대한한방내과학회지 제21권 1호(2000년 8월)
Korean J.Orient.Int. Med. 2000;21(1):13-22

排氣飮이 人間의 腸管 上皮細胞에서 Oxidant에 의해 誘發된 細胞死亡과 DNA 損傷에 미치는 영향

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Effect of Baegieum (BGU) on Oxidant induced cell death in human intestinal epithelial cells

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목 적 : 本 硏究는 排氣飮이 人間의 腸管內에서 酸化物에 의해 誘發된 細胞의 死亡 및 DNA의 損傷을 防止할수 있는지를 檢證하기 위한 實驗이다.

방 법 : 培養된 人體腸管 細胞系列인 Caco-2 細胞에서 細胞의 死亡는 trypan blue의 소실정도에 의해서 평가했으며, DNA의 損傷은 double stranded DNA의 파괴정도를 측정하여 평가하였다. H_2O_2는 標本 酸化劑로 사용되었다.

결 과 : H_2O_2에 노출된 세포들의 細胞死亡 정도는 노출시간과 용량에 비례하여 증가하는 양상을 보였다. 排氣飮은 H_2O_2에 의해 誘發되는 세포파괴를 방지하였고, 0.05-1%의 농도범위에 걸쳐서는 그 효과가 용량에 비례하여 증가하는 양상을 보였다. H_2O_2에 의해 誘發된 細胞損傷은 catalase (hydrogen peroxide scavenger enzyme)와 deferoxamine (iron chelator)에 의해 억제되었다. 그러나 강력한 抗酸化劑인 DPPD는 H_2O_2에 의해 誘發되는 細胞損傷에는 영향을 주지 못했다. H_2O_2에 의해 誘發된 脂質의 過酸化는 排氣飮과 DPPD에 의해 억제되었다. H_2O_2에 의해 誘發된 DNA의 손상은 排氣飮에 의해 방지되었으며 용량에 의존하는 양상을 보였다. H_2O_2에 의해 誘發된 DNA의 손상은 catalase와 deferoxamine에 의해 억제되었지만 DPPD는 억제시키지 못했다. 排氣飮은 H_2O_2에 의해 誘發된 ATP의 소실을 회복시켰다. 이러한 실험결과는 H_2O_2에 의해 誘發된 細胞의 損傷은 脂質의 過酸化와는 다른 독립적인 기전에 의해 일어남을 나타낸다.

결 론 : 이러한 결과들로 볼 때 Caco-2 細胞에서 排氣飮이 抗酸化作用보다는 다른 기전을 통하여 Caco-2 세포안에서 酸化劑에 의해 誘發된 細胞의 死亡와 DNA의 損傷을 방지할 수 있다는 것을 가리킨다. 따라서 본 硏究는 排氣飮이 反應性酸素基에 의해 매개된 人體 胃腸管疾患의 治療에 사용할 수 있을 可能性이 있음을 제시하고 있다.

Key Word : Oxidant, intestinal epithelial cell, Baegieum

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 molecular 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, thus impairing cellular function and leading to cell death.

ROS contribute to gastrointestinal injury in various pathological conditions such as ischemia-reperfusion injury[4-6], certain types of drug-induced gastroenteropathy[7-10], necrotising 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].

Baegieum, which is prescribed by

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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]. Baegieum 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. Baegieum is one of the most useful drugs and effective treatment for various gastrointestinal disease[23].

This study was undertaken to determine whether BGU, an oriental medicine, protects against ROS-induced cell death and to examine whether its efficacy was associated with its antioxidant action in small intestine using the human-derived cultured intestinal epithelial cell line Caco-2 as a model which has been extensively employing in studies to characterize intestinal transport function[24,25].

II. Materials and Methods

1. BGU extract preparation

BGU(320g 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 46g. The dried extract was dissolved in Hank's balanced salt solution (HBSS, Sigma Co. USA) just before use.

2. Culture of Caco-2 cells

Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained by serial passages in 75-cm^2 culture flasks (Costar, Cambridge, MA). The cells were grown in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Sigma Chemical Co.) containing 10% fetal bovine serum at 37℃ in 95% air/5% CO_2 incubator. When the cultures reached confluence, subculture was prepared using a 0.02% EDTA-0.05% trypsin solution. The cells were grown on 24-well tissue culture plates in DMEM/F12 medium containing 10% fetal bovine serum. All experiments started 3-4 days after plating when a confluent monolayer culture was achieved. Cells were treated with hydrogen peroxide (H_2O_2) in HBSS without serum in the presence or absence of BGU.

Table 1. Prescription of Baegieum

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

3. Measurement of cell death

Cells were grown to confluence in 24-well dishes, incubated in the HBSS containing H_2O_2 for 120 min at 37℃ in 95% air/5% CO_2, and then harvested using 0.025% trypsin. Cells were incubated with 4% trypan blue solution. Cells failing to exclude the dye were considered nonviable, and the data are expressed as percentage of nonviable cells.

4. Measurement of DNA double-strand breaks

DNA strand break was measured by the DNA precipitation assay[26]. Confluent cells grown in 24-wells were labelled in the presence of 0.25 uCi/ml [^{3}H]methylthymidine for 24 hr. The cells were thoroughly washed with HBSS, and treated with H_2O_2 in the presence or absence of BGU. After treatment, the cells were washed with HBSS and lysed in effendorf tube with 0.5ml of lysis buffer (10 mM Tris/HCl, 10 mM EDTA, 50 mM NaOH, 2% SDS, pH 12.4), followed by addition of 0.5 ml of 0.12 M KCl. The lysate was

incubated for 10 min at 65℃, followed by a 5 min cooling-and-precipitation period on ice. A DNA-protein K-SDS precipitate was formed under these conditions, from which low-molecular-mass brCaco-2en DNA was released. This DNA was recovered in the supernatant from a 10 min centrifugation at 200g, 10℃, and transferred into a liquid scintillation vial containing 1 ml of 200 mM HCl. The precipitation pellet (intact double-stranded DNA) was solubilized in 1 ml of water at 65℃. The tube was rinsed with 1 ml of water, and 8ml of scintillation fluid was added to each vial. The amount of double-stranded DNA remaining was calculated for each sample by dividing the d.p.m. value of the pellet by the total d.p.m. value of the pellet plus supernatant and multiplying by 100. The extent of DNA damage was expressed as the ratio of single stranded DNA to total stranded DNA (double stranded + single stranded).

5. Measurement of ATP content

ATP levels in Caco-2 cells were measured by a luciferin-luciferase assay. After an exposure to oxidant stress, cells were solubilized with 500 *ul* of 0.5% Triton X-100 and acidified with 100 *ul* of 0.6 M perchloric acid and placed on ice. The cell suspension was then diluted with 10 mM potassium phosphate buffer containing 4 mM $MgSO_4$ (pH 7.4) and 100 *ul* of 20mg/ml luciferin-luciferase was added to 10 *ul* of diluted sample. Light emission was recorded at 20 s with a luminometer (MicroLumat LB96P, Berthold, Germany). Protein content was determined on an aliquot of cell suspension.

6. Lipid peroxidation measurement

Lipid peroxidation was estimated by measuring the renal cortical content of malondialdehyde (MDA) according to the method of Uchiyama and Mihara[27]. Cells were 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 2,000g 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 tetraethylacetal standards. MDA values were expressed as pmoles per mg protein. Protein was measured by the method of Bradford[28].

7. Chemicals

[^{3}H]methylthymidine were purchased from Amersham International (Amersham, UK). Hydrogen peroxide (H_2O_2), deferoxamine, and catalase were purchased from Sigma Chemical (St. Louis, MO, USA). N,N' -diphenyl-p-phenylenediamine (DPPD) was obtained from Aldrich Chemical (Milwaukee WI, USA). All other chemicals were of the highest commercial grade available.

8. 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

1. Time course of cell death in Caco-2 cells subjected to H_2O_2

In order to determine the time course of H_2O_2-induced cell injury, Caco-2 cells were exposed to 0.5 mM H_2O_2, and cell viability was determined at various time points (0-180 min). The significant loss of cell viability as determined by trypan blue exclusion was present 30 min after exposure of cells to H_2O_2, with irreversible cell injury increasing up to 180 min (Fig. 1). However, cell death was significantly prevented by addition of 0.1% BGU.

When cells were exposed to various concentrations (0.05-0.5 mM) of H_2O_2 for 120 min, cell death was increased in a dose-dependent manner. The significant loss of cell viability was observed at 0.1 mM

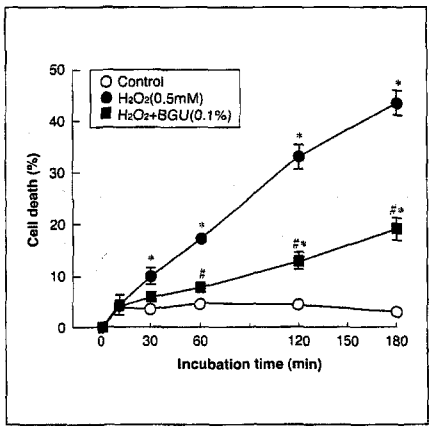

Fig 1. Time course of H_2O_2 effect on cell death in Caco-2 cells. Cells were incubated for various times in medium containing 0.5 mM H_2O_2 in the presence or absence of 0.1% Baegieum (BGU). Cell death was measured by a trypan blue exclusion assay. Data are mean ± SE of four experiments. *p<0.05 compared with control, #p<0.05 compared with H_2O_2 alone.

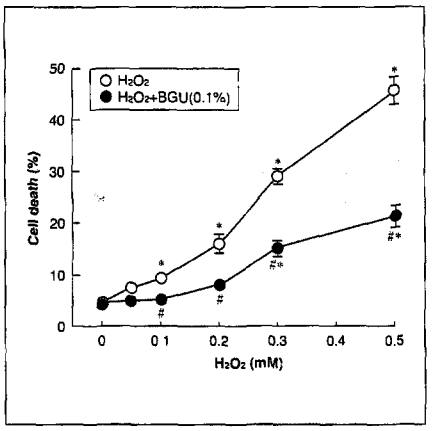

Fig 2. Dose-dependency of H_2O_2 effect on cell death in Caco-2 cells. Cells were incubated for 120 min in medium containing various concentrations of H_2O_2 in the presence or absence of 0.1% Baegieum (BGU). Cell death was measured by a trypan blue exclusion assay. Data are mean ± SE of four experiments. *p<0.05 compared with the absence of H_2O_2, #p<0.05 compared with H_2O_2 alone.

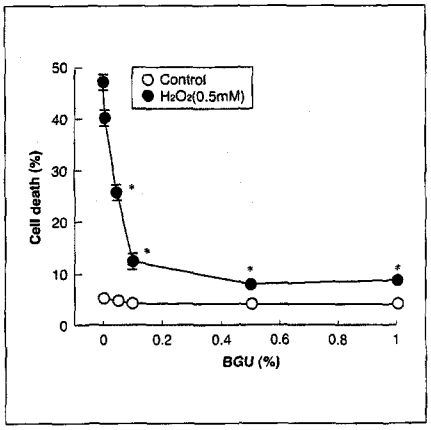

Fig 3. Dose-dependency of Baegieum (BGU) effect on H_2O_2-induced cell death in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of various concentrations of BGU. Cell death was measured by a trypan blue exclusion assay. Data are mean ± SE of five experiments. *p<0.05 compared with H_2O_2 alone.

H_2O_2 (9.38 ± 1.03 vs. 5.37 ± 0.66% in control). Such changes were significantly prevented by addition of 0.1% BGU (Fig. 2).

2. Dose-dependency of protective effect of BGU against H2O2-induced cell death in Caco-2 cells

In order to determine dose-dependency of the protective effect of BGU, cells were treated with 0.5 mM H_2O_2 in the presence of various concentrations of BGU. As shown in Fig. 3, BGU prevented H_2O_2-induced cell death in dose-dependent fashion and a significant protection was present at 0.05% (25.38 ± 2.06 vs. 46.94 ± 2.08% in H_2O_2 alone). When BGU concentrations were increased up to 0.5 and 1%, cell death was decreased 7.98 ± 1.38 and 8.46 ± 1.4%, respectively, which were not different from the control. However, BGU did not exert any effect in normal cells untreated with H_2O_2.

Effect of other well-known antioxidants on H_2O_2-induced cell death were examined to compare with that of BGU. The results are summarized in Fig. 4. Catalase (200 units/ml), the hydrogen peroxide scavenger enzyme, prevented completely cell death induced by 0.5 mM H_2O_2. Similar results were observed with deferoxamine (5 mM), the iron chelator, suggesting involvement of an iron-dependent mechanism in H_2O_2-induced cell death. By contrast, a potent antioxidant DPPD at 20u M did not affect the loss of cell viability induced by H_2O_2, indicating that H_2O_2-induced cell death is not associated with lipid peroxidation.

3. Effects of BGU and antioxidant on H_2O_2-induced lipid peroxidation in Caco-2 cells

The failure of DPPD effect on H_2O_2-induced cell death may be due to that it does not block H_2O_2-induced lipid peroxidation in Caco-2 cells. To test this possibility, we examined if H_2O_2 induces lipid peroxidation and the effect was

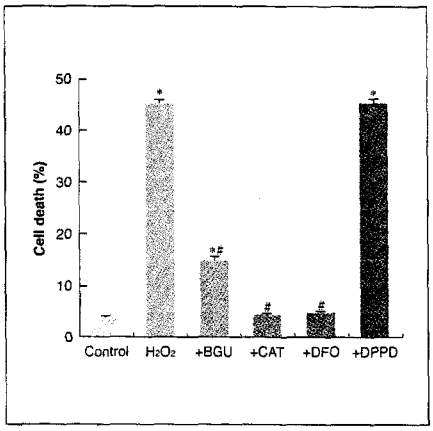

Fig 4. Effects of Baegieum (BGU), hydrogen peroxide scavenger, and antioxidants on H_2O_2-induced cell death in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of 0.1% BGU, 500 units/ml catalase (CAT), 5 mM deferoxamine (DFO), and 0.01 mM N,N'-diphenylphenylene diamine (DPPD). Cell death was measured by a trypan blue exclusion assay. Data are mean ± SE of five experiments. *p<0.05 compared with the control; #p<0.05 compared with H_2O_2 alone.

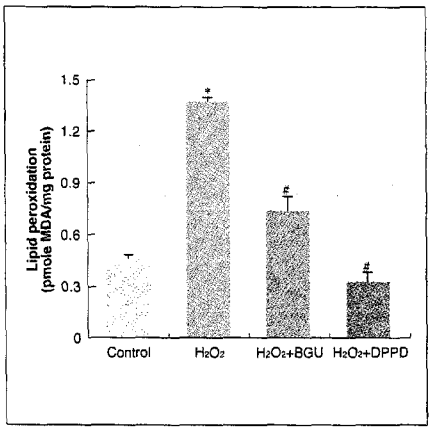

Fig 5. Effect of Baegieum (BGU) and antioxidant on H_2O_2-induced lipid peroxidation in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of 0.1% BGU and 0.01 mM N,N'-diphenylphenylene diamine (DPPD), and then lipid peroxidation was measured. Data are mean ± SE of five experiments. *p<0.05 compared with the control (cont); #p<0.05 compared with H_2O_2 alone.

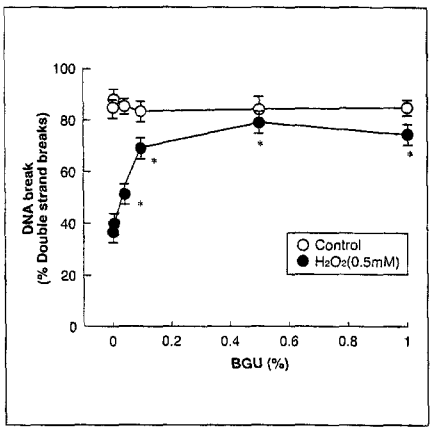

Fig 6. Dose-dependency of Baegieum (BGU) effect on H_2O_2-induced DNA damage in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of various concentrations of BGU, and then DNA damage was measured. Data are mean ± SE of four experiments. *p<0.05 compared with H_2O_2 alone.

altered by BGU and DPPD. Exposure of cells to 0.5 mM H_2O_2 caused an increase in lipid peroxidation, which could be prevented by 0.1% BGU and 20 uM DPPD (Fig. 5).

4. Effect of BGU on H_2O_2-induced DNA damage in Caco-2 cells

In order to examine if BGU exerts the beneficial effect against DNA damage induced by oxidants, DNA breaks were measured in cells treated with H_2O_2 in the presence of various concentrations of BGU. As shown in Fig. 6, exposure of cells to 0.5 mM H_2O_2 resulted in a significant increase in DNA damage as evidenced by a decrease in double stranded DNA (36.93 ± 5.96 vs. 85.07 ± 6.03% in the control). These changes were prevented by BGU and its effect was dose-dependent. Double stranded DNA was increased from 36.93 ± 5.96% to 55.9 ± 7.02, 69.94 ± 5.88, 79.98 ± 5.93, and 75.39 ± 6.34% by addition of 0.05, 0.1, 0.5, and 1% BGU, respectively. At concentrations higher than 0.1%, thus, BGU prevented completely DNA damage induced by 0.5 mM H_2O_2.

As expected, H_2O_2-induced DNA damage was prevented by catalase. Similar results were observed with deferoxamine. However, DPPD did not affect the DNA damage (Fig. 7). Such results are consistent with those in cell death.

5. Effect of BGU on H_2O_2-induced ATP deletion in Caco-2 cells

It has been known that oxidants result in decreases in cell ATP content which may lead to cell death[29]. Thus, BGU exerts the

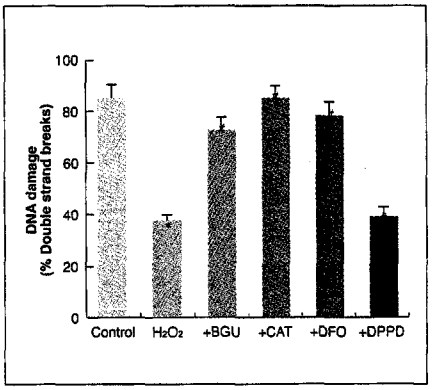

Fig 7. Effects of Baegieum (BGU), hydrogen peroxide scavenger, and antioxidants on H_2O_2-induced DNA damage in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of 0.1% BGU, 500 units/ml catalase (CAT), 5 mM deferoxamine (DFO), and 0.01 mM N,N'-diphenylphenylene diamine (DPPD). Data are mean ± SE of five experiments. *p<0.05 compared with the control; #p<0.05 compared with H_2O_2 alone.

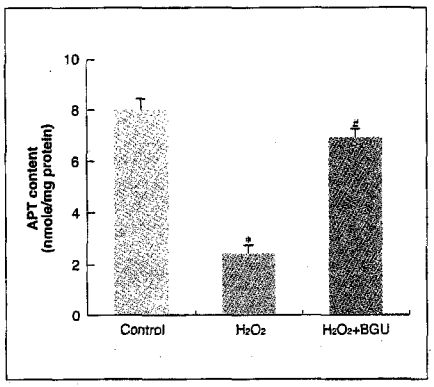

Fig 8. Effects of Baegieum (BGU), hydrogen peroxide scavenger, and antioxidants on H_2O_2-induced ATP depletion in Caco-2 cells. Cells were incubated for 120 min in medium containing 0.5 mM H_2O_2 in the presence or absence of 0.1% BGU. Data are mean ± SE of four experiments. *p<0.05 compared with the control; #p<0.05 compared with H_2O_2 alone.

protective effect against H2O2-induced cell death by preventing ATP depletion. To test the possibility, cellular ATP content was measured in Caco-2 cells exposed to 0.5 mM H_2O_2 in the presence or absence of 0.1% BGU. As shown in Fig. 8, H_2O_2 decreased ATP content, which was restored by BGU and was not significantly different from the control.

IV. Discussion

A growing body of evidence suggesting that ROS are implicated in the pathogenesis of stress- and chemically-induced gastrointestinal injury[30]. 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[31,32].

Baegieum(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 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.

Baegieum 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 BGU exerts protective effect against oxidant-induced cell death in human intestinal cells using Caco-2 cells. In this study, H_2O_2 increased cell death in a time- and dose-dependent manner in Caco-2 cells as measured by a trypan blue exclusion assay (Figs. 1 and 2). BGU prevented H_2O_2-cell death in a dose-dependent manner (Fig. 3). Cell death induced by 0.5 mM H_2O_2 was completely prevented by 500 units/ml catalase. This effect was expected because catalase is a scavenger enzyme of hydrogen peroxide (Fig. 4).

Iron appears to be the critical in the cytotoxic effect of H_2O_2 in Caco-2 cells as the iron chelator deferoxamine was markedly protective (Fig. 4), indicating that H_2O_2-induced cell killing is resulted from an iron-dependent mechanism. The source of iron, how it becomes biologically available, and the mechanism of action of deferoxamine on this system remain unknown. Gannon et al.33) have presented evidence that, in oxygen radical mediated cell death, the source of iron is the target cell itself. They demonstrated that stimulated neutrophils were cytotoxic for endothelial cells in an iron dependent manner. Pretreatment of the neutrophils with deferoxamine did not protect against the cytotoxicity. However, pretreatment of the endothelial cells with deferoxamine was significantly protective in a time and concentration dependent fashion. Whether BGU exerts the protective effect against H_2O_2-induced cell death by an action mechanism similar to deferoxamine remains to be defined.

Iron chelators have been shown to be protective in several in vivo models of tissue injury[34,35]. Although the role of iron is not completely understood [36,37], the protective effect of iron chelators has been generally taken as evidence for the participation of hydroxyl radical in cell injury, because a trace metal such as iron appears to be critical for the generation of hydroxyl radical from H_2O_2 via metal-catalyzed Haber-Weiss reaction[38]. Thus, if BGU scavenges hydroxyl radicals, it could prevent H_2O_2-induced cell killing. Interestingly, a potent antioxidant DPPD did not affect H_2O_2-induced cell death(Fig. 4).

Although lipid peroxidation of cell membrane has been considered to be an evidence for oxidant-induced cell injury[38], the role that lipid peroxidation plays as a critical event in the pathogenesis of oxidant-induced cell injury has not been clearly established[39-41]. Lipid peroxidation can be a result or an epiphenomenon of cell death rather than a cause of cell injury[38]. To determine whether H_2O_2 leads to cell death via a lipid peroxidation-dependent mechanism, Caco-2 cells were treated with H_2O_2 in the presence of antioxidants. DPPD have been reported to effectively prevent oxidant-induced cell injury in renal proximal tubular cells[42] and renal cortical slices[43]. If H_2O_2-induced cell injury was caused by lipid peroxidation, both the cell death and lipid peroxidation should be prevented by DPPD. In the present study, however, despite ^{H2O2}-induced lipid peroxidation was completely blocked by DPPD (Fig. 5), the cell death was not prevented (Fig. 4). These results suggest that H2O2-induced cell death is not mediated by lipid peroxidation in Caco-2 cells. H_2O_2-induced lipid peroxidation may be a result of the cell injury rather than a mechanism by which the cell death is induced[38]. Although the results of the present study showed that BGU inhibits H_2O_2-induced lipid peroxidation, therefore, its protective effect against H_2O_2-induced cell death may be attributed to a mechanism other rather than antioxidant action.

DNA is an important cellular and molecular target of oxidant stress. Oxidant stress results in DNA damage by induction of single-strand breaks, by base modification, or by the induction of apoptosis[38,44-46]. However, whether DNA damage leads to cell killing is controversial. Various investigators reported that DNA damage plays a central role in cell death[38,44,47], whereas DNA damage is not the primary mediators of cell death following oxidative

stress in renal epithelial cells[48] and heptocytes[49]. The present study demonstrated that BGU, catalase, and deferoxamine at concentrations that effectively decrease H_2O_2-induced cell death prevented H_2O_2-induced DNA damage (Figs. 6 and 7). Similarly to cell death, however, DPPD did not prevent H_2O_2-induced DNA damage. This may suggest that DNA damge is linked to cell death in Caco-2 cells.

It has been demonstrated that ATP levels decline and ATP metabolites are lost from cells as an early response to oxidant injury[50]. Such changes may lead to cell death. In the present study, H_2O_2 decreased significantly ATP levels, which was prevented by addition of BGU (Fig. 8).

The present study demonstrated that BGU exerts beneficial effect against H_2O_2-induced cell death and DNA damage in Caco-2 cells. The underlying mechanism of BGU protective effect is not clear from the results of the present study. In the present study, effect of BGU is mimic that of catalase and deferoxamine rather than DPPD. Therefore, BGU may act as a H_2O_2 scavenger and/or as an iron chelator rather than as antioxidant. Although the precise mechanism remains to be explored, the results of the present study provide extensive information on the underlying mechanism of ROS-induced cell death, 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 Baegieum (BGU) exerts beneficial effect against cell death and DNA damage induced by oxidants in human intestine. Cell death was evaluated by trypan blue exclusion and DNA damage was estimated by measuring double stranded DNA breaks in Caco-2 cells, cultured human intestinal cell line. Hydrogen peroxide (H_2O_2) were used as a model oxidant.

1. Exposure of cells to H_2O_2 induced increases in the loss of cell viability in a time and dose-dependent fashion.

2. BGU prevented H_2O_2-induced cell death and its effect was dose-dependent over concentration range of 0.05-1%.

3. H_2O_2-induced cell death was prevented by catalase, the hydrogen peroxide scavenger enzyme, and deferoxamine, the iron chelator. However, a potent antioxidant DPPD did not affect H_2O_2-induced cell death.

4. H_2O_2 caused lipid peroxidation, which was inhibited by BGU and DPPD.

5. H_2O_2 caused DNA damage in a dose-dependent manner, which was prevented by BGU.

6. H_2O_2-induced DNA damage was prevented by catalase and deferoxamine, but not DPPD.

7. BGU restored ATP depletion induced by H_2O_2.

8. These data suggest that H_2O_2-induced cell death results from a lipid peroxidation-independent mechanism.

These results indicate that BGU prevents cell death and DNA damage induced by oxidants in Caco-2 cells possibly by a mechanism other rather than an antioxidant action. In addition, 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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