Note

Effect of Ginsenosides and Red Ginseng Water Extract on Tumor Necrosis Factor-α Production by Rat Peritoneal Macrophages

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The effects of ginsenosides and extracts containing melanoidins, which are components of red ginseng, on TNF-α production by rat peritoneal macrophages were examined. The ginsenosides by themselves had no effect on TNF-α production, whereas water extract containing melanoidins, which are Maillard reaction products, stimulated TNF-α production by rat peritoneal macrophages. The total ginsenoside fraction and ginsenoside Rc increased TNF-α production, whereas water extract containing melanoidins, which are components of red ginseng, stimulated TNF-α production with LPS, as did stimulation by ginsenosides with the water extract. These results suggest that for a healthy immune response it might be better to consume red ginseng itself, which contains a mixture of ginsenosides, polysaccharides and Maillard reaction products, rather than ingesting pure ginseng alone. Red ginseng as an immuno-modulator may be effective in defending against infections or tumors.

Keywords: red ginseng, macrophage, tumor necrosis factor, ginsenoside, polysaccharide, melanoidin

Materials and Methods

Materials Soluble starch (Wako Pure Chemical Industries Ltd., Osaka), Bacto-peptone (Difco Laboratories Co, Detroit, MI), and lipopolysaccharide (LPS, Sigma Chemical Co., St. Louis, MO), were used. Ginsenosides: Fig. 1 shows the chemical structures of the ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) used in this study. Samples of ginsenosides derived from Korean red ginseng were kindly provided by the Korea Ginseng and Tobacco Research Institute (Taejeon, Korea). An appropriate amount of each one was dissolved in 50% (v/v) ethanol prior to use.

Animals Male Sprague-Dawley (SD) rats weighing 300–500 g were purchased from Charles River Japan Co. (Atsugi, Japan).

Preparation of melanoidin fractions Melanoidin fractions of panax ginseng were extracted from Korean red ginseng powder three times with water while stirring for 24 h at cold-room temperature. The extract was centrifuged, the clear supernatant fluid was concentrated to a suitable volume, and dissolved constituents were precipitated by addition of 5–6 volumes of ethanol. A portion of the resulting sample was dialyzed against tap water for 3 days and against distilled water for 1 day. The non-dialyzed portion was freeze-dried (sample L), and the dialyzed portion was fractionated by chromatography using a Bio-Gel P-2 column with water elution (samples S-1 and S-2).

Macrophage collection Macrophages were collected by the method described previously (Watanabe et al., 1990). Briefly, a mixture of soluble starch and Bacto-peptone (5% w/v each) was injected intraperitoneally into male SD rats (dosing volume: 5 ml per 100 g body weight). Four days thereafter, the rats were sacrificed by cutting the carotid artery under diethyl ether anesthesia and peritoneal cells were harvested. The cells were suspended in RPMI 1640 medium (Gibco, NY) supplemented with

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10% fetal calf serum (FCS, Filtron Co., Victoria, Australia). The macrophages were seeded at 1×10^5 cells per well in a 96-well microplate (Falcon, #3072) and incubated for 2 h at 37˚C in an atmosphere of 5% carbon dioxide in air. Then, the plates were washed three times with Hanks balanced salt solution (HBSS) to wash out non-adherent cells, and then the cells were incubated for 24 h in RPMI-1640 containing 10% FCS.

Stimulation of macrophages and measurement of TNF-α
After 24-h of incubation, the macrophages were washed with HBSS 3 times and suspended in HBSS in each well. The samples of ginseng were added to the wells and LPS or melanoidins were added to stimulate TNF-α production. After 6-h of incubation, the supernatants were removed to estimate TNF-α production levels. The supernatant samples were stored in a freezer at −30˚C until measurement of the TNF-α concentration by enzyme linked immunosorbent assay (ELISA) This was performed using a rat TNF-α ELISA kit (R&D Systems Inc., Minneapolis, MN).

Statistical analysis
The extent of promotion of TNF-α release by various ginsenosides in each separated experiment was calculated and the values were compared using the unpaired Student’s t-test. Significance was denoted by p<0.05.

Fig. 1. Chemical structures of the ginsenosides used in the experiments.
Results and Discussion

Properties of melanoidin fractions From 100 g of Korean red ginseng powder, 5.4 g of sample L, 3.83 g of sample S-1, and 4.51 g of sample S-2 were finally obtained. According to Sephadex G-25 gel filtration, the molecular weight of sample L, S-1, and S-2 was 4400–4800, 3700–4000, and 1800–2100, respectively. Sugar composition was: sample L: xylose 44.4%, glucose 42.9%, S-1; xylose 43.8%, glucose 52.1%, S-2; xylose 35.1%, fructose 9.5%, glucose 48.6%, respectively. These samples were used for the trypsin inhibitory activity test, which is a test for high molecular weight melanoidin (Hirano et al., 1994). Trypsin inhibition activity of these fractions as soybean trypsin inhibitor equivalent was under 0.001%. From these results it was determined these fractions contain mainly polysaccharides and a small amount of low molecular weight melanoidins: L (Mw. ca. 1800–2100), and almost no high molecular weight melanoidin.

Effect of ginsenosides on TNF-α production with or without concomitant stimulation with LPS Figure 2 shows the effects of added melanoids on TNF-α production by the rat macrophages. The ginsenosides had no effect on TNF-α production in the absence of LPS (data not shown). While, the extract fractions induced TNF-α production in a dose-dependent manner. The order of potency of the extract fractions in stimulating the production was S-1 > S-2 > L. To compare the potency of the effect on TNF production, we defined the effective dose as 600, which indicated the concentration eliciting half of the maximum production (about 1200 pg/ml). The EC50 of fractions S-1, S-2, and L were 5.29, 7.10, and 50.36 μg/ml, respectively. From these results, it was clear that the low molecular weight fraction was more effective in stimulating TNF-α production by the rat peritoneal macrophages. The effective dose 600 of the S-1 fraction was lower than that of the L fraction.

To identify ginsenosides effective in the synergism production of TNF-α, we performed experiments of LPS stimulation in the macrophages. Figure 3 shows the amount of TNF-α production by macrophages upon addition of ginsenosides with concomitant LPS stimulation. Stimulation of LPS alone (1 μg/ml) induced 770 pg of TNF-α production. The total ginsenoside fraction (TS), and some of the individual ginsenosides examined, Rb1, Rb2, and Rc, enhanced the LPS-induced TNF production. The effects of TS and Rc were stimulatory in the range of 10 to 100 μg/ml. Other ginsenosides showed no enhanced effect, but also little inhibitory effect. Among the effective ginsenosides, with the exception of TS, Rc showed the most effective production.

Increasing effects of ginsenosides and extract fractions on TNF-α production Figure 4 shows the increasing effects of some ginsenosides on TNF-α production induced by S-1 and S-2 fractions of the extracts, with the exception of the L-fraction. The increase effect of TS was significantly greater than that observed for Rc, which means that the TS fraction may contain other ginsenosides which have synergistic effects. It seems that the activity of the TS fraction may be the sum of the activity of the individual ginsenosides (Fig. 3). Especially, Rc ginsenoside...
showed a increasing effect with LPS. Considering the chemical structure of the ginsenosides, only Rc has a terminal ribose moiety (Fig. 1), that may be the reason for the strong increase effect observed.

There are reports of the synergistic effects of ginseng in combination with other active agents. Doda et al. (1999) reported that American ginseng extract and the breast cancer therapeutic agent estradiol synergistically inhibited MCF-7 breast cancer cell growth. Chung et al. (1998) reported that ginsenoside Rg1 with cAMP synergistically induced glucocorticoid receptor-mediated transcription. In the present study, these synergistic effects seem similar to the down-regulatory effect described in the report on glucocorticoid receptor-mediated transcription (Chung et al., 1998).

It is known that there is almost no absorption of indigestable melanoidin into the body at the lumen of intestine (Faist & Erbersdobler, 2001). However, in the intestine these melanoidins may be effective in gut immunity, for example, immunoregulation and immunologic tolerance in the intestine.

In conclusion, it is evident that ginseng is effective in stimulating TNF-α production by rat peritoneal macrophages. The consumption of ginseng by itself, which contains a mixture of ginsenosides, polysaccharides and Maillard reaction products, may be more effective than ingesting a pure ginsenoside. It seems that in some cases red ginseng has an inhibitory effect and in other it has a stimulatory effect. More detailed investigation is necessary to determine the combined effects of defense function against infection and tumor.

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References