol/L in LPS-treated animals, whereas it was not measurable in the placebo group.

Quantification of ADC-Value and T2-relxation-time

As compared to baseline values, ADC and T2-relaxation-time in cortex and basal ganglia showed no significant differences in LPS-treated animals. No abnormal ADC- and T2-values were detectable in animals treated with saline. (Figure 1).

Post-mortem analysis

No significant differences in brain water content ($\%H_2O$) could be detected between both groups (LPS: right hemisphere 74 ± 3 % vs. left hemisphere 74 ± 2 %; placebo: right hemisphere 76 ± 5 % vs. left hemisphere 76 ± 4 %; p = n.s.).

Discussion

The present data point against a significant role of a brain edema to explain reports on an early uncoupling of the neurovascular coupling mechanism in the present endotoxic shock rat model [6]. Whereas uncoupling was found as early as 60 min after LPS application, brain edema were lacking up to 3.5 h after sepsis induction in the same animal model (male CD-rats, same strain and weight class) using the same LPS-charge in identical doses. The present in-vivo MRT-data are corroborated by the in-vitro quantification of the absolute brain water content at the end of the experiments. Comparisons between different sepsis models can not be easily undertaken. Occurrence of brain edema in a coecal ligature and puncture model was demonstrated to begin approximately 8 h after sepsis induction [11, 14]. An edema in cortical regions as well as brain stem nuclei was not found [14]. However, a recent study found edema in the frontal cortex but not cerebellum 3 h after i. p. LPS administration [15]. Since rats were housed in their cages for the 3 h a direct comparison cannot be driven to the present study in which rats were invasively monitored and treated. Perfusion deficits due to middle cerebral artery occlusion lead to more severe changes in the brain stem nuclei rather than cortex [21]. The brain stem is supplied by functional terminal arteries, whereas the blood supply to the cortex can be compensated from collateral pathways [22].

The absence of any brain edema in the present study demands careful consideration of the model and the adequacy of LPS dosage. Further studies with longer investigation times are merited to determine the beginning of brain edema. However, longer investigation times will be hampered by the high toxin dose used leading to progressive circulatory and pulmonary failure resulting in insufficient oxygen and substrate supply of organs after 4 h of sepsis induction (unpublished material). Methodical artifacts are unlikely since the techniques and procedures are highly standardised and have previously been used to determine subtle changes of the brain water content [21–24]. Although experiments show intraindividual variation concerning the time course and severity of a sepsis syndrome, the LPS model appears to be more reliable than a coecal ligature and puncture model. Narrow weight classes of rats, artificial ventilation, intensive monitoring as well as correction for blood gases, blood pressures

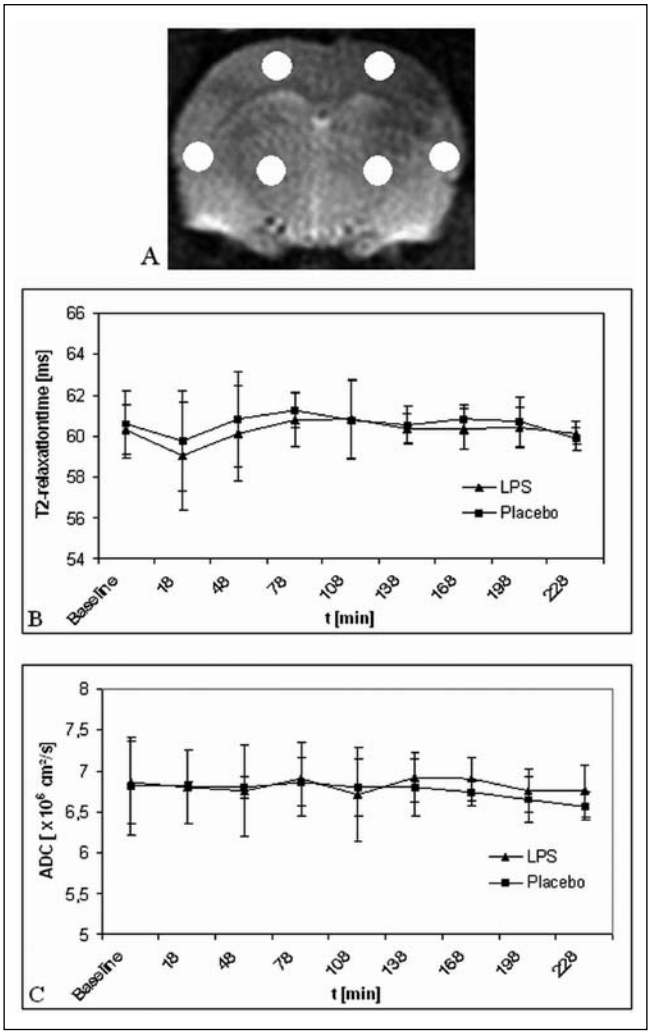

Fig. 1: A. Diffusion weighted imaging was used to quantify ADC in regions of interest (white circles). Positioning of the ROIs for T2RT-quantification was done likewise. **B.** Time-course of T2RT, a parameter that represents the amount of protons within tissue and thus correlates well with the brain water content. T2RT [ms] was averaged over all ROI´s on all slices. **C.** Time-course of ADC, a parameter that represents the amount of cytotoxic brain edema. ADC [$x10^6 cm^2/s$] was averaged over all ROI´s on all slices. In LPS-treated animals (triangles) T2RT as well as ADC did not change significantly as compared to baseline and was almost identical to the time-course of non-septic control animals (quadrangles). These findings indicate the absence of vasogenic and cytotoxic brain edema in the acute (≤3.5 h) phase of LPS-induced sepsis.

have further increased the standardization of experiments. The same toxin (charge) and toxin-dose was used in previous investigations and resulted in a reproducible endotoxic shock syndrome [6]. The initial drop in blood pressure is a typical effect due to intravenous LPS-administration. Special care was undertaken that the blood pressure levels did not drop below the lower limit of cerebral autoregulation which was determined between 50 and 60 mmHg mean arterial blood pressure in rats [25, 26]. Succeeding norepinephrine administration kept blood pressure levels within the physiologic range. Although we cannot exclude a protective effect of norepinephrine on the occurrence of a brain edema the administration resembles the situation in clinical conditions. However, in case of a blood-brain barrier breakdown, which is an initial finding of brain edema occurrence, norepinephrine would have resulted in a vasoconstriction [27]. Such an effect was also not seen in the T2-scans as compared to controls. Lactate levels increased and pH dropped in LPS treated animals as expected from previous reports.

The MRI-technique used in the present study is sensitive to subtle changes in cytotoxic and vasogenic brain edema. Cytotoxic edema is defined as a shift of extracellular volume into the intracellular compartment, caused by malfunctioning of energy-dependant Na-/K-transporter systems. This type of edema can be found under various conditions, i. e. in the hyperacute phase of cerebral ischemia and potentially also could be expected in the acute phase of LPS-induced sepsis. Diffusion weighted imaging allows mapping of the apparent diffusion coefficient ADC, an MRI-parameter that is sensitive for fluid shifts between the extra- and the intracellular space. Previous in-vivo studies indicated that a significant ADC-decrease can be detected within the first 5 minutes following occlusion of the middle cerebral artery as well as in animals suffering from experimental sinus thrombosis or subclinical brain edema caused by transcranial therapeutic ultrasound. Similar correlations were reported for excitotoxin-induced or osmotic-manipulated cytotoxic cell swelling and water ADC decrease. These findings illustrate the high sensitivity of this technique with respect to the detection of cytotoxic brain edema [21, 23, 24, 28, 30–33, 38].

Vasogenic brain edema, in contrast, is defined as a net water influx from the intravasal into the intercellular compartment. A breakdown of the blood-brain barrier thus is a prerequisite for this type of brain edema, which is likewise conceivable under conditions of LPS-induced sepsis. MR-imaging of the T2-relaxation time allows non-invasive quantification of the number of protons within a region of interest. This parameter has been shown to correlate closely with the amount of brain water content (assessed with the wet-dry-method) and with the amount of brain swelling. In-vivo imaging of the hyperacute phase of focal cerebral ischemia indicated a significant increase in T2RT as early as 25 minutes following stroke onset and thus demonstrates the sensitivity of this method for the detection of vasogenic brain edema [17, 20, 21, 33–38].

To estimate the sensitivity of our MRI-technique for the detection of subtle edema formation, we used data from a previous study in which we investigated potential side-effects of transcranial 20 kHz-ultrasound, a new therapeutic approach to improve arterial recanalization during thrombolysis in acute stroke [38]. In this study, healthy rats received ultrasound treatment for 20 minutes with 20-kHz Doppler. Ultrasound energy was varied between 0.0 and 1.1 W/cm^2. ADC decreased significantly at 0.5 and 1.1 W/cm^2, indicating cytotoxic edema. T2-RT increased significantly in the 0.5 and 1.1 W/cm^2 group, consistent with vasogenic edema. No changes were detectable in the low-power output group (0.2 W/cm^2). The sample size here was n = 6 per group [37]. These data were used to calculate power analyses to detect the effect sizes. Effect size was calculated for dependent variables, using the formula:

$$\varepsilon' = \frac{\mu 1 - \mu 2}{\sigma\sqrt{1-r}},$$

where $\mu 1 - \mu 2$ is the mean difference between time points, is their common standard error and r is the empirical correlation coefficient between the time points. Regarding the ADC decrease after 24 hours, as compared to baseline, we found an effect size of $\varepsilon' = 4.0$, and regarding the T2-RT increase, as compared to baseline, the effect size was $\varepsilon' = 3.7$. These effect sizes were attained in a small sample size of 6 rats. Considering in advance that in the current study effect sizes might be somewhat lower, we selected an even larger sample size in the present study.

Therefore, both sequences should have detected subtle edema formation in the present investigation. However, neither ADC- nor T2RT-quantification revealed any formation of cytotoxic or vasogenic brain edema within the first 3.5 h after induction of sepsis. These findings are corroborated by the direct quantification of brain water content, which likewise did not differ between LPS- and placebo-treated animals.

Summary

We assume that brain edema is not an early factor explaining the occurrence of the uncoupling and microcirculatory dysregulation of the neurovascular coupling mechanism in an endotoxic rat shock model. Neither the ADC coefficient nor T2-sequences were altered due to the induction of a sepsis syndrome. Special care has to be taken regarding blood pressure levels. The initial drop of mean arterial blood pressure levels to approximately 70 mmHg led to a shift in the ADC values, which was reversible after blood pressure resauration. Progressive failure of macrocirculation and insufficient blood supply to the brain might lead to energy depletion of neurons and resultant ADC changes. Our findings further underline the importance of early control of blood pressure in the occurrence of a sepsis syndrome.

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