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排氣飮이 Mepirizole에 의해 유발된 토끼의 위장관 손상에 미치는 영향

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Effect of Baegi-eum (BGU) on mepirizole-induced gastrointestinal tissue injury in rabbit

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목적 : 본 연구는 排氣飮이 토끼의 위장관내에서 화학물질에 의해 유발된 장관의 궤양에 유효한 효과를 발휘할 수 있는지를 검증하기 위한 실험이다.

방법 : 토끼 5마리를 한 군으로 하여 정상군과 체중 1kg당 200mg 분량의 mepirizole을 경구 투여한 군과 100 mg/kg의 排氣飮(경구투여)과 800Units/kg 분량의 catalase(정맥주사)를 mepirizole을 경구투여하기 2시간 전에 각각 전처치한 군으로 나누었다. Mepirizole을 경구 투여한 후 각각 24hr와 48hr에 토끼를 희생시켜 위, 십이지장부의 궤양성 병변을 관찰하였다.

결과 : Mepirizole을 경구투여하여 위장 및 십이지장 기부의 궤양성 병변이 유발되었다. 排氣飮(경구투여)과 catalase(정맥주사)를 전처치하였을 경우 궤양의 크기가 현저하게 줄어들었다. Mepirizole은 십이지장 점막에서 지질의 과산화를 증가시키는데 이는 수산화기와 관련되어 있음을 시사한다. 排氣飮과 catalase를 전처치함으로써 mepirizole에 의해 유발된 지질의 과산화가 현저하게 억제되었다. 형태학상의 연구에서도 mepirizole의 처치에 의한 십이지장의 손상과 排氣飮에 의한 방지효과가 나타났다.

결론 : 이러한 결과들로 볼 때 반응성산소기는 mepirizole에 의해 유발된 위장관 궤양의 병리변화 형성에 주요한 영향을 미치며 排氣飮이 항산화작용을 통해 궤양의 형성을 억제하는 역할을 하고 있음을 나타낸다. 따라서 본 연구는 排氣飮이 반응성산소기에 의해 매개된 인체 위장관질환에 치료적 역할을 할 수 있음을 제시하고 있다.

Key Word : mepirizole, Baegi-eum, catalase, antioxidant, ulcer

I. Introduction

Free radicals are unstable chemical entities that contain an unpaired electron in their outer orbital and are in general very reactive[1]. Free radicals participate in oxidation/reduction reactions with neighboring compounds in order to regain thermodynamic and electrochemical stability. Oxygen free radicals are continually produced in the mitochondrial electron transport chain of respiring cells as a consequence of the incomplete reduction of molecu- lar oxygen[2]. The reactive oxygen species (ROS) that are formed in this process include superoxide anion (O2-), hydrogen peroxide, and hydroxyl radical (-OH). Excess production of ROS may exceed cellular cytoprotective mechanisms and has been shown to be highly toxic to cells[3]. ROS-induced cytotoxicity occurs by the oxidation of constituent proteins, carbohydrates, lipids, and nucleic acids, rhus impairing cellular function and leading to cell death.

ROS contribute to gastrointestinal injury in various pathological conditions such as ischemia-reperfu- sion injury[4-6], certain types of drug-induced gastroenteropathy[7-10], necrotis-ing enterocolitis[11], experimental colitis[12-15], and inflammatory bowel diseases[16-19]. Thus, agents that efficiently scavenge ROS may protect the gastrointestinal damage induced by noxious chemicals[20,21].

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Table 1. Prescription of Baegi-eum.

Herbal name	Scientific name	Weight
Chun Pi(陳皮)	*Deliciosa Perioapium*	8g
Mu Xiang(木香)	*Costi Radix*	8g
Zhi Ke(枳殼)	*Ponciri Fructus*	8g
Hou Po(厚朴)	*Magnoliae Cortex*	8g
Ze Xie(澤瀉)	*Alismatis Rhizoma*	8g
Wu Yao(烏藥)	*Linderae Radix*	8g
Huo Xiang(藿香)	*Anisamelis Herba*	8g
Xiang Fu(香附子)	*Cyperi Rhizoma*	8g
Shan Zha(山査)	*Crataegi Fructus*	8g
Bo He(薄荷)	*Menthae Folium*	8g
Jue Ming Zi(決明子)	*Cassiae Torae Semen*	8g
	Total amount	88g

Baegi-eum(排氣飮), which is prescribed by Shen Jinao(沈金鰲) a physician (1717-1776) of the Qing Dynasty, author of "Shen's Work on the importance of Life Preservation (沈氏尊生書)"[22]. Baegi-eum is indicated to enhance the function of the stomach and to resolve phlegm, and to check upward adverse flow qi, air, or gas, and for treating symptoms such as cough, vomiting, abdomen pain. Baegi-eum is one of the most useful drugs and effective treatment for various gastrointestinal disease[23].

Mepirizole, a nonsteroidal antiinflammatory drug, is known to be a duodenal ulcerogen[24], and its effect is mediated by generation of ROS[25].

Thus, this study was undertaken to determine whether Baegi-eum (BGU), an oriental medicine, protects against mepirizole-induced ulcers and to examine whether its efficacy was associated with its antioxidant action in rabbits[26].

II. Materials and Methods

BGU extract preparation

BGU (352g of crushed crude drugs) was extracted with 3000 ml distilled water at 100 ℃ for 2 hr and the total extract was evaporated under reduced pressure to give 46 g. The dried extract was dissolved in Hank's balanced salt solution (HBSS, Sigma Co. USA) just before use.

Induction of mepirizole-inducd ulcers

New Zealand white male rabbits weighing 2-3 kg were used. Animals were fasted for 24 hr prior to experiments but had free access to drinking water. Stomach and duodenal ulcers were induced by oral administration of mepirizole at a dose of 200 mg/kg body weight.

Mepirizole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In other experiments, animals were pretreated with catalase (800 Units/kg, i.v.) 2 hr before administration of mepirizole. Control groups received an equivalent volume of the vehicle alone.

Assessment of ulcers

After sacrificing animals, a segment and stomach and duodenum was removed and opened with a longitudinal incision. The ulcer index was determined by calculating the area (square millimeters) of the ulcerative and erosive lesions measured with a dissecting microscope at a magnification of x10.

Measurement of lipid peroxidation

Lipid peroxidation was estimated by measuring the tissue content of malondialdehyde (MDA) according to the method of Uchiyama and Mihara[27]. The stomach and duodenal mucosa was scraped off using two glass slides. The mucosal tissue was homogenized in ice-cold 1.15% KCl (5% wt/vol). A 0.5 ml aliquat of homogenate was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid.

The mixture was heated for 45 min on a boiling water bath. After addition of 4 ml of n-butanol the contents were vigorously vortexed and centrifuged at 2,000 g for 20 min. The absorbance of the upper, organic layer was measured at 535 and 520 nm with a diode array spectrophotometer (Hewlett Packard, 8452A), and compared with freshly prepared malondialdehyde tetraethy-

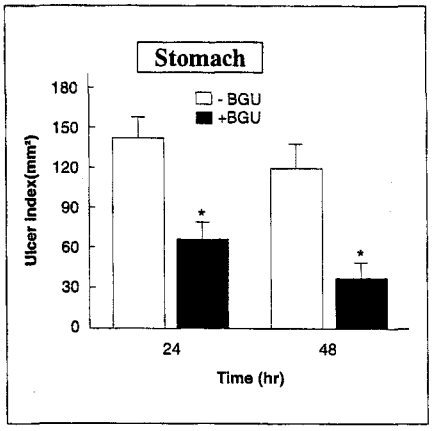

Fig. 1. Effect of Baegi-eum (BGU) on ulcer index in the stomach after the oral administration of mepirizole at a dose of 200 mg/kg body weight. Mepirizole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In BGU pretreatment groups, animals received the oral administration of BGU at a dose of 100 mg/kg body weight 2 hr before administration of mepirizole. Data are mean ± SE of five animals in each group. *p<0.05 compared with the absence of BGU pretreatment (-BGU).

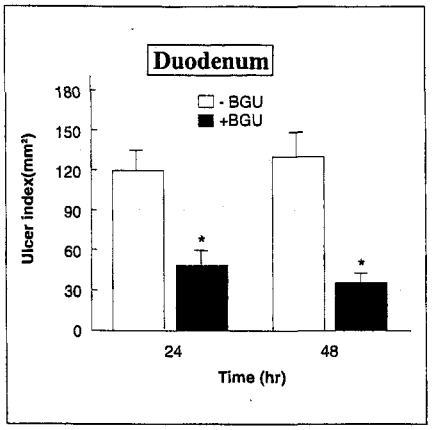

Fig. 2. Effect of Baegi-eum (BGU) on ulcer index in the duodenum after the oral administration of mepiri- zole at a dose of 200 mg/kg body weight. Mepirizole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In BGU pretreatment groups, animals received the oral administration of BGU at a dose of 100 mg/kg body weight 2 hr before administration of mepirizole. Data are mean ± SE of five animals in each group. *p<0.05 compared with the absence of BGU pretreatment (-BGU).

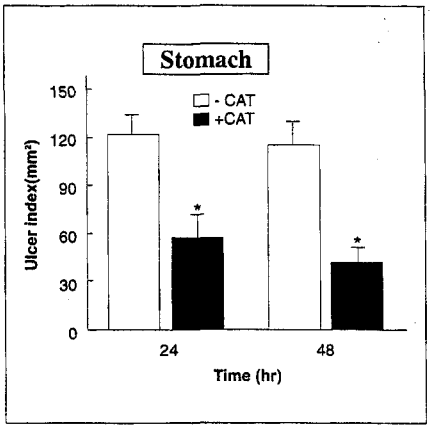

Fig. 3. Effect of catalase (CAT) on ulcer index in the stomach after the oral administration of mepirizole at a dose of 200 mg/kg body weight. Mepirizole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In catalase pretreatment groups, animals received the intra venous administration of CAT at a dose of 800 Units/kg body weight 2 hr before administration of mepirizole. Data are mean ± SE of five animals in each group. *p< 0.05 compared with the absence of CAT pretreatment (-CAT).

lacetal standards. MDA values were expressed as pmoles per mg protein. Protein was measured by the method of Bradford[28].

Morphological analysis

Duodenal tissue was removed 24 hr after administration of mepirizole, fixed in formaldehyde and embed ded in paraffin. Thin sections were processed and stained with hematoxylin and eosin.

Chemicals

Mepirizole and catalase were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals were of the highest commercial grade available.

Statistical analysis

The data are expressed as mean ± SE and the difference between two groups was evaluated using Student' s t-test. A probability level of 0.05 was used to establish significance.

III. Results

Effects of BGU and catalase on mepirizole-induced ulcers

Animals were sacrificed 24 and 48 hr after administration of 200 mg/kg mepirizole. The ulcer index was determined in stomach and duodenum. Simple or multiple ulcer lesions were developed 24 hr after oral administration of mepirizole and remained unchanged even after 48 hr. The ulcer index in the stomach was 143 ± 15.2 and 120 ± 18.3 mm^2 24 and 48 hr after administration of mepirizole alone, respectively.

However, pretreatment with BGU significantly prevented the formation of ulcers (Fig. 1). Similar ulceration in the duodenum was present, showing ulcer index of 120 ± 15.0 and 130 ± 19.1 mm^2, 24 and 48 hr after administration of mepirizole alone, respectively. Pretreatment of BGU also exerted a significant protective effect against the duodenal ulcers induced by

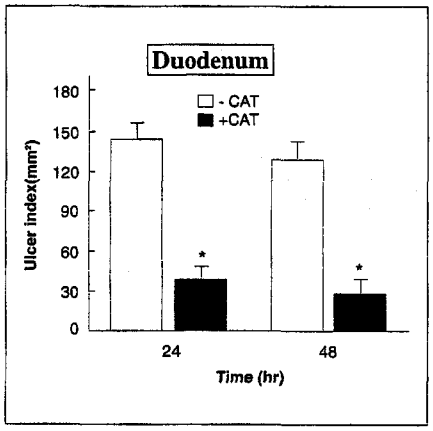

Fig. 4. Effect of catalase (CAT) on ulcer index in the duodenum after the oral administration of mepirizole at a dose of 200 mg/kg body weight. Mepirizole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In CAT pretreatment groups, animals received the intra venous administration of CAT at a dose of 800 Units/kg body weight 2 hr before administration of mepiri zole. Data are mean ± SE of five animals in each group. *p<0.05 compared with the absence of CAT pretreatment (-CAT).

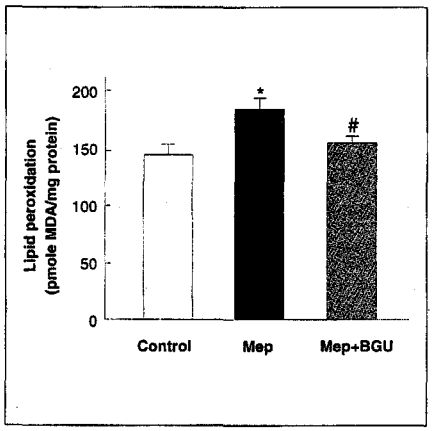

Fig. 5. Effect of Baegi-eum (BGU) on lipid peroxidation in the mucosa of duodenum after the oral adminis tration of mepirizole at a dose of 200 mg/kg body weight. Mepiri-zole was suspended in 1 ml of 0.5% carboxymethylcellulose solu tion. In BGU pretreatment groups, animals received the oral administration of BGU at a dose of 100 mg/kg body weight 2 hr before administration of mepiri zole. The duodenal tissues were obtained 24 hr after administration of mepirizole. Data are mean ± SE of five animals in each group. *p< 0.05 compared with control; #p<0.05 compared with mepiri-zole (Mep) alone.

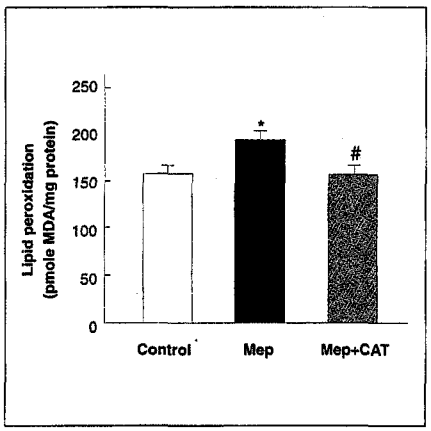

Fig. 6. Effect of catalase (CAT) on lipid peroxidation in the mucosa of duodenum after the oral administration of mepirizole at a dose of 200 mg/kg body weight. Mepiri-zole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In CAT pretreatment groups, animals received the intravenous administration of CAT at a dose of 800 Units/kg body weight 2 hr before administration of mepirizole. The duodenal tissues were obtained 24 hr after administration of mepirizole. Data are mean ± SE of five animals in each group. *p<0.05 compared with control; #p<0.05 compared with mepirizole (Mep) alone.

mepirizole (Fig. 2).

In order to determine whether mepirizole induces ulcers via generation of ROS, animals were pretreated with catalase, a hydrogen peroxide scavenger. Similarly to BGU, catalase also siginificantly inhibited formation of ulcers induced by mepirizole in stomach and duodenum. The ulcer index in the stomach was 122 ± 12.0 and 116 ± 14.3 mm^2 24 and 48 hr after administration of mepirizole alone, respectively, which was significantly prevented by pretreatment of catalase (57 ± 15.1 and 42 ± 9.7 mm^2) (Fig. 3). In duodenum, the ulcer index was 143 ± 12.9 and 129 ± 14.2 mm^2 24 and 48 hr after administration of mepirizole alone, respectively, and the value in animals pretreated with catalase was 38 ± 10.7 and 29 ± 11.0 mm^2(Fig. 4).

To further confirm the role of ROS in formation of ulcers induced by mepirizole, lipid peroxidation was determined in animals treated with mepirizole for 24 hr with or without pretreatment of BGU. When animals were treated with mepirizole alone, lipid peroxidation in duodenal mucosa increased from 145.37 ± 8.93 pmole MDA/mg protein to 185.28 ± 9.04 pmole MDA/mg protein. Such an increase in lipid peroxidation was prevented by BGU (155.92 ± 5.67 pmole MDA/mg protein) (Fig. 5). Similar protective effects were obtained with pretreatment of catalase (Fig. 6).

The formation of duodenal ulcers induced by mepirizole was evaluated by histological findings. As shown in (Fig. 7B), in the duodenum necrosis and detachment of villi and necrosis

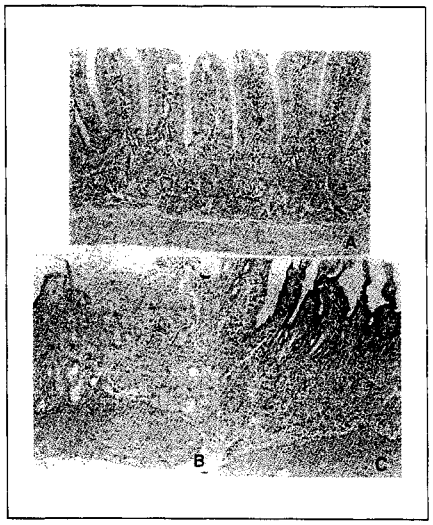

Fig. 7. Effect of Baegi-eum (BGU) on morphological changes in the duodenum after the oral administration of mepirizole at a dose of 200 mg/kg body weight. Mepiri - zole was suspended in 1 ml of 0.5% carboxymethylcellulose solution. In BGU pretreatment groups, animals received the oral administration of BGU at a dose of 100 mg/kg body weight 2 hr before administration of mepirizole. The duodenal tissues were obtained 24 hr after administration of mepirizole. A, control; B, mepirizole treatment; C, mepirizole treatment after BGU pretreatment. Hematoxylin-eosin staining. x 66.

of goblet cells and Brunner's glands in mucosa and submucosa were apparent as compared with the normal control animals (Fig. 7A). But when animals were treated with mepirizole after pretreatment of BGU, detachment of villi was ameliorated (Fig. 7C).

IV. Discussion

A growing body of evidence suggesting that ROS are implicated in the pathogenesis of stress- and chemically-induced gastrointestinal injury[29]. A potent antioxidant may serve as a possible preventive intervention for gastrointestinal injury. In recent times therefore, the search for natural antioxidants and other preparations of plant origin to achieve this objective has been intensified.

Medical herbs continue to play an important therapeutic role in the treatment of human ailments. In fact, plant-derived drugs exert the intensive influence on the practice of Western medicine. Approximately 120 drugs are obtained from plants, a large number of therapeutic activities are mediated by these drugs, and a host of the drugs currently in use are still obtained from plants in which they are synthesized. Examples include steroids, cardiotonic glycosides, anticholinergics, analgesics, antimalarials, and anticancer agents[30].

Baegi-eum(排氣飮), which is prescribed by Shen Jinao(沈金鰲) a physician(1717-1776) of the Qing Dynasty, author of "Shen's Work on the importance of Life Preservation(沈氏尊生書)"[22]. Prescription with the effects of strengthening the function of the stomach and resolving phlegm, and checking upward adverse flow qi, air, or gas, and for treating such symptions as cough, vomiting, abdomen pain. Chun Pi(陳皮) and Ze Xie(澤瀉), which are the components of BGU have been used to promote circulation, of qi and digestion, to remove dampness and phlegm. Mu Xiang(木香) and Zhi Ke(枳殼), which are the components of BGU have been used to promote flow of qi and relieve pain, to warm the middle-jiao and normal functioning of the stomach. Bo He(薄荷) used to dispel wind and heat and promote eruption. Shan Zha(山査) used to remove food stagnancy and blood stasis. Xiang Fu(香附子) used to smooth the liver and regulate the circulation of qi and to normalize menstruation and relieve pain. Huo Xiang(藿香) used to an aromatic to disperse dampness, as a stomachic, antiematic, and diaphoretic. Wu Yao(烏藥) used to promote circulation of qi and ease pain, to dispel cold and warm kidney. Hou Po(厚朴) used to an agent to promote circulation of qi and remove dampness, and to relieve asthma. Jue Ming Zi(決明子)used to remove heat from the liver and improve acuity of vision, and as laxative to relieve constipation[31]. Baegi-eum is indicated in the treatment of patients with anorexia, vomiting, diarrhea, abdominal pain due to hypofunction of the spleen and stomach with obstruction in the channels[23].

The present study was undertaken to determine whether DGT exerts protective effect against the intestinal injury induced by ROS. It has been known that a nonsteroid antiinflammatory drug mepirizole induces duodenal ulcers and its effect are prevented by ROS scavengers[25], suggesting that mepirizole induces

ulcers through generation of ROS.

The present study showed that the oral administration of mepirizole at a dose of 200 mg/kg produced deep perforated ulcers in the stomach and the proximal duodenum as evidenced by ulcer index and morphological evaluation, similar to previous studies[24,25]. Such changes were significantly prevented by oral pretreatment of BGU at a dose of 100 mg/kg. The protective effect of BGU was supported by morphological studies.

Since mepirizole has been reported to induce duodenal ulcers via generation of ROS[25], the effect of catalase, a hydrogen peroxide scavenger, was determined. When inflammatory cells such as polymorphonuclear leukocytes and macro phages were activated, these cells release enzymaltically synthesized superoxide and its dismutation product H_2O_2 into the surrounding medium. Thus, the hydrogen peroxide is considered as normal physiological products with wide spread occurrence in both the interior and exterior milieu of the cell[32]. In the present study, mepirizole-induced ulcers were prevented by pretreatment of catalase. These results suggest that generation of hydrogen peroxide may play an important role in formation of ulcers induced by mepirizole, a results consistent with reports in rats by Iinuma et al[25]. They reported that catalase decreased to almost normal control levels the ulcer index in animals treated with mepirizole.

In the presence of a transient metal such as iron, hydroxyl radical will be generated from hydrogen peroxide by metal-catalyzed Haber-Weiss reaction, and the hydroxyl radicals have been known to be initiator of lipid peroxidation[32]. In the present study, lipid peroxidation significantly increased in mucosa of duodenum treated with mepirizole and such changes were prevented by catalase. These data indicate that hydroxyl radicals generated from hydrogen peroxide may mediate formation of duodenal ulcers. Similar results were obtained with BGU. These results suggest that the protective effect of BGU against mepirizole-induced ulcers may be the result of its antioxidant action.

The present study demonstrated that mepirizole induces formation ulcers in stomach and duodenum and the ulcers are prevented by pretreatment of BGU and catalase. Since mepirizole increases lipid peroxidation in mucosa of duodenum and its effect is inhibited by BGU and catalase, BGU may inhibit formation of ulcers through ROS scavenging effect and antioxidant action. Although the precise mechanism remains to be explored, the results of the present study provide extensive information on the underlying mechanism of mepirizole-induced ulcers, and suggest that BGU may be useful in treatment and prevention of gastrointestinal injuries mediated by ROS.

V. Conclusion

This study was undertaken to determine whether Baegi-eum (BGU) exerts beneficial effect against intestinal ulcers induced by chemicals in rabbits. Oral administration of mepirizole at a dose of 200 mg/kg resulted in ulcer lesions in the stomach and the proximal duodenum.

1. Pretreatment of BGU (100 mg/kg, orally) and catalase (800 Units/kg, i.v.) significantly decreased the size of ulcers.

2. Mepirizole increased lipid peroxidation in the mucosa of duodenum, suggesting an involvement of hydroxyl radicals.

3. Pretreatment of BGU and catalase significantly inhibited lipid peroxidation induced by mepirizole.

4. Morphological studies showed that mepirizole treatment causes duodenal injury and its effect is prevented by BGU.

These results indicate that ROS plays an important role in the pathogenesis of formation of gastrointestinal ulcers induced by mepirizole and BGU exerts the protective effect against the formation of ulcers via antioxidant action. The present study suggests that BGU may play a therapeutic role in the treatment of human gastrointestinal diseases mediated by ROS.

VI. References

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