

Anti-proliferation Effects of Isorhamnetin Isolated from *Persicaria thunbergii* on Cancer Cell Lines

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Abstract – Isorhamnetin from the aerial parts of *Persicaria thunbergii* has been reported to have anti-tumor activity mediated by the inhibition of farnesyl protein transferase. In this study, we investigated the anti-proliferation effects of isorhamnetin on NIH3T3, K-RAS, H-RAS and SW620 cells, and it showed anti-proliferative effects in a dose-dependent manner with IC₅₀ value 4.1, 7.9, 20.2, and 22.4 µg/ml, respectively.

Keywords – Isorhamnetin, *Persicaria thunbergii*, anti-proliferative activity

Introduction

Isorhamnetin is a flavonol compound which has been occasionally isolated from plant. Various pharmacological activities of isorhamnetin have been reported including inhibitory effects of telomerase (Yang *et al.*, 2004), farnesyl protein transferase (Oh *et al.*, 2005), prolyl endopeptidase (Sultanova *et al.*, 2004), and xanthine oxidase (Nagao *et al.*, 1999). And also demonstrated anti-salmonella (Phadungkit and Luanratana, 2006) and anti-tumor (Teng *et al.*, 2006) activities as well as protective effects on H₂O₂-induced chromosomal damage to WIL2-NS cells (Saito *et al.*, 2004) and cardiovascular effects (Ibarra *et al.*, 2002). Isorhamnetin has various characteristics that make it a potential anti-cancer compound. These functions include cell cycle regulation and induction of tumor cell apoptosis (Hibasami *et al.*, 2005; Yang *et al.*, 2004). Here, in the present study, we evaluated the effects of isorhamnetin on anti-proliferative activity in NIH3T3, K-RAS, H-RAS and SW620 cells.

Experimental

General procedure – TLC was carried out on Merck

precoated silica gel F₂₅₄ plates, with Kiesel gel 60 (230-400 mesh, Merck) used as the silica gel. Sephadex LH-20 was used for the column chromatography (Pharmacia, 25 - 100 µm). The column used for LPLC was Lobar-A (Merck Lichroprep Si 60, 240 - 10 mm). ¹H- and ¹³C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. All other chemicals and solvents were analytical grade and used without further purification.

Plant materials – The aerial parts of *P. thunbergii* were collected and air-dried in October 2005 at Samrye, Jeonbuk, Korea. A voucher specimen was deposited in the herbarium of the college of pharmacy, Woosuk University (WSU-05-010).

Extraction and isolation – The dried plant material (300 g) was extracted with MeOH at room temperature and filtered. The filtrate was evaporated *in vacuo* to give a dark brownish residue. The resultant methanolic extract (95 g) was followed by successive solvent partitioning to give *n*-hexane (4 g), CHCl₃ (8 g), EtOAc (3 g), *n*-BuOH (18 g) and H₂O soluble fractions. Silica gel column chromatography of the EtOAc soluble fraction with CHCl₃-EtOAc-MeOH (10:1:1) gave three fractions (fr.1-fr.3). The major fraction fr.2 was rechromatographed on the Sephadex LH-20 column (MeOH) and purified by Lobar-A column chromatography (CHCl₃-EtOAc, 6:1) to yield compound **1** (16 mg).

Isorhamnetin (1) yellow powder; mp. 303 - 304 °C;

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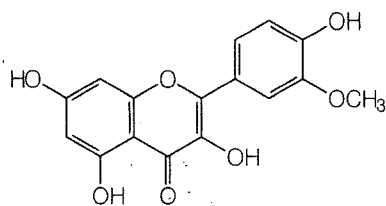


Fig. 1. Structure of isorhamnetin.

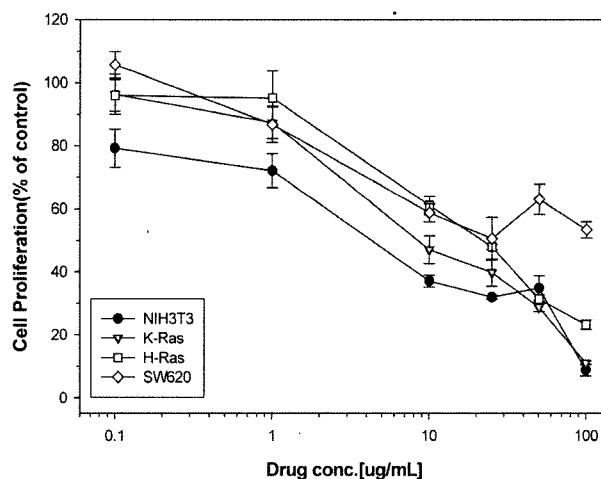
$^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz) δ : 12.43 (1 H, s, 5-OH), 7.73 (1 H, d, $J=1.9$ Hz, H-2'), 7.66 (1 H, dd, $J=8.0$, 1.9 Hz, H-6'), 6.91 (1 H, d, $J=8.0$ Hz, H-5'), 6.46 (1 H, d, $J=1.8$ Hz, H-8), 6.18 (1 H, d, $J=1.8$ Hz, H-6), 3.82 (3 H, s, OCH₃); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6): 176.0 (C-4), 164.1 (C-7), 160.8 (C-5), 156.4 (C-9), 148.9 (C-3'), 147.6 (C-4'), 146.8 (C-2), 136.0 (C-3), 122.1 (C-1'), 121.9 (C-6'), 115.7 (C-5'), 111.8 (C-2'), 103.2 (C-10), 98.4 (C-6), 93.8 (C-8).

Cell lines and culture – NIH3T3 vector cells and H-ras and/or K-ras transformed NIH3T3 cells were grown in DMEM (Gibco/BRL) supplemented 10% heat-inactivated FBS (Gibco/BRL). SW620 cells was grown in RPMI1640 (Gibco/BRL) supplemented 10% heat-inactivated FBS (Gibco/BRL). Cell cultures were maintained at 37 °C in a humidified incubator containing 5% CO₂. Geneticin was used for selection and maintenance of stable transformants. In experiments, cells were seeded at a density of 3×10^5 per 10-cm dish. After incubation for 24 h, cells were treated with each compound.

Cell proliferation assay – Cells were seeded at a density of 5,000 cells/well in a 96-well microtiter plate. Cells were counted with a hemocytometer. After 24 hr, cells were replenished with fresh complete medium containing compounds or 0.1% DMSO. After incubation for 48 hr, cell proliferation reagent WST-1 (Roche) was added to each wells. The amount of WST-1-formazan produced was measured at 450 nm by ELISA Reader (Bio-Rad). All data were the mean \pm SD of three independent determination.

Results and Discussion

In the course of our search for anti-cancer compounds from natural products, it was observed that isorhamnetin isolated from the aerial parts of *Persicaria thunbergii* has been showed to have anti-tumor activity mediated by the inhibition of farnesyl protein transferase (Oh *et al.*, 2005). The farnesyl protein transferase inhibitors have been reported as a promising target for the development of anti-cancer agent in *ras*-activated cancer cell lines (Gibs *et al.*, 1994). We investigated the proliferation inhibitory



NIH3T3 : mouse fibroblast cell line
 K-RAS : K-*ras* transformed NIH3T3 cell line
 H-RAS : H-*ras* transformed NIH3T3 cell line
 SW620 : colon cancer cell line (K-*ras* mutation)

Fig. 2. Inhibitory effects of isorhamnetin on the proliferation of NIH3T3, K-RAS, H-RAS, and SW620 cells.

effects of isorhamnetin on NIH3T3 (mouse fibroblast cell line), K-RAS (K-*ras* transformed NIH3T3 cell line), H-RAS (H-*ras* transformed NIH3T3 cell line) and SW620 cells (colon cancer cell line, K-*ras* mutation), and it showed anti-proliferative effects in a dose-dependent manner with IC₅₀ value 4.1, 7.9, 20.2, and 22.4 µg/ml, respectively. But isorhamnetin didn't show the selective inhibition of cell proliferation of *ras*-transformed NIH3T3 cells in comparison with that of normal NIH3T3 cells (Fig. 2). The flavonoids, having flavan nucleus, one of the most diverse and widespread groups of natural products are known to have beneficial effects, such as antioxidative effect, tumor cell growth inhibitory activity, and activity in induction of apoptosis in cancer cell lines. Therefore, flavonoids have attracted attention as chemopreventive agents (Iwashita *et al.*, 2000). It was reported that some flavonoids showed their antitumoral effects to inhibit topoisomerase, which involved the growth and proliferation of tumor cells, and regulate apoptosis and cell cycle of tumor cells (Reiss *et al.*, 1990). Quercetin, frequently isolated from plants, has been found to inhibit the proliferation of human U138MG glioma cell (Braganhol *et al.*, 2006), bladder cancer cell (Ma *et al.*, 2006), and human microvascular endothelial cells (Fan *et al.*, 2003). Kaempferol has been shown to inhibit the proliferation of human leukemic mast cell (Alexandrakis *et al.*, 2003), and human breast cancer cell (Hung, 2004). Genistein has been found to inhibit the proliferation of human breast cancer cell (Shon *et al.*, 2006), and colon cancer HT-29

by interchain pressure (full line, $\tau_c=1462$ s).

In this contribution, we reconsider the Molecular Stress

cell (Yu *et al.*, 2004). Baicalein has been found to inhibit the proliferation of human myeloma cell (Ma *et al.*, 2005), and vascular smooth muscle cell (Huang *et al.*, 1994). Isorhamnetin, as a flavonoid having inhibitory effect on farnesyl protein transferase and anti-proliferation activity, may be a resource for the developing of anti-tumor agents. But further extensive study should be followed.

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