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Rescue of *Helicobacter pylori* –induced Cytotoxicity by Red Ginseng; Basic and clinical implications

박수진, 여말희, 진주현, 조정선, 이기명, 정재연, 조성원, 함기백

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Backgrounds Ginseng, the root of *Panax ginseng* C.A. Meyer, has been used as one of the major herbs in Oriental medicine, based on its rejuvenating and revitalizing action for thousands of years. Practically even in United States, ginseng has been found to be one of the best selling dietary supplements due to the growing recognition of their benefits in health, although its exact biological mode of action was not fully identified.

Especially, in the field of microbiology and immunology, several studies have demonstrated that ginseng exerted anti-oxidant, anti-bacterial growth or anti-inflammatory effects. Ginsenoside Rb1, one of the primary ginsenoside components, has been known to inhibit lipopolysaccharide (LPS)-induced expression of the proinflammatory cytokine, tumor necrosis factor-α (TNF-α) and acidic polysaccharides from ginseng has been reported to inhibit the adhesion of *Helicobactor pylori (H. pylori)* to human gastric epithelial cells. Also the growth inhibiting effect of ginseng fraction, panaxatriol (PT) against *H. pylori* was suggested *in vitro* with an MIC of 50 μg/ml.

In addition to these antimicrobial action of ginseng ingredients, it has been documented that ginseng have distinctive actions on the cell growths in various carcinoma cells, which are through growth inhibition or cytotoxic effects in some cell lines like human mealanoma, A375-S2 or hepatoma SK-HEP-1, prostate carcinoma, LNCaP, but growth stimulating or protective effects in some cell lines like neuroblastoma, SHSY5Y, or keratinocyte, HaCaT were also reported. In an aspect of chemoprevention, apoptosis inducing action would be chosen, but in the situation of damaging and destructive conditions, growth-enhancing action is required. Thus it was far from clear whether or not RGE have advantage on cell growth in gastric epithelia. Moreover, the potent roles of red ginseng extracts (RGE) upon *H. pylori*-infected gastric epithelial cells have never been explored *in vitro* or *in vivo*.

The microaerophilic bacterium, *H. pylori* has been proposed as 'class I carcinogens', which plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma. Emerging data have proposed that the mechanism of *H. pylori* provoked gastric damages includes active gastric inflammation with infiltration of neutrophils, which could cause DNA damage to the adjacent cells through several cytokines such as IL-1β, IL-2, IL-6, IL-8, and TNF-α, whose expression is controlled by gene transcriptional factors such as NF-κB or AP-1.

Aim and Methods Taken together, based on the anti-inflammatory, anti-adhesion, and

growth modulating activity against microbes, we hypothesized that RGE, which is steamed, dried, and more concentrated ginseng, might protect gastric epithelial cells against *H. pylori*. The gastroprotective effect of RGE against experimental *H. pylori* infection was demonstrated using coculture system of AGS cells and *H. pylori*, and then mechanisms of gastroprotection of red ginseng was explored, in detail, including apoptosis, cytokine secretion or the changes of signal transduction leading to altered gene transcription.

Results & Discussion RGE significantly attenuated either *H. pylori*-induced DNA damage assessed by Comet assay or apoptosis measured by DNA fragmentation. Inactivation of ERK1/2 signaling and the attenuation of caspase-3 activation and PARP cleavage were revealed with RGE against *H. pylori* infection. RGE decreased *H. pylori*-stimulated IL-8 gene expression, which was resulted from the transcriptional regression of NF-κB. Conclusively, RGE showed significant gastroprotective effects against *H. pylori*-associated gastric mucosal cell damages, suggesting red ginseng could be used as medicinal phytonutrient against *H. pylori*–infection in the stomach.

Red ginseng extract (RGE), medicinal phytonutrient, efficiently rescued gastric mucosal cells from proinflammatory activation as well as apoptosis exerted by live bacteria, *H. pylori*. With the lesser than 100 μg/ml of RGE pretreatment showed the significant protective effects using co-culture system with *H. pylori* in gastric epithelia assessed by trypan blue dye exclusion assay. And significant genomic protection of RGE

against *H. pylori*-induced DNA damages assessed by Comet assay, very useful way of evaluating the individual DNA stability, was demonstrated in this study. Also the responsible protective mechanisms of RGE against bacteria infection were revealed that inactivation of signal transductions, which led to inhibition of apoptosis and decreased DNA binding of NF-κB resulting in attenuation of IL-8 mRNA.

Taken together, RGE has significant protective activity against *H. pylori* infection in AGS cells and we can add already documented anti-adhesion or anti-microbial actions of ginseng with the following underlying mechanisms; **1) Rescue from *H. pylori*-induced cytotoxicity, 2) Significant avoidance from *H. pylori*-induced DNA damages, 3) The inhibition of *H. pylori*-induced apoptotic cell death accompanied by ERK-1/2 inactivation, 4) Transcriptional repression of NF-κB with subsequent inhibition of proinflammatory IL-8 mRNA, 5) Significant cytoprotection activities against *H. pylori* infection.** However, these studies focusing on whole extract of ginseng, commercially available type of ginseng in general, used to predict the potential effect of specific individual constituents derived from ginseng, but that additional, confirmatory studies using specific components need to be elucidated. Conclusively, our results suggested that red ginseng could be a potent novel source of clinical application to prevent the miserable *H. pylori*-related gastric diseases including gastric cancer.

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참고문헌

1. Bae EA, Han MJ, Baek NI, Kim DH: In vitro anti-*Helicobacter pylori* activity of panaxytriol isolate from ginseng. Arch Pharm Res 24: 297-299, 2001
2. Li SH, Chu Y: Anti-inflammatory effects of total saponins of Panax notoginseng. Zhongguo Yao Li Xue Bao 20:551-554, 1999
3. Belogortseva NI, Yoon JY, Kim KH: Inhibition of *Helicobacter pylori* hemagglutination by polysaccharide fractions from roots of Panax ginseng. Planta Med 66: 217-220, 2000
4. International Agency for Research on cancer. Schistosomes liver flukes and *Helicobacter pylori*. Monograph 61. Lyon. France: IARC, 1994
5. Kikuchi S: Epidemiology of *Helicobacter pylori* and gastric cancer. Gastric Cancer 5: 6-15, 2002
6. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* Infection and the Development of Gastric Cancer. N Engl J Med 345: 784-789, 2001
7. Israel DA, Peek RM: Pathogenesis of *Helicobacter pylori*-induced gastric inflammation. Aliment Pharmacol Ther 15:1271-1290, 2001
8. Hahm KB, Lee KJ, Kim JH, Cho SW, Chung MH: *Helicobacter pylori* infection, oxidative DNA damage, gastric carcinogenesis, and reversibility by rebamipide. Dig Dis Sci 43: 72S-77S, 1998

9. Naito Y, Yoshikawa T: Molecular and cellular mechanisms involved in *Helicobacter pylori*-induced inflammation and oxidative stress. Free Radic Biol Med 33: 323-336, 2002

10. Everett SM, White KL, Drake IM, Schorah CJ, Calvert RJ, Skinner C, Stringer MD, Miller D, Dixon MF, Chalmers DM, Axon AT: The effect of *Helicobacter pylori* infection on levels of DNA damage in gastric epithelial cells. Helicobacter 7:271-80, 2002

11. Mitsuno Y, Yoshida H, Maeda S, Ogura K, Hirata Y, Kawabe T, Shiratori Y, Omata M: *Helicobacter pylori* induced transactivation of SRE and AP-1 through the ERK signaling pathway in gastric cancer cell. Gut 49: 18-22, 2001

12. Sivam GP: Protection against *Helicobacter pylori* and other bacterial infections by garlic. J Nutr 131:1106S-8S, 2001

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