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Short Communication

Inhibitory Activities of *cis*-Hinokiresinol from *Trapa pseudoincisa* on FPTase, PRL-3, and NO-Production

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Abbreviations: COX, cyclooxygenase; FPTase, farnesyl protein transferase; LPS, lipopolysaccharide; MS, mass spectrometry; MTT assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Thiazolyl Blue Tetrazolium Bromide) assay; NMR, nuclear magnetic resonance; NO, nitric oxide; NOS, nitric oxide synthase; PGs, prostaglandins; PRL-3, phosphatase of regenerating liver-3; TNF-α, tumor necrosis factor-α

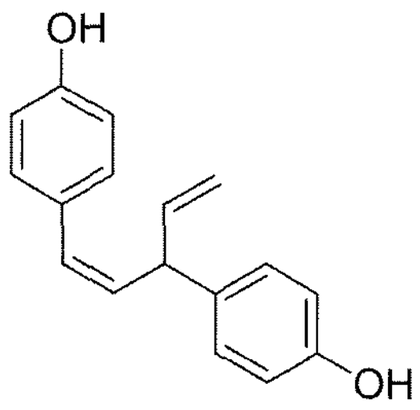
Cancer remains the second leading cause of death in most countries, increasing the need for the development of novel and effective treatments. Ras FPTase is an enzyme catalyzing the transfer of the farnesyl group from farnesyl pyrophosphate onto cysteine 186 at the C-terminal of the Ras protein. This is an essential step for the membrane association of Ras which is critical for triggering the *ras* oncogene toward the tumor formation. When the farnesylation of these proteins is blocked, their oncogenic activity is abolished [Oliff, 1999]. Recent works have demonstrated that specific inhibitors of the FPTase showed excellent efficacy *in vivo* against the solid tumors in nude mice [Kwon *et al.*, 1996; Lee *et al.*, 2000].

One of the characteristics of the cancer cells is their high metastatic index. Metastasis is the neoplastic process responsible for most deaths attributed cancer, because the primary tumors can usually be surgically removed. That is why, despite the recent advances in diagnostic and therapeutic measures, the prognosis of the colorectal cancer patients with distant metastasis still remains poor. In addition, many colorectal cancer patients suffer from the unexpected development of occult metastases after the curative resection of their primary tumors [Weiss, 2000]. Therefore, studies have been performed to clarify the molecular mechanisms involved in metastasis and to identify the specific biomarkers of the colorectal cancer metastasis [Choi *et al.*, 2006]. Recently, Saha *et al.* reported that PRL-3 was frequently overexpressed in the liver metastases, but expressed at lower levels in the primary tumors and normal colorectal epithelium. Furthermore, PRL-3 is the only gene consistently overexpressed in all 18 of the cancer metastases examined, with essentially undetectable PRL-3 expression in the normal colorectal epithelia and an intermediate expression in the advanced primary cancers. These studies suggest the possibility that the activity of PRL-3 phosphatase is a key contributing factor to the acquisition of metastatic properties of the tumor cells [Peng *et al.*, 2004].

Trapa pseudoincisa Nakai (Hydrocaryaceae), an aquatic annual herb found in Korea, Japan, and China, has been used for the remedy of several diseases including quadriplegia, diarrhea, and gastric ulcers [Jung and Shin, 1990]. Prior analytical results reported that some sterols were effective against the ascites sarcoma [Irikura *et al.*, 1972]. The methanol extracts maintained antioxidant qualities, and *cis*-hinokiresinol scavenged the 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) cation and superoxide anion radicals, inhibited low density lipoprotein-oxidation [Song *et al.*, 2007], and exhibited anti-allergic effect [Bae *et al.*, 2006]. Described herein are the isolation of a norlignan and its biological activities such as the inhibitory activity on FPTase, the anticancer activity against PRL-3, and the anti-inflammatory activity on the lipopolysaccharide-induced release of NO production.

Materials and Methods

***cis*-Hinokiresinol.** The procedure for the isolation and the structural determination of *cis*-Hinokiresinol from *T. pseudoincisa* was previously reported by the authors [Song *et al.*, 2007].



cis-hinokiresinol

Fig. 1. Chemical structure of *cis*-hinokiresinol isolated from *Trapa pseudoincisa*.

FPTase inhibition assay. FPTase assay was carried out using the method as described in the literature, and 2-hydroxycinnaldehyde was used as a positive control [Kwon *et al*., 1996].

PRL-3 inhibition assay. PRL-3 assay was measured using the method as described in the literature [Choi *et al*., 2006].

Nitrite and MTT assays. The accumulated nitrite, an oxidative product of NO, was measured in the culture medium using the Griess reaction, and MTT assay was performed as described previously [Jung *et al*., 2005].

Results and Discussion

To search for the biologically active materials from *T. pseudoincisa*, the aerial parts of the plant were extracted with MeOH and partitioned using several solvents. The successive repeated silica gel or ODS column chromatography on the obtained fractions led to the isolation of a norlignan, *cis*-hinokiresinol (yield; 7.3×10^{5}%), and the identification of the structure was performed on the basis of several spectroscopic analyses including NMR and MS [Song *et al*., 2007].

Anticancer activities. the results showed the extract of *T. pseudoincisa* strongly inhibited FPTase, 90% at 100 μg/mL. A norlignan isolated from the plant, *cis*-hinokiresinol, inhibited the activity of FPTase by 33.1±1.1% at 198 μM, and its IC_{50} value was determined as 233 μM, which was almost half of the activity than that of a well known FPTase inhibitor, 2-hydoxycinnamaldehyde (IC_{50}=121 μM) [Kwon *et al*., 1996]. *cis*-Hinokiresinol also exhibited PRL-3 inhibitory activity by 16.1% as compared to the negative control at 198 μM. Therefore, *cis*-hinokiresinol should be studied further for the development of a new material for the protection against cancer.

Anti-inflammatory activity. NO is produced by iNOS in macrophage, hepatocyte, and renal cells under the stimulation of LPS, TNF-α, interleukin-1 or interferon-γ [Nathan, 1992]. To determine the effect of *cis*-hinokiresinol on the NO production in RAW 264.7 cells, the cells were treated with LPS (1 μg/mL) in the presence and the absence of *cis*-hinokiresinol. The production of NO was measured using the method of Griess [1879]. LPS (1 μg/mL) induced the NO_2^- production by approximately 8-folds, and this induction was significantly inhibited by *cis*-hinokiresinol with the IC_{50} value of 39.4 μM. The cytotoxic effect of *cis*-hinokiresinol was evaluated by MTT assay in the absence and the presence of LPS. It was evident that the exposure concentrations lower than 396 μM of *cis*-hinokiresinol were not cytotoxic to the RAW264.7 cells. The cell viability of RAW 264.7 cells was higher than 50% when the cells were incubated in the medium containing 396 mM of *cis*-hinokiresinol for 24 h. *cis*-Hinokiresinol exhibited almost equal inhibitory activity on the NO production as the positive inhibitor, 1-N^6-(1-Iminoethyl)lysine (IC_{50}=25.6 μM). Chronic inflammations and infections lead to the up-regulation of a series of enzymes and signaling proteins in the affected tissues and cells. Among these pro-inflammatory enzymes, the inducible forms of NOS and COX, which are responsible for increasing the levels of NO and PG, respectively, are known to be involved in the pathogenesis of many chronic diseases including multiple sclerosis, Parkinson's and Alzheimer's diseases, and colon cancer [Heiss *et al*., 2001].

In conclusion, the findings of this study suggest that *T. pseudoincisa* as well as its isolated compound, *cis*-hinokiresinol, might be useful for the treatments of inflammation and cancer.

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