Investigation of the interactive effects between emodin and genistein in rat vascular smooth muscle cell line A-10

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Summary

Interactive effects between emodin and genistein were investigated using the rat vascular smooth muscle cell line A-10. Genistein significantly retarded the proliferation. While emodin alone also retarded the proliferation, its effect was reversed in the presence of genistein. It appeared that emodin cancelled the effect of genistein. Exposure to high concentrations of genistein resulted in a reduction of TNF-α induced MCP-1 secretion. Concomitant treatments of emodin and genistein had more effect on TNF-α induced MCP-1 secretion compared to emodin treatment alone. A slight decrease in TIMP-2 secretion was observed in 100 μM genistein-treated cells, and the results of concomitant treatments of emodin and genistein showed the same tendencies as those seen with MCP-1 secretion. Consequently, it is likely that their effects on MCP-1 and TIMP-2 secretions were additive. With regard to MCP-1 and TIMP-2 secretions, it is suggested that emodin and genistein share the same signal transduction pathways, and thus reinforce the signal transduction to exert the additive effects. Conversely, emodin appeared to interfere with the effect of genistein on proliferation, suggesting that the mechanism of inhibition of proliferation is different from those of the other two phenomena.

Key words: emodin, genistein, MCP-1, TIMP-2

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Introduction

Potentially beneficial food functionalities of plant products have been the focus of considerable attention. So far, most of the food functionalities of plant products have been studied using single compound. However, because we take multiple plant products in our daily life, to evaluate the benefit of plant product properly, the effects of combinations of plant products should be addressed.

We previously showed the anti-atherogenic potential of emodin1,2), an anthraquinone from Chinese herbs and certain fungi. Emodin retarded the proliferation of rat vascular smooth muscle cell A-101), and reduced the secretions of MCP-1 and TIMP-2 in A-10 cells2). In addition, the anti-atherogenic activities of another plant product genistein have been reported1,3-6). In this study, therefore, we treated A-10 cells with emodin and genistein concomitantly, and examined the interactive effects between these two compounds.
**Materials and Methods**

**Chemicals and Cells** Emodin and human tumor necrosis factor (TNF)-α were purchased from Calbiochem-Novabiochem Corporation (Germany). Genistein was purchased from Wako Pure Chemical Industries (Japan). Fetal calf serum (FCS) and dextran-coated charcoal were purchased from JRH Biosciences Inc. (USA) and Sigma-Aldrich (USA), respectively. Cell Proliferation ELISA, BrdU (colorimetric) was purchased from Roche Diagnostics (Switzerland). The rat aortic myoblast cell line A-10 was purchased from American Tissue Culture Collection (U.S.A.). A-10 cells were cultured in phenol red-free Dulbecco’s Modified Eagles medium containing 10% FCS. FCS was treated with charcoal overnight before use.

**Treatments of Cells and Assays** The method used to measure 5-bromo-2-deoxyuridine (BrdU) incorporation during DNA synthesis was identical to that of Nagashima and Goto. Two times 10^3 A-10 cells in 100 μl with chemicals in dimethyl sulfoxide were placed in each well of a 96-well microtiter plate for BrdU incorporation (Cell Proliferation ELISA, BrdU (colorimetric)). Cell proliferation was examined after 24 hr-culture. The methods used to measure monocyte chemotactic protein (MCP)-1 and tissue inhibitor of metalloproteinases (TIMP)-2 were identical to those of Nagashima et al. Approximately 7.5 x 10^4 cells in 0.5 ml of medium with or without 25 ng/ml (1.43 pM) human TNF-α were cultured in each well of a 24-well culture plate for 24 hr followed by collection of medium. Collected media were centrifuged at 1,000 x g for 5 min to remove cell debris, and the resulting supernatants were subjected to assays. The levels of MCP-1 and TIMP-2 were quantified using Monocyte chemoattractant protein-1 [(r)MCP-1], rat ELISA system (Amasham Biosciences Corp., U.S.A.) and Tissue inhibitor of metalloproteinases-2 (TIMP-2), human, ELISA system (Amasham Biosciences Corp.), respectively.

**Statistics** Data were analyzed statistically by the two tailed Student’s t-test, and expressed as mean ± S.D.

**Results**

Fig. 1A shows the effects of concomitant treatments of emodin and genistein on BrdU incorporation. As we reported previously, high concentration (100 μM) of emodin inhibited proliferation considerably, and the treatment of genistein alone caused a marked dose-dependent retardation of A-10 cell proliferation (Fig. 1A). The difference between the percent activities of 0 μM and 100 μM genistein-treated samples was 98.4%. On the other hand, in the presence of genistein, emodin boosted the proliferation slightly (25 μM) or markedly (50 μM and 100 μM) (Fig. 1A). At 50 μM and 100 μM emodin, the differences between the percent activities of 0 μM and 100 μM genistein-treated samples decreased to 78.2% and 1.5% respectively. As a result, it appeared that all the lines converged on 50% activity of control at 100 μM emodin (Fig. 1A). Interpretation of these results is that emodin cancelled the effect of genistein. At 100 μM emodin, its effect was predominant, and all the values were similar regardless of the presence of genistein.

Table 1 shows the inhibitory effect of genistein on 25 ng/ml TNF-α induced MCP-1 secretion in A-10 cells. Exposure to high concentrations of genistein (50 μM and 100 μM) resulted in a
significant reduction of MCP-1 secretion (Table 1). Compared to emodin, however, its effect was moderate. Our results are consistent with previous reports that genistein inhibits MCP-1 secretion.

As shown in Fig. 1B, concomitant treatments of emodin and genistein reduced TNF-α induced MCP-1 secretion considerably more than emodin treatment alone, and the effect of genistein appeared to be dose-dependent. It is conceivable that their effects were additive and emodin did not interfere with the effect of genistein. These results (Fig. 1B) were totally different from those of BrdU incorporation (Fig. 1A).

No one has reported the effect of genistein on TIMP-2 secretion. Thus, we investigated the effect of genistein on TIMP-2 secretion for the first time using A-10 cells (Table 2). A slight but statistically significant (p < 0.05) decrease in TIMP-2 secretion was observed in 100 μM genistein-treated samples (Table 2). Kim et al. reported the effect of genistein on TIMP-2 mRNA levels, but not TIMP-2 secretion. According to their results, genistein down-regulated and up-regulated the expression of TIMP-2 in rat NK cells and Jurkat T leukemia cells, respectively.

With regard to TIMP-2 secretion (Fig. 1C), although obvious dose-dependent effect of genistein was not observed, the results were basically the same as those of MCP-1 secretion (Fig. 1B). Consequently, it appeared that their effects on TIMP-2 secretion were additive.
Discussion

In many cases, combination of the independent treatments results in the additive effects. Verma et al., for instance, reported that genistein and curcumin exhibited additive inhibitory effects on pesticide-induced proliferation using human breast cancer cell MCF-7\(^{11}\). Likewise, Tanos et al. showed that tamoxifen enhanced the antiproliferative effect of genistein in human dysplastic epithelial breast cell lines\(^{12}\). We now report that the effects of concomitant treatments of emodin and genistein on MCP-1 and TIMP-2 secretions exhibited the same tendencies (Fig. 1B, 1C). We suggest that in these four cases, genistein and its counterparts share signal transduction pathways, and therefore reinforce the signal transduction to exert the additive effects. On the contrary, emodin appeared to interfere with the effect of genistein on proliferation (Fig. 1A), suggesting that the mechanism of inhibition of proliferation is different from those of the other two phenomena in A-10 cells. It is noteworthy that emodin is reported to exert the inhibition of kinase activities\(^{13-16}\). It is possible that emodin inhibited certain kinases, and this inhibition antagonized the signal transduction of genistein. With respect to MCP-1 and TIMP-2 secretions, presumably different signal transduction pathway(s) to those involved in cell proliferation dominated, therefore, emodin boosted rather than interfered the effects of genistein.

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References

ラット血管平滑筋細胞におけるエモジンとゲニスチンの効果の相互作用の検討

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エモジンとゲニスチンの効果の相互作用をラットの血管平滑筋細胞であるA-10を用いて検討した。ゲニスチンは細胞増殖を顕著に阻害した。エモジンの単独処理は細胞増殖を阻害したが、ゲニスチン存在下ではエモジンは逆の効果を示した。このことは、エモジンがゲニスチンの効果を打ち消すためと考えられた。高濃度のゲニスチンへの暴露は、腫瘍性因子（TNF）の誘導性の単球走化性タンパク質（MCP-1）の分泌を減少させた。エモジンとゲニスチンの同時処理がTNFの誘導性のMCP-1分泌に与える影響は、エモジン単独処理に比べて強く、ゲニスチンの処理によって、メタボロマーゼ酵素細胞因子（MCP-1）の分泌がわずかに減少した。また、エモジンとゲニスチンの同時処理の結果は、TNFの分泌と同様の傾向を示した。従って、これらの化学物質のエモジンとゲニスチンの分泌に与える影響は、相加的であると考えられた。エモジンとゲニスチンの分泌に関しては、エモジンとゲニスチンが同じシグナル伝達経路を共有し、シグナル伝達を強化した結果、相加効果を現していると考えられた。これに対し、エモジンは細胞増殖に対するゲニスチンの影響を妨害すると考えられるので、細胞増殖阻害は他のエモジンの現象とは異なるメカニズムで起こると考えられた。

キーワード：エモジン、ゲニスチン、MCP-1、TIMP-2