Induction of Cytokines by a Phenylpropanoid Glycoside Acteoside

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A phenylpropanoid glycoside acteoside was found to induce interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) in macrophage-like cell line J774.A1 at 1—100 ng/ml. In addition, when the stimulatory action of acteoside was studied using the bovine glomerular endothelial cell line GEN-T, it stimualted IL-6 production. These stimulatory activities were not abolished by treatment with polymixin B, which inactivates lipopolysaccharide (LPS), indicating that the action was not a contamination of LPS.

Key words acteoside; phenylpropanoid; interleukin-1; interleukin-6; tumor necrosis factor-α

Acteoside is aphenylpropanoid glycoside and is widely distributed in the plant kingdom. Many studies have reported that acteoside shows enzyme inhibitory activities against 5-lipoxygenase,[1] protein kinase C,[2] and cyclic-AMP phosphodiesterase[3] *in vitro*. In addition, previous *in vivo* studies found antiinflammatory,[4] antianoxia,[5] antihypertensive,[6] immunosuppressive,[7] antinephritic,[8] and antihepatotoxic activity.[9] We have also found that acteoside isolated from *Scutellaria salvifolia* Bentham (Lamiaceae) and *Phlomis armeniaca* Willd. (Lamiaceae) shows cytotoxic activity against cancer cells at the relatively high concentration of more than 10 μg/ml.[10—12] In this study, we investigated in detail the biological activities of acteoside using the mouse macrophage-like cell line J774.A1 and bovine glomerular endothelial cells (GEN-T) and found that it had activities similar to lipopolysaccharide (LPS) at a lower concentration than previously reported.

J774.A1 cells (5×10^5 cells/ml) were incubated in RPMI 1640/10% FCS and primed with interferon (IFN)-γ (10 U/ml) for 2 h, followed by incubation with varying amounts of acteoside for 48 h. GEN-T cells (5×10^5 cells/ml) were also treated in the same manner with J774.A1, except for priming with IFN-γ. The final culture medium were used for determining tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 concentrations secreted by J774.A1 and GEN-T cells. TNF-α and IL-1β were measured by ELISA (Bio source) and IL-6 was determined by proliferation of IL-6-dependent MH-60.BSF-2 hybridoma cells (kindly provided by Dr. Toshio Hirano, Osaka University).

The structure of acteoside is depicted in Fig. 1. When IFN-γ-primed J774.A1 cells were incubated with increasing doses of acteoside for 48 h, dose-dependent production of TNF-α, IL-1β, and IL-6 was observed, as shown in Fig. 2. Significant amounts of TNF-α (630.2±27.0 ng/ml), IL-1β (267.0±33.1 pg/ml), and IL-6 (79.3±10.3 pg/ml) were secreted by 1 μg/ml of acteoside. IL-6 production was detected at a concentration of 1 ng/ml (about 1.6 nM). This activity may be the most potent ever reported in *in vitro* studies of acteoside. Although the data are not shown, pretreatment of acteoside with polymyxin B (10 μg/ml), which inactivates LPS,[13] did not affect cytokine induction. This means that this action of acteoside was not due to the contamination with LPS.

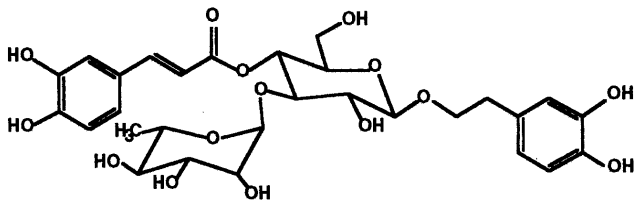

Fig. 1. Structure of Acteoside

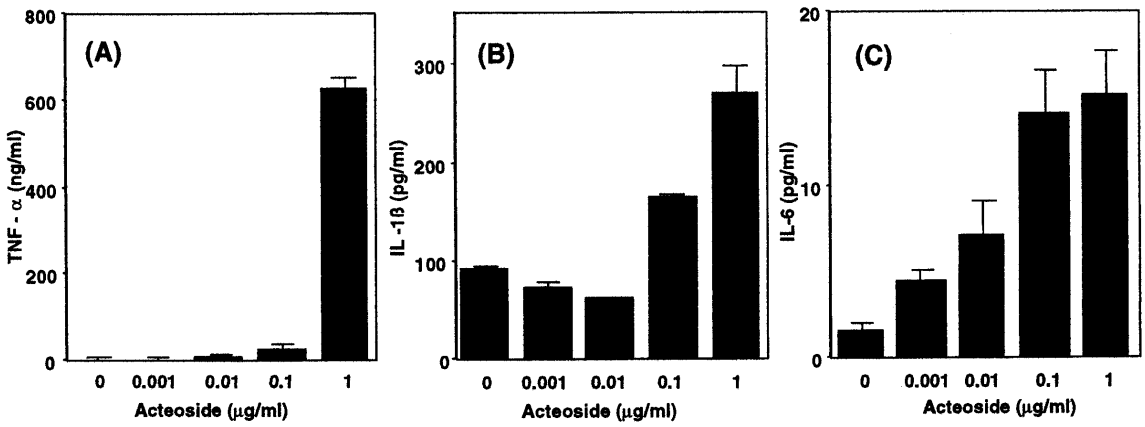

Fig. 2. Dose-Dependent Production of (A) TNF-α, (B) IL-1β, and (C) IL-6 by J774.A1 Cells

J774.A1 cells (5×10^5 cells/ml) were pretreated with IFN-γ (10 μ/ml) for 2 h and then incubated for another 48 h in the presence of varying doses of acteoside. The resulting medium was used for the assay of (A) TNF-α and (B) IL-1β by ELISA and of (C) IL-6 by proliferation of IL-6-dependent MH-60. BSF-2 cells. Data are represented as the mean±S.E. of 6 wells.

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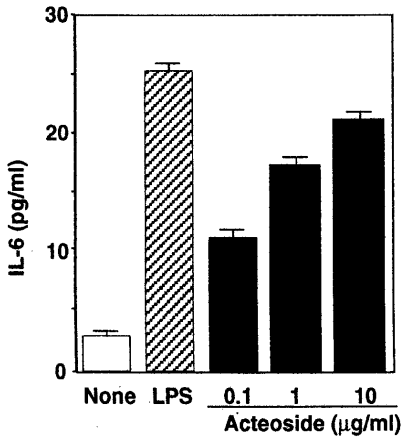

Fig. 3. Dose-Dependent Production of IL-6 by GEN-T Cells

GEN-T cells (5×10^5 cells/ml) were incubated with medium alone, LPS (10 μg/ml), or increasing doses of acteoside for 48 h. IL-6 in medium was determined by the proliferative response of IL-6-dependent MH-60. BSF-2 cells. Data represented as the mean±S.E. of 6 wells.

Next we determined the effect of acteoside on IL-6 production in GEN-T cells. As shown in Fig. 3, acteoside was able to induce IL-6 production in GEN-T cells at similar doses as in J774.A1 cells. These results in terms of cytokine induction indicate that acteoside activates macrophage-like cells and endothelial cells, similar to LPS. Substances reported to activate macrophages or endothelial cells to date were restricted to microbes and some cytokines in addition to plant-derived polysaccharides, lectins, and tannin.[14] In this respect, acteoside could be the first plant-derived substance with low molecular weight (M.W. 624) found to activate macrophages and endothelial cells. Although the data are not shown, the study of the structure–activity relationship indicated that caffeic acid, a component of acteoside, did not show cytokine-inducing activity. In addition, caffeic acid phenethyl ester, which is more hydrophobic than caffeic acid, was reported to be a potent and specific inhibitor of activation of a nuclear transcription factor, NF-κB,[15] which plays an important role in induction of inflammatory cytokines such as IL-1, IL-6, and TNF-α. These results suggest that the cytokine-inducing activity might be unique to acteoside, but not the product of its degradation. We previously reported that acteoside shows cytotoxic activity against several tumor cells with IC_{50}s more than 10 μg/ml. J774.A1 and GEN-T cells were killed by acteoside at concentrations higher than 50 μg/ml, a concentration much higher than that reported in this study. It is evident that its cytotoxicity depends on the generation of hydrogen peroxide in the culture (data not shown). In contrast, cytokine-inducing activity was not mediated by hydrogen peroxide (data not shown), thus suggesting that cytokine-inducing activity and cytotoxic activity induce different types of signals in cells. In conclusion, acteoside shows biphasic biological activity at different concentrations; that is, cytokine-inducing activity at a fairly low concentration and cytotoxic activity at a high concentration.

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