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Inhibitory Effect of Lignans from the Rhizomes of *Coptis japonica* var. *dissecta* on Tumor Necrosis Factor-α Production in Lipopolysaccharide-stimulated RAW264.7 Cells

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The inhibitory effect of 10 lignan constituents isolated from the rhizomes of *Coptis japonica* var. *dissecta* on tumor necrosis factor (TNF)-α production in lipopolysaccharide (LPS)-stimulated macrophage cell line (RAW264.7 cells) has been studied. Among them, pinoresinol, woorenoside-V and lariciresinol glycoside showed significant inhibitory activities in the range from 37% to 55% at the concentration of 25 μg/ml. The results are first report that the lignans isolated from *Coptis japonica* inhibit TNF-α production, and suggest that the lignan components may partly participate in antiinflammatory and antiallergic effect of *Coptis japonica* through the inhibition of TNF-α production.

Key words : TNF-α, RAW264.7, LPS, *Coptis japonica*, Lignan, Pinoresinol, Woorenoside, Lariciresinol glycoside, ELISA

INTRODUCTION

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine that is primarily produced by activated macrophages (Vileek and Lee, 1991). In addition to its role in cell-mediated immune responses, TNF-α enhances the production of other cytokines by autocrine stimulation (Hensel *et al.*, 1987) and induces the production of prostaglandin E_2 (PGE_2) by synovial fibroblast-type cells and of prostaglandin I_2 (PGI_2) by endothelial cells (Dayer *et al.*, 1986; Manogue *et al.*, 1992; Rossi *et al.*, 1985). The PGs in turn further promote inflammation (Dayer *et al.*, 1986; Manogue *et al.*, 1992; Rossi *et al.*, 1985). Therefore, TNF-α has been implicated in many human diseases as a main mediator. For instance, TNF-α participates in the pathogenesis of rheumatoid arthritis and allergic inflammation (Firestein, 1994; Manogue *et al.*, 1992). LPS derived from Gram-negative bacteria also induces the production of TNF-α by macrophages, which then serves as a key mediator in septic shock (Firestein *et al.*, 1994; Mohler *et al.*, 1994; Novgrodiski *et al.*, 1994).

Because of its pivotal role in inflammatory disease, a significant effort has been focused on developing therapeutic agents that interfere with TNF-α production or action. These have included tyrphostin-related tyrosine kinase inhibitors (Novgrodiski *et al.*, 1994), pentoxifylline (Han *et al.*, 1990), thalidomide (Moreira *et al.*, 1993), various inhibitors of TNF-α processing (McGeehan *et al.*, 1994; Mohler *et al.*, 1994), a family of carbocyclic nucleosides (Bradshaw *et al.*, 1995; Firestein *et al.*, 1994; Parmely *et al.*, 1993; Sajjadi *et al.*, 1996), and new antiinflammatory benzylamide derivatives (Lang *et al.*, 1995).

There is an increasing amount of data indicating that various compounds such as lignans and flavonoids derived from plants may possibly possess various biological activities including antiviral, anticancer, antiallergic and immunoregulatory activities (Havsteen *et al.*, 1983; Hirano *et al.*, 1991; Hirano *et al.*, 1994; Sankawa *et al.*, 1985; Schröder *et al.*, 1990; Torrance *et al.*, 1979). Lignans including machilin A, meso-dihydroguaiaretic acid and (-)-sesamin possess a potent suppressive activity on mitogen-induced proliferation of lymphocytes *in vitro,* but not by the cytotoxicity (Hirano *et al.*, 1991). Previously we reported that some lignans isolated from Flos Magnoliae showed an inhibitory activity against TNF-α production from murine macrophage cell line (Chae *et al.*, 1997).

In this study, lignans isolated from rhizomes of *Coptis japonica* that has been used for Korean traditional me-

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dicine were investigated to find their inhibitory effect on TNF-α production using murine macrophage cell line (RAW264.7 cells) stimulated by lipopolysaccharide (LPS).

MATERIALS AND METHODS

Materials

Coptis japonica was obtained from a drug store (Dongyang Yakup Co.) in Korea as a herbal medicine which can be clinically used for Korean traditional prescription. Ten lignans were prepared using the extraction method reported by Yoshikawa and his coworkers (1995) (shown in Fig. 1 and Fig. 2). RAW264.7 cells are an Abelson leukemia virus-transformed murine monocyte-macrophage line obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Fetal bovine serum, RPMI1640, penicillin and streptomycin were obtained from GIBCO Lab. (Grand Island, NY, U.S.A.). LPS (*Escherichia coli* 0111:B4), hydrocortisone, dexamethasone, prednisolone, pentoxifylline and theophylline were products of Sigma Chem. Co. (St. Louis, MO, U.S.A.). Murine TNF-α ELISA kit was purchased from Amersham (Little Chalfont, Buckinghamshire, U.K.).

Preparation of samples

The lignans were dissolved in the mixture of propylene glycol, ethanol, and dimethyl sulfoxide (89.9:10.0:0.1). Samples were diluted in RPMI1640 medium (the final concentration of vehicle is less than 0.1 %). Preliminary test showed that 0.1% vehicle does not interfere with TNF-α production.

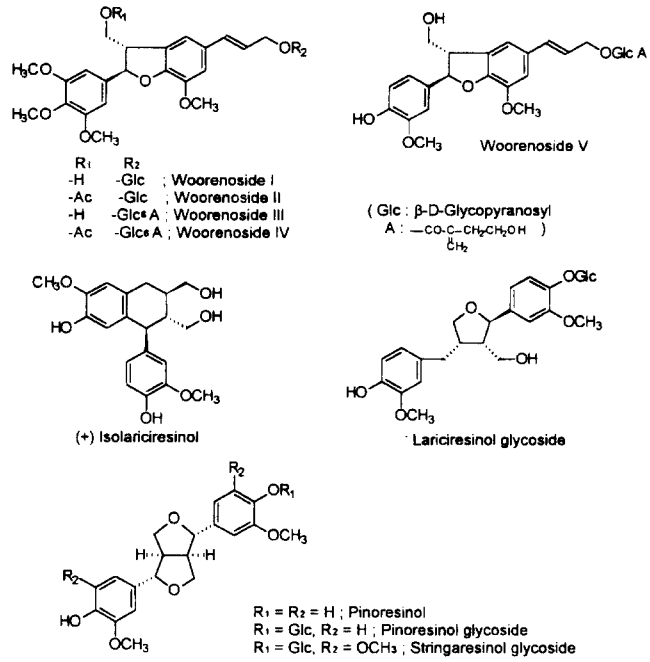

Fig. 1. Structures of lignans isolated from rhizomes of *Coptis japonica*.

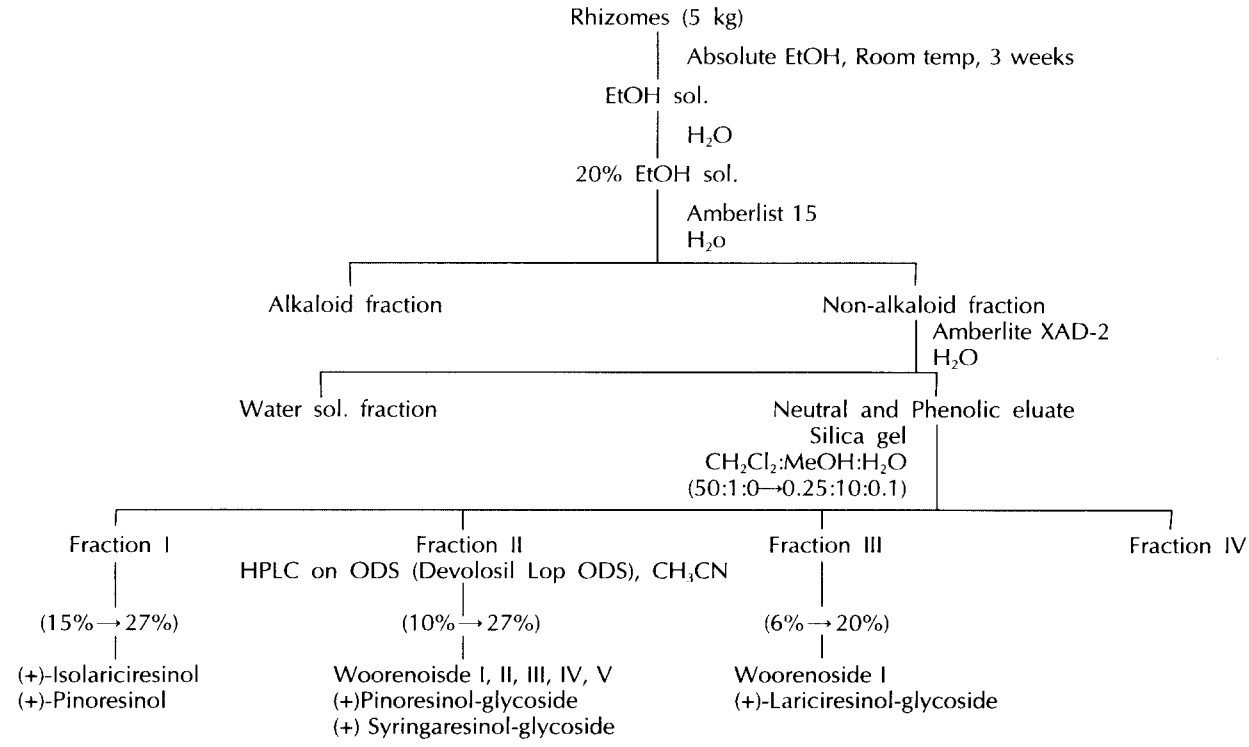

Fig. 2. Flow chart for isolation of lignans from *Coptis japonica*.

TNF-α production *in vitro*

RAW264.7 cells were maintained in RPMI1640 supplemented with 100 U/ml of penicilline and 100 μg/ml of streptomycin, and 5% fetal bovine serum. Cells were grown at 37°C in humidified air with 5% CO_2.

Before stimulation with LPS (1 μg/ml) and testing samples, RAW264.7 cells (1×10^6 cells/ml) were incubated for 18 hours in 24 well plates in the same condition. Stimuli and test samples were then added to the wells for 6 h, which was optimal for TNF-α production in our conditions. Supernatants were then collected and assayed for TNF-α content. Cell viability was assessed by Trypan blue dye exclusion and was always more than 95%.

TNF-α assay

Samples stored at -70°C were thawed and diluted appropriately, and TNF-α concentrations were determined by ELISA.

Statistical analysis

All values expressed as mean±SEM were obtained from three or four separated experiments. The Student's *t*-test for unpaired observation between control and experimental samples was carried out for statistical evaluation of the difference; p values of 0.05 or less were considered as statistically significant.

RESULTS AND DISCUSSION

RAW264.7 cells contained 0.5 ng/ml to 1 ng/ml of TNF-α as a basal level. When RAW264.7 cells were stimulated with 1 μg/ml of LPS for 6 h, TNF-α produced about 65 ng/ml in culture medium, more than 60~120-fold amount than the basal level. So, this condition was applied to analyze suppressive effect on TNF-α production by lignans and standard drugs.

Effect of antiinflammatory drugs and cyclic AMP phosphodiesterase (cAMP PDE) inhibitors on TNF-α production as a standard drug in LPS-stimulated RAW 264.7 cells was estimated and compared with testing samples (Table I). Steroid drugs such as dexamethasone, hydrocortisone and prednisolone significantly inhibited TNF-α production at 1 μM.

Cyclic AMP (cAMP) has an inhibitory effect on TNF-α production in the transcriptional level (Sajjadi *et al.*, 1996; Taffet *et al.*, 1989; Han *et al.*, 1990). The drugs that enhance intracellular cAMP level such as adenylate cyclase activators (forskolin and PGE_2), cell permeable cAMP (dibutylcyclic AMP and 8-bromo-cAMP) and cAMP PDE inhibitors (pentoxifylline and theophylline) also inhibit the TNF-α production in macrophage. In our results, pentoxifylline and theophylline decreased TNF-α production as 25.4% and 12.1% at 100 μM, respectively.

Table I. Inhibitory effect of standard drugs (anti-inflammatory drugs and cAMP phosphodiesterase (PDE) inhibitors) on TNF-α production in LPS-stimulated RAW264.7 cells

Class of compounds	Name of drug	% Inhibition
Steroids[1]	Dexamethasone	47.1±1.5**
	Hydrocortisone	40.1±6.1**
	Prednisolone	18.8±5.7*
cAMP PDE inhibitors[2]	Pentoxifylline	25.4±2.5**
	Theophylline	12.1±5.2

The RAW264.7 (1×10^6 cells/ml) cells stimulated with 1 μg/ml of LPS produced about 65 ng/ml of TNF-α and contained 0.5 ng/ml to 1 ng/ml of TNF-α as a basal level.
Data represent mean±SEM of 3 observations.
[1]All compounds were tested at 1 μM.
[2]All compounds were tested at 100 μM.
*: P<0.05 compared to control.
**: P<0.01 compared to control.

Coptis japonica in this study was selected by its frequent use in Korean traditional prescriptions for inflammation-related diseases and by a part of an elucidation on inhibitory activity of herbal midicines on TNF-α production. In our previous results, inhibitory potency of the total ethanol extract of *Coptis japonica* and its all solvent fractions except water layer strongly exhibited at 100 μg/ml and 50 μg/ml as a final concentration (Data not shown). On the basis of these results we tried to search the inhibitory constituents in *Coptis japonica* from solvent fractions.

Previous phytochemical studies on rhizomes of *Coptis japonica* mainly showed the presence of alkaloids including berberin, magnofiorine, sanguinarine, norsanguinarine, oxysanguinarine and 6-acetonyl-5,6-dihydorsanguinarin, and phenolic compounds from polar layer (Otsuka *et al.*, 1983; Yahara *et al.*, 1985). Recently, however, Yoshikawa and his coworkers (1995) reported that several novel and known lignans were widely distributed in non-alkaloid fraction from the total ethanol extract using column chromatography. However, the biological activities of their lignans were not confirmed yet. We previously reported that the lignans isolated from activity-guided fractionation of Flos Magnoliae effectively inhibit TNF-α production (Chae *et al.*, 1997). Because of their importance, therefore, we became to investigate the inhibitory effect of these lignans in view of TNF-α production.

The inhibitory effect of 10 lignans (25 μg/ml) isolated from *Coptis japonica* on TNF-α production is summarized in Table II. The inhibitory percent of each compound was in the range from -9.9% to 55.3%. Among them, pinoresinol exhibited the strongest inhibitory activity whose IC_{50} value is below 25 μg/ml (69.8 μM) which shows more effective than that of cAMP PDE inhibitors (IC_{50}=250~500 μM) but not steroid drugs (IC_{50}=5~40 μM).

Table II. Inhibitory effect of lignans isolated from *Coptis japonica* on TNF-α production in LPS-stimulated RAW264.7 cells

Compound[1]	% Inhibition
Woorenoside I	11.4±2.4
Woorenoside II	23.4±4.0*
Woorenoside III	19.8±3.8*
Woorenoside IV	30.4±2.1**
Woorenoside V	38.6±3.6**
(+)-Isolariciresinol	-9.9±5.5
Lariciresinol glycoside	36.8±1.8**
Pinoresinol	55.3±1.9**
Pinoresinol glycoside	9.4±5.8
Syringaresinol glycoside	6.1±8.3

The RAW264.7 (1×10^6 cells/ml) cells stimulated with 1 μg/ml of LPS produced about 65 ng/ml of TNF-α and contained 0.5 ng/ml to 1 ng/ml of TNF-α as a basal level.
[1]All compounds were tested at 25 μg/ml.
Data represent mean±SEM of 3 observations.
*: $P<0.05$ compared to control.
**: $P<0.01$ compared to control.

Pinoresinol is a well known lignan of furofuran type which is distributed widely in the plant kingdom. Pinoresinol and its derivatives show a variety of biological activities such as antioxidative effect, analgesic effect on writhing symptoms, and inhibitory activity of platelet activating factor (Okuyama *et al.*, 1995). However, because of low cytotoxicity against various cancer cell lines, pinoresinol was not cytotoxic against P-388 lymphocytic leukemia test system (Torrance *et al.*, 1979).

The structures of the lignans are so heterogenous that an apparent structure-activity relationship could not be obtained among the compounds described in the present study. In case of woorenoside derivatives, however, acetyl group of R_1 and A moiety of R_2 may play an important functional role in the inhibitory activity.

Thus, the results suggest that lignan constituents may partly participate in antiallergic and antiinflammatory effects of *Coptis japonica* through inhibition of TNF-α production. The inhibitory mechanism of these lignans such as pinoresinol, woorenoside-V and lariciresinol glycoside are not clear, and hence further studies related on their inhibitory mechanism and other biological activities are required. In addition, the toxicity of pinoresinol needs to be precisely evaluated *in vitro* and acute or subacute toxicity test *in vivo*.

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