藥學碩士學位論文

Effects of capsaicin on pro-inflammatory and anti-inflammatory signaling in the stomach of transient receptor potential type vanilloid 1 (TRPV1) wild type and knockout mice

TRPV1 wild type과 knockout 마우스 위장세포의 염증 및 항염증 신호전달에 미치는 캡사이신의 효과

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서울大學校 大學院

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Abstract

Effects of capsaicin on pro-inflammatory and anti-inflammatory signaling in the stomach of transient receptor potential type vanilloid 1 (TRPV1) wild type and knockout mice

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Gastric cancer is the fourth most common cancer in men and fifth in women, and the second leading cause of cancer mortality in the rate of gastric cancer in the world. There are several factors that contribute to gastric cancer development. These include *Helicobacter pylori* infection, high salt diet, and smoking. Frequent consumption of hot spicy food has been speculated to be a risk factor for the gastric cancer, but the data in the literature are still conflicting and discordant. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a major pungent principle of hot chili pepper which

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is one of the most frequently consumed spices throughout the world, especially in Korea, Southeast Asian and Latin-American countries. The transient receptor potential type vanilloid 1 (TRPV1) is known as the capsaicin receptor activated not only by capsaicinoids, but also other stimuli including heat (>43℃). However, the roles of TRPV1 and capsaicin in gastric carcinogenesis are still controversial. In the present study, I investigated the effects of capsaicin on gastric inflammation in TRPV1 wild type (WT) and knockout (KO) mice. TRPV1 WT and KO mice were fed normal diet or 0.05% capsaicin diet for 36 weeks. TRPV1 KO mice fed normal diet showed significantly elevated expression of cyclooxygenase-2 (COX-2) than did the WT animals. In contrast, the expression level of heme oxygenase-1 (HO-1) with an anti-inflammatory function was reduced in the TRPV1 KO mice. COX-2 up-regulation and HO-1 down-regulation in the TRPV1 KO mice were attenuated by capsaicin administration. AMP-activated protein kinase (AMPK), which functions as a cellular energy sensor, has been shown to mediate critical anti-inflammatory effects. The expression level of phosphorylated-AMPK was elevated in capsaicin-treated TRPV1 KO mice. These findings suggest capsaicin may exert anti-

inflammatory effects in mouse stomach via TRPV1.

Keywords: Transient Receptor Potential type Vanilloid 1 (TRPV1), capsaicin, cyclooxygenase-2 (COX-2), AMP-activated protein kinase (AMPK), heme oxygenase-1 (HO-1)

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Introduction

Gastric cancer is a disease caused by an abnormal growth of malignant cells in the stomach tissues [1]. The rate of gastric cancer survival is decreasing due to the development of endoscopy and frequently physical examination leading to the early detection and cancer treatment. [2]. However, gastric cancer is still the second most frequently occurring disease-related mortality in the world [3]. According to the report from the World Cancer Research Fund International in 2008, the Republic of Korea had the highest rate of gastric cancer in the world. There are exogenous and endogenous factors that contribute to the development of gastric cancer. Hot chili pepper consumption has been suspected as a risk factor for the development of stomach cancer. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide, **Fig. 1**) is a major pungent and irritating ingredient of hot chili pepper [4]. The effects of capsaicin and hot chili pepper on carcinogenesis are controversial [5]. Some studies demonstrated that capsaicin has carcinogenic or co-carcinogenic effects [6] and promotes gastric cancer progression [7]. However, other studies indicated that

capsaicin exerts chemoprotective effects on experimentally induced carcinogenesis [8] and induces apoptosis in various types of tumor cells, including human hepatoma [9], colon cancer [10] and myeloid leukemia [11] cells. Capsaicin is the most well known agonist of TRPV1. [12]. TRPV1 (**Fig. 2**), an ion channel, is primarily distributed in sensory neurons involved in the transmission and modulation of pain [13]. Therefore, the research of TRPV1 has mainly focused on pain and neuron, and there is a paucity of data on the role of TRPV1 in carcinogenesis.

Cyclooxygenase (COX) is an enzyme responsible for production of important biological mediators collectively called prostanoids. Constitutive isoform (COX-l) is considered a housekeeping enzyme, whereas COX-2 is induced by inflammatory stimuli including tissue damages [14]. COX-2, a rate-limiting enzyme for prostanoid biosynthesis, is involved in differentiation, cell growth and inflammation-associated tumorigenesis [15, 16]. Elevated COX-2 expression has been detected in several common human malignancies, including stomach [17], oesophageal [18], pancreatic [19] and colorectal [20] carcinomas.

c-Jun, a component of activator protein (AP-1) that is transcription factor, homo- or heterodimerizes with c-Fos or basic region-leucine zipper proteins to organize AP-1 [21, 22]. The up-regulation of COX-2 can be induced by stimulation of the AP-1 pathway [22].

Nuclear factor-κB (NF-κB) has also been known to drive inflammation to cancer [23, 24]. The NF-κB exists in either homo- or heterodimer of RelB, p65 (RelA), p52, c-Rel and p50 subunits. In most cells, the p50/p65 heterodimer is the most abundant functionally active subunit [25]. NF-κB is a key transcription factor that induces COX-2 expression.

Heme oxygenase-1 (HO-1), an inducible protein in response to stress, has anti-inflammatory, anti-proliferative and potent antioxidant functions [25, 26]. The level of HO-1 is up-regulated by numerous stressors such as reactive oxygen species, heat, and ultraviolet irradiation [27]. However, no study has been reported regarding the relationship of HO-1 expression and capsaicin intake in the stomach of mice.

AMP-activated protein kinase (AMPK) is an enzyme that plays a

central role in cellular energy homeostasis and signaling to metabolic stress [28, 29]. After AMPK is phosphorylated at the threonine 172 site, the enzyme is activated and can modulate numerous energy-conserving cellular responses [30]. It has been also reported that AMPK can inhibit inflammatory response [28, 31].

Based on these findings, I attempted to investigate the effect of capsaicin on pro-inflammatory and anti-inflammatory signaling in the stomach of TRPV1 WT and KO mice.

Materials and Methods

Materials

Female C57BL/6 mice (6 weeks of age) were purchased from Central Lab. Animal Inc. (Seoul, Korea). A couple of TRPV1 KO mice were obtained from Prof. Uhtaek Oh in Seoul National University. Capsaicin was purchased from Sigma Aldrich Inc. (St. Louis, MO, USA). Antibody against COX-2 was a product of neomarkers (Neomarkers, Fremont, CA, USA). Antibody against HO-1 was a product of Stressgen (Ann Arbor, MI, USA). RPMI 1640 medium and fetal bovine serum were purchased from Gibco BRL (Grand Island, NY, USA). Horseradish peroxidase-conjugated anti-rabbit, mouse or goat secondary antibodies were obtained by Zymed Laboratories (San Francisco, CA, USA). Pico EPD Western blot detection kit was purchased from ELPIS (Republic of Korea). All other chemicals used during experiment were obtained in the purest form available commercially.

Cell culture

AGS cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and a 100 ng/ml penicillin/streptomycin/fungizone mixture at 37℃ in humidifying atmosphere of 5% CO_2 and 95% air. The cells were plated at an appropriate density according to each experimental scale.

Animal treatment

The animals were kept in plastic cages under controlled conditions of temperature (23℃±2℃), humidity (50%±10%), and light (12/12-h light/dark cycle). C57BL/6 mice were acclimated for 7 days. A total of 20 mice were divided into 4 experimental groups. Each group consists of 5 mice. In the control group, mice were kept with a ground basal diet. In the capsaicin only diet group, mice were given 0.05% capsaicin-containing ground diet. In the high salt diet group, mice were given 7.5% NaCl-containing ground diet. In the capsaicin and salt combination diet group, mice were given 0.05% capsaicin and 7.5% NaCl-containing ground diet. TRPV1 KO mice were equally treated with C57BL/6 mice.

Tissue lysis and protein extraction

Stomach tissues were lysed with ice cold lysis buffer [150 mM NaCl, 0.5% Triton-X 100, 50 mM Tris–HCl (pH 7.4), 20 mM ethylene glycol tetra-acetic acid (EGTA), 1 mM dithiothreitol (DTT), 1 mM Na3VO4 and protease inhibitors, 1mM phenylmethylsulfonylfluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA)-free cocktail tablet] followed by periodical vortex for 30 min at 0 ℃. Lysates were centrifuged at 13,000 rpm for 15 min at 4 ℃. Supernatants were collected and stored at -70 ℃.

Preparation of cytosolic and nuclear extracts from mouse stomach

According to previous work in our laboratory, cytosolic extracts were obtained from lysates dissolved in hypotonic buffer A [10 mM HEPES (pH 7.8), 10 mM KCl, 1.5 mM MgCl2, 0.5 mM DTT, 0.2 mM EDTA and 0.2 mM phenylmethylsulfonylfluoride (PMSF)] with NP-40 by homogenizing and vortex every 10 min for 1 h. After centrifugation at 13,000 rpm for 15 min, supernatants (the cytosolic extracts) collected

and stored at -70°C. The pellets were washed with hypotonic buffer A to remove remaining cytosolic components. Then pellets were resuspended in hypertonic buffer C [20 mM HEPES (pH 7.8), 20% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF] for 30 min on ice and centrifuged at 13,000rpm for 15min. The supernatant containing nuclear proteins was collected and stored at -70℃ after determination of the protein concentration by Bradford method using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot analysis

After collecting lysates of tissues or cells, the total protein concentration of was quantified by using bicinchoninic acid (BCA) protein assay kit. Lysates of tissues and cells (30–50 μg protein) were boiled in sodium dodecyl sulfate (SDS) sample buffer for 3 min before electrophoresis on 7-10% SDS–polyacrylamide gel (SDS-PAGE). They were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane. Blots were incubated in fresh blocking

buffer (0.1% Tween-20 in tris-buffered saline (TBST) containing 5% non-fat dry milk, pH 7.4) for 1 h followed by incubation with appropriate primary antibodies in TBST with 3% BSA. After washing with TBST three times, blots were incubated with horseradish peroxidase-conjugated secondary antibody in TBST with 3% non-fat dry milk for 2 h at room temperature. Blots were washed again three times with TBST buffer, and were detected with enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Histological evaluation

Mouse stomach parts were cut and washed with phosphate-buffered saline (PBS). Specimens of inner cross section parts of the stomach were fixed with 10% buffered formalin and embedded in paraffin. Each section was stained with hematoxylin and eosin (H&E). The fixed sections were examined by light microscopy for the presence of lesions.

Statistical analysis

Except for the data on body weight change expressed as the mean ± standard deviation (SD), all the other values were expressed as the mean ± standard error (SE) of at least three independent experiments. Statistical significance was determined by the Student's *t*-test and $p < 0.05$ was considered to be statistically significant.

Results

The high dose of capsaicin or salt induces expression of COX-2 and c-Jun in AGS cells.

COX-2 has been known to be a pro-inflammatory enzyme that plays an important role in the inflammation. c-Jun is one of well-known transcription factors that regulate COX-2 expression. Thus, I first examined whether capsaicin or salt could affect the expression of this pro-inflammatory enzyme in the human gastric cancer AGS cell line. When AGS cells were treated with capsaicin or salt, the level of COX-2 expression was increased in a concentration-dependent manner (**Fig. 3A, 3B**). To investigate whether COX-2 induction was mediated via activation of AP-1 signaling, I conducted Western blot analysis with nuclear extracts which were treated with capsaicin or salt. c-Jun was also increased in a time dependent manner (**Fig. 3C, 3D**).

The High dose of capsaicin induces inflammatory effects in mouse

stomach.

To examine effects of capsaicin and salt to stomach of mice *in vivo*, C57BL/6 mice were divided into 4 groups and treated with normal diet, 7.5% salt-containing diet, 0.05% capsaicin-containing diet and 0.05% capsaicin plus 7.5% salt combination diet (**Fig. 4**). The mice were sacrificed after 12 weeks. As shown in **Figure 5A**, COX-2 expression was highly increased in the capsaicin diet group compared with that in other groups. To understand whether COX-2 expression was mediated through the activation of the NF-κB signaling pathway, I checked expression of p65 in mouse stomach tissues. As shown in **Figure 5B**, the level of p65 was elevated in the stomch of mice fed capsaicin diet. In addition, HO-1 expression was decreased in the same group (**Fig. 5C**). These results suggest that high dose of capsaicin might induce inflammation in mouse stomach.

I also evaluated the histological changes in H&E stained-mouse stomach tissues (**Fig. 6**). Microscopic observation displayed the features of mouse stomach mucosa (stomach lining). In the normal diet group, the gastric mucosa exhibits no histologic abnormality (**Fig. 6A**).

In the 7.5% salt diet group, the mucosa was shown to be slightly damaged compared to that of the normal diet group (**Fig. 6B**). As shown in **Figure 6C**, the mucosa was depleted by 0.05% capsaicin diet. Lastly, in the 0.05% capsaicin and 7.5% salt combination diet group, the mucosa was also shown to be slightly damaged (**Fig. 6D**). These results are consistent with Western blotting data.

Capsaicin diet has different effects on body weight changes in TRPV1 WT and KO mice.

To investigate effects of capsaicin to stomach of TRPV1 WT and KO mice, mice were divided into 4 groups and treated with normal diet or 0.05% capsaicin-containing diet. The mice were sacrificed after 36 weeks each diet (**Fig. 7**). In TRPV1 WT mice, capsaicin diet was shown to have the effects that mimic calorie restriction. Based on the results shown in **Figure 9**, there was a variation in the body weight increase between normal diet and capsaicin diet groups in WT mice. On the other hand, in TRPV1 KO mice, normal diet and capsaicin diet had no significant effect on the proportion of body weight increase. Taken

together, the body weight change of mice caused by capsaicin diet might be dependent in the presence of TPRV1.

Capsaicin might have pro-inflammatory and anti-inflammatory effects in the stomach of TRPV1 WT and KO mice.

To examine effects of long-term intake of capsaicin in the stomach of TRPV1 WT and KO mice, the mice were divided into 4 groups and treated with normal diet or 0.05% capsaicin-containing diet. This diet was maintained for 36 weeks (**Fig. 7**). As shown in **Figure 10**, the levels of COX-2 expression were not significantly different between normal diet and capsaicin diet groups in the stomach of TRPV1 WT mice. Interestingly, COX-2 expression was increased in the absence of TRPV1. It is suggested that TRPV1 itself might have a protective effect in the stomach of mouse. The elevated COX-2 expression was attenuated by capsaicin administration in the stomach of TRPV1 KO mice (**Fig. 10A)**. I performed Western blot analysis with nuclear extracts of TRPV1 WT and KO mice to investigate the possible involvement of AP-1 signaling related in COX-2 expression. The

pattern of c-Jun expression was corresponding to that of COX-2 expression. It was proposed that AP-1 signaling can play a role in the induction of COX-2 related to capsaicin diet. As shown in **Figure 10C**, in TRPV1 WT mice, the level of HO-1 expression was decreased by capsaicin diet. In the absence of TRPV1, HO-1 expression was down-regulated compared to its TRPV1 WT counterpart. Furthermore, the decreased HO-1 expression was attenuated by capsaicin administration. AMPK is known to have an inhibitory effect on inflammatory stress [28], capsaicin diet had no effect on the expression of the phosphorylated form of AMPK (p-AMPK) in TRPV1 WT mice. In contrast, when TRPV1 was silenced, p-AMPK was down-regulated. The expression level of p-AMPK constitutively decreased in the stomach of TRPV KO mice was restored by capsaicin administration (**Fig. 10D**). It is speculated that TRPV1 itself might have a protective effect in the mouse stomach by maintaining the proper activation of AMPK signaling. In addition, TRPV1 WT mice fed capsaicin-containing diet showed no signs of inflammatory damage in the stomach.

I also performed histological evaluation in H&E stained-stomach tissues (**Fig. 11**). Microscopic observation displayed the features of mouse stomach mucosa (stomach lining) after feeding normal diet and 0.05% capsaicin-containing diet for 36 weeks. In the TRPV1 WT mice fed normal diet, the gastric mucosa was shown to have no injury (**Fig. 11A**). In TRPV1 WT mice given capsaicin-containing diet, the mucosa was shown to be slightly damaged (**Fig. 11B**). As shown in **Figure 11C,** in the normal diet group of TRPV1 KO mice, the mucosa was destroyed. However, the destruction was attenuated by capsaicin administration in TRPV1 KO mice (**Fig. 11D**).

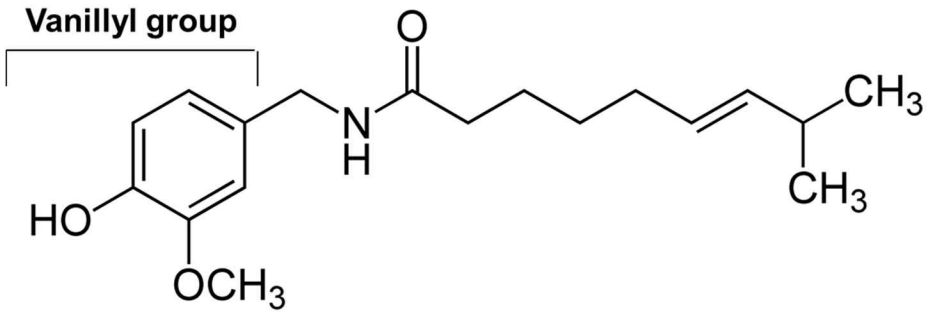

Figure 1. The chemical structure of capsaicin (8-methyl-*N*-vanillyl-6-nonenamide)

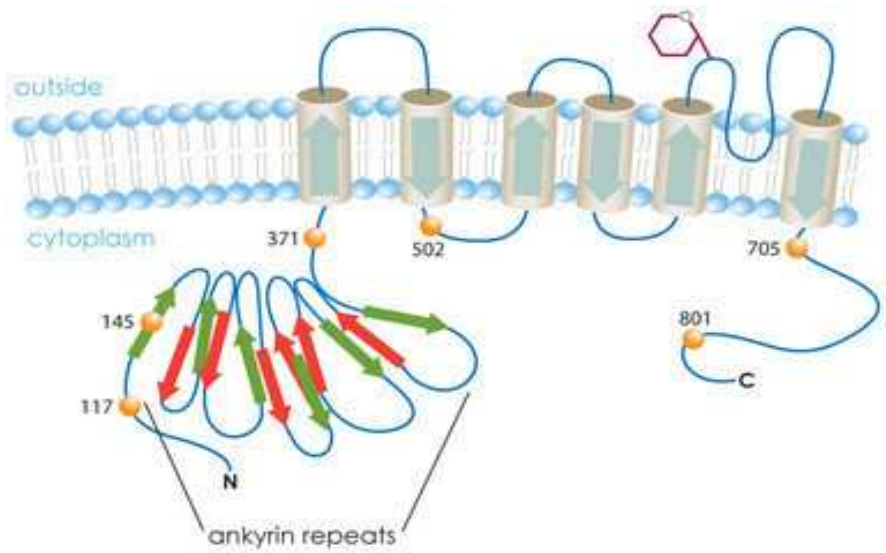

Figure 2. The assembly of TRPV1. TRPV1 is predicted to have six transmembrane domains, including an *N*-terminal six ankyrin repeat domains [32].

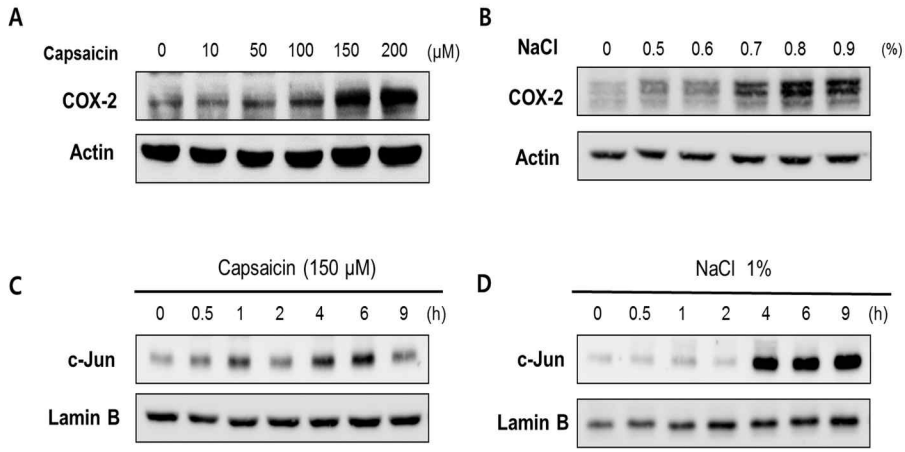

Figure 3. The effects of capsaicin and salt on expression of COX-2 and c-Jun in gastric cancer (AGS) cells. AGS cells were treated with the indicated concentrations of capsaicin or NaCl. **A and B,** Cells were incubated for 6 h to measure COX-2 expression. **C and D,** Cells were incubated with 150 μM of capsaicin or 1% of NaCl to measure c-Jun expression. The protein levels of COX-2 and c-Jun were determined by Western blot analysis.

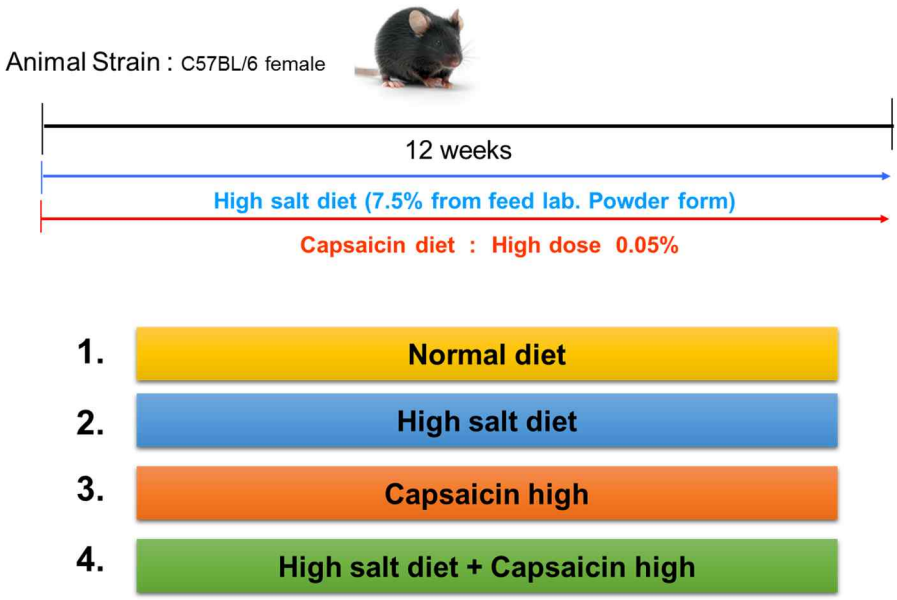

Figure 4. Experimental design for investigating effects of capsaicin and/or salt in mouse stomach. Female C57BL/6 mice were divided into 4 groups. Each group has 3 mice. Animals in the first group were fed normal diet for 12 weeks. Animals in the second group were given 7.5% of NaCl-containing diet. Animals in the third group received diet containing 0.05% of capsaicin. Animals in the last group were fed NaCl and capsaicin combination diet.

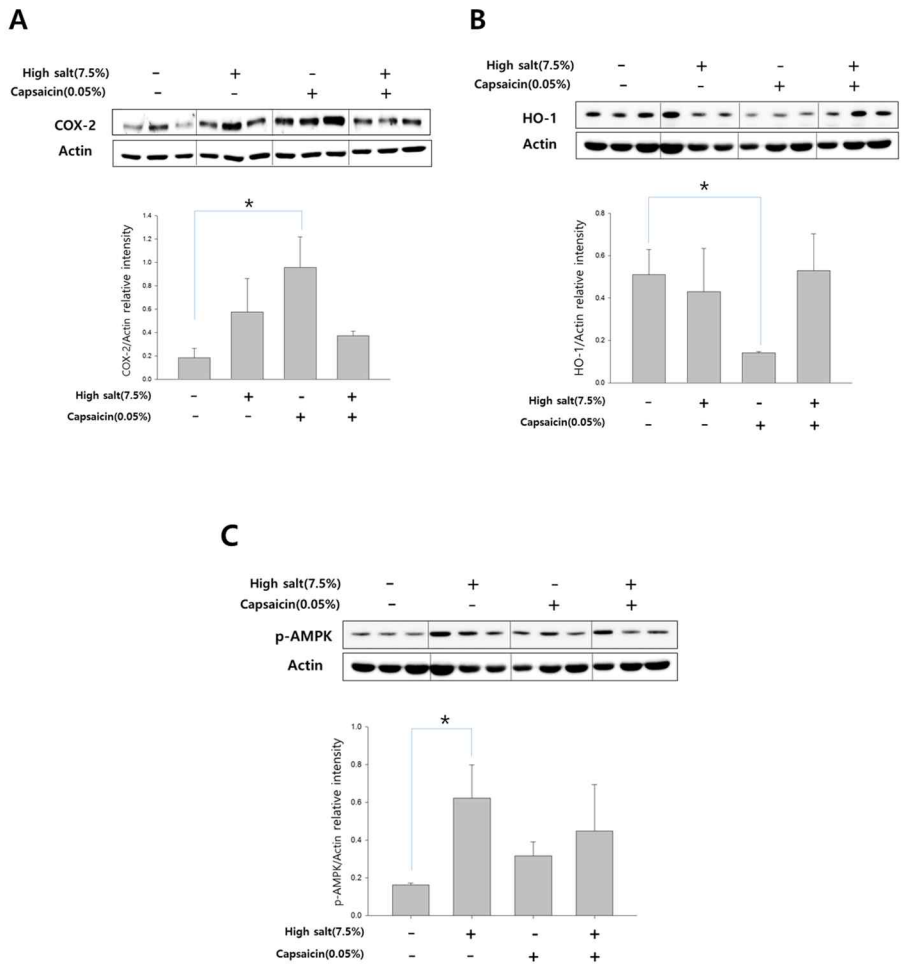

Figure 5. Effects of capsaicin and salt on expression of COX-2, p65 and HO-1 in mouse stomach. Animals were treated as described in **Figure 4**. Stomach tissues were collected and lysed in ice-cold cell lysis buffer. Protein extracts (30 μg) were loaded onto a 7% SDS-PAGE, electrophoresed and

subsequently transferred onto PVDF membrane. Immunoblots were probed with rabbit polyclonal COX-2 antibody. Quantification of COX-2 immunoblots was normalized to that of actin followed by statistical analysis of image density. All of the relative expression levels are presented means ± SE. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

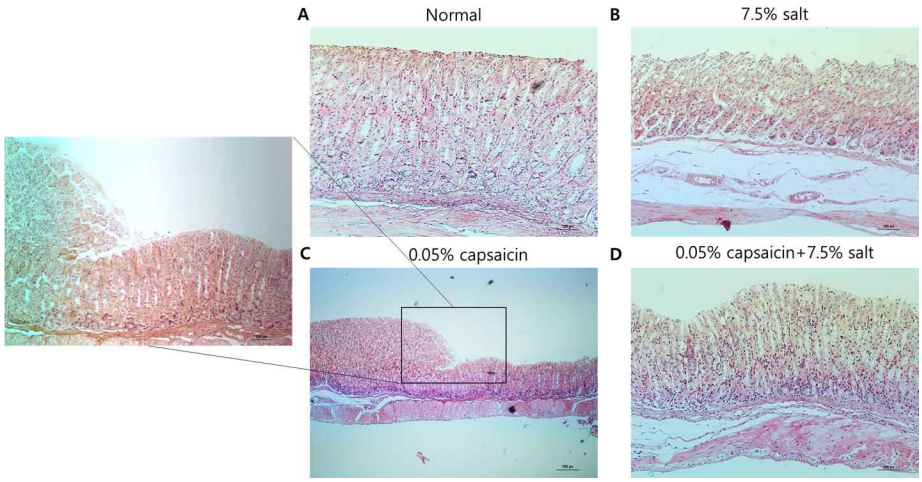

Figure 6. Histological images of mouse stomach tissues from C57BL/6 mice fed diet containing capsaicin, salt or both. Specimens of stomach tissues were fixed with formalin and embedded in paraffin and H&E. Magnifications X 100.

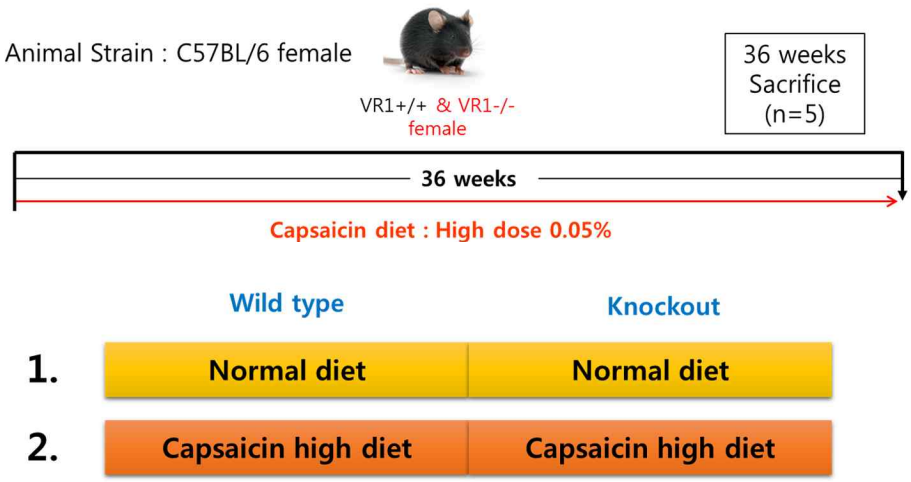

Figure 7. Experimental design for comparing effects of capsaicin in the stomach TRPV1 WT and KO mice. Female TRPV1 WT and KO mice were divided into 4 groups, each consisting of 5 animals. TRPV1 WT and KO mice were fed either normal diet or diet containing 0.05% capsaicin for 36 weeks.

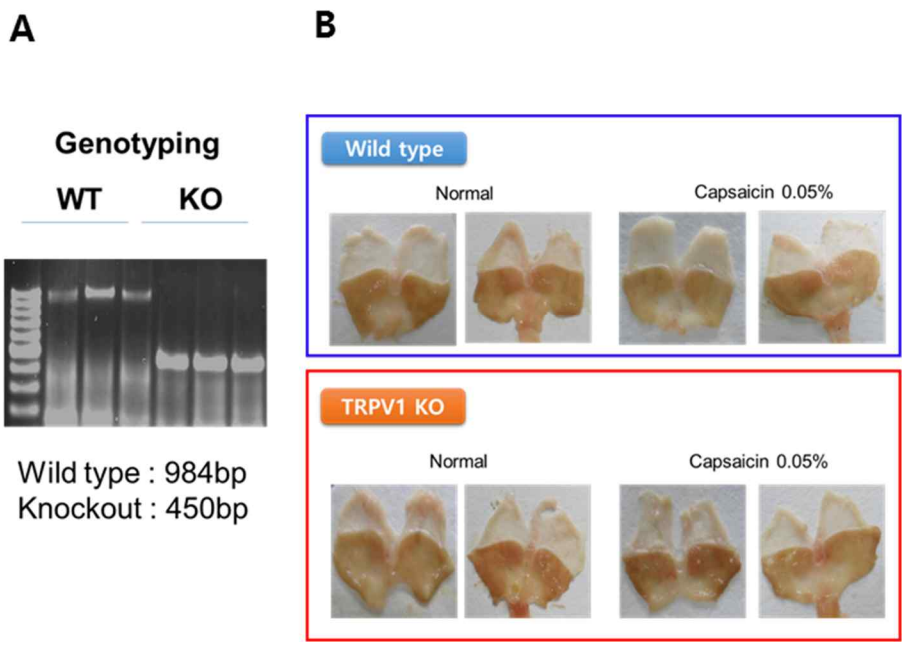

Figure 8. Gross evaluation of stomach tissues from TRPV1 WT and KO mice stomach. A, Genotyping of TRPV1 WT and KO mice. Genomic DNA was isolated from the tails of the animals and subjected to PCR amplification by using specific primers to verify TRPV1 WT and KO mice. The amplified DNA was size fractionated on a 1.5% agarose. **B,** Macroscopic views of the stomach in TRPV1 WT and KO mice.

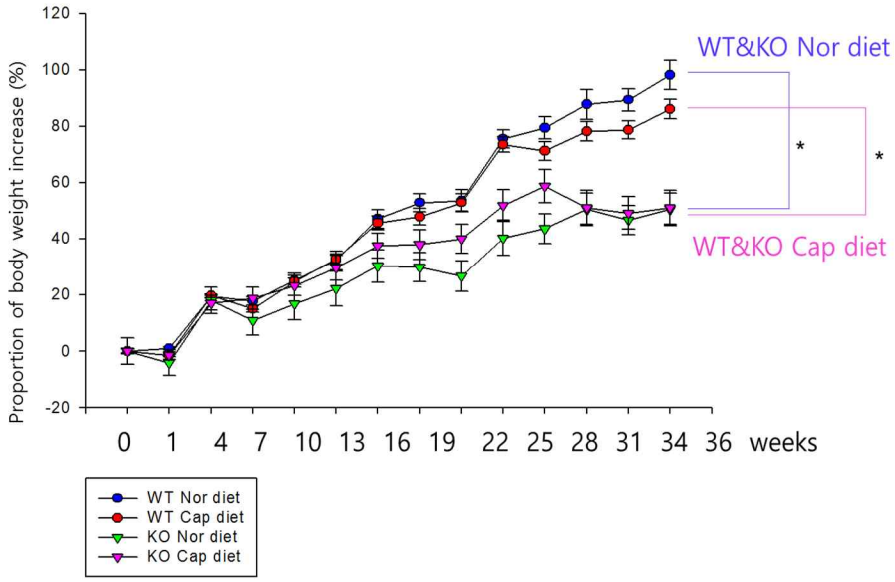

Figure 9. Effects of capsaicin on the body weight change in TRPV1 WT and KO mice. The TRPV1 WT and KO mice were treated as described in **Figure 7** for 36 weeks. Body weight was measured every week. All of the relative expression levels are presented means ± SD. * $p<0.05$

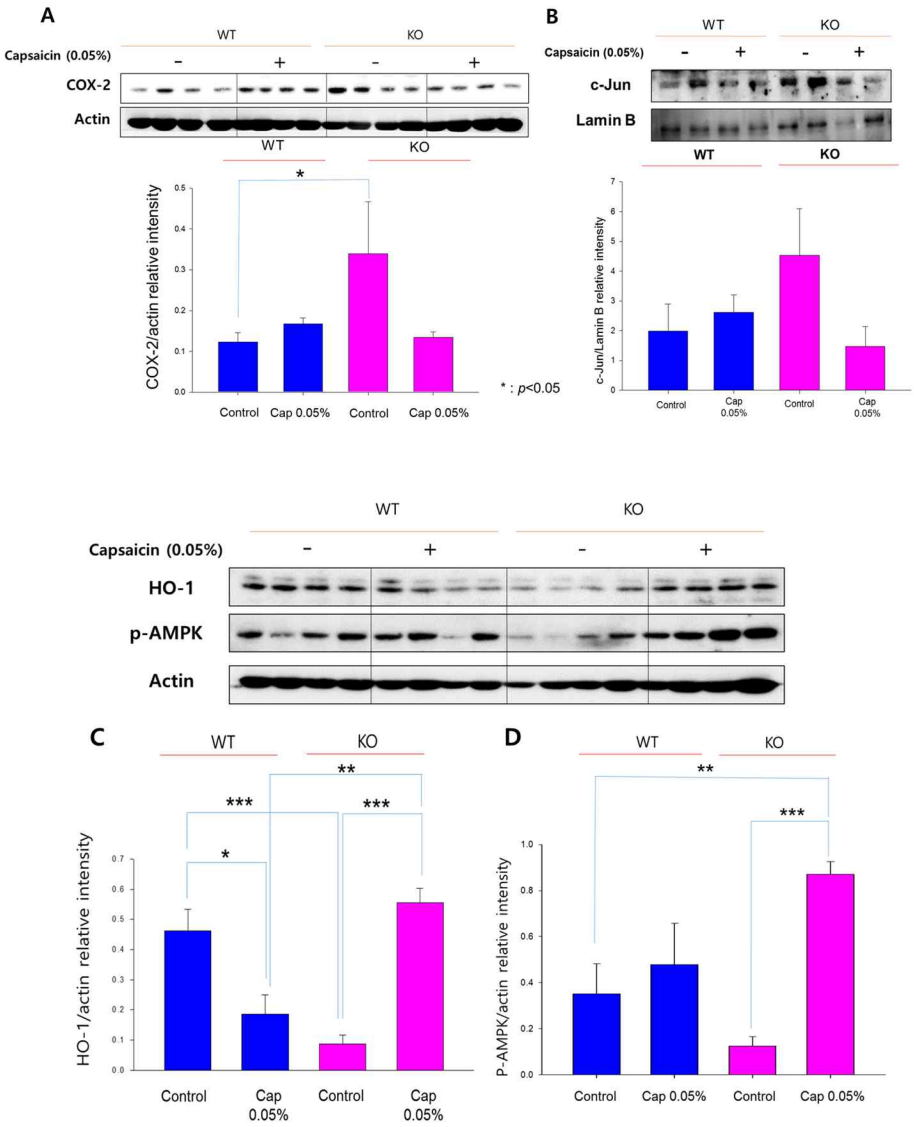

Figure 10. Effects of capsaicin on expression of COX-2, c-Jun, HO-1 and p-AMPK in the stomach of TRPV1 WT and KO mice. A, Western blot

analysis of COX-2 expression in TRPV1 WT and KO mice after capsaicin administration. **B**, Western blot analysis of c-Jun expression with nuclear extracts of TRPV1 WT and KO mice after capsaicin administration. **C**, Western blot analysis of HO-1 expression in TRPV1 WT and KO mice after capsaicin administration. **D**, Western blot analysis of p-AMPK expression in TRPV1 WT and KO mice after capsaicin administration. All of the relative expression levels are presented means ± SE. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

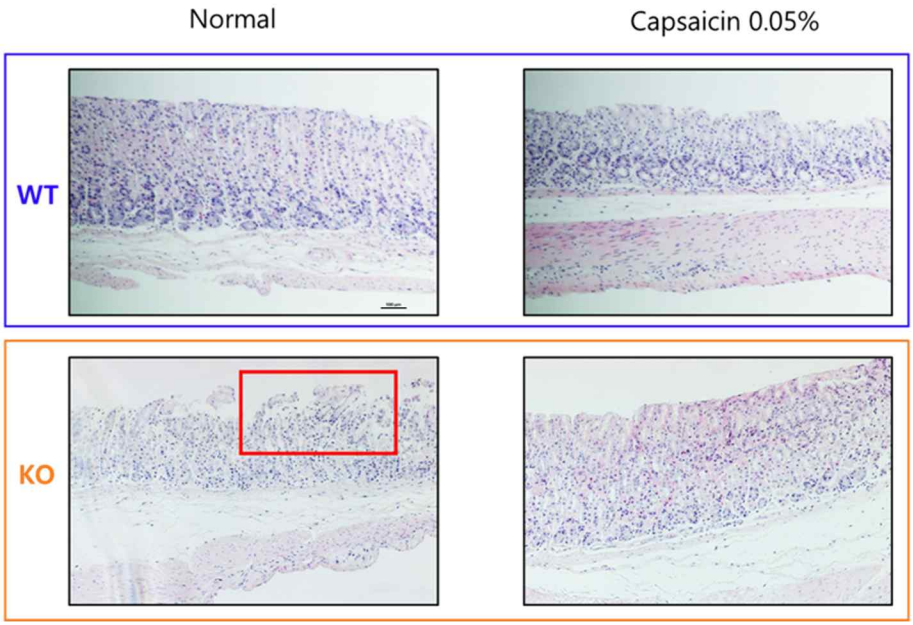

Figure 11. Histological images of mouse stomach tissues from TRPV1 WT and KO mice fed capsaicin diet. Specimens of stomach tissues were fixed with formalin and embedded in paraffin and H&E. Magnifications X 100.

Discussion

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a principal pungent and irritating component of hot chili peppers which belong to the genus *capsicum* [33, 34]. Although capsaicin has been widely consumed for a long period of time, it is being still debated whether its topical use or intake is completely safe [34]. Some studies suggested that capsaicin might be mutagenic [35] and can stimulate the proliferation of cancer cells [36]. In contrast, other studies indicated that capsaicin has anti-proliferative activities in various cancer cells [37, 38] and induces apoptosis or cell-cycle arrest [39, 40]. In this study, I investigated whether capsaicin has pro-inflammatory or anti-inflammatory activities in the stomach of mice when given as a diet.

TRPV1, known as a capsaicin receptor, is mainly expressed in both central and peripheral nervous systems [41]. So, there have been studies on both expression and distribution of TRPV1 [42, 43]. Early studies have demonstrated that activation of TRPV1 plays important roles in detection, transmission and modulation of pain [41, 44]. However, there has been paucity of data demonstrating the physiologic

functions of TRPV1 in the stomach of mice treated with capsaicin. This prompted me to investigate the effects of capsaicin and TRPV1 on pro-inflammatory and anti-inflammatory status in the stomach tissue of mice and also in cultured gastric epithelial cells.

Cyclooxygenase (COX) has been known to be present at least in two isoforms that are COX-1 and COX-2. COX-1 is a housekeeping enzyme and is constitutively expressed in most tissues. COX-1 is considered to be responsible for the biosynthesis of prostaglandins (PG) that are essential for homeostatic functions, maintaining the gastric mucosa integrity, protecting the stomach lining and mediating normal platelet functions [45, 46]. In contrast, COX-2 is an inducible enzyme and is selectively induced in response to pro-inflammatory cytokines in activated macrophages and other cells at the sites of inflammation [47]. Therefore, in this study, I determined COX-2 as a representative inflammatory marker in the stomach of mice.

HO-1 is the rate-limiting enzyme in heme catabolism. HO-1 catalyzes a step which leads to the degradation of free heme, generating biliverdin, carbon monoxide (CO) and free iron (Fe^{2+}) [48, 49]. HO-1 is

a stress-response protein that is induced when cells are challenged by various environmental and internal stressors. HO-1 has cytoprotective effects against oxidative damage during inflammation [49, 50].

Several studies have demonstrated that capsaicin has a carcinogenic effect. For example, it was reported that capsaicin (0.01% in drinking water for 6 weeks) promotes the development of enzyme-altered foci in the liver of rats treated with diethylnitrosamine (DEN) [51]. Swiss albino mice were fed 0.03% capsaicin in semisynthetic powdered diet for their lifespan. Histopathologically, benign polypoid adenomas of the cecum were developed in mice [52]. In rats fed diets-containing hot chili pepper, the occurrence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine–induced gastric cancer was slightly higher than that observed in the control group [53]. The present study was aimed to determine whether a high dose of capsaicin exert an inflammatory effect in the stomach of mice. In addition, it has been reported that high salt diet can cause gastritis and may promote development of gastric cancer. Thus, salt was also administered as another factor to accelerate inflammation in the mouse stomach. In this study, it was shown that capsaicin diet for 12

weeks led to damage of gastric mucosa. Additionally, short-term administration of dietary capsaicin increased the levels of inflammatory markers in the stomach of mice. Although it was expected that salt might accelerate inflammation of the stomach, results from this study are not supportive. In my opinion, the reason is that the mice fed salt or combination of capsaicin and salt diet drank much more water than mice fed normal diet. TPRV1 WT and KO mice fed long-term capsaicin diet (for 36 weeks) showed results discordant with those observed in mice fed short-term capsaicin diet, I expected that degree of inflammation in the stomach of mice fed long-term capsaicin diet (for 36 weeks) would be more prominent than the mice fed short-term capsaicin diet (for 12 weeks). However, contrary to my expectation, the damage caused by silencing of TRPV1 gene was shown to be severe. Notably, the damage was attenuated by capsaicin administration.

In conclusion, this study suggests that short-term capsaicin diet might have acute response to stomach, but long-term capsaicin diet might induce adaptive response to stomach. In addition, effects caused by the silencing of TRPV1 were attenuated by long-term capsaicin diet.

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국 문 초 록

위암은 남성에게는 네 번째 그리고 여성에게는 다섯 번째로 빈번하게 발병하는 질병이며, 위암은 암 사망률에 있어서는 세계에서 두 번째로 가장 중요한 원인으로 대두 되고 있다.

2008년 World Cancer Research Fund International 조사에 의하면, 대한민국은 세계에서 가장 높은 위암 발병률을 나타낸다고 보고 되었다. 위암 발달에 영향을 미치는 여러 가지 요인들이 존재하는데, 주요 요인으로는 헬리코박터 파일로리 균 감염, 높은 농도의 소금 섭취 그리고 흡연 등이 있다. 매운 음식의 빈번한 섭취 역시 위암 발달의 위험 요인으로 여겨지고 있지만, 이에 대한 문헌상의 자료들은 일치되지 않고 있으며, 여전히 논쟁의 여지가 존재한다고 보고되고 있다.

캡사이신은 고추 속 식물의 매운 맛을 내는 주요한 성분으로, 세계에서 가장 자주 사용되는 향신료 중 하나이다. 특히, 라틴 아메리카와 남아시아 국가들에서 주로 소비되고 있다. 캡사이신의 수용체로 알려져 있는 transient receptor potential type vanilloid 1 (TRPV1)은 캡사이신과 같은 자극뿐 만 아니라, 43℃ 이상의 열과 vanilloid 구조를 갖는 화합물 등에 의해 활성화된다고 알려져 있다. 그러나 위의 발암 과정에 있어 캡사이신과 TRPV1의 역할은 여전히 논란의 여지가 많은 상태다. 따라서, 본 연구에서는 TRPV1 WT 과 KO 쥐들을 이용하여 위염에 미치는 캡사이신의 영향을 검토하였다.

TRPV1 WT과 KO 쥐들에게 36주 동안 일반사료 또는 0.05%의 캡사이신이 섞인 사료를 섭취시켰다. 그 결과, 일반사료를 먹인 TRPV1 KO 쥐들의 위에서는 같은 조건의 TRPV1 WT 쥐들의 위에 비해 cyclooxygenase-2 (COX-2)의 발현이 확연히 증가함을 확인하였다. 반대로, 항 염증 기능을 갖는 heme oxygenase-1 (HO-1)의 발현수준은 일반사료를 먹인 TRPV1 WT 쥐들의 위에 비해 TRPV1 KO 쥐들의 위에서 감소됨을 확인하였다. TRPV1 KO 쥐들의 위에서 COX-2 유전자 발현의 증가와 HO-1 유전자 발현의 감소는 캡사이신 섭취에 의해 그 효과 약화되는 것을 확인하였다. 세포의 에너지 센서의 기능을 하는 AMP-activated protein kinase (AMPK) 역시 중요한 항 염증 효과를 조절한다고 보고된 바 있다. 캡사이신이 섞인 사료를 먹인 같은 조건의 TRPV1 WT 쥐들의 위에 비해 TRPV1 KO 쥐들의 위에서 인산화 된 AMPK의 발현 수준이 증가함 역시 확인하였다.

이러한 실험 결과들을 바탕으로, 캡사이신은 TRPV1의 유무에 따라 위에서 상이한 영향을 나타내며, 장기간의 캡사이신 섭취는 TRPV1 KO 쥐들의 위에서 보호 효과를 나타내는 것으로 사료된다.