Inhibition of NF-κB ameliorates sepsis-induced downregulation of aquaporin-2/V₂ receptor expression and acute renal failure in vivo

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Höcherl K, Schmidt C, Kurt B, Bucher M. Inhibition of NF-κB ameliorates sepsis-induced downregulation of aquaporin-2/V₂ receptor expression and acute renal failure in vivo. Am J Physiol Renal Physiol 298: F196–F204, 2010. First published October 14, 2009; doi:10.1152/ajprenal.90607.2008.—Acute renal failure (ARF) is frequently associated with polyuria and urine concentration defects and it is a severe complication of sepsis because it increases the mortality rate. Inhibition of NF-κB activation has been suggested to provide a useful strategy for the treatment of septic shock. However, the impact on sepsis-induced ARF is still unclear. Therefore, we examined the effect of pyrrolidine dithiocarbamate (PDTC) and of small interfering RNA (siRNA) silencing NF-κB on sepsis-induced downregulation of vasopressin V₂ receptors and aquaporin (AQP)-2 channels using a cecal ligation and puncture (CLP) mouse model. CLP caused a time-dependent downregulation of renal vasopressin V₂ receptor and of AQP2 expression without alterations in plasma vasopressin levels. Renal activation of NF-κB in response to CLP was attenuated by PDTC pretreatment, which also attenuated the downregulation of V₂ receptor and AQP2 expression. Furthermore, a strong nuclear staining for the NF-κB p50 subunit throughout the whole kidney in response to CLP was observed. siRNA against NF-κB p50 attenuated the CLP-induced nuclear translocation of the p50 subunit and the CLP-induced downregulation of V₂ receptor and AQP2 expression. Additionally, PDTC and siRNA pretreatment inhibited the CLP-induced increase in renal TNF-α and IL-1β concentration and NO synthase (NOS)-2 mRNA abundance. Moreover, PDTC and siRNA pretreatment ameliorated CLP-induced hypotension and ARF. Our findings suggest that NF-κB activation is of importance for the downregulation of AQP2 channel and vasopressin V₂ receptor expression during sepsis. In addition, our data indicate that NF-κB inhibition ameliorates sepsis-induced ARF.

cecal ligation and puncture; kidney; inflammation; vasopressin; cytokines

SEPSIS-CAUSED MULTIORGAN failure remains still a leading cause of death in patients of intensive care units with a mortality rate higher than 50% (17, 21). Acute renal failure (ARF) is a critical complication of sepsis, with an incidence of ~20–50% (23, 24). Sepsis-induced ARF clearly worsens the survival prognosis during sepsis; the mortality is higher in patients with septic ARF than in those whose renal failure did not result from sepsis (13, 19). ARF can be nonoliguric or even polyuric in as much as one-third of septic patients (4, 10). However, up to now, there are no effective therapies to treat or prevent sepsis-induced ARF, because the pathogenesis of ARF in sepsis remains incompletely understood (1).

Endotoxemia in animals can also cause nonoliguria or polyuria (1, 11, 33). Renal water reabsorption is regulated by the neuropeptide vasopressin, which binds to vasopressin V₂ receptors located on the basolateral membrane of the renal collecting duct principal cells, leading to an increase in total aquaporin-2 (AQP2) expression and to a translocation of AQP2 channels from intracellular storage vesicles to the apical membrane in both animal models and cell culture systems. This event causes an increase in water permeability of renal principal cells (20). It has been shown that endotoxemia decreases vasopressin V₂ receptor and AQP2 expression in vivo, which may account for the decrease in urine osmolality despite alterations in plasma levels of vasopressin (7, 35).

Cecal ligation and puncture (CLP) is an experimental approach commonly used for the examination of the pathogenesis of sepsis, because the resulting polymicrobial sepsis mimics many features of human sepsis (36). This animal model leads to an activation of an inflammatory cascade, with increased formation of proinflammatory cytokines. Subsequently, these cytokines induce a variety of genes, including nitric oxide synthase-2 (NOS-2), which are responsible for alterations in regional and systemic hemodynamics and the impairment of organ function (30, 32, 37–40). We and others found that the induction of proinflammatory cytokines, like tumor necrosis factor (TNF)-α and interleukin (IL)-1β, is critical in the pathogenesis of endotoxemia-induced ARF (25, 26, 28, 29). The increased formation of proinflammatory cytokines strongly depends on the activation of NF-κB proteins (14). Indeed, inhibition of NF-κB by pyrrolidine dithiocarbamate (PDTC), a potent inhibitor of NF-κB (31), has been shown to attenuate the formation of proinflammatory cytokines, to prevent the development of systemic hypotension, and to improve survival in endotoxemic animals (9, 12, 15, 16). Since it has been found that endotoxemia strongly activates renal NF-κB in vivo (6), NF-κB may be an interesting target for sepsis-induced ARF. However, the effect of NF-κB inhibition on sepsis-induced ARF still remains to be established. In addition, it is has recently been found that NF-κB may negatively regulate the transcription of AQP2 in renal collecting duct cells (8), suggesting a potential direct effect of NF-κB for the downregulation of AQP2 expression during endotoxemia (7, 33, 35). The present study was therefore undertaken to examine the effect of NF-κB inhibition on sepsis-induced ARF and on sepsis-induced downregulation of V₂ receptor and AQP2 expression.

METHODS

Animal model. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal protection committee. Male C57BL/6J mice (20 to 25 g) were obtained from Charles River (Sulzfeld, Germany). CLP was performed as recently described with modifications (27). Under sevoflurane anesthesia, a 1.5-cm midline incision was made and the cecum was exposed. A 4–0 silk ligature was placed 1.0 cm from the cecal tip. The cecum was punctured twice with an 18-gauge needle and gently squeezed to
confirm leakage of cecal contents. In sham-treated animals, a similar procedure was performed but without CLP. The abdominal incision was closed in two layers with 4–0 silk sutures and, to ensure an adequate fluid resuscitation, animals received a subcutaneous injection of prewarmed isotonic NaCl solution (1 ml) directly after the intervention. Animals (n = 6 per group) were killed 12, 24, and 36 h after CLP. In addition, mice received a single dose of PDTC (100 mg/kg ip; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) 2 h before CLP treatment.

**Measurement of mean arterial pressure, renal and blood parameters.** Mice were anesthetized with Sevoflurane, using a Trajan 808 (Dräger, Lübeck, Germany). The right femoral artery was cannulated for continuous monitoring of mean arterial pressure (MAP; Siemens SC 9000, Munich, Germany). Plasma levels of creatinine and of urea were determined by commercially available kits (BioAssay Systems, Hayward, CA). Since the stress of anesthesia induces the release of vasopressin, plasma AVP levels were determined from blood samples rapidly taken from non-anesthetized animals in a separate experiment by radioimmunoassay.

**Cell culture.** Mouse cortical collecting duct (mpkCCDc14) cells were obtained from Alain Vandewalle (Institut National de la Santé et de la Recherche Médicale U773, Centre de Recherche Biomédicale Bichat-Beaujon, Paris, France) (2). The cells were seeded on perme-
able filters (Millicell-PCF, 0.4-µm-pore size, 1-cm² growth area; Millipore, Carrigtwohill, Cork, Ireland) and grown in culture medium until confluent (DMEM:Ham’s F₁₂, 1:1 vol/vol; 60 nmol/l sodium selenite, 5 µg/ml transferrin, 2 mmol/l glutamine, 50 mmol/l dexamethasone, 1 mmol/l triiodothyronine, 10 ng/ml EGF, 5 µg/ml insulin, 20 mmol/l α-glucose, 2% fetal calf serum, and 20 mmol/l HEPES, pH 7.4) and then in serum- and hormone-free medium for 48 h before use. Cells were treated with LPS (100 ng/ml; Sigma-Aldrich Chemie GmbH), PDTC (40 µM), or IL-1β (50 ng/ml; PreproTech, Hamburg, Germany) for 8 h.

mRNA extraction and real-time PCR analysis. Total RNA was reverse transcribed into cDNA according to standard protocols as described previously (18). Real-time PCR for AQP2, vasopressin V₂, adenylyl cyclase (AC) 6, and GAPDH was performed in a LightCycler (Roche, Mannheim, Germany). All PCR experiments were performed using the LightCycler DNA Master SYBR Green I kit provided by Roche Molecular Biochemicals (Mannheim, Germany). The amplification program consisted of 1 cycle of 95°C with 10-min hold (“hot start”) followed by 40 cycles of 15 s 95°C, 5 s 60°C, and 20 s 72°C. Amplification was followed by melting curve analysis to verify the accuracy of the amplification. To verify the correctness of the amplicon, a negative control with water instead of cDNA was run with every PCR to assess specificity of the reaction. To verify the accuracy of the amplification, PCR products were further analyzed on ethidium bromide-stained 2% agarose gel. The standard curves for AQP2, vasopressin V₂, and GAPDH were quantified relative to β-actin mRNA as described previously in detail (18). In brief, a standard calibration curve was made. The Roche software uses the second derivative maximum method to calculate the fractional cycle numbers where the fluorescence rises above background [crossing point (Cp)] that is the point at which the rate of change of fluorescence is fastest. For the standard curve, Cps are plotted vs. log concentration for the standards. This standard curve was used to estimate the concentration of each sample. The standard curves for AQP2, vasopressin V₂, and β-actin were saved in coefficient files, which were used by the relative quantification software from Roche to calculate the mRNA levels relative to β-actin.

Protein preparation and tissue concentrations of cytokines. Whole kidneys were homogenized in ice-cold homogenization buffer containing 250 mM sucrose, 1 mM EDTA, 0.1 mM PMSF, 0.7 µg/ml pepstatin, 0.5 µg/ml leupeptin, 1 µg/ml aprotinin, and 10 mM Tris-HCl buffer followed by centrifugation with 500 g for 15 min at 4°C. The resultant supernatant was centrifuged at 10,000 g for 30 min at 4°C. The supernatant was used for determination of tissue cytokine concentration, and the resultant pellet was reconstituted in blotting buffer and used for Western blotting. Nuclear extracts from whole kidneys were prepared using a commercially available kit (Active Motif, Rixensart, Belgium). Immunoblotting for GAPDH was used as a cytoplasmatic marker in a separate experimental setting. Tissue concentrations of TNF-α and IL-1β were determined using ELISA kits (R&D Systems, Minneapolis, MN) and set into proportion to total protein.

NF-κB activation assay. NF-κB p65 activity was quantified by using the TransAM Assay (Active Motif). In brief, a 96-well plate to which an oligonucleotide, containing an NF-κB consensus binding site, has been immobilized was incubated with 30 µg of nuclear extracts from whole kidneys followed by an antibody that is directed against the NF-κB p65 subunit and a secondary horseradish peroxidase (HRP)-conjugated antibody. The optical density (OD) values of the HRP reaction from each sample were normalized with the OD values of positive control samples (Raji nuclear extract).

Immunoblotting. Protein samples were electrophoretically separated on 10% polyacrylamide gels and transferred to nitrocellulose membranes, which were blocked overnight in 5% nonfat dry milk diluted in Tris-buffered saline with 0.1% Tween-20, and then incubated for 1 h at room temperature with antibodies against AQP2 (Calbiochem, Merck Biosciences, Darmstadt, Germany; 1:2,000),

Fig. 3. Effect of PDTC (100 mg/kg ip) on CLP-induced downregulation of V₂ (A) and AQP2 (B) mRNA expression in the kidney 24 h after induction of CLP. C, D: immunoblots of AQP2 and β-actin 24 h after CLP. Values are related to signals that were obtained for β-actin and given as percentage of vehicle-treated sham animals. Data are means ± SE of 6 mice per group. *P < 0.05 vs. vehicle-treated sham animals. #P < 0.05 vs. CLP.
GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1,000), β-actin (Sigma Aldrich; 1:5,000), or NF-κB p50 (Santa Cruz Biotechnology; 1:2,000). After being washed, the membrane was incubated for 2 h with the secondary antibody (Santa Cruz Biotechnology; 1:2,000) and subjected to a chemiluminescence detection system.

**Immunohistochemistry for AQP2, NF-κB p50 and p65.** Kidneys from sham-operated and CLP-treated mice were fixed in 4% paraformaldehyde solution by retrograde perfusion through the abdominal aorta. Immunolabeling was performed on 5-μm paraffin sections as described previously (34). In brief, after being boiled in a microwave oven followed by cooling, kidney sections were incubated with an anti-NF-κB p50 antibody (Santa Cruz Biotechnology; 1:1,000), anti-NF-κB p65 antibody (Novus Biologicals, Littleton, CO; 1:100), or an anti-AQP2 antibody (Santa Cruz Biotechnology; 1:600) overnight at 4°C, followed by incubation with a secondary antibody. As a negative control, we used the secondary antibody without incubation with the primary antibody.

**Statistical analyses.** Data were analyzed by ANOVA with multiple comparisons followed by the t-test with Bonferroni adjustment. P < 0.05 was considered significant.

**RESULTS**

**CLP decreases AQP2 and V2 receptor mRNA expression.** Plasma arginine vasopressin (AVP) concentration of control mice was 2.8 ± 0.4 pg/ml and was not altered in mice subjected to sham or CLP surgery (Fig. 1A). Polymicrobial sepsis, induced by CLP, time-dependent decreased renal AQP2 and V2 receptor mRNA abundance. AQP2 mRNA was downregulated to 46, 26, and 29% of control levels at 12, 24, and 36 h after CLP, respectively. Vasopressin V2 receptor mRNA levels were decreased to 26, 17, and 21% of control levels at 12, 24, and 36 h after CLP, respectively (Fig. 1B). AC type 6 mRNA was not altered 12 and 24 h after CLP surgery, but was decreased to 70% of control levels 36 h after CLP treatment (Fig. 1B). Neither renal GAPDH nor β-actin mRNA was significantly altered by CLP (Fig. 1B). Since the expression of β-actin was more robust than the expression of GAPDH after CLP, the expression of AQP2 and vasopressin V2 mRNA in subsequent experiments was quantified relative to β-actin mRNA. Abundant AQP2 immunoreactivity was observed in the apical region and throughout the cytoplasm of collecting duct cells in sham-operated mice (Fig. 1, C and E). In kidneys from CLP-treated mice, there was a marked reduction in the AQP2 staining after 24 h. The cytoplasmatic immunoreactivity of AQP2 in the renal cortex and the renal medulla was clearly decreased by CLP. The remaining labeling of AQP2 was associated with the apical plasma membrane of collecting ducts and was clearly lower in the renal cortex but not in the inner medulla (Fig. 1, D and F).

**Nuclear factor-κB activation in the kidney was inhibited by PDTC.** We were further interested in the renal activation of NF-κB. Twelve hours after CLP, nuclear staining for the NF-κB p65 subunit was observed throughout the whole kidney (Fig. 2, A–D). We further examined the effect of the NF-κB inhibitor PDTC on NF-κB activation in the kidney. NF-κB activation in nuclear extracts from whole kidneys was increased 3.6-fold 12 h after CLP surgery compared with sham-operated animals. Administration of PDTC at a dose of 100 mg/kg had no influence on basal NF-κB activity, but clearly inhibited the activation of NF-κB in response to CLP (Fig. 2C).
PDTC attenuates CLP-induced downregulation of AQP2 and V2 mRNA expression. We further investigated the effect of the NF-κB inhibitor PDTC on CLP-induced downregulation of AQP2 and V2 mRNA expression. Administration of PDTC alone had no influence on the expression of V2 receptor and AQP2 mRNA compared with control levels. However, PDTC attenuated the CLP-induced downregulation of V2 receptor and of AQP2 expression (Fig. 3, A and B). V2 receptor mRNA substantially increased from 14 to 58% compared with vehicle-treated, sham-operated animals (Fig. 3A). Furthermore, PDTC attenuated CLP-induced downregulation of AQP2 mRNA abundance from 24 to 78% compared with vehicle-treated, sham-operated animals (Fig. 3B). Densitometric analysis of immunoblots of AQP2 and β-actin protein from whole kidneys showed that AQP2 protein expression was decreased to 58% of control values 24 h after CLP surgery and that PDTC attenuated the CLP-induced decrease of AQP2 protein expression to 85% (Fig. 3, C and D).

CLP activates the NF-κB p50 subunit in the kidney. We were further interested in the specific role of the NF-κB p50 subunit for the CLP-induced downregulation of AQP2 and V2 receptor expression. Therefore, we first investigated the renal activation of the p50 subunit. Sham-operated animals displayed only a weak cytosolic immunoreactivity for p50 (Fig. 4, A and C). Twelve hours after CLP, we found a strong nuclear immunoreactivity for the p50 subunit throughout the whole kidney (Fig. 4, B and D). To examine the specific role of the p50 subunit, we pretreated mice with small interfering RNA (siRNA) silencing NF-κB p50/p105 mRNA. To test the effectiveness of our siRNA approach, we analyzed the renal mRNA expression of NF-κB p50/p105 and found that sole treatment with siRNA decreased the basal expression of NF-κB p50/p105 mRNA to ~60% of control levels (Fig. 4E) and that CLP increased NF-κB p50/p105 mRNA ~6.5-fold. This increase was clearly attenuated by pretreatment with siRNA silencing NF-κB p50/p105 (Fig. 4E). To verify the specificity of the siRNA targeting p50/p105 mRNA, we also investigated the mRNA expression of the p52/p100 and p65 subunit. CLP increased NF-κB p52/p100 and p65 mRNA ~4.0- and 5.6-fold, respectively (Fig. 4E). Pretreatment with siRNA silencing NF-κB p50/p105 did not influence basal or CLP-induced levels of NF-κB p52/p100 and p65 mRNA (Fig. 4E). We further investigated the effect of pretreatment with siRNA silencing NF-κB p50/p105 on the CLP-induced nuclear translocation of the p50 subunit. Immunoblotting of GAPDH in cytoplasmatic and nuclear extracts from whole kidneys was used as a cytoplasmatic marker to exclude cytoplasmatic contamination of the nuclei preparation (Fig. 4F). We found that the CLP-induced nuclear translocation of the p50 subunit was inhibited by siRNA pretreatment (Fig. 4F). Notably, scrambled siRNA did not influence the expression of NF-κB p50/p105 mRNA (data not shown).

Silencing of NF-κB p50/p105 mRNA attenuates CLP-induced downregulation of AQP2 and V2 expression. We further investigated the effect of siRNA pretreatment on CLP-induced downregulation of AQP2 and V2 mRNA expression. Administration of siRNA alone had no influence on the expression of V2 receptor and AQP2 mRNA compared with sham-operated animals (Fig. 5, A and B). However, siRNA pretreatment attenuated the CLP-induced downregulation of V2 and AQP2 mRNA (Fig. 5, A and B). Immunoblotting of AQP2 revealed the attenuated downregulation of AQP2 by siRNA pretreatment (Fig. 5, C and D). Scrambled siRNA did not influence the expression of V2 receptor and AQP2 channel mRNA (data not shown).

Fig. 5. Effect of siRNA pretreatment on V2 (A) and AQP2 (B) mRNA expression in the kidney 24 h after CLP treatment. C, D: immunoblots for AQP2 and β-actin from vehicle-treated sham mice, from CLP-treated mice, and from siRNA-pretreated CLP mice. Values are related to signals obtained for β-actin and given as % of vehicle-treated sham animals. Shown are means ± SE of 6 animals per group. *P < 0.05 vs. vehicle-treated sham animals. #P < 0.05 vs. CLP.
NF-κB inhibition inhibits CLP-induced renal tissue cytokine formation and renal NOS-2 mRNA expression. We further determined the effect of PDTC and of siRNA pretreatment on the induction of proinflammatory cytokines and on renal NOS-2 mRNA expression in response to CLP. We found that pretreatment with PDTC or siRNA inhibited the CLP-induced increase in renal tissue concentrations of TNF-α and IL-1β (Fig. 6, A and B). In addition, pretreatment with PDTC or siRNA also attenuated the CLP-induced increase in renal NOS-2 mRNA (Fig. 6C).

NF-κB inhibition improves CLP-induced hypotension and renal dysfunction. In addition, we examined the influence of PDTC or siRNA pretreatment on CLP-induced changes in cardiovascular and renal function. MAP was decreased from 81 ± 5 to 53 ± 6 mmHg 24 h after CLP (Fig. 7A). Neither PDTC nor siRNA pretreatment affected MAP. However, pretreatment with PDTC or with siRNA attenuated the fall in MAP in response to CLP (Fig. 7A). Plasma creatinine and urea levels were increased from 0.41 ± 0.06 and 43.1 ± 4.2 to 1.58 ± 0.16 and 120 ± 9.2 mg/dl, respectively, 24 h after CLP (Fig. 7, B and C). Sole pretreatment with siRNA or with PDTC did not influence plasma creatinine and plasma urea levels. Pretreatment with siRNA or with PDTC attenuated the CLP-induced rise in plasma creatinine and plasma urea concentration to 0.58 ± 0.11 or 0.54 ± 0.08 and to 58.3 ± 6.1 or 55.2 ± 7.8 mg/dl, respectively (Fig. 7, B and C).

LPS and IL-1β downregulate AQP2 and V2 mRNA expression in mpkCCDc14 cells. To examine a possible direct effect of sepsis-induced inflammation on AQP2 and vasopressin V2 receptor expression, we incubated mpkCCDc14 cells with LPS or IL-1β. We found that vasopressin V2 receptor mRNA and AQP2 mRNA were downregulated to 74 and 55% of control levels 8 h after the addition of LPS, respectively. Similarly, IL-1β decreased vasopressin V2 receptor and AQP2 mRNA to ~77 and 53% of control levels, respectively. Treatment of mpkCCDc14 cells with PDTC also decreased vasopressin V2 receptor mRNA and AQP2 mRNA to 81 and 59% of control levels, respectively, but prevented a further decrease in AQP2 and vasopressin V2 receptor mRNA by LPS and IL-1β (Fig. 8, A and B).

DISCUSSION

We demonstrated that inhibition of NF-κB by PDTC and by siRNA improved sepsis-induced ARF and attenuated the downregulation of vasopressin V2 receptor and AQP2 expression.

As an in vivo model for severe experimental inflammation, we used the CLP model, which caused a pronounced fall in MAP and renal dysfunction, as measured by plasma creatinine and urea levels, indicating the validity of our sepsis model (5). Pretreatment with PDTC or with siRNA clearly attenuated the fall in MAP and the rise in plasma creatinine and urea levels, suggesting that inhibition of NF-κB may be a useful strategy to prevent sepsis-induced ARF. In line with this, PDTC treatment has been shown to have beneficial effects in other kidney diseases (3, 22) and in endotoxemic animals (9, 12, 15, 16). Similar to previous reports, where the effect of LPS in rodents was studied, we found that also CLP caused a time-dependent decrease of vasopressin V2 receptor mRNA and of AQP2 gene expression (7, 35), suggesting that this downregulation may be a general effect during inflammation. We also found a marked reduction in cytosolic AQP2 labeling in response to CLP. Our finding that the remaining labeling of AQP2 was associated with apical membranes of collecting duct cells may suggest that AQP2 trafficking is not strongly impaired in septic kidneys, especially in the inner medulla. We were further interested in the pathway by which CLP leads to a downregulation of vasopressin V2 receptor and AQP2 expression. It is well-known that sepsis initiates an inflammatory cascade. Within this cascade, the activation of NF-κB is thought to play a central role in the pathophysiology of sepsis (14). In line with previous observations, we found a strong renal activation of NF-κB in response to CLP (5). This activation is crucial for the induction of a wide range of NF-κB-
responsive proinflammatory genes such as proinflammatory cytokines, like TNF-α and IL-1β, and NOS-2, which are well-described important mediators during inflammation (21). Confirming previous observations, we found that PDTC inhibits the induction of these proinflammatory cytokines and also of NOS-2, likely by the inhibition of NF-κB activation, which is supported by our observation of an attenuated NF-κB activation by PDTC (9, 12, 15, 16). We now found that PDTC also attenuates the CLP-induced downregulation of the vasopressin V2 receptor and of AQP2 gene expression, suggesting that the activation of NF-κB per se or the increased formation of cytokines is of importance for the CLP-induced downregulation of AQP2 and also of the vasopressin V2 receptor. Interestingly, it has been found that NF-κB can directly decrease the expression of AQP2 in vitro (8). Since activation of NF-κB in response to LPS has been described even for renal collecting duct cells (6), a direct effect of NF-κB inhibition on CLP-induced downregulation of AQP2 may be possible.

Although it has been shown that PDTC inhibits the LPS-induced nuclear translocation of the NF-κB subunits p65 and p50, the specificity and selectivity of PDTC must be questioned (12). Therefore, we performed experiments with siRNA against NF-κB in subsequent experiments to avoid possible side effects of PDTC, like the scavenging of oxygen radicals, for example. Since in vitro data suggest that the reduced AQP2 transcription in response to LPS derives from a decreased p65 binding and an increased binding of p50 and p52 homo- and/or heterodimers (8), we decided to examine the role of the p50 subunit for CLP-induced ARF. Using immunohistochemistry, we found a strong nuclear staining for the p50 subunit throughout the whole kidney after CLP. To investigate the role of the p50 subunit for the observed downregulation of AQP2 and of vasopressin V2 receptor expression, we then pretreated mice with siRNA silencing NF-κB p50/p105 mRNA. This treatment reduced basal mRNA levels of NF-κB p50/p105 and inhibited the CLP-induced increase of NF-κB p50/p105 mRNA levels, indicating the effectiveness of our approach. In addition, pretreatment with siRNA clearly attenuated the nuclear translocation of the p50 subunit. Moreover, this approach attenuated the downregulation of AQP2 and of vasopressin V2 receptor expression.

Fig. 7. Effect of PDTC (100 mg/kg ip) or siRNA pretreatment on mean arterial pressure (MAP; A), plasma creatinine concentration (B), and plasma urea concentration (C) 24 h after CLP. Data are means ± SE of 6 mice per group. ★P < 0.05 vs. vehicle-treated sham animals. #P < 0.05 vs. CLP.

Fig. 8. Effect of LPS (100 ng/ml), IL-1β (50 ng/ml), and PDTC (40 μM) on V2 (A) and AQP2 (B) mRNA expression in mpkCCD c14 cells. Data are means ± SE of 4 independent experiments per group after 8 h of incubation. ★P < 0.05 vs. vehicle-treated control.
expression in response to CLP, suggesting that the NF-κB p50 subunit is of importance for sepsis-induced downregulation of AQ2 and of vasopressin V₂ expression in vivo. Although our in vivo data for AQ2 expression cannot really prove the recently published in vitro data (8), they are in excellent congruence with them. Since it has been recently shown that siRNA against the subunits p65, p50, and p52 also decreases basal AQ2 transcription, these in vitro findings further suggest that NF-κB is not only of importance for the downregulation of AQ2 in response to LPS, but also for the basal transcriptional activity of AQ2 (8). In line with these findings, we found that LPS, IL-1β, and the NF-κB inhibitor PDTC decreased the expression of AQ2. However, LPS did not further decrease AQ2 expression during inhibition of NF-κB in vitro. Thus, one may conclude from these in vitro experiments that there may be a direct link between NF-κB and AQ2 regulation. Moreover, one may speculate from our data that the NF-κB pathway may also be of importance for the downregulation of the vasopressin V₂ receptor in the kidney. On the other side, one may suggest that the observed downregulation of AQ2 could also be secondary to the decrease in vasopressin V₂ receptor expression. However, this suggestion seems to be unlikely, since LPS-induced downregulation of AQ2 has also been observed ex vivo, independent of the effect of vasopressin (8). In addition, since neither PDTC nor siRNA affected the basal levels of AQ2 and vasopressin V₂ receptor expression in vivo, one may also assume from our data that the NF-κB pathway is not of major relevance for the basal expression of these two genes in vivo.

In conclusion, our findings provide evidence that the activation of NF-κB likely mediates the downregulation of AQ2 channel and vasopressin V₂ receptor expression during sepsis. Furthermore, we conclude from our findings that inhibition of NF-κB ameliorates sepsis-induced ARF. Therefore, NF-κB may be an interesting target for the treatment of sepsis-induced ARF.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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