Short Communication

Radicicol, an Agent Inducing the Reversal of Transformed Phenotypes of src-Transformed Fibroblasts

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Recent extensive studies on viral and cellular oncogenes have shown that many kinds of protein-tyrosine kinases are important in fundamental cellular regulation such as growth factor-mediated signal transduction, cell division control, and cell differentiation.1-2 The malfunctioning of the protein-tyrosine kinases has also been found to be one of the causes of cell transformation and carcinogenesis. For example, in Rous sarcoma virus (RSV)-infected rat fibroblast cells, morphological changes are induced by a single transforming gene, src, whose product, p60src, has protein kinase activity specific to tyrosine residue.3-4 To discover new types of antitumor agents, we conducted a screening program for microbial agents that convert the transformed morphology of RSV-infected rat fibroblast cells, SR-3Y1-2 (referred to as SR), to the normal morphology. As a result of screening, we found a potent activity in the culture broth of a fungus, strain KF9. The active agent was identified as radicicol (synonymous with monorden), which had been reported as a macrocyclic fungal antibiotic.5-6 Here, we report the isolation and identification of radicicol as an agent showing a novel biological activity on the src-transformed cell line.

Strain KF9 was isolated from a soil sample from Tottori-ken, Japan. It was cultivated in a 5-liter flask containing 1 liter of 1% glucose, 0.5% Polypepton, 0.3% malt extract (Difco), and 0.3% yeast extract (Difco); pH 6.0, at 26°C for 7 days on a rotary shaker at 115 rpm. The active compound was extracted with acetone from the wet cells (240 g) and was chromatographed on a silica gel column chromatography (Merck, Kieselgel 60, 3 x 15 cm) eluted with CHCl3-methanol (98:2). The active fractions were collected and further purified by successive HPLC; first, with a column of Senshu Pak Aquasil SS (8 x 250 mm) using a solvent system composed of CHCl3-HCOOH (100:0.5), and second, with a reverse phase column of Senshu Pak SSC-ODS-H-3251 (8 x 250 mm) using methanol-water (7:3). The final active fraction was evaporated to dryness to give 32 mg of pure colorless needles called KF9-A substance.

The molecular formula of KF9-A was C18H18ClO6 by EI and HRFAB-MS data (MW 364.782). The 1H NMR spectrum indicated the presence of two phenolic hydroxy protons (δH = 6.7, δH = 11.2), methine protons of an epoxide (δH = 2.96, 1H, ddd, J = 2.8, 11 Hz, 1H, s), vinyl protons (δH = 5.84, 1H, d, J = 2, 5, 11 Hz; δH = 6.05, 1H, d, J = 16 Hz; δH = 6.19, 1H, d, J = 8, 11 Hz; δH = 7.47, 1H, dd, J = 11, 16 Hz) and methylene protons (δH = 0.07, 1H, m, J = 15 Hz; δH = 2.43, 1H, ddd, J = 15 Hz). The IR spectrum (KBr disc) had a broad band at 3500 cm⁻¹ ascribable to a phenolic hydroxyl group. UVmax was 265 and 315 nm in methanol. All these data indicated that this compound is identical to radicicol. This was confirmed by direct comparison of the 13C NMR spectral data of KF9-A with that of authentic radicicol (data not shown).

![Chemical Structure of KF9-A (Radicicol)](image)

Fig. 1. Chemical Structure of KF9-A (Radicicol).

![Effects of Radicicol on the Morphology of SR Cells](image)

Fig. 2. Effects of Radicicol on the Morphology of SR Cells. Shown are a control culture of SR cells (A), a culture treated with 0.5 μg/ml of radicicol for 12 hr (B), and another control culture of 3Y1 rat fibroblasts which is the normal counterpart of SR cells (C).
Reversal of src-Transformed Phenotypes by Radicicol

Monoclonal src antibody (Oncogene Science, Inc.) and anti-phosphotyrosine antibody (PY20, ICN Immunobiological). Tyrosine-phosphorylated proteins were detected using streptavidin biotinylated peroxidase complex and an ECL Western blotting detection system (Amersham). As shown in Fig. 3A, radicicol reduced the amount of the phosphorylated form of p60SRC in a dose-dependent manner. On the other hand, a pulse-labeling experiment of the radicicol-treated cultures with [35S]methionine showed that synthesis of p60SRC was not inhibited significantly by the antibiotic treatment (Fig. 3B). Thus, it is most likely that the reversal of the transformed phenotype of SR cells caused by radicicol is due to the inhibition of p60SRC in vivo.

A variety of microbial metabolites, such as herbimycin, herbstatin, and genistein with no structural similarity, have been reported as inhibitors of tyrosine kinases in vivo. Here we add another agent with a different structure, radicicol, which shows a potent activity in vivo. We assume that the agent may be useful in elucidating the role of the src oncogene in the complicated mechanisms of signal transduction cascades and possibly in cancer chemotherapy.

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References