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Anti-inflammatory effect of a selective 11β-hydroxysteroid dehydrogenase type 1 inhibitor via the stimulation of heme oxygenase-1 in LPS-activated mice and J774.1 murine macrophages

Sung Bum Park [a], Ji Seon Park [b], Won Hoon Jung [a], Hee Youn Kim [a], Hyun Jung Kwak [a], Jin Hee Ahn [a], Kyoung-Jin Choi [a], Yoon-Ju Na [a], Sunhwa Choi [a], Sang Dal Rhee [a, c], Ki Young Kim [a, c, d, *]

[a] Bio & Drug Discovery Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong-gu, Daejeon 305-600, Republic of Korea
[b] Department of Human and Environmental Toxicology, University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 305-333, Republic of Korea
[c] Graduate School of New Drug Discovery and Development, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea
[d] Department of Medicinal Chemistry and Pharmacology, University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 305-333, Republic of Korea

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ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive cortisone to the active cortisol. 11β-HSD1 may be involved in the resolution of inflammation. In the present study, we investigate the anti-inflammatory effects of 2-(3-benzoyl)-4-hydroxy-1,1-dioxo-2H-1,2-benzothiazine-2-yl-1-phenylethanone (KR-66344), a selective 11β-HSD1 inhibitor, in lipopolysaccharide (LPS)-activated C57BL/6J mice and macrophages. LPS increased 11β-HSD1 activity and expression in macrophages, which was inhibited by KR-66344. In addition, KR-66344 increased survival rate in LPS treated C57BL/6J mice. HO-1 mRNA expression level was increased by KR-66344, and this effect was reversed by the HO competitive inhibitor, ZnPP, in macrophages. Moreover, ZnPP reversed the suppression of ROS formation and cell death induced by KR-66344. ZnPP also suppressed animal survival rate in LPS plus KR-66344 treated C57BL/6J mice. In the spleen of LPS-treated mice, KR-66344 prevented cell death via suppression of inflammation, followed by inhibition of ROS, iNOS and COX-2 expression. Furthermore, LPS increased NFκB-p65 and MAPK phosphorylation, and these effects were abolished by pretreatment with KR-66344. Taken together, KR-66344 protects against LPS-induced animal death and spleen injury by inhibition of inflammation via induction of HO-1 and inhibition of 11β-HSD1 activity. Thus, we concluded that the selective 11β-HSD1 inhibitor may provide a novel strategy in the prevention/treatment of inflammatory disorders in patients.

1. Introduction

Glucocorticoids are stress hormones that can exert a wide spectrum of physiological effects by modulating immune and inflammatory responses, and regulating energy metabolism, cardiovascular homeostasis, and the stress response (1). Chronically-elevated glucocorticoid levels are reliant on glucocorticoid pre-receptor metabolism regulated by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) rather than on circulating glucocorticoid levels themselves (2). 11β-HSD1 is widely expressed and functions as an endoplasmic reticulum-associated enzyme of an NADPH-dependent oxoreductase, converting inactive cortisone to the active glucocorticoid cortisol (3).

* Corresponding author. Bio & Drug Discovery Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong-gu, Daejeon 305-600, Republic of Korea. Fax: +82 42 861 0770.
E-mail address: kykim@krict.re.kr (K.Y. Kim).
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Identification and targeting of central molecules involved in the integration of inflammatory and metabolic responses appear to have potential for use as a therapeutic approach in the treatment of inflammatory diseases and metabolic syndromes. Accordingly, the possible involvement of 11β-HSD1 in the resolution of inflammation has been reported (4). Cytokines upregulate 11β-HSD1 activity in inflammatory-responsive cells including macrophages (5,6), T, B, and dendritic cells (5,7), and adipocytes (8). A selective 11β-HSD1 inhibitor inhibits obesity and inflammation in diet-induced obese mice (9), and suppresses the expression of diabetes-regulating genes (10). Thus, modulation of 11β-HSD1 activity selectively in immune-inflammatory cells may present a new therapeutic strategy for chronic inflammatory disease.

Heme oxygenase-1 (HO-1) is the inducible isoform of heme oxygenase, which is a microsomal enzyme with the function of oxidative degradation of heme (11). Induction of HO-1 in endothelial cells protect against oxidative stress (12) and cilostazol suppressed the production of anti-inflammatory cytokines and molecules via the inhibition of NF-κB activation, through a mechanism involving the up-regulation of cyclicAMP-dependent protein kinase activation-coupled Nrf2-linked HO-1 expression in macrophages (13). Thus, selective HO-1 induction may represent an adaptive response that increases cellular resistance to inflammation.

In this study, we elucidate the anti-inflammatory effects of KR-66344, a selective 11β-HSD1 inhibitor in LPS-treated murine J774.1 macrophages and C57BL/6J mice. KR-66344 may provide protection against LPS-induced cell death, animal death and spleen injury by inhibiting inflammation through the decrease in ROS formation and inflammatory cytokines release, followed by the inhibition of NFκB-p65, ERK, JNK, and p38 MAPK phosphorylation, iNOS and COX-2 expression, and induction of HO-1 expression as a result of the inhibition of 11β-HSD1 activity and expression.

2. Materials and methods

2.1. Animals and drug administration

All animal experiments were carried out using 10-week-old male C57BL/6J mice according to the *Guidelines for Animal Experimentation* according to the established guidelines of the *Institutional Animal Care and Use committee* of the Korea Research Institute of Chemical Technology.

Three or four groups of 5 up to 10 mice were randomly divided into: control, LPS, LPS plus KR-66344, and/or LPS plus KR-66344 and zinc protoporphyrin (ZnPP) groups. Mice were pretreated with KR-66344 (30 mg/kg body weight, i.p.) or 30 mg/kg KR-66344 plus ZnPP (30 mg/kg body weight, i.p) for 2 h, followed by LPS (50 or 100 mg/kg, i.v.) treatment for 6 h (western blotting, PCR and plasma NO analysis) or 3 days (animal survival analysis). Animals were weighed regularly to allow accurate dosing.

2.2. Cell culture

Mouse reticulum cell sarcoma-derived J774.1 macrophages (ATCC #TIB-67) were maintained in RPMI-1640 medium (Gibco/Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2.

2.3. In vitro assay for 11β-HSD1 activity

Macrophages were seeded at a density of 2.5×10^4 cells/well into 96-well plates, and incubated in medium containing 160 nM cortisone, 20 nM NADPH and 50 ng/ml LPS in KR-66344 or carbenoxolone (CBX) for 24 h. Small aliquots of the reaction mixtures were removed and subjected to a homogeneous time-resolved fluorescence cortisol assay in accordance with the manufacturer's instructions (Nihon Schering, Tokyo, Japan). The IC_{50} values of the compounds were determined from concentration-dependent inhibition curves using the GraphPad Prism software (GraphPad Software Inc., La Jolla, CA).

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Expression studies were carried out using gene-specific primers for mouse 11β-HSD1, IL-6, IL-1β, HO-1, and GAPDH. All primers were designed using the Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/). PCR primers for amplification were designed based on the following sequences: 11β-HSD1: sense, 5′- gggataattgacgccctagc-3′; antisense, 5′-tgaggcaggactgttctaag-3′, IL-6: sense, 5′-agttgccttcttgggactga-3′; antisense, 5′-tgcacgatttcccagagaac-3′, IL-1β: sense, 5′-caggcaggcagtatcactca-3′; antisense, 5′-agctcatatgggtccgacag-3′, HO-1: sense, 5′- tccttgtaccatatctacacggcc-

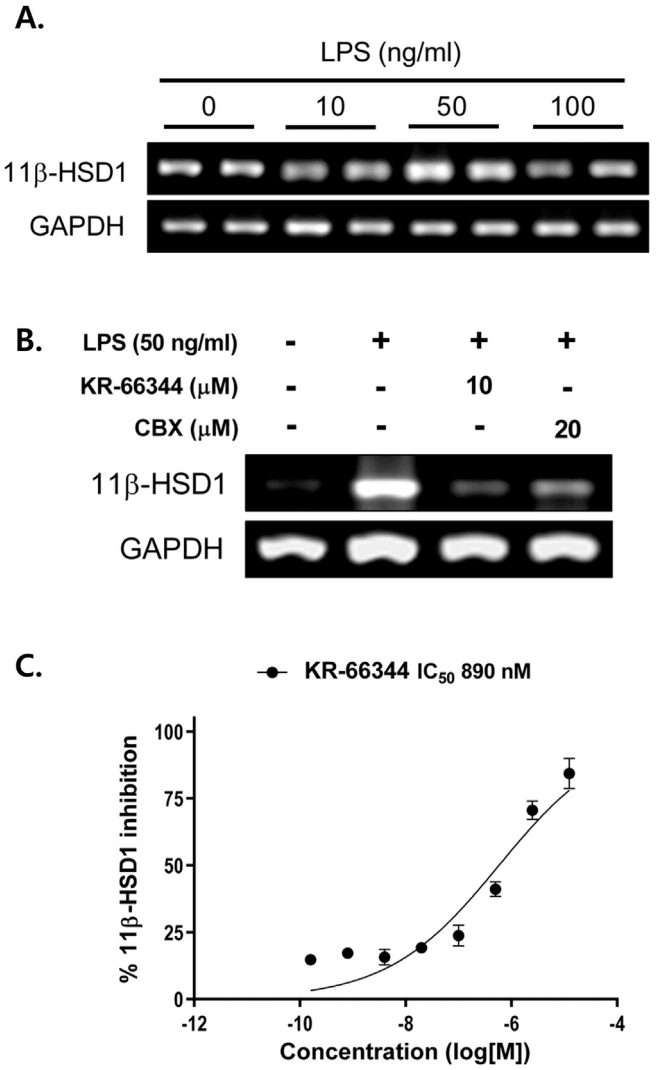

Fig. 1. Effect of KR-66344 on 11β-HSD1 expression and inhibitory activity in LPS-treated J774.1 macrophages. (A and B) 11β-HSD1 mRNA expression was determined by RT-PCR and GAPDH was used as a control. Macrophages were incubated with various concentrations of LPS (10–100 ng/ml) (A) and KR-66344 and CBX (B) for 24 h. (C) The 11β-HSD1 inhibitory activity was determined using a cortisol concentration assay. The 11β-HSD1 enzyme inhibitory activity is expressed as the mean ± S.E.M. of two independent experiments performed in quadruplicate.