Anti-inflammatory Effect of MeOH Extracts of the Stem of Polygonum multiflorum in LPS-stimulated Mouse Peritoneal Macrophages

Dong Seok Cha¹ and Hoon Jeon¹²*
¹College of Pharmacy, Woosuk University, Chonbuk 565-701, Korea
²Center for Healthcare Technology Development, Woosuk University, Chonbuk 565-701, Korea

Abstract – Polygonum multiflorum Thunb has been widely used as a traditional medicine for the treatment of lots of diseases. In macrophages, nitric oxide is released as an inflammatory mediator and has been proposed to be an important modulator of many pathophysiological conditions in inflammation. In the present study, it was investigated that the inhibitory effects on NO and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and the mechanism of down-regulation of immune response by 85% methanol extracts of PM in mouse (C57BL/6) peritoneal macrophages. Extracts of PM (0.1, 1 mg/ml) suppressed NO production and showed inhibition of pro-inflammatory cytokines like TNF-α, IL-6 and it attenuated iNOS and COX-2 expression via down-regulation of NF-κB activation. The present results indicate that the 85% methanol extracts of PM has an inhibitory effect on the production of NO through down-regulation of iNOS expression in LPS stimulated mouse peritoneal macrophages and therefore may be beneficial in diseases related to macrophage-mediated inflammatory disorders.

Keywords – Nitric Oxide, NF-κB, iNOS, COX-2, Polygonum multiflorum Ramulus

Introduction

Inflammation is characterized by redness, heat, swelling, pain and dysfunction of the organs. It is first response of the immune system to infection or irritation and may be referred to as the innate cascade including various cells and cytokines. The inflammatory response is out of proportion to the external irritation. Thus, the result can be more damage to the body than the agent itself would have produced. Many types of autoimmune diseases and allergies such as asthma, rheumatoid arthritis and multiple sclerosis are example of excessive inflammatory responses (Rakel et al., 2005).

Macrophages play a central role in host defense and maintenance as a major immune cell in inflammation, since they are concerned in not only natural immunity but specific acquired immunity. Lipopolysaccharide (LPS) is a component of the outer cell membrane of gram-negative bacteria. It is an endotoxin, which induces septic shock and stimulates the production of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukins, prostanoids and leukotrienes (Chen et al., 2005; Erridge et al., 2002; Hewett et al., 1993). The stimulation of macrophages with LPS also induces expression of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Cao et al., 2006).

Expression of an iNOS, which closely related with up-regulation of nuclear factor kappa B (NF-κB), result in catalyze the abundant production of NO from L-arginine and molecular oxygen (Jeon et al., 2006). High levels of NO have been described in a variety of pathophysiological processes including various forms of circulatory shock (Szabo et al., 1995), inflammation (MacMicking et al., 1997) and carcinogenesis (Ohshima et al., 1994). NO up-regulates the release of inflammatory mediators by mouse macrophages (Marcinkiewicz et al., 1995).

It is widely acknowledged that a number of pro-inflammatory cytokines such as TNF-α and IL-6 play an important role in the regulation of immune response (Lee et al., 2005). TNF-α and IL-6, which macrophages release simultaneously by pathogenic germs or toxicants, lead to secondary immune response such as proliferation of T and B cells and killing of microorganisms (Shaeter et al., 1993; Haslberger et al., 1992). It is well known that
NO, synthesized by iNOS, is released from macrophage intimately correlated with the pathophysiology in inflammation and lots of diseases (Kim et al., 2005; Thiemermann et al., 1990) and increased expression of iNOS and its catalytic activity has been observed in several human tissues and in chemically-induced animal tumors and also in inflammatory disorders (Goldstein et al., 1998; Wilson et al., 1998; Amb’s et al., 1998).

Another enzyme that plays an important role in mediating inflammation is COX-2. The inducible isoform, COX-2, is induced by LPS, certain serum factors, growth factors, cytokines and is a predominant cyclooxygenase at a site of inflammation (Thiemermann et al., 1990; Shaeter et al., 1993). Expression of the inducible enzymes such as iNOS, COX-2 and cytokines in macrophages is regulated mainly at the transcriptional level, particularly by NF-κB and it plays a key role in inflammatory responses, cell growth, cell adhesion and apoptosis (Baeuerle et al., 1996; Verma et al., 1995). NF-κB exists in the cytoplasm of unstimulated cells as a latent form and is bound to IκB, the inhibitory protein (Karin et al., 2000). LPS stimulate NF-κB activation by phosphorylation and degradation of IκB-α and then NF-κB is translocated to the nucleus (Sanchez-Perez et al., 2002). It binds to DNA at the κB site in the promoter region of target genes and activates gene expression (Chen et al., 1995). In mammalian cells, regulation of iNOS expression is predominantly governed by the ubiquitously expressed NF-κB which is required for the inducible expression of genes associated with inflammatory responses (Kim et al., 2005). Therefore, inhibition of signal transduction proteins in the pathways leading to activation of NF-κB is now widely recognized as a reasonable strategy to inflammatory disease. Since NO production, related enzymes, pro-inflammatory cytokines might cause inflammatory damage, many studies about inflammation focused to find materials which selective modulate these inflammatory mediators from traditional plant-derived medicines (Lee et al., 2005).

The dried stem of Polygonum multiflorum Thunb. (Polygonaceae) has been widely used crude drugs as an oriental medicine, with beneficial effects in numerous diseases, including insomnia, hyperhidrosis in Korea and China. The major types of chemical constituents, stilbenes and anthraquinones, were analyzed from Polygonum multiflorum. The root of P. multiflorum have been found to possess antioxidant (Chi et al., 2002), myocardial protective (Yim et al., 1998), neuroprotective (Wang et al., 2006; Li et al., 2005), anti-aging (Xiao et al., 1993), estrogenic (Kang et al., 2006; Zhang et al., 2005), cognitive enhancing (Um et al., 2006; Chan et al., 2003), antimutagenic activity (Zhang et al., 1999), arthritic pain and skin infections (Li et al., 2003). It also has been demonstrated that 2,3,5,4′-tetrahydroxystilbene-2-O-beta-D-glucoside, an active component of P. multiflorum have protective effects on experimental colitis (Wang et al., 2008) and inhibitory effects on experimental inflammation and cyclooxygenase 2 activity (Zhang et al., 2007). However, the anti-inflammatory mechanism of the stem of P. multiflorum are not clearly understood.

In the present study, the inhibitory effect of the MeOH extracts of Polygoni multiflori Ramulus (PM) on NO and cytokines such as TNF-α, IL-6 was investigated in LPS stimulated mouse peritoneal macrophage cell. For clear the evident mechanism of NO suppression, the author assessed the effect of PM on expression level of iNOS, COX-2 and NF-kB.

**Experimental**

**Preparation of the stem of PM**—The plant materials were purchased from Wansanyakupsa (Jeonju, South Korea) in October 2005. A voucher specimen (WME005) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University. An extract was obtained twice from the dried sample (250 g) with 6,000 ml of 85% MeOH under ultrasonification for 2 h. It was evaporated and lyophilized to yield an MeOH extract of PM (Yield : 4.185%), which was then stored at -20°C until use.

**Peritoneal macrophage culture**—TG-elicited macrophages were harvested 3 – 4 days after i.p. injection of 2.5 ml TG to the mice and isolated. Using 8 ml of HBSS containing 10 U/ml heparin, peritoneal lavage was performed. Then, the cells were distributed in DMEM, which was supplemented with 10% heat-inactivated FBS, in 4-well tissue culture plates (3 × 10⁶ cells/well) incubated in an atmosphere of 5% CO₂, washed three times with HBSS to remove non-adherent cells and equilibrated with DMEM that contained 10% FBS before treatment.

**3-(3,4-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay**—Cell respiration, an indicator of cell viability, was performed by the mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan, as described by Mosmann (Mosmann et al., 1983). The extent of the reduction of MTT to formazan within cells was quantified by measuring the optical density (OD) at 540 nm using an automated microplate reader (GENios,