Evaluation of Immunotoxicity of Shizukaol B Isolated from *Chloranthus japonicus*

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Abstract

Dimeric sesquiterpenoid shizukaol B (SKB) was isolated from *Chloranthus japonicus* Sieb. Except that SKB inhibited adhesion molecule expression in monocytes and endothelial cells, no more biological and pharmacological activity of SKB had been reported until now. In this study, we examined immunosuppressive activity of SKB. SKB strongly inhibited lipopolysaccharide (LPS)-induced B cell proliferation with IC₅₀ of 137 ng/ml, but slightly or not concanavalin A-induced T cell proliferation, LPS-induced macrophage NO production, and LPS-induced dendritic cell maturation. As a mechanism, SKB strongly induced apoptotic death of B cells, but not other cell types. These results suggested that SKB induced toxicity-mediated immunosuppression against B cells.

Key Words: Shizukaol B, *Chloranthus japonicus*, Immunosuppression, Cytotoxicity, B cells

INTRODUCTION

The genus *Chloranthus* has been taxonomically placed in the Chloranthaceae and has long been used as folk medicine for their antitumor, antifungal, and anti-inflammatory activities (Kuang et al., 2008). The components of genus *Chloranthus* are volatile oil, simple coumarins, amide alkaloids and sesquiterpene lactones having a lindenane skeleton named shizukanolides and chloranthalactones (Kuang et al., 2009). *Chloranthus japonicus* is a perennial herb that grows in the southern part of Korea, Japan, and China (Kwon et al., 2006). As a biologically active substance from this plant, dimeric sesquiterpenoid shizukaol B (SKB) inhibited expression of several adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, in HL-60 and human umbilical vein endothelial (HUVEC) cells, suggesting that shizukaol B might inhibit monocytes adhesion to endothelial cells, which was a critical step in the pathogenesis of atherosclerosis (Kwon et al., 2006). Except this data, there were no reports on the pharmacological or biological activity of SKB until now. In this study, we investigated immunosuppressive activity of SKB and demonstrated that SKB might induce apoptosis-mediated immunosuppression. Of note was that SKB preferentially induced apoptosis in B cells, but not T cells, macrophages, and dendritic cells.

MATERIALS AND METHODS

Materials

Female C57BL/6 mice (6-8 weeks old) were obtained from Korea Research Institute of Bioscience and Biotechnology (Chungbuk, Korea). Mice were housed in specific pathogen-free conditions at 21-24°C and 40-60% relative humidity under a 12-hr light/dark cycle. All animals were acclimatized for at least 1 week prior to the experiments. The experimental procedures used in this study were approved by the Chungbuk National University Animal Experimentation Ethics Committee. The dried roots of *Chloranthus japonicas* Sieb. (Chloranthaceae) were extracted three times with MeOH at room temperature for 3 days (Kwon et al., 2006). The dried roots of *Chloranthus japonicas* Sieb. (Chloranthaceae) were extracted three times with MeOH at room temperature for 3 days (Kwon et al., 2006). The MeOH extract was separated into two fractions using CHCl₃ as the non-aqueous phase. The CHCl₃ fraction was loaded on a silica gel column (Merck, 230-400 mesh) and eluted successively with 70% MeOH. The

Citation

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Received Aug 9, 2010 Revised Sep 28, 2010 Accepted Oct 7, 2010

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fraction was subjected to semi-preparative HPLC and gave shizukaol B (SKB) (Fig. 1).

Isolation of spleen cells

Spleen cells were obtained from specific pathogen free C57BL/6 mice (female, 6-7 weeks) and were freed of red blood cells by lysis buffer treatment (Han et al., 2001). Splenic B cells were isolated by negative depletion by using biotinylated antibodies to CD4, CD8, GR-1, and CD11c (BD Pharmingen) and Dynabeads M-280 Streptavidin (Invitrogen, Carlsbad, CA, USA). Splenic T cells were isolated by negative depletion using biotinylated antibodies to B220, GR-1, and CD11c (Han et al., 2005b). Purity was typically >90%. Cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (HyClone, Logan, UT, USA), 2 mM L-glutamine and 50 μM 2-mercaptoethanol (Sigma, St. Louis, MO, USA).

Generation of primary macrophages and dendritic cells

Bone marrow (BM) cells were obtained from femurs and tibias of C57BL/6 mice. After red blood cells were lysed, whole BM cells (2×10⁶ cells/ml) were cultured in 100-mm² culture dishes in 10 ml/dish of complete medium containing 10 ng/ml M-CSF to generate primary macrophages or 2 ng/ml GM-CSF (R&D Systems, Minneapolis, MN, USA) to make dendritic cells (DC). On day 3, another 10 ml of fresh complete medium containing 10 ng/ml M-CSF (or 2 ng/ml GM-CSF) was added, and half of the medium was changed on day 6. On day 8, non-adherent and loosely adherent cells were harvested by vigorous pipetting and used as primary macrophages or DCs (Kim et al., 2007). Primary macrophages recovered from these cultures were generally >85% F4/80⁺, but not CD3⁺ and B220⁺ (Shi et al., 2004). Cells were treated with LPS and SKB for 24