G0/G1 Cell Cycle Arrest and Activation of Caspases in Honokiol-mediated Growth Inhibition of Human Gastric Cancer Cells

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Abstract – Honokiol, a naturally occurring neolignan mainly found in Magnolia species, has been shown to have the anti-angiogenic, anti-invasive and cancer chemopreventive activities, but the molecular mechanism of actions has not been fully elucidated yet. In the present study, we investigated the effect of honokiol on the growth inhibitory activity in cultured SNU-638 human gastric cancer cells. We found that honokiol exerted potent anti-proliferative activity against SNU-638 cells. Honokiol also arrested the cell cycle progression at the G0/G1 phase and induced the apoptotic cell death in a concentration-dependent manner. The cell cycle arrest was well correlated with the downregulation of Rb, cyclin D1, cyclin A, cyclin E, and CDK4 expression, and the induction of cyclin-dependent kinase inhibitor p27. The increase of sub-G1 peak by honokiol was closely related to the induction of apoptosis, which was evidenced by the induction of DNA fragmentation, the cleavage of poly(ADP-ribose) polymerase, and the sequential activation of caspase cascade. These findings suggest the cell cycle arrest and induction of apoptosis might be one possible mechanism of actions for the anti-proliferative activity of honokiol in human gastric cancer cell.

Keywords – Honokiol, Growth inhibition, Cell cycle arrest, Apoptosis, Human gastric cancer cells

Introduction

Although the incidence and mortality of gastric cancer has been declined in Western countries, particularly in USA and Europe, gastric cancer is still the second-ranked most common cancer worldwide. Especially, gastric cancer is the most prevalent malignant neoplasm and the leading cause of cancer-related human death in Asian countries including Korea, China and Japan (Whelan et al., 1993; Roukos, 2006). The excessive intake of salt and Helicobacter pylori infection have been considered one of major etiologic factors to induce the gastric carcinogenesis (Miller et al., 1994; Tsugane et al., 1994). The strategy for prevention and treatment of gastric cancer with vegetables and dietary antioxidants is also proposed for reducing the risk of gastric carcinogenesis (Wu et al., 1999). Therefore, there is still needed to identify and procure promising chemotherapeutic agents for better treatment of advanced malignancy of gastric cancer.

Honokiol is a neolignan type phytochemical found in the bark, leaf, and seed cones of the magnolia tree, and has been shown to exhibit a variety of important biological effects such as antithrombosis, antimicrobial and anxiolytic activity (Chang et al., 1998; Kuribara et al., 2000; Zhang et al., 2007). Many studies have also demonstrated the potential of natural honokiol to mediate the strong antioxidant, anti-inflammatory, or potent cancer chemopreventive effects in carcinogenesis (Lin et al., 2006; Munroe et al., 2007). In addition, honokiol inhibited the growth of several human cancer cell lines, including human lung squamous carcinoma, promyelocytic leukemia, breast, and colon cancer cells (Hirano et al., 1994; Yang et al., 2002; Chen et al., 2004; Wolf et al., 2007). In our recent study we also reported that the growth inhibition of breast cancer cells by honokiol was associated with the down-regulation of c-Src/EGFR-mediated cell signaling activation (Park et al., 2009). In view of the growth inhibitory potential of honokiol against cancer cells, we further designed to investigate the cellular mechanisms of honokiol on the growth of human gastric cancer cells. Here, we report for the first time that honokiol inhibited the growth of human gastric cancer
cells and remarkably induced arrest at the G0/G1 of the cell cycle, and also induced apoptosis in human gastric (SNU-638) cancer cells.

**Experimental**

**Chemicals** – Trichloroacetic acid (TCA), sulfon rhodamine B, propididum iodide, trypsin inhibitor, RNase A, and anti-β-actin primary antibody were purchased from Sigma (St. Louis, MO, USA). Rosewell Park Memorial Institute medium 1640 (RPMI 1640), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100X), trypsin-EDTA solution (1X) and antibiotic-antimycotic solution (PSF) were from GIBCO-BRL (Grand Island, NY, USA). Rabbit polyclonal anti-CDK4, anti-cyclin A, anti-caspase-3, anti-p27 antibody, horseradish peroxidase (HRP)-conjugated anti-mouse IgG, and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit monoclonal anti-Rb, anti-caspase-8, rabbit anti-caspase-9, and anti-phospho-Rb (Ser 807/811) primary antibody were obtained from Cell Signaling (Danvers, MA, USA). Mouse monoclonal anti-PARP, and anti-cyclin E was from BD Biosciences (San Diego, CA, USA).

Honokiol, magnolol, obovatol, and 4-methoxyhonokiol (Fig. 1A) isolated from the bark of *Magnolia obavata* were provided from Dr. KiHwan Bae (Chungnam National University, Korea).

**Cell culture** – Human gastric carcinoma SNU-638 cells, obtained from the Korean Cell Line Bank (Seoul, Korea), were cultured in RPMI supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B. Cells were maintained at 37°C in humidified atmosphere with 5% CO2.

**Evaluation of growth inhibitory potential** – SNU-638 cells (5 × 10⁴ cells/mL) were treated with various concentrations of test compound for 3 days. After treatment, cells were fixed with 10% TCA solution, and cell proliferation was determined with sulforhodamine B (SRB) protein staining method (Lee et al., 1998a). The result was expressed as a percentage, relative to solvent-treated control incubations, and the IC₅₀ values were calculated using non-linear regression analysis (percent survival versus concentration).

**DNA fragmentation assay** – SNU-638 cells were plated in 100-mm culture dish at a density of 1 × 10⁶ cells/dish. Twenty-four hours later, fresh media containing test sample were added to cultured dishes. After 24 h, the cells were washed with PBS and lysed in buffer containing 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, and 1.0% NP-40. After centrifugation, 1% SDS and RNase A