**α₂-Adrenoceptor agonist dexmedetomidine protects septic acute kidney injury through increasing BMP-7 and inhibiting HDAC2 and HDAC5**

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**Hsing CH, Lin CF, So E, Sun DP, Chen TC, Li CF, Yeh CH. α₂-Adrenoceptor agonist dexmedetomidine protects septic acute kidney injury through increasing BMP-7 and inhibiting HDAC2 and HDAC5. Am J Physiol Renal Physiol 303: F1443–F1453, 2012. First published August 29, 2012; doi:10.1152/ajprenal.00143.2012.—Bone morphogenetic protein (BMP)-7 protects septic acute kidney injury (AKI). Dexmedetomidine (DEX), an α₂-AR agonist, has anti-inflammatory effects. We investigated the protective effects of DEX on sepsis-induced AKI and the expression of BMP-7 and histone deacetylases (HDACs). In vivo, the effects of DEX or trichostatin A (TSA, an HDAC inhibitor) on TNF-α, monocyte chemotactic protein (MCP)-1, BMP-7, and HDAC mRNA expression in LPS-stimulated rat tubular epithelial NRK52E cells, was determined using real-time PCR. In vivo, mice were intraperitoneally injected with DEX (25 μg/kg) or saline immediately and 12 h after cecal ligation and puncture (CLP) surgery. Twenty-four hours after CLP, we examined kidney injury and renal TNF-α, MCP-1, BMP-7, and HDAC expression. Survival was monitored for 120 h. LPS increased HDAC2, HDAC5, TNF-α, and MCP-1 expression, but decreased BMP-7 expression in NRK52E cells. DEX treatment decreased the HDAC2, HDAC5, TNF-α, and MCP-1 expression, but increased BMP-7 expression and acetyl histone H3 expression, whose effects were blocked by yohimbine, an α₂-AR antagonist. With DEX treatment, the LPS-induced TNF-α expression and cell death were attenuated in scRNAi-NRK52E but not BMP-7 RNAi-NRK52E cells. In CLP mice, DEX treatment increased survival and attenuated AKI. The expression of HDAC2, HDAC5, TNF-α, and MCP-1 mRNA in the kidneys of CLP mice was increased, but BMP-7 was decreased. However, DEX treatment reduced those changes. DEX reduces sepsis-induced AKI by decreasing TNF-α and MCP-1 and increasing BMP-7, which is associated with decreasing HDAC2 and HDAC5, as well as increasing acetyl histone H3.

dexmedetomidine; sepsis; acute kidney injury; bone morphogenetic protein-7; histone deacetylase 2; histone deacetylase 5

Sepsis is characterized by a systemic inflammatory response to infection, with its severe form associated with multiple organ failure (42). The combination of acute kidney injury (AKI) and sepsis represents a serious medical problem in the intensive care unit and is associated with 70% mortality (29). Despite growing understanding of the pathophysiological mechanisms of sepsis-induced AKI, pharmacological strategies for preventing and treating AKI during sepsis are relatively limited.

Bone morphogenetic protein (BMP)-7 is crucial in nephrogenesis during development and is present in the precursor cells of the nephron, although its expression is repressed in the proximal tubular cells after maturation of the kidney (49, 51). BMP-7 reduces the severity of ureteral obstruction-induced renal injury (19), acute ischemic renal injury (48), diabetic nephropathy (50), and lupus nephritis (53). The suppressive effects of BMP-7 on TNF-α-induced inflammatory responses during nephropathy suggest that BMP-7 might provide a therapeutic target for treating acute or chronic kidney injuries (3, 11, 17).

Dexmedetomidine (DEX) is an α₂-adrenoceptor (α₂-AR) agonist used as an analgesic and sedative agent (1). The α₂-AR agonists provide a prophylactic option in treating sepsis (18, 30, 35). Although DEX decreased production of inflammatory cytokines and increased survival in septic mice (16, 45), the underlying molecular mechanisms remain unclear. In the kidney, α₂-AR agonists mediate blood flow (2) and reduce Na+ and water transport (39). DEX produces its neuroprotective (25, 40) and renoprotective (12) effects via α₂-AR.

Changes in histone acetylation, controlled by histone deacetylases (HDACs) and histone acetyltransferase (HAT), regulate gene transcription by altering chromatin structure (28). In the kidney, HDAC2 or HDAC5 induction in response to inflammation (28) or ischemia (27), respectively, contributes to renal damage. HDAC5 downregulation contributes to histone reacetylation and BMP-7 induction in the recovery phase of ischemia/reperfusion in the mouse kidney (27). The induction of HDAC2, accompanied by a decrease in histone acetylation, was observed in kidneys injured after ureteral obstruction. HDAC inhibitors, such as trichostatin A (TSA), attenuate macrophage infiltration and fibrotic changes in tubulointerstitial injury (28).

This study evaluates the potential effects of DEX treatment on preventing AKI in septic mice and regulating cytokines, including tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, and BMP-7, together with HDAC-2, HDAC-5, and acetyl histone H3.

**MATERIALS AND METHODS**

**Cell culture and reagents.** A rat kidney tubular epithelial cell line, NRK52E (American Type Culture Collection, Manassas, VA), was maintained in DMEM supplemented with 10% FBS. Approximately 60% confluent NRK52E cells were cultured in normal medium or treated with 100 ng/ml LPS (Escherichia coli B555; Sigma-Aldrich, St. Louis, MO), in combination with DEX (Hospira, Lake Forest, IL),

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Table 1. Primer pairs used in this study

<table>
<thead>
<tr>
<th>Factor</th>
<th>Primer Sequence (5'-3')</th>
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<tbody>
<tr>
<td>BMP-7</td>
<td>CTGGATGCGCCAAGACATCAA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>GAGTCAAGAAGGCTTTAGCAGGGC</td>
</tr>
<tr>
<td>MCP-1</td>
<td>ACCCTCGCTCATGTCCATTAC</td>
</tr>
<tr>
<td>HDAC2</td>
<td>GGGTACAGAAGGAGGCGGCGG</td>
</tr>
<tr>
<td>HDAC5</td>
<td>CTGGGAGGTTGAGGACAGCGG</td>
</tr>
<tr>
<td>β-actin</td>
<td>GCTGGAAAGGTTGAGGACAGCGG</td>
</tr>
</tbody>
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BMP, bone morphogenetic protein; MCP-1, monocyte chemotactic protein-1; HDAC2, 5, histone deacetylase 2, 5.
induced more TNF-α/H9251/TNF-α combined with DEX or TSA for 12 h, and then examined treated scRNAi- and BMP-7 RNAi-NRK52E cells with LPS of DEX and TSA on BMP-7-knockdown NRK52E cells. We of BMP-7 in LPS-induced cytokine expression and the effect stimulated NRK52E cells (Fig. 1). We next determine the role of DEX on TNF-α, MCP-1, and BMP-7 expression in LPS-fell but BMP-7 expression rose. Yohimbine blocked the effects of DEX and LPS TSA-treated cells: TNF-α expression fell (Fig. 1 DEX-). The opposite occurred in LPS expression rose (Fig. 1 BMP-7). With DEX or TSA treatment, LPS-induced TNF-α, MCP-1, and BMP-7 expression fell in LPS-stimulated NRK52E cells (Fig. 1). We next determine the role of BMP-7 in LPS-induced cytokine expression and the effect of DEX and TSA on BMP-7-knockdown NRK52E cells. We treated scRNAi- and BMP-7 RNAi-NRK52E cells with LPS combined with DEX or TSA for 12 h, and then examined TNF-α levels in cultured supernatants using ELISA. LPS induced more TNF-α protein in the cultured supernatants of BMP-7 RNAi-NRK52E cells than of scRNAi-NRK52E cells (Fig. 2). With DEX or TSA treatment, LPS-induced TNF-α expression was attenuated in scRNAi- but not in BMP-7 RNAi-NRK52E cells (Fig. 2).

Cell survival was higher in DEX-treated NRK52E cells. BMP-7 regulates cytokine expression and protects kidney injury in inflammatory responses (3, 11, 17). We next investigated the effect of DEX on BMP-7-mediated renal cell protection. We treated scRNAi- and BMP-7 RNAi-NRK52E cells with LPS combined with DEX or BMP-7 for 24 h. Morphological observations showed that LPS induced cell death in both scRNAi- and BMP-7 RNAi-NRK52E cells (Fig. 3A), which was attenuated by BMP-7. Notably, DEX treatment reduced the cell death in scRNAi- but not BMP-7 RNAi-NRK52E cells (Fig. 3A). An MTT assay showed that the cell viability in LPS-stimulated BMP-7 RNAi-NRK52E cells was markedly lower than in scRNAi-NRK52E cells (21.0 ± 3.9, P < 0.05) (Fig. 3B). BMP-7 treatment reduced LPS-induced cell death in both scRNAi- and BMP-7 RNAi-NRK52E cells; however, DEX reduced cell death in scRNAi-
but not BMP-7 RNAi-NRK52E cells (Fig. 3B). These data indicated that DEX reducing LPS-induced renal cell death is associated with BMP-7 expression.

Survival time was longer and AKI incidence lower in DEX-treated polymicrobial septic mice. We next investigated the effect of DEX on septic AKI in vivo. Twenty-four hours after CLP surgery, the mice showed characteristics of sepsis-lethargy, piloerection, diarrhea, huddling, and malaise, and developed AKI. Plasma levels of urea, creatinine, and CRP (Fig. 4, A–C, respectively) in CLP mice were significantly higher than in control mice but significantly lower in CLP+DEX and CLP+TSA mice; however, CRP, urea, and creatinine levels in CLP+DEX+yohimbine mice were as high or higher than in control mice. Histological examination revealed focal tubular epithelial swelling, shortened brush border, and vacuolar degeneration in both the cortex (Fig. 5A, b) and medulla (Fig. 5A, f) of CLP mice. The tubular damage score in the cortex (Fig. 5B) and medulla (Fig. 5C) of CLP+DEX- and CLP+TSA-treated mice was lower than in CLP-only mice. We further determined the effect of DEX treatment on the survival of the CLP mice. DEX+CLP mice, both in early and late DEX treatment, survived significantly longer than CLP mice (Fig. 4D).

Inflammatory cytokines were lower and BMP-7 was higher through 2-AR. In the kidneys of CLP mice, TNF-α and MCP-1 mRNA expression was higher (Fig. 6, A and B), but BMP-7 mRNA expression was lower (Fig. 6C) than in control mice. However, in CLP+DEX- and CLP+TSA-treated mice, TNF-α, and MCP-1 mRNA expression in the kidneys was lower but BMP-7 mRNA expression was higher than in CLP mice. In CLP+DEX+yohimbine mice, TNF-α and MCP-1 mRNA expression levels were significantly higher, but BMP-7 levels were lower than in CLP+DEX mice. Immunohistochemical staining showed that BMP-7 was strongly or moder-
ately stained in the tubular cells of the control and CLP mice (Fig. 6, D, a and D, c, respectively), but weakly stained in CLP mice (Fig. 6, D, b). Taken together, the effects of DEX on expression of inflammatory cytokines and BMP-7 in kidneys of septic mice are, at least in part, through 2-AR.

HDAC2 and HDAC5 levels were lower, but histone H3 acetylation was higher in DEX-treated septic mice. HDAC2 or HDAC5 induction in inflammation contributes to renal damage (28). HDAC5 downregulation contributes BMP-7 induction and reduces ischemic kidney injury (27). Our data also showed that inflammation and renal cell death were lower by DEX or TSA treatment. We hypothesized that DEX protects renal cell injury in sepsis by inhibiting HDAC expression. Thus, we investigated the effect of DEX on HDAC regulation in sepsis. In vitro, HDAC2 and HDAC5 mRNA expression was higher in LPS-treated NRK52E cells (Fig. 7, A and B) but lower in LPS+DEX- and LPS+TSA-treated NRK52E cells. Acetyl histone H3 expression was significantly lower in LPS-treated NRK52E cells than in controls (Fig. 7C), but higher in LPS+DEX- and LPS+TSA-treated NRK52E cells. The effects of DEX on expression of HDAC2, HDAC5, and acetyl histone H3 were inhibited by yohimbine (Fig. 6). In vivo, HDAC2 and HDAC5 mRNA expression (Fig. 8, A and B) was higher in the kidneys of CLP mice than in controls, but not in CLP+DEX and CLP+TSA mice. Acetyl histone H3 expression in the kidneys of mice was lower after CLP surgery but...
higher after DEX treatment (Fig. 8C). Yohimbine also inhibited the effects of DEX on HDAC2, HDAC5, and acetyl histone H3 expression in kidneys of CLP mice (Fig. 8).

**DISCUSSION**

The present study performed CLP surgery on mice, causing lethal peritonitis by microbial infection. Using this valid animal model for human sepsis (43), we showed that DEX treatment reduced mortality in septic mice, which is consistent with previous reports (16, 43, 45). DEX also ameliorated sepsis-induced AKI by decreasing inflammatory cytokine expression and increasing BMP-7 expression. In this study, we focus on the kidney protection effect of DEX during sepsis. Our in vitro experiments showed that LPS increased HDAC2, HDAC5, TNF-α, and MCP-1 expression, but decreased BMP-7 expression in kidney tubular epithelial cells. DEX treatment decreased the HDAC2, HDAC5, TNF-α, and MCP-1 expression, but increased the BMP-7 and acetyl histone H3 expression. In vivo, the expression of HDAC2, HDAC5, TNF-α, and MCP-1 mRNA in the kidneys of CLP mice was increased, but BMP-7 was decreased. However, DEX treatment reduced those changes. Therefore, we considered that DEX reduces sepsis-induced AKI by decreasing TNF-α and MCP-1 and increasing BMP-7, which is associated with decreasing HDAC2 and HDAC5, as well as increasing acetyl histone H3. Our data demonstrated that DEX directly protected kidney during sepsis. On the other hand, the nervous system may regulate the exaggerated innate immune response via different pathways, such as cholinergic pathway activated by vagus nerve stimulation, α- or β-adrenergic receptor activation during sepsis (22). DEX might play its immune modulating effect by activating a cholinergic pathway. The role of DEX in vagus stimulation during sepsis needs further investigation.

In the kidney, BMP-7 helps to maintain the structure and function of podocytes, mesangial cells, and tubular epithelial cells (14, 49, 51), but it disappears quickly when the kidney is

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**Fig. 5. Effects of DEX treatment on renal histological changes of CLP mice.**

A: kidney histology in control, CLP, CLP+DEX, and CLP+TSA mice (n=6 in each group) as the treatments described in MATERIALS AND METHODS. Kidney sections were stained with hematoxylin and eosin, and representative histology is shown. The tubular damage score was evaluated in the cortex (B) or the medulla (C). Data are expressed means ± SD. *P < 0.05 compared with the control group. #P < 0.05 compared with the LPS group.
damaged, which facilitates pathological progression (19, 23, 48, 50, 53). BMP-7 also has anti-inflammatory effect by downregulating inflammatory mediators (26, 48). Elevating BMP-7 could be a therapeutic strategy for kidney disease (17, 23). Renal cell death is critical in the pathogenesis of AKI during sepsis (36). The present study identified that DEX treatment increased BMP-7 expression in the kidneys of CLP mice. LPS-induced renal cell death is critical in the pathogenesis of AKI during sepsis (36). In LPS-treated BMP-7 knock-down NRK52E cells, DEX did not protect the LPS-induced cell death, which indicated that the effect of DEX on renal cell protection is related to BMP-7 expression. Previous reports (5, 12) showed that DEX-induced cell survival correlated with a decreasing cleaved caspase-3 expression, activating pAKT survival signal, and reducing HMGB1 release. In addition, BMP-7 could be induced in the recovery phase after ischemia via epigenetic mechanisms, contributing to the regeneration of the organ (6, 27, 46). Inflammatory response plays a major role in ischemic AKI, and inflammatory cascades can be augmented dramatically by the generation of a number of potent mediators by the ischemic proximal tubule, such as induction of MCP-1, TNF-α, IL-6, and IL-1β. BMP-7 also is induced in postischemic tubules (46) and protects against ischemic AKI by decreasing MCP-1, IL-8, IL-6, and IL-1β in cultured proximal tubule cells (11), which suggest BMP-7 may be a therapeutic agent for kidney disorders involving inflammation and ischemic damage of proximal tubular epithelial cells.

We noted that DEX or TSA reduced the histological injury score in CLP mice 24 h after surgery, but the change levels were not as in serum creatinine. We consider that the histology injury score and functional data may be not at the same impairment level at this time point in this study.

TNF-α and MCP-1 amplify acute tubular injury during sepsis-induced AKI (36, 42). DEX may attenuate the production of TNF-α and MCP-1 during sepsis (16, 45). In this study, DEX decreased TNF-α and MCP-1 expression in LPS-stimulated NRK52E cells and the kidneys of CLP-mice. These findings indicate that DEX may reduce sepsis-induced AKI by decreasing TNF-α and MCP-1. In this study, BMP-7-knock-down NRK52E cells were more susceptible to LPS-induced cell death than scRNAi-transfected NRK52E cells, and the DEX treatment that protected them against the cell death was BMP-7 dependent. We considered that DEX protects renal cell in sepsis by increasing BMP-7 and reducing inflammatory cytokines. In this study, DEX did not alter TNF-α, MCP-1, and BMP-7 expression in NRK52E cells without LPS stimulation (data not shown). In contrast, DEX might expand its epigenetic effect on BMP-7 and TNF-α expression in LPS-stimulated NRK52E cells. Other factors involved in regulation of BMP-7 expression by DEX, such as changes in chromatin structure of the BMP-7 promoter region, require further clarification. Given the in vitro experiment of silencing BMP-7 that exacerbate LPS-induced cell death, we thus considered that expression of BMP-7 during DEX treatment may play an important role, at least in part, in reducing septic AKI.

Several organ-protective effects of α2-AR agonist have been reported (2, 12, 25, 40). The effects on presynaptic nerve terminals contribute to the immunomodulatory effects of α2-AR stim-
ulation in vivo (8, 13, 47). α2-AR agonists increase the urine flow rate and improve perioperative renal function because they are vasodilators and widely distributed in the kidney (9, 21). Yohimbine is an α2-AR antagonist with a high affinity for the α2A, α2B, and α2C ARs (24). In this study, yohimbine used alone did not affect the renal function in CLP mice (data not shown). Yohimbine inhibited renal protection provided by DEX against TNF-α, MCP-1, and BMP-7 expression in CLP mice and LPS-stimulated NRK52E cells. Therefore, DEX may prevent sepsis-induced AKI and increase tubular cell survival by increasing BMP-7 expression via the α2-AR. Which subtype of α2-AR is responsible for the renal protective effect of DEX needs to be further investigated.

Histone acetylation, mediated by HATs and HDACs, is a vital process in the epigenetic regulation of gene expression (31, 38). When HATs are activated, or HDACs are suppressed, histones are acetylated, and the chromatin structure becomes relaxed. Transcriptional regulators are then able to access the promoter regions of genes and regulate their expression (38). HDAC inhibitors are used as anticancer (7, 15) and antifibrogenic agents (34, 37), whose mechanisms compose the transcriptional regulation of specific genes. The HDAC inhibitor TSA upregulates BMP-7 expression via chromatin remodeling in tubular epithelial cells during epithelial-to-mesenchymal transition (52). Inhibiting HDAC5 contributed to histone reacetylation and BMP-7 induction during the recovery phase of ischemia/reperfusion in the mouse kidney (27). We administered a subhypnotic dose of DEX (4) to CLP mice and found that DEX may act like HDAC inhibitors by inhibiting HDAC2 and HDAC5 mRNA expression and increasing histone H3 acetylation, which regulates TNF-α, MCP-1, and BMP-7 expression. The effect of TSA on acetyl histones seems more profound than the effect of DEX, although it was not statistically significant. We suggested that HDACs may be partially responsible for the effect of DEX. Taken together, our study showed that the effect of DEX on reducing inflammation in sepsis may be involved in epigenetic regulation of cytokine expression.

Fig. 7. Effects of DEX on HDAC2, HDAC5, and histone H3 in LPS-stimulated NRK52E cells. HDAC2 (A) and HDAC5 (B) mRNA expression in LPS-stimulated NRK52E cells combined with DEX (10 μmol/l) or TSA (300 ng/ml) treatment. C: above the graph are representative images of acetyl histone H3 in NRK52E cells analyzed using Western blot analysis. Quantified protein levels are in the graph. All groups: n = 3. Data are expressed as means ± SD. *P < 0.05 compared with the control group. #P < 0.05 compared with the LPS group.
The α2-AR is a G protein-coupled receptor that transduces signals via catalyzing the dissociation of the Gα and Gβγ subunits, thus modulating the subunits’ downstream effectors (33). Gβγ binds to the C terminus of HDAC5 (44). Although we suggest that DEX could regulate HDAC2 and HDAC5 mRNA expression and histone H3 acetylation through α2-AR, whether DEX modulates cytokines by directly promoting the formation of the Gβγ-HDAC2 or Gβγ-HDAC5 complex warrants further investigation.

In conclusion, DEX treatment reduced AKI in CLP mice. During sepsis, DEX increased BMP-7 but decreased TNF-α and MCP-1 expression via the α2-AR, which is associated with a decrease in HDAC2 and HDAC5 expression in the kidney. This study provides evidence of possible epigenetic effects of DEX on renal protection during sepsis. The widely used sedative agent DEX might have potential therapeutic use in reducing AKI in sepsis.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

REFERENCES


