Antifibrotic Activity of Manassantin B from *Saururus chinensis* in HSC-T6 Hepatic Stellate Cells

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**Abstract** — Manassantin B, a dilignan isolated from *Saururus chinensis*, significantly inhibited proliferation in HSC-T6 cells in concentration- and time-dependent manners. In addition, treatment of HSC-T6 cells with manassantin B changed cell morphology from flattened myofibroblast membrane morphology, representing activation state, to slender shape, representing quiescent state. Furthermore, manassantin B effectively reduced collagen content in HSC-T6 cells. These results suggested that manassantin B exerted antifibrotic activity in HSC-T6 cells, in part, via inhibition of cell proliferation and decrease of collagen production.

**Key words** — manassantin B, antifibrotic, *Saururus chinensis*, HSC-T6 hepatic stellate cells, collagen

**Introduction**

Hepatic fibrosis represents a wound-healing process of the liver in response to acute and chronic insults such as ethanol, viral infection, cholestasis and metabolic diseases (Friedman, 2003). Hepatic stellate cells (HSCs) have been well characterized in liver fibrosis. After a fibrogenic stimulus, HSCs transform from quiescent vitamin A storing cells to activated myofibroblast-like cells (Friedman, 2000). In addition, two major events occur after HSC activation, which substantially contribute to their active role in liver fibrosis. First, activated HSCs are the primary cell type responsible for increased synthesis and deposition of extracellular matrix (ECM) proteins in the liver. Second, they show high proliferative property thereby effectively increasing the population of fibrogenic cells and amplifying the fibrotic response (Tsuchida *et al*., 2006). Therefore, HSCs are considered to play a key role in the pathogenesis of liver fibrosis, which has directed the approach of developing antifibrotic drugs towards targeting HSCs (Wu and Zem, 2000).

*Saururus chinensis* Baill. (Saururaceae) is a perennial herb that has been used for many years to treat edema, jaundice and gonorrhea in folk medicine (Chung and Shin, 1996). We have been interested in diverse therapeutic potentials of *S. chinensis*. In our previous work on *S. chinensis*, we isolated lignans, sesquilignans, dilignans, flavonoids and alkaloid, including five new compounds, from the aerial and/or underground parts of *S. chinensis* (Sung *et al*., 1997; Sung and Kim, 2000; Sung *et al*., 2001). In addition, we reported the neuroprotective, hepatoprotective and anti-inflammatory activities of the constituents of *S. chinensis* (Sung *et al*., 2000; Lee *et al*., 2003a; Kim *et al*., 2004). Recently, our further study on *S. chinensis* afforded manassantin derivatives from the underground parts of *S. chinensis* (Sung, 2006). Manassantins are dilignans that have been reported to exert diverse biological activities, including antitumor and anti-inflammatory effects (Hodges *et al*., 2004; Lee *et al*., 2003b). To date, however, there were no previous studies on antifibrotic activity of manassantins. Thus, in the present study, we attempted to elucidate antifibrotic activity of manassantin B from *S. chinensis* by employing HSC-T6 immortalized rat hepatic stellate cell lines as an *in vitro* system (Fig. 1).

**Experimental**

**Chemical** — Supplement for cell culture and other reagents used in the study were obtained from Sigma-Aldrich Co. and 5-bromo-2'-deoxyuridine (BrdU) assay kit was purchased from Roche Diagnostics Co., Manassantin B was isolated from the underground parts of *S. chinensis* and its purity was higher than 95.0% (Sung, 2006).

**Culture of HSC-T6 hepatic stellate cells** — An immortalized rat hepatic stellate cell line, HSC-T6 (Vogel
et al., 2000), was kindly provided by Prof. S.L. Friedman (Columbia University, New York). HSC-T6 cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin and 100 µg/mL streptomycin at 37°C in a humidified atmosphere of 95% air-5% CO₂. HSC-T6 cells were treated with vehicle or manassantin B for 48 hr or as indicated.

Assessment of cell proliferation—Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effect on cell proliferation was measured by BrdU incorporation assay according to manufacturer’s protocol.

Measurement of collagen content—Collagen content was quantified by Sirius Red-based colorimetric assay (Tullberg-Reinert and Jundt, 1999). Cultured HSC-T6 cells were washed with PBS, followed by fixation with Bouin’s fluid for 1 hr. After fixation, the fixation fluid was removed and the culture dishes were washed by immersion in running tap water for 15 min. The culture dishes were air dried and stained by Sirius Red dye reagent for 1 hr with mild shaking on a shaker. Thereafter, the solution was removed and the cultures were washed with 0.01 N HCl to remove non-bound dye. The stained material was dissolved in 0.1 N NaOH and absorbance was measured at 550 nm against 0.1 N NaOH as a blank.

Statistical analysis—The evaluation of statistical significance was determined by the Student’s t-test with a value of p < 0.05 or less considered to be statistically significant.

Results and discussion

High proliferative activity is one of the major characteristics of HSC activation (Friedman, 2000). Therefore, we first investigated the effect of manassantin B on cell proliferation in HSC-T6 cells to assess antifibrotic activity of manassantin B. In our culture system, activated HSCs in serum supplemented DMEM showed high proliferative activity. However, as shown in Fig. 2A, treatment of HSC-T6 cells with manassantin B decreased the cell viability in concentration- and time-dependent manners. When HSC-T6 cells were treated with 20 µM manassantin B for 48 hr, the cell viability was decreased up to 40% compared to that of control. Manassantin B also significantly decreased cell proliferation in HSC-T6 cells as measured by BrdU incorporation (Fig. 2B).

Next we evaluated the morphology of HSC-T6 cells cultured in the presence or absence of manassantin B.