

Effects of Crude Ginseng Saponin on the Thromboxane Synthesis in Lipopolysaccharide-stimulated Macrophages

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Abstract—Crude ginseng saponin fraction reduced the production of thromboxane A₂ in the lipopolysaccharide-stimulated macrophages. Several kinds of crude saponins showed variant potency that might be caused by the compositional difference of ginseng saponins. From the metabolic labeling experimental data, this reduction of thromboxane A₂ formation, at least in part, resulted from the reduction of protein synthesis of inducible isozyme of cyclooxygenase(COX-2). This activity may be resulted from the fact that ginseng saponins have steroidal moiety in their structures.

Keywords □ ginseng, saponin, thromboxane A₂, cyclooxygenase

The eicosanoids are the products of oxidative metabolism of 20-carbon fatty acids, in particular arachidonic acid (C20 : 4, ω -6), and they have potent and diverse biological activities, each having a distinct biological profile. They act as mediators or modulators of various physiological and pathological events, for example in the pathogenesis of inflammation, asthma, thrombosis, etc. Therefore, compounds which could modulate the metabolism of arachidonic acid have a wide therapeutic potentials. Prostaglandin H (PGH) synthase/cyclooxygenase (EC 1.14.99.1) is a rate-limiting enzyme in eicosanoids metabolism. Recently, a mitogen-inducible form of cyclooxygenase (COX-2) was discovered, which is encoded by 4-kilobase mRNA, whereas the first discovered enzyme (COX-1) encoded by 2.8-kilobase mRNA (Xie *et al.*, 1991). These two forms of cyclooxygenase may have different patho-physiological functions, eventhough they catalyze the same reaction of converting arachidonate to prostaglandin H₂ (PGH₂). The COX-1 produce prostaglandins involved in cellular housekeeping function (Smith *et al.*, 1991) whereas COX-2 may produce prostanoids involved in inflammation and/or mitogenesis. This was supported by the fact that the biosynthesis of COX-2 is selectively stimulated by mediators of inflammation including lipo-

polysaccharide (LPS) and that expression is inhibited by glucocorticoids in rat alveolar macrophages (Lee *et al.*, 1992).

Ginseng is one of the most important traditional medicine in Korea and Lee *et al* (1989) already reported the effects of ginseng saponins on the biosynthesis of prostaglandins in cell free system. As a part of our ongoing study on the anti-inflammatory components from medicinal plants, we have found the inhibitory effect of crude ginseng saponin (*Panax ginseng* C.A. Mayer) on prostaglandin synthesis in LPS-stimulated macrophages. And this result was found to be, at least in part, due to the reduced expression of COX-2.

Materials and Methods

Plant Material

Dry root (3 kg) of Korean ginseng aged six years were extracted three times with 6 liters of hot methanol, and the combined extracts (400 g) were suspended in water and extracted with water saturated *n*-butanol to afford crude saponin fraction (70 g).

Isolation of Macrophages

Sprague-Dawley rats, weighing 160~180 g, were supplied by National Institute of Safety Research. Alveolar macrophages were collected by bronchoalveolar lavage as described by Chandler and Fulmer (1987). Ce-

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cells in RPMI 1640 media (Gibco) were allowed to adhere to 24-well plates for 2 hours (37°C, 5% CO₂) in the presence of 300 µM of aspirin to inactivate the endogenous cyclooxygenase (COX-1).

Radioimmuno Assay of TXB₂

Macrophages were incubated in RPMI containing 3% fetal bovine serum with or without LPS (10 µg/ml, Difco) and effectors. After incubation of 16 h, spent media was removed to assess the amount of thromboxane B₂ (TXB₂), which is the major of COX-derived metabolite of arachidonic acid in rat alveolar macrophages. Radioimmunoassay was performed as described elsewhere (Hwang *et al.*, 1988). The cells were treated with 0.5N-NaOH for the quantification of protein by the method of Bradford's (1976). The quantitative analysis of RIA result was done with PGM-2 program.

Metabolic Labeling and Immunoprecipitation of Cyclooxygenase

Rat alveolar macrophages (2 × 10⁶ cells/well) were preincubated for 12h in RPMI 1640 medium containing LPS (10 µg/ml) and effectors, and then further incubated with 100 µCi of [³⁵S]-methionine (1,139 Ci/mol, ICN) for 4 h. Cells were washed three times with PBS, pH 7.4, and solubilized as described elsewhere (Lee *et al.*, 1992). Precleared cell lysates were immunoprecipitated with antibody specific for COX-2 followed by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). The protein in the gels were fixed in a solution of 30% methanol and 10% acetic acid and treated with Enhance (DuPont-New England Nuclear) for 2 h. Gels were dried in a gel drier and exposed to XAR-5 film (Kodak) at -70°C.

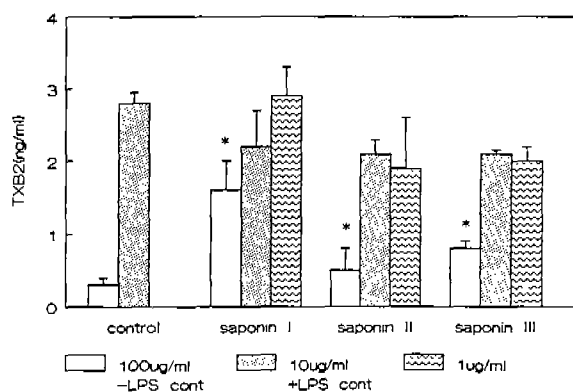


Fig. 1. Effects of several preparations of crude ginseng saponin on the accumulated levels of TXB₂ (ng/ml) synthesized from endogenous arachidonic acid in LPS-stimulated macrophages. Results are the mean ± S.E. (n=3-6). *Significantly different from (+) LPS control (p<0.05)

Results and Discussion

Rat alveolar macrophages were treated with LPS to enhance the prostaglandin production and TXB₂ was determined as the final metabolite in the arachidonic acid cascade. As mentioned above, COX-2 is known to be responsible for the various pathologic processes including inflammation (Vane, 1994). Thus, COX-2 is recognized as one of the major sites of pharmacological interventions to control the inflammation. Although prostaglandin synthesis could be affected by several enzymatic steps from arachidonate release by phospholipase A₂ (PLA₂) to each isomerases, we assumed that the amount of TXB₂ released into the cell culture media reflected COX-2 activity because LPS-induced prostaglandin production was paralleled by the variation of COX-2 activity (Lee *et al.*, 1992) and not by PLA₂ activity (data not shown) in our experimental conditions.

As shown in Fig. 1, three different preparations of crude ginseng saponins showed inhibitory effect against the accumulated amounts of TXB₂ in LPS-stimulated macrophages. Each preparations showed somewhat variable potency, and this result might be caused by the compositional difference of crude saponin fraction. Fig. 2 showed the concentration dependency of saponin fraction III on the accumulated level of TXB₂. At the final concentration of 1,000 µg/ml, thromboxane level was reduced to 36% of the amount of LPS control on the basis of same protein amount of cell. Dexamethasone used as positive control inhibited the thromboxane synthesis up to 27% of LPS control.

Generally ginseng saponin is respected as active principles of ginseng and ginseng saponin has steroidal moiety in their structure. Lee *et al.* (1992) proposed

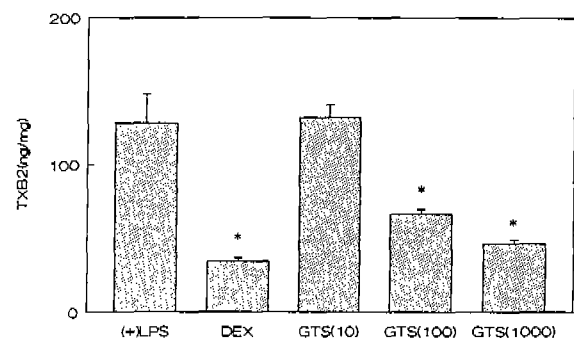


Fig. 2. Effects of several concentrations of crude ginseng saponin III on the accumulated levels of TXB₂ (ng/mg of used cell protein) synthesized from endogenous arachidonic acid in LPS-stimulated macrophages. Results are the mean ± S.E. (n=3-6). *Significantly different from (+) LPS control (p<0.01)

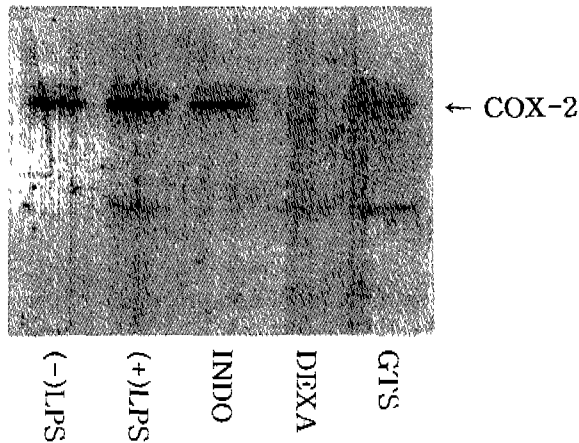


Fig. 3. Immunoprecipitation of *de novo* synthesized COX-2 in rat alveolar macrophages. Lane 1: (-) LPS control, Lane 2: (+) LPS control, Lane 3: indomethacin (2 μ M) and LPS, Lane 4: dexamethasone (2 μ M) and LPS, Lane 5: ginseng total saponin (100 μ g/ml) and LPS

that the treatment of dexamethasone specifically inhibited the induction of COX-2 in the LPS-stimulated macrophages. In our preliminary data, ginseng total saponin inhibited the COX enzymatic activity in LPS-stimulated macrophages under the same condition as previous report (Lee *et al.*, 1992). In order to find out the step on which ginseng saponin have an effect to decrease the level of TXB₂, experiments of metabolic labeling was performed. The metabolic labeled protein by the incubation with [³⁵S] methionine was immunoprecipitated by the treatment of peptide antibody which were made against the specific peptide sequences of COX-2. As shown in Fig. 3, ginseng saponin treated well showed reduced *de novo* synthesis of COX-2 protein. Treatment of dexamethasone completely inhibited the induction of COX-2 by LPS, while indomethacin did not (same result as the previous report by Sanduja *et al.*, 1990). This means that the reduced level of accumulated TXB₂ was resulted from the decrease of LPS-induction of COX-2 by the treatment of ginseng saponin. This effect of same pattern with dexamethasone may be explained by the fact that those have similar structural moiety which may compete against same receptor. In consideration of the side effect of COX-1 specific inhibitor such as irritation of stomach lining and toxic effects on kidney, COX-2 specific inhibitor is promising candidate as antiinflammatory agents (Mitchell *et al.*, 1993). Recently, Matsuda *et al* (1990) reported that ginsenoside Ro have anti-inflammatory activity, but ginsenoside Ro is not active in our system. So with the anticipation of development of antiinflammatory agent having the minimized side

effect, we are performing the further experiments to find out COX-2 specific inhibitor from ginseng and its mechanism of inhibitory action.

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