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Inhibitory Effect of Shihogayonggolmoryeu-tang (SGYMT) on MMP-2 and MMP-9 Gelatin Zymography

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[Purpose] The proteolytic matrix metalloproteinases (MMPs) is play a fundamental role in wide variety of pathologic conditions that involve connective tissue destruction including osteoarithritis and rheumatoid arithritis, tumor metastasis and angiogenesis, and atherosclerosis. The 72-kDa gelatinase A (MMP-2) and 92-kDa gelatinase B (MMP-9) is the most widely distributed in the connective tissue cells including endothelial cell, smooth muscle cell, fibroblast, osteoblasts as well as invasive tumor cells. In this study, we investigated to efficacy of SGYMT on MMP-2 and MMP-9 activation in cancer cell line and isolated smooth muscle cell (rSMCs) from descending aorta of rat.

[Materials and Methods] The six formulations (Chodung-san, Daesunggi-tang, Mokbanggi-tang, Danggi-tang, Zagancho-tang and Shihogayonggolmoryeu-tang) in this study were used. The cells were used normal human liver cell (Chang) and liver cancer cell line (Hep-3B, SK-Hep 1), and rSMCs. The cells were cultured in 6 well plates for 24 hrs in 0.4% FBS medium before treated formulations, and than cultured 24 hrs in treated formulations. Secreted MMP-2 and MMP-9 activity was investigated in conditioned media. MMP activity secreted by cells was determined in SDS-polyacrylamide gel co-polymerized with gelatin (1 mg/ml), using 4% staking and 7.5% separating gels. After washing, gels were incubated at 24 hrs in 20 mM NaCl, 5 mM $CaCl_2$ and 50 mM Tris-HCl buffer (pH 7.4) for MMP detection or in the same metal-independent protease activity. Gels were fixed, stained with Coomassie blue. Lytic bands were quantitated with Gel-Print system (Core Bio Corp., Korea).

[Results and Conclusion] The Chang cell line was secreted to MMP-2 protease, MMP-9 in Hep-3B, but SK-Hep 1 cell line was secreted to MMP-2 and MMP-9 protease. The Daesunggi-tang and SGYMT were inhibited to MMP-2 and MMP-9 activation in cancer cell and rSMCs compared with non-treated cells. The inhibitory effect of formulations showed non-specificity in cell lines, and MMP-2 and MMP-9. IC50 values of SGYMT on MMP-2 and MMP-9 activity are 57.2 μg/ml and 34.2 μg/ml, respectively. From these results, SGYMT treatment may offer protection against post angioplasty restenosis, generating SMCs migration and proliferation.

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