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Inhibition of Red Ginseng on 5-Hydroxyeicosatetraenoic Acid (5-HETE) Biosynthesis from Arachidonic Acid in *Helicobacter Pylori*-infected Gastric Cells*

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***Helicobacter pylori (H. pylori)* infection rapidly stimulated either COX-2 or 5-LOX and released arachidonic acid metabolites that have been considered as pivotal mediators in *H. pylori*-induced inflammatory responses. To determine whether red ginseng extract (RGE) can suppress the biosynthesis of 5(S)-hydroxyeicosatetraenoic acids (HETE), a precursor metabolite of leukotrienes B4 (LTB_4) in *H. pylori*-provoked inflammatory responses in gastric epithelial cells, the biosynthesis of monohydroxy fatty acids was measured using radioactive arachidonic acid and validated by RP-HPLC using non-radioactive AA as substrate in AGS cells cocultured with *H. pylori* (ATCC 43504) with or without pretreatment of RGE. Among three known major HETEs, *H. pylori* infection specifically induced the biosynthesis of ^{14}C-5(S)-HETE rather than the complex of ^{14}C-15S- /^{14}C-12(S)-HETE from ^{14}C-AA, concomitantly obtained by HPLC ($p<0.01$). RGE, 1 to 100 μg/ml, selectively suppressed *H. pylori*-stimulated ^{14}C-5(S)-HETE production implying the attenuation of 5-lipoxygenase activity, of which was similar to known LOX inhibitor NDGA (10 μM) ($p<0.01$). However, the amount of 5(S)-HETE was significantly reduced by higher dose of RGE (100 μg/ml) ($p<0.05$). These results indicated that LOX pathway might be one of principle pathogenic mechanisms of *H. pylori* and red ginseng could be a nutraceutical against *H. pylori* infection through inhibiting action of LOX activity.**

Key words: *Helicobactor pylori*, Red Ginseng, 5(S)-HETE, LOX, Nutraceutical

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INTRODUCTION

H. pylori infection is associated with several pathological alterations of stomach, including peptic ulcer, chronic atrophic gastritis, and mucosal-associated lymphoid tissue lymphoma, even gastric cancer.[1-4] Mechanistically and pathologically, all of these gastric lesions after *H. pylori* infection were closely associated with the initiation, perpetuation and chronic persistence of gastric inflammations, predisposed to diverse molecular changes associated with either epigenetic or genetic alterations[5], leading to crossroads of inflammation and carcinogenesis.

A dramatic change in gastric mucosal phospholipid composition and elevation of the metabolites of various eicosanoids from epithelial membrane phospholipids has been shown by the colonization of *H. pylori* in the gastric mucosa, which has been related to the development of several inflammatory host responses in infected gastric environment[5-6]. This is further proved by the fact that the eradication of *H. pylori* reduces eicosanoids synthesis, resulting in the normalization of gastric mucosal phospholipid content and its fatty acid composition, which may consequently render the gastric barrier function to be normalized[7-8]. Concomitantly, infection of epithelial cells with *H. pylori* leads to rapid generation of intracellular calcium concentration and initiates calcium signaling along with the generation of adenosine 3', 5'-cyclic monophosphate and guanosine 3', 5'-cyclic monophosphate[9], of which alterations appear to enhance either the expressions or the activities of cytosolic phospholipase A2 ($cPLA_2$) via Gαi/Gαo proteins or the p38 MAP kinase pathway[9-11]. Consequently, prostaglandins (PGs) and monohydroxy fatty acids are then de novo synthesized via cyclooxygenase (COX) and lipoxygenase (LOX) pathway, respectively[12]. Several studies showed that COX-2 is closely associated with diverse clinical

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manifestations of *H. pylori*-associated diseases and COX-2 inhibition with NSAID could ameliorate either gastric inflammation or even *H. pylori*-induced gastric carcinogenesis in vitro and *in vivo*[13-14]. However, the metabolic conversion to hydroxy fatty acids via LOX pathway after *H. pylori* infection has not been fully explored yet.

H. pylori infection rapidly released mediators and chemokines including IL-8, iNOS, and COX-2. Particularly, IL-8 could stimulate lipid metabolites through modulating LOX activities[15-19] and *H. pylori*-provoked cytokine mediated-inflammatory responses can be neutralized by blocking 5-LOX that metabolized arachidonic acid (AA) into 5(S)-hydroxyeicosatetraenoic acid (HETE), a precursor metabolite of leukotrienes B_4 (LTB_4), one of powerful chemotactic factors for neutrophils. These facts suggested that a molecule, which can regulate LOX activity, could be the potential way of either eliminating gastric inflammations or extensively preventing inflammation-associated carcinogenesis. In spite of abundant evidences about the COX-2 expressions and its activities after *H. pylori* infection, only scant evidence has been accumulated about LOX and none has shown that LOX inhibitor could be intervened in preventing *H. pylori*-associated inflammatory signaling transduction of gastric epithelial cells.

Lately, we have reported the protective roles of Red Ginseng Extract (RGE) from *H. pylori*-associated cytotoxicity through the inhibitory actions of both MAPK and redox sensitive transcriptional activity[25], but we have another concerns and curiosity about the action of RGEon LOX pathway. Few documents are available for the action of ginseng extract on LOX pathway, though some phytochemicals like polyphenols or agentlike nordihydroguaiaretic acid (NDGA) are known to inhibit LOX activities specifically. Based on the reports that individual ginsenoside isolate or Panaxynol isolated from ginseng can inhibit the rise of intracellular calcium caused by arachidonic acid or calcium ionophore A23187 and shows anti-platelet activity through the inhibition of thromboxane formation[21-22] and the findings that ginsenoside Rh1 also inhibited COX-2 protein expression in RAW 264.7 cells with the inactivation of the transcription factor, NF-κB[23] and Rg3, a major ginsenoside derived from heat-processed ginseng inhibited phorbol ester-induced COX-2 expression, NF-κB activation and tumor promotion[24], we hypothesized that RGE might influence LOX pathway and could impose the modulation of gastric inflammation in *H. pylori* infection.

Here, in order to know the influence of *H. pylori* infection on LOX pathway and the efficacy of RGE on modulating arachidonate-5-LOX-inflammatory link in gastric epithelium after *H. pylori* infection,we measured the LOX activities by both reverse phase-high performance liquid chromatography (RP-HPLC) and thin layer chromatography (TLC) after RGE pretreatment.

MATERIALS AND METHODS

1. Chemicals and Reagents

^{14}C-arachidonic acid (^{14}C-AA, 50 mCi/mmol) was purchased from Perkin Elmer (Boston, MA). Standards for eicosanoids metabolites, 15(S)-HETE, 12(S)-HETE and 5(S)-HETE were all purchased from Cayman Chemical Co. (Ann Arbor, MI). Calcium ionophore, A23187 and nordihydroguaiaretic acid (NDGA) were obtained from Sigma (St. Louis, MO).

2. Cells, Bacterial Strains, and Growth Conditions

AGS cells, human gastric adenocarcinoma cells, were purchased from American Type Culture Collection (ATCC, Rockville, MD). They were cultured in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) supplemented with 25 mM HEPES, 2 mM L-glutamine (Life Technologies, Stockholm, Sweden), and 10% fetal calf serum (FCS, HyClone, Logan, UT), antibiotics and antimycotics in humidified environment at 37℃ in 5 % CO_2. *H. pylori* (ATCC 43504, cag PAI and vac A positive) was grown on blood agar plates with 7 % sheep blood under microaerophilic conditions generated with Anaeropack Campylo® (Mitsubishi Gas Chemicals Co., Tokyo, Japan) at 37℃ for 5 days. For inoculation of the bacteria, *H. pylori* were resuspended in PBS to an A450 of 1.2 units, which corresponds to a bacterial concentration of 5×10^8 CFU/ml and cocultured with AGS cells at the concentration of 5×10^7 CFU/ml.

3. Treatment of Cells

RGE was kindly provided by the Korean Ginseng Corp. (Seoul, Korea), which was dissolved in phosphate-buffered saline (PBS, pH 7.4). AGS cells (1×10^7/ml) were pretreated with several concentrations of RGE (1, 10, 100 μg/ml) for 24 h and then followed with inoculation of *H. pylori* (5×10^7 CFU/ml, MOI (multiplicity of infection) was 5. NDGA was resuspended in DMSO and diluted in the culture medium to final concentration of 10 μM.

4. Bioassay of Lipoxygenase Activity

The bioassay of arachidonate lipoxygenase activity

was done using a moderate modification of a previously described method[25], besides each LOX incubation contained ^{14}C-AA (0.01 μCi) and 25 μg protein of cell lysates in a 100 μl reaction mixture which contained 100 mM Tris-HCl buffer, pH 7.4 with 1 mM EDTA and 2 mM reduced-glutathione.

5. Extraction of HETEs From AGS Cells

After appropriate treatment, AGS cell monolayer was incubated in 2 ml of RPMI 1640 supplemented with 10 % FBS in the presence of 2.0 μM calcium ionophore A23187 in DMSO (at a final concentration of 0.02%) at 37 ℃. The eicosanoids extraction from AGS cells was performed by the method of Wescott *et al.*[26] using a slight modification. After 1 h of incubation with A23187, culture media were removed and cells were added 3 ml of ice-cold methanol. Methanolic pellets were transferred to the glass tube and then two volumes of ice-cold chloroform were added. Eicosanoids were extracted by vigorous vortexing for 1 min, and then centrifuged for 2 min. The chloroform phase was evaporated to dryness under nitrogen, and the residue was stored at -70 ℃ pending eicosanoid separation by RP-HPLC.

6. Identification of Lipoxygenase Metabolites in Human Gastric Epithelial Cells by RP-HPLC

Separation of eicosanoids was accomplished according to the method previously described[25] and cochromatography with authentic mono-HETE standards for 15(S)-HETE, 12(S)-HETE, and 5(S)-HETE wasused with each sample to control for any variation in retention time and elution with methanol and H_2O (76:24, *v/v*) adjusted to pH 3.0 with acetic acid, isocratically at a flow rate of 1.4 ml/min for 30 min and monitored at 234 nm with a Hewlet Packard spectrophotometer.

7. Statistical Analysis

Results were expressed as the means S.D. The data were analyzed by the one-way analysis of variance (ANOVA) and statistical significance between groups was determined by Duncan's multiple range tests. Statistical significance was accepted within $p<0.05$.

RESULTS

1. Effect of *H. Pylori* Exposure on the Biosynthesis of HETEs from AA in Gastric Cells

H. pylorik generated significant amounts of ^{14}C-5(S)-HETE rather than the complex of ^{14}C-15S-/^{14}C-12(S)-HETE from 14C-AA, implying that bacterial infection might lead to increase 5-LOX enzyme activities, which was reversed by the pretreatment of a LOX inhibitor NDGA in coculture system of *H. pylori* and gastric AGS cell (Fig. 1). Because the data in Fig. 1 was obtained with co-elution of ^{14}C-15S-/^{14}C-12(S)-HETE and ^{14}C-5(S)-HETE by TLC separation, we re-sorted them with RP-HPLC to confirm and quantify the amounts of 15(S)-HETE and 12(S)-HETE in the AGS cells (Fig. 2). Without bacterial infection, the relative ratio of the biosynthesis of 5(S)-HETE: 12(S)-HETE: 15(S)-HETE was 1: 0.45: 0.5 in AGS cells, but *H. pylori* infection significantly increased 5(S)-HETE biosynthesis more than threefold. On the other hand, no significant changes werefound on the biosynthesis of either 15(S)-HETE or 12(S)-HETE even after *H. pylori* infection in gastric cells (Fig. 2). Therefore, *H. pylori* infection particularly activated 5(S)-HETE generations in AGS cells among LOX metabolites.

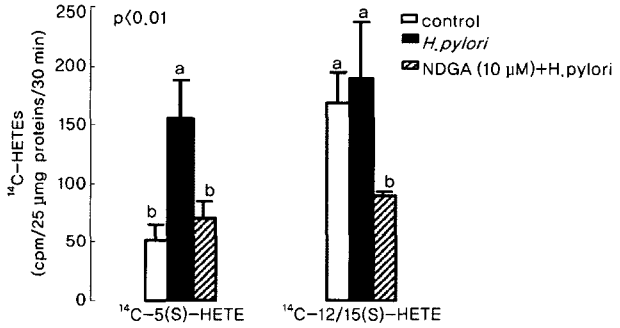

Fig. 1. Selective stimulation of 5(S)-HETE generation in AGS cells after *H. pylori* infection.

AGS cells were cocultured with *H. pylori* (ATCC 43504 strain, 5×10^7 CFU/ml) for 24 h with or without RGE or LOX inhibitor (NDGA) as described in the "Materials & Methods" section. *H. pylori* infection selectively induced the biosynthesis of ^{14}C-5(S)-HETE metabolite from ^{14}C-AA by more than threefold, while no significant change of ^{14}C-12/15(S)-HETE synthesis was shown. *H. pylori*-induced 5-LOX activity was reversed by pretreatment of LOX inhibitors, NDGA (10 μM) for 24 h.

2. Suppressions of RGE on the Biosynthesis of 5(S)-HETE in *H. Pylori*-infected AGS Cell

Since *H. pylori* triggered the biosynthesis of 5(S)-HETE (Figs. 1 and 2), whether RGE modulates 5-LOX activity and production of 5(S)-HETE or not was further examined. AGS cells pretreated with several doses of RGE (1-100 μg/ml) significantly suppressed the production of ^{14}C-5(S)-HETE from prelabeled-AA ($p<0.01$, Fig. 3), implying that RGE could modulate the attenuation of 5-lipoxygenase activity, of which was similar to known LOX inhibitor NDGA (10 μM) ($p < 0.01$). RP-HPLC results showed that the amount of 5(S)-HETE tended to

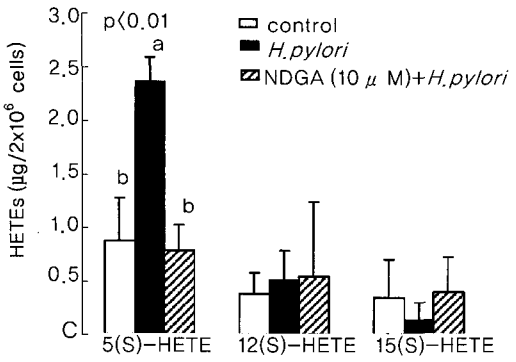

Fig. 2. Identification of monohydroxy fatty acid profile of AGS cells after *H. pylori* infection.

Eicosanoids extracted from *H. pylori*-infected AGS cells were identified by RP-HPLC analysis using the co-chromatography with authentic mono-HETE standards for 15(S)-HETE, 12(S)-HETE, and 5(S)-HETE. Major eicosanoid was 5(S)-HETE followed by 12(S)-HETE and 15(S)-HETE (1:0.45:0.5) in unstimulated AGS cells. *H. pylori* infection triggered dramatic production of 5(S)-HETE by threefold compare to non-infected cells, which was blocked by pretreatment of selective 5-LOX inhibitor, NDGA (10 μM) for 24 h. However, no significant changes of 12(S)-HETE and 15(S)-HETE were noted even after bacterial infection or LOX inhibitor administration

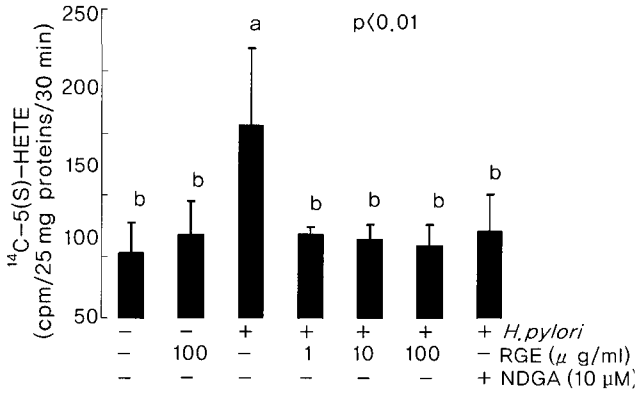

Fig. 3. RGE reversed selective biosynthesis of 5(S)-HETE in *H. pylori* infected AGS cells.

RGE (1, 10, 100 μg/ml, respectively) pretreatment for 24 h strongly suppressed *H. pylori*-stimulated ^{14}C-5(S)-HETE biosynthesis from ^{14}C-AA to the basal level of 5(S)-HETE as similarly as LOX inhibitor (NDGA). Results are represented the means±S.D. of three independe

be reduced by RGE in a dose dependent manner and significant reduction was observed by higher dose of RGE (100 μg/ml) ($p<0.05$). NDGA didnot effectively reduce the 5(S)-HETE production from arachidonic acids (Fig. 4).

DISCUSSION

Current study explored for the first time that *H. pylori* induced the shunting of 5(S)-HETE biosynthesis among LOX metabolites from AA in gastric epithelia. Also RGE suppressed *H. pylori*-induced pro-inflammatory 5(S)-HETE production in gastric epithelia paralleled its effect as inhibitor of AA metabolism *in vitro*. We elicited previously that the overall actions of RGE couldbe

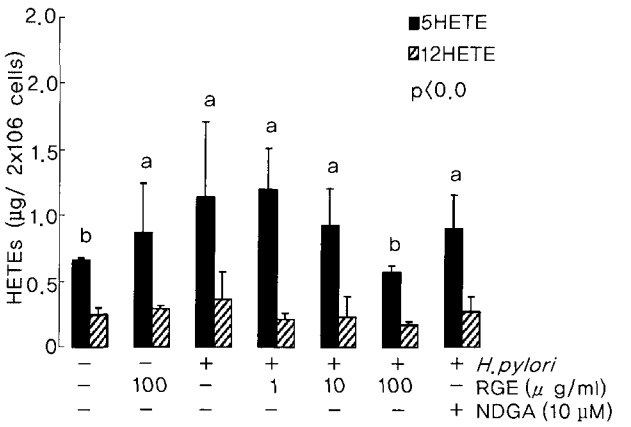

Fig. 4. Effect of RGE pretreatment on monohydroxy fatty acid profile in *H. pylori*-infected AGS cells

Eicosanoids extracted from *H. pylori*-infected AGS cells were identified by RP-HPLC analysis using the co-chromatography with authentic mono-HETE standards for 15(S)-HETE, 12(S)-HETE, and 5(S)-HETE as described in the "Materials & Methods" section. Dose dependent inhibition of *H. pylori*-stimulated 5(S)-HETE synthesis by pretreatment of RGE (1, 10 and100 μg/ml) for 24 h was observed, of which effects were better than selective LOX inhibitor, NDGA

summarized as significant protection of gastric epithelial cells by reducing proinflammatory mediators such as IL-8 and 5(S)-HETE via transcriptional and transactivational modification and could extensively contribute to the prevention of atrophic gastritis and gastric carcinoma[27]. Hence several studies showed that NSAID played significant chemopreventive effects against *H. pylori*-induced gastric carcinogenesis through attenuating COX activities[28] and our study stressed that LOX activity could influence the inflammatory or carcinogenic activities related to *H. pylori* infection, we inferred that modulation of both activities will be required to avoid from deleterious outcomes of *H. pylori* infection. Besides of inhibitory effects of RGEon 5-LOX expressions and activities, non-saponin fraction of Red Ginseng is generally reported to reduce calcium ionophore stimulated- calcium release in polymorphonuclear leukocyte[29], polyphenol or protopanaxatriol (PT) from *Panax ginseng* C.A. Mayer have been documented to inhibit PLA_2 by 49% at the concentration of 60 μg/ml and directly inhibit LTB_4 synthesis (IC_{50} 3.22 μM) *in vitro*[30]. Therefore, it is possible that RGE may modulate *H. pylori*-stimulated AA metabolism at the level of intracellular calcium concentration or calcium signaling and consequent cascades.

Specifically, *H. pylori* provoked-5-LOX-derived leukotrienes production of human gastric epithelial cells[31] contributed to active gastric inflammation with infiltration of neutrophils[32] and even relating gastric carcinogenesis through DNA damage to the adjacent cells through several arachidonate metabolites controlled by extracellular signal regulated kinases, ERK-1 and -2[33], or c-Jun N-terminal

kinase, JNK[34]. Our observations could be supplemented with the findings of Kimura *et al.*,[30] that there is a positive correlation between LT levels and neutrophil infiltration in *H. pylori*-infected gastric mucosa and the LOX inhibitor, AA-861, exerts beneficial effect in response to *H. pylori* infection both *in vitro* and *in vivo*.

It has long been known that normal gastric epithelium produces considerable levels of certain PGs, but is very limited in the ability to produce LTs, of which productions arelimited to certain inflammatory cells. According to Shimakura and Boland[35], under basal condition, gastric adenocarcinoma cell line AGS, the same cell line used in the current experiment, produced LTD_4 as its principal eicosanoid metabolite followed by 6-keto-PGE_1, LTC_4, LTB_4, 12-HETE, TXB_2, PGF_2, PGE_2, 12-HHT, 15-HETE, and PGD2, but after stimulation with calcium ionophore, the principal eicosanoid metabolites became 12-HETE, followed by LTB4, LTC4, PGF2, PGE2, 6-keto PGF1, TXB2, LTD4, 15-HETE and PGD2, suggesting that 5-LOX might be more active than COX in gastric epithelial cells. Their ability to metabolize AA differed according to cell context and each celltype may have a unique ability to produce PGs, HETEs, LTs, and other related eicosanoids. In non-cancerous gastric epithelial cell, RGM cell, we found that 12(S)-HETE and 15(S)-HETE were main metabolites from AA in unstimulated condition, but was shunting to 5(S)-HETE when *H. pylori* infection was added. On the other hand, certain gastric cancerous epithelial cells, SNU 16 and AGS cell line, generally produce 5(S)-HETE under the basal condition among known LOX metabolites and 5(S)-HETE generation is more highly stimulated by *H. pylori* infection. All of these facts signify that *H. pylori* infection might activate AA metabolism to 5-LOX pathway rather than COX pathway in both non-cancerous and cancerous gastric cells, but the modulation of LOX activity after *H. pylori*infection might be more critical in either modulation of ensuing inflammation or gastric carcinogenesis than COX pathway in some aspects.

Since both *H. pylori* and 5-LOX are responsible for carcinogenesis, modulation of 5-LOX activity could be defined as chemoprevention in more extensive meaning. Longstanding, uncontrolled persistence of inflammation imposes the critical steps to carcinogenesis in several clinical diseases including *H. pylori*-associated gastric carcinogenesis, Barrett's esophagus-associated esophageal cancer, colitic cancer, and cholangiocarcinoma, for which oxidative stress, gene mutation, and overwhelming inflammatory cascade might be quite responsible. AA metabolism results in the formation of reactive oxygen species and other free radicals, which can be modulated by certain exogenous antioxidants. In fact, dietary modifications by selected nutritional and botanical agents, for instance, NDGA, epigallocatechin-3-gallate (EGCG), curcumin, quercetin, and omega-3 fatty acids, favorably influence eicosanoid production and effectively strengthen the enzymatic line of defense against oxidative molecules through retaining NF-κB in the cytoplasm by an inhibitor of NF-κB, IκB αprotein.[36-39]. Based on the several studies reported on the anti-oxidative effect of ginseng against lipid peroxidation[40], this is one critical point of mechanisms of RGE upon AA metabolism through 5-LOX inhibition.

Pananx Ginseng has been demonstrated to have anti-microbial, anti-adhesive activity against *H. pylori* [41-42]. Ginsenoside Rb1 isolated from Red Ginseng significantly decreased histamine and leukotriene production in a dose-dependent manner during mast cell activation[21] as well as LPS-induced proinflammatory cytokine production[43]. Recently, dietary pigment, curcumin appears to block *H. pylori*-induced NF-κB activation, IL-8 gene expression, the cell scattering response 36), and modulate AA metabolism including PLA2, COX, and 5-LOX[44]. We found that RGE can regulate *H. pylori*- induced NF-κB activation and subsequent expression of IL-8 in gastric AGS cells.

In summary, RGEelicited the down-regulation of proinflammatory intermediate 5(S)-HETE synthesis, which was stimulated by *H. pylori* infection in gastric epithelia. Although further detailed clinicaltrials or the studies documenting what component of RGE is attributable to theses efficacy should be followed, RGEcould be a potent functional dietary source against pathogenic inflammatory processes initiated by *H. pylori* infection.

Literature Cited

1) Kikuchi S. Epidemiology of *Helicobacter pylori* and gastric cancer. *Gastric Cancer* 5:6-15, 2000
2) International Agency for Research on cancer. Schistosomes liver flukes and *Helicobacter pylori*. Monograph, pp.61, Lyon, 1994
3) Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper *RJ. Helicobacter* pyloriinfection and the development of gastric cancer. *N Engl J Med* 345:784-789, 2001
4) Hahm KB, Kim DH, Lee KM, Lee JS, Surh YJ, Kim YB, Yoo BM, Kim JH, Joo HJ, Cho YK, Nam KT, Cho SW. Effect of long-term administration of rebamipide on *Helicobacter*

pylori infection in mice. *Aliment Pharmacol Ther* 18:S24-38, 2003

5) Israel DA, Peek RM. Pathogenesis of *Helicobacter pylori-induced* gastric inflammation. *Aliment Pharmacol Ther* 15:1271-1290, 2001
6) Nakaya A, Wakabayashi H, Imamura L, Fukuta K, Makimoto S, Naganuma K, Orihara T, Minemura M, Shimizu Y, Nagasawa T, Hamazaki T, Watanabe A. *Helicobacter pylori* alters n-6 fatty acid metabolism and prostaglandin E_2 synthesis in rat gastric mucosal cells. *J Gastroenterol Hepatol* 16:1197-1205, 2001
7) Wakabayashi H, Orihara T, Nakaya A, Miyamoto A, Watanabe A. Effect of *Helicobacter pylori infection* on gastric mucosal phospholipid contents and their fatty acid composition. *J Gastroenterol Hepatol* 13(6):566-571, 1998
8) Orihara T, Wakabayashi H, Nakaya A, Fukuta K, Makimoto S, Naganuma K, Entani A, Watanabe A. Effect of *Helicobacter pylori* eradication on gastric mucosal phospholipid content and its fatty acid composition. *J Gastroenterol Hepatol* 16(3):269-75, 2001
9) Marlink KL, Bacon KD, Sheppard BC, Ashktorab H, Smoot DT, Cover TL, Deveney CW, Rutten MJ. Effects of *Helicobacter pylori* on intracellular Ca^{2+} signaling in normal human gastric mucous epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 285(1):163-176, 2003
10) Nardone G, Holicky EL, Uhl JR, Sabatino L, Staibano S, Rocco A, Colantuoni V, Manzo BA, Romano M, Budillon G, Cockerill FR 3rd, Miller LJ. *In vivo* and *in vitro* studies of cytosolic phospholipase A_2 expression in *Helicobacter pylori* infection. *Infect Immun* 69(9):5857-5863, 2001
11) Pomorski T, Meyer TF, Naumann M. *Helicobacter pylori*-induced prostaglandin E_2 synthesis involves activation of cytosolic phospholipase A_2 in epithelial cells. *J Biol Chem* 276(1):804-810, 2001
12) Steinberg G, Slaton WH Jr, Howton DR, Mead JF. Metabolism of essential fatty acids. Incorporation of linoleate into arachidonic acid. *J Biol Chem* 220(1):257-264, 1956
13) Scheiman JM, Greenson JK, Lee J, Cryer B. Effect of cyclooxygenase-2 inhibition on human *Helicobacter pylori* gastritis: mechanisms underlying gastrointestinal safety and implications for cancer chemoprevention. *Aliment Pharmacol Ther* 17(12):1535-1543, 2003
14) Nam KT, Hahm KB, Oh SY, Yeo M, Han SU, Ahn B, Kim YB, Kang JS, Jang DD, Yang KH, Kim DY. The selective cyclooxygenase-2 inhibitor nimesulide prevents Helicobacter pylori-associated gastric cancer development in a mouse model. *Clin Cancer Res* 10(23):8105-13, 2004
15) Etienne A, Soulard C, Touvay C, Clostre F, Braquet P. *In vivo* anti-allergic and anti-inflammatory effects of drugs that inhibit lipoxygenase activity *in vitro*. *Int J Tissue React* 7(6):459-62, 1985
16) Meier RW, Niklaus G, Dewald B, Fey MF, Tobler A. Inhibition of the arachidonic acid pathway prevents induction of IL-8 mRNA by phorbol ester and changes the release of IL-8 from HL 60 cells: differential inhibition of induced expression of IL-8, TNF-alpha, IL-1 alpha, and IL-1 beta. *J Cell Physiol* 165(1):62-70, 1995
17) Denizot Y, Godard A, Raher S, Trimoreau F, Praloran V. Lipid mediators modulate the synthesis of interleukin 8 by human bone marrow stromal cells. *Cytokine* 11(8):606-610, 1999
18) Sharma SA, Tummuru MK, Blaser MJ, Kerr LD. Activation of IL-8 gene expression by *Helicobacter pylori* is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. *J Immunol* 160:2401-2407, 1998
19) Schroder JM. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. *J Exp Med* 170(3):847-863, 1989
20) Aoki Y, Qiu D, Zhao GH, Kao PN. Leukotriene B4 mediates histamine induction of NF-kappaB and IL-8 in human bronchial epithelial cells. *Am J Physio* l274:L1030-1039, 1998
21) Teng CM, Kuo SC, Ko FN, Lee JC, Lee LG, Chen SC, Huang TF. Antiplatelet actions of panaxynol and ginsenosides isolated from ginseng. *Biochim Biophys Acta* 990(3):315-320, 1989
22) Park HJ, Rhee MH, Park KM, Nam KY, Park KH. Effect of non-saponin from Panax ginseng on cGMP and thromboxane A2 in human platelet aggregation. *J Ethnopharmco* l49:157-162, 1995
23) Park EK, Choo MK, HanMJ, Kim DH. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. *Int Arch Allergy Immunol* 133(2):113-120, 2004
24) Keum YS, Han SS, Chun KS, Park KK, Park JH, Lee SK, Surh YJ. Inhibitory effects of the ginsenoside Rg3 on phorbol ester-induced cyclooxygenase-2 expression, NF-kappaB activation and tumor promotion. *Mutat Res* 523(4):75-85, 2003
25) Park S, Choue RW, Cho Y, Ziboh VA. Regional biosynthesis of prostaglandins and hydroxyeicosatetraenoic acids from arachidonic acid in the rat stomach tissue. *Prostaglandins Leukot Essent Fatty Acids* 68(1):35-42, 2003
26) Wescott JY, Balazy M, Stenmark KR, Murphy RC. Analysis of leukotriene D4 in human lung lavage by HPLC, RIA and mass spectrometry. *Adv Prostaglandin Thromboxane Leukot Res* 16:353-361, 1986
27) Park S, Won YM, Cho JS, Jin JH, Hahm KB. Rescue of *Helicobacter pylori* induced Cytotoxicity by Red Ginseng. *Dig Dis Sci* 50(7):1218-1227; 2004
28) Nam KT, Hahm KB, Oh SY, Yeo M, Han SU, Ahn B, Kim YB, Kang JS, Jang DD, Yang KH, Kim DY. The selective cyclooxygenase-2 inhibitor nimesulide prevents *Helicobacter pylori*-associated gastric cancer development in a mouse model. *Clin Cancer Res* 10(23):8105-8113, 2004
29) Choi HJ, Han HS, Park JH, Son JH, Pae JH, Choi C. PLA2 inhibiting, Anticancer effect of polyphenol rich fractions from *Panax Ginseng* C. A. Meyer. *J Korean Soc Agric Chem Biotechnol* 46(3):251-6, 2003
30) Kimura S, Fukuda T, Arakawa T, Kobayashi K. Leukotriene levels of *Helicobacter pylori*-infected gastric mucosa. *Nippon Rinsho* 51(12):3159-3162, 1993

31) Marleau S, Fruteau de Laclos B, Sanchez AB, Poubelle PE, BoRGat P. Role of 5-lipoxygenase products in the local accumulation of neutrophils in dermal inflammation in the rabbit. *J Immunol* 163(6):3449-3458, 1999
32) Konturek JW, Bielanski W, Konturek SJ, Domschke W. Eradication of *Helicobacter pylori* and gastrin-somatostatin link in duodenal ulcer patients. *J Physiol Pharmacol* 47(1) :161-175, 1996
33) Ghosh J. Inhibition of arachidonate 5-lipoxygenase triggers prostate cancer cell death through rapid activation of c-jun N-terminal kinase. *Biochem Biophys Res Commun* 307:342-349, 2003
34) Tseng HJ, Chan CC, Chan EC. Sphingomyelinase of Helicobacter pylori-induced cytotoxicity in AGS gastric epithelial cells via activation of JNK kinase. *Biochem Biophys Res Commun* 314(2):513-518, 2004
35) Shimakura S, Boland CR. Eicosanoid production by the human gastric cancer cell line AGS and its relation to cell growth. *Cancer Res* 52(7):1744-1749, 1992
36) Foryst-Ludwig A, Neumann M, Schneider-Brachert W, Neumann M. Curcumin blocks NFκB and the motogenic respinse in infected epithelial cells. *Biochem Biophys Res Commun* 316:1065-1072, 2004
37) Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids; old and new aspects of a class of natural therapeutic drugs. *Life Sci* 65:337-53, 1999
38) Wallace JM. Nutritional and botanical modulation of the inflammatory cascade-eicosanoids, cyclooxygenases, and lipoxygenases-as an adjunct in cancer therapy. *Integr Cancer Ther* 1(1):7-37, 2002
39) Lee KM, Yeo M, Choue JS, Jin JH, Park SJ, Cheong JY, Lee KJ, Kim JH, Hahm KB. Protective mechanism of epigallocatechin-3-gallate against *Helicobacter pylori*-induced gastric epithelial cytotoxicity via the blockage of TLR-4 signaling. *Helicobacter* 9(6):632-642, 2004
40) Kim YK, Guo Q, Packer L. Free radical scavenging activity of Red Ginseng aqueous extracts. *Toxicology* 172(2):149-156, 2002
41) Lee JH, Park EK, Uhm CS, Chung MS, Kim KH. Inhibition of *Helicobacter pylori* adhesion to human gastric adenocarcinoma epithelial cells by acidic polysaccharides from Artemisia capillaris and Panax ginseng. *Planta Med* 70(7):615-619, 2004
42) 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
43) Smolinski AT, Pestka JJ. Modulation of lipopolysaccharide-induced proinflammatory cytokine production in vitro and *in vivo* by the herbal constituents apigenin (chamomile), ginsenoside Rb (1) (ginseng) and parthenolide (feverfew). *Food Chem Toxicol* 41:1381-1390, 2003
44) Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, Lee MJ, Yang CS. Modulation of arachidonic acid metabolism by curcumin and related beta-diketone derivatives: effects on cytosolic phospholipase A_2, cyclooxygenases and 5-lipoxygenase. *Carcinogenesis* 25(9):1671-169, 2004