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The role of MAP kinase phosphatase-1 in the protective mechanism of dexamethasone against endotoxemia

Xianxi Wang, Leif D. Nelin, Joshua R. Kuhlman, Xiaomei Meng, Stephen E. Welty, Yusen Liu *

Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Ohio, USA
Department of Pediatrics, The Ohio State University College of Medicine, Columbus, Ohio, USA

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ABSTRACT

Aims: We have previously shown that glucocorticoids induce the expression of MAP kinase phosphatase (Mkp)[a]-1 in innate immune cells. Since Mkp-1 is a critical negative regulator of the innate immune response, we hypothesize that Mkp-1 plays a significant role in the anti-inflammatory action of glucocorticoids. The specific aim of the present study is to understand the role of Mkp-1 in the anti-inflammatory function of glucocorticoids.

Main methods: Wild-type and *Mkp-1*$^{-/-}$ mice were treated with different doses of dexamethasone and then challenged with different doses of lipopolysaccharide (LPS). The survival and blood cytokines were assessed. The effects of dexamethasone on cytokine production in wild-type and *Mkp-1*$^{-/-}$ primary macrophages *ex vivo* were also examined.

Key findings: We found that dexamethasone induced the expression of *Mkp-1 in vivo*. Dexamethasone treatment completely protected wild-type mice from the mortality caused by a relatively high dose of LPS. However, dexamethasone treatment offered only a partial protection to *Mkp-1*$^{-/-}$ mice. Dexamethasone attenuated TNF-α production in both wild-type and *Mkp-1*$^{-/-}$ mice challenged with LPS, although TNF-α production in *Mkp-1*$^{-/-}$ mice was significantly more robust than that in wild-type mice. Dexamethasone pretreatment shortened the duration of p38 and JNK activation in LPS-stimulated wild-type macrophages, but had little effect on p38 or JNK activation in similarly treated *Mkp-1*$^{-/-}$ macrophages.

Significance: Our results indicate that the inhibition of p38 and JNK activities by glucocorticoids is mediated by enhanced *Mkp-1* expression. These results demonstrate that dexamethasone exerts its anti-inflammatory effects through both Mkp-1-dependent and Mkp-1-indepent mechanisms.

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Introduction

Glucocorticoids, such as dexamethasone, are potent anti-inflammatory drugs frequently prescribed for the treatment of various inflammatory diseases, including asthma, chronic obstructive pulmonary disease, and acute respiratory distress syndrome (Barnes, 1998; Joyce et al., 2001; Rhen and Cidlowski, 2005; Saklatvala, 2002). In addition to these chronic inflammatory diseases, glucocorticoids have also been used for the treatment of severe sepsis and septic shock in patients in the intensive care unit (Annane, 2005). Clinical trials have indicated that low dose glucocorticoids alleviate the systemic inflammatory response, reduce the duration of shock, and favorably affect survival in patients with septic shock (Annane et al., 2002). In animal models of endotoxic shock, prophylactic treatment with dexamethasone attenuates the production of inflammatory cytokines including TNF-α and IL-1β, and prevents shock and mortality (Berry and Smythe, 1964; Spink and Anderson, 1954). Although glucocorticoids have been used in clinical medicine as anti-inflammatory drugs for more than half a century, the underlying anti-inflammatory mechanisms are still not fully understood (Rhen and Cidlowski, 2005; Saklatvala, 2002). Glucocorticoids can modulate the expression of genes involved in inflammation in both positive and negative manners (Saklatvala, 2002). Although transcriptional repression of pro-inflammatory cytokine and chemokine genes by activation of nuclear glucocorticoid receptors is widely accepted to be the primary anti-inflammatory mechanism, induction of anti-inflammatory genes may also contribute to the therapeutic activity (Barnes, 1998; Joyce et al., 2001; Rhen and Cidlowski, 2005; Saklatvala, 2002). Glucocorticoids can induce the expression of IκB and IL-10 (Goulding, 2004; Rhen and Cidlowski, 2005). Both IκB and IL-10 play an important role in antagonizing inflammatory cascades. IκB inhibits the transcription factor NF-κB by forming inactive heterotrimer with NF-κB, a master

Abbreviations: MAP, mitogen-activated protein; Mkp, MAP kinase phosphatase; LPS, lipopolysaccharides; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; TNF, tumor necrosis factor; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; PBS, phosphate-buffered saline; ANOVA, analysis of variance; NF-κB, nuclear factor-κB; IκB, inhibitor-κB; GR, glucocorticoid receptor.

* Corresponding author. Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205, USA. Tel.: +1 614 722 3073; fax: +1 614 355 3455.
E-mail address: yusen.liu@nationwidechildrens.org (Y. Liu).

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regulator in the transcription of a variety of cytokine and chemokine genes. IL-10 can inhibit the production of inflammatory cytokines and promote the resolution of inflammation.

Previously, we and several other laboratories have independently demonstrated that glucocorticoids induce MAP kinase phosphatase (MKP)-1 in macrophages, mast cells, and transformed epithelial cells (Chen et al., 2002; Kassel et al., 2001; Lasa et al., 2002). Moreover, we have reported that the capacity of a given synthetic glucocorticoid to induce *Mkp-1* in cultured immortalized macrophages is associated with the relative anti-inflammatory potency of the synthetic corticosteroid (Zhao et al., 2005). However, whether Mkp-1 plays a significant role in the anti-inflammatory action of glucocorticoids *in vivo* has not been clearly defined. Very recently, a number of studies conducted using *Mkp-1* knockout mice have demonstrated that Mkp-1 is responsible for the inactivation of p38 and JNK in innate immune cells, and is a critical negative regulator of inflammatory cytokine biosynthesis *in vivo* (Chi et al., 2006; Hammer et al., 2006; Salojin et al., 2006; Zhao et al., 2006).

In this report, we examined the contribution of Mkp-1 in the protective and anti-inflammatory action of glucocorticoids using both cultured macrophages and mice. We found that dexamethasone exerts its anti-inflammatory action through both Mkp-1-dependent and Mkp-1-independent mechanisms. Mkp-1 is required for the optimal anti-inflammatory activity of dexamethasone. In the absence of Mkp-1, dexamethasone fails to accelerate the dephosphorylation of p38 and JNK, and is less effective in preventing endotoxic shock and mortality. These results indicate that Mkp-1 induction constitutes a part of host-protective and anti-inflammatory mechanisms of glucocorticoids. Our results also support notion that full anti-inflammatory activity of glucocorticoids required both Mkp-1-dependent inhibition on MAP kinases and Mkp-1-independent mechanisms.

Materials and methods

Animals

The generation of *Mkp-1* knockout mice has been described previously (Dorfman et al., 1996). Cryopreserved embryos of *Mkp-1* knockout mice (*Mkp-1*$^{+/-}$ and *Mkp-1*$^{-/-}$) were kindly provided by Bristol–Myers Squibb Pharmaceutical Research institute and were regenerated into mice in The Jackson Laboratory (Bar Harbor, ME). These mice were bred in-house to yield both wild-type and *Mkp-1*$^{-/-}$ mice. These mice were maintained on Harlan Tecklad irradiated diet (Harlan Sprague Dawley, Indianapolis, IN) at 24 °C with relative humidity between 30 and 70% on a 12-h day–night cycle. All of the animals received humane care in accordance with the guidelines of the National Institutes of Health and were sacrificed by CO_2 inhalation. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Columbus Children's Research Institute.

LPS and dexamethasone administration

Wild-type and *Mkp-1*$^{-/-}$ mice were injected intraperitoneally with the designated doses of LPS dissolved in phosphate-buffered saline (PBS). Dexamethasone was first dissolved in 2-Hydroxypropyl-β-cyclodextrin solution (Sigma, St. Louis, MO), and then diluted in PBS prior to intraperitoneal injection. Mice were sacrificed, and blood was harvested by cardiac puncture for determination of serum TNF-α, IL-6, and IL-10 levels.

Isolation, culture, and treatment of peritoneal macrophages

Thioglycollate-elicited peritoneal macrophages were isolated from wild-type or *Mkp-1*$^{-/-}$ mice by peritoneal lavage as described previously (Shepherd et al., 2004; Zhao et al., 2006). Briefly, each mouse was injected intraperitoneally with 2 ml of 3% Brewer Thioglycollate Medium (BD Diagnostic, Sparks, MD). Four days later cells in the peritoneum were harvested by lavage with cold RPMI 1640 medium (Invitrogen, Carlsbad, CA) containing 5% FBS (HyClone Laboratories, Logan, UT). Peritoneal cells were recovered by centrifugation, resuspended in RPMI 1640 medium containing 5% FBS, and plated onto tissue culture plates. Cells were allowed to adhere for 2 h, washed free of nonadherent cells, and maintained overnight in RPMI 1640 medium containing 5% FBS. The next day, macrophages were pretreated with either dexamethasone or vehicle (DMSO dissolved in PBS) for 15 min, and then stimulated with LPS (100 ng/ml) for the indicated periods of time. Cells were harvested in lysis buffer as previously described (Shepherd et al., 2004).

Western blotting and ELISA

Western blot analysis was conducted using ECL reagent (Amersham Biosciences) essentially as described previously (Chen et al., 2002). Mkp-1 protein levels were assessed using a rabbit polyclonal antibody (Catalog number: Sc-10199, Santa Cruz Biotechnology, Santa Cruz, CA). Phosphorylated ERK, JNK, and p38 were detected using rabbit polyclonal phospho-specific antibodies purchased from Cell Signaling Technology (Beverly, MA). Total p38 was detected using a monoclonal antibody (BD Transduction Laboratories, San Jose, CA). β-Actin was detected using a monoclonal antibody purchased from Sigma. ELISA was performed as previously described using commercial kits according to manufacturers' recommendations (Zhao et al., 2006).

Northern blotting

Total RNA was isolated using STAT-60 (Tel-Test, Friendswood, TX). Northern blot analysis was carried out using a full-length mouse *Mkp-1* cDNA as a probe as described previously (Barnes, 1998). The membrane was stripped and reprobed with GAPDH cDNA to normalize for RNA loading.

Statistical analysis

Cytokine production was compared between wild-type and *Mkp-1*$^{-/-}$ cells or mice using one-way analysis of variance (ANOVA) and a modified *t*-test. Differences in survival after LPS challenge between wild-type and *Mkp-1*$^{-/-}$ mice with or without dexamethasone treatment were determined by Kaplan–Meier analysis. All the tests were performed using SPSS 13.01 software (SPSS Inc., Chicago, IL). A P value less than 0.05 was considered significant.

Results

Effect of dexamethasone on LPS-induced mortality in wild-type and Mkp-1$^{-/-}$ *mice*

To determine the role of Mkp-1 in the protective effect of dexamethasone against endotoxic shock, *Mkp-1*$^{-/-}$ mice and their wild-type littermates were given either 30 mg/kg dexamethasone or vehicle intraperitoneally. Thirty minutes later these mice were given LPS (20 mg/kg body weight) intraperitoneally. Animal survival was monitored periodically, using death or a moribund state as end-point criteria. Wild-type mice, that were pretreated with vehicle, exhibited 100% mortality by 156 h after challenge with 20 mg/kg LPS (Fig. 1A). Prophylactic treatment with dexamethasone at a dose of 30 mg/kg completely prevented mortality (different from vehicle, $P<0.001$, $n=11$). All *Mkp-1*$^{-/-}$ mice that were pretreated with vehicle died by 24 h after LPS challenge (*Mkp-1*$^{-/-}$ different from wild-type, $P<0.001$, $n=11$). The median survival time of this group was 17 h. Prophylactic treatment of the *Mkp-1*$^{-/-}$ mice with dexamethasone at the same dose

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