Inhibitory Effects of 4-Guanidinobutyric Acid against Gastric Lesions

In Young Hwang and Choon Sik Jeong*

College of Pharmacy, Duksung Women's University, Seoul 132-714, Republic of Korea

Abstract
This study examined the inhibitory effects of 4-guanidinobutyric acid (4GBA), an alkaloid, against gastric lesions by assessing the inhibition of Helicobacter pylori (H. pylori) and gastric cancer cells. Acute and chronic gastritis were also observed using HCl/ethanol (EIOH) and indomethacin-induced gastric lesion models, respectively. 4GBA inhibited the growth of H. pylori in a dose-dependent manner, and showed acid-neutralizing capacity. In the pylorus ligated rats, 4GBA decreased the volume of gastric secretion and gastric acid output slightly, and increased the pH. 4GBA at a dose of 100 mg/kg reduced the size of HCl/EIOH-induced gastric lesions (70.8%) and indomethacin-induced gastric lesions (38.8%). The antigastritic action of 4GBA might be associated with the acid-neutralizing capacity, anti-H. pylori action, and decreased volume of gastric secretion. These results suggest that 4GBA might be useful in the treatment and/or protection of gastritis.

Key Words: 4-guanidinobutyric acid, Helicobacter pylori, Cytotoxicity, Anti-oxidant, Gastric lesion

INTRODUCTION
4-Guanidinobutyric acid (4GBA), an alkaloid included in guanidino compounds, is present in the mammalian brain, herbal medicines, fish and shellfish (Tachikawa and Hosoya, 2011). 4GBA has stimulatory effects on monocytes and granulocytes (Schepers et al., 2010) (Fig. 1). Recurring gastritis and gastric ulcers are generally caused by an imbalance between aggressive factors (i.e., gastric acid, pepsin, stimulation of the vagus nerves, secretion of gastrin, and increasing the number of parietal cells) and protective factors (i.e., bicarbonate ion, mucus productivity, mucus secretion, and prostaglandins) (Shay et al., 1945). The gastric mucosal barrier is exposed to a range of aggressive factors, but is normally protected by unique protective mechanisms. Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin/chemotherapeutic agents and Aspirin, can cause gastric lesions, such as hemorrhages and ulcers by stimulating the gastric mucosal barrier directly (Elakim et al., 1995). Ethanol (EIOH) damages the stomach by accelerating the mucous membrane penetrability and inhibiting active transport. Reactive oxygen species (ROS), one of the aggressive factors, leads to acute and chronic inflammation in the stomach (Leirisalo-Repo et al., 1993). H. pylori is an important pathogen associated with stomach cancer, chronic gastritis and ulceration in the stomach and duodenum by producing toxic agents (Leunk et al., 1988; Sarosiek et al., 1989; Correa, 1992; Slomiany 1992). The gastric mucosa infected with H. pylori has higher levels of ROS, which induce DNA damage (Drake et al., 1998; Arend et al., 2005). Antiacids are effective in accelerating the healing of duodenal and gastric ulcers due to the neutralization of gastric luminal acid (Tarnawski et al., 1995). HCl/EIOH-induced gastric lesions appear to be produced by the direct irritation of the gastric mucosal barrier (Seiki et al., 1990). EIOH induces long ulcers and petechial lesions within a relatively short period of time, which makes this technique suitable for screening anti-ulcer drugs. The continuous decrease in acid-neutralizing capacity and rapid acid movement into the duodenum, coupled with the hyper-secretion of pepsinogen, leads to abnormal acid secretion (Tarnawski et al., 1985). Acute and chronic gastritis appear to be generated from the over-secretion of gastric juices. The inhibition of acid secretion is believed to be the most important factor for treating gastric ulcers and gastritis. This study examined the effects of 4GBA using a range of methods including an evaluation of its anti-H. pylori activity, anti-oxidant effects using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, reducing power and acid-neutralizing capacity. The cytotoxicity of 4GBA was evaluated against human gastric cancer cell lines. The effects of 4GBA on HCl/EIOH- and indomethacin-induced gastritis models and on gastric secretion were also investigated.
MATERIALS AND METHODS

Reagents and laboratory equipments

Brucella broth, bacto agar, horse serum, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT), sodium bicarbonate, positive control including ascorbic acid, hydrotalcite, ampicillin and cimetidine were obtained from Sigma (Sigma-Aldrich Inc., MO, USA). The cell culture medium and reagents, such as RPMI 1640, fetal bovine serum (FBS), penicillin/streptomycin, and trypsin-EDTA were purchased from Gibco (Invitrogen Inc., NY, USA). The other solvents were purchased from Duksan pure Chemical Co. Ltd. (Kyunggi-do, Korea). All other reagents were of pharmaceutical or analytical grade.

The equipment included a pH meter (IQ Scientific Instruments, Inc), clean Bench (Johnsam Co.), CO2 incubator (Olympus), autoclave (Duksan Chem. Co.), micropipette (Gilson Co.), centrifuge 5810R (Eppendorf), high speed centrifuge (Sorvall RT-6000), liquid nitrogen Dewars (CHART MVE), and UV-spectrophotometric plate reader (ASYS UVM340).

Anti-H. pylori activity

The inhibitory effect of 4GBA on the growth of H. pylori (ATCC, Rockville, MD, USA) was examined by modifying the method reported by Kim et al. (2003). 600 μl of the sample was mixed with 5.4 ml of brucella agar medium containing 7% horse serum in a petri dish. H. pylori (5×106 CFU) was seeded into the media and incubated for 3 days in a 37°C incubator (AnaeroPak Campylo: 85% N2, 10% CO2, 5%, O2). The viability of H. pylori was determined from the colony counts after 3 days incubation. Ampicillin was used as the positive control.

Cell culture and cytotoxicity assay for gastric cancer cell lines

SNU638 and AGS gastric cancer cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured with RPMI-1640 containing 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) in a 5% CO2 humidified incubator at 37°C. For the subculture, the SNU638 and AGS cells were rinsed twice with phosphate buffered saline (PBS, pH 7.4) to remove all traces of the serum (which can inhibit trypsin) and subdivided using 0.05% trypsin with 0.53 mM EDTA.

The cytotoxicity of 4GBA to SNU638 and AGS cells (gastric cancer cell lines) was examined using a MTT assay. The inhibitory effect of 4GBA on the growth of SNU638 and AGS gastric cancer cell lines for Safety Evaluation of Drugs (Notification No. 2000-63) issued by the Korea Food and Drug Administration.

Animals

Male Sprague–Dawley rats, weighing 190 to 200 g, were purchased from Samtako, Kyunggi-do, Korea, and acclimatized to standard laboratory conditions (22 ± 2°C, 55 ± 5% humidity and 12 h light/dark cycle) for 14 days in the animal facility at Dusung Women's University. All experimental procedures for the rats were carried out in accordance with the Guidelines of the Care and Use of Laboratory Animals, Dusung Women's University. The animals were allowed access to food (standard pellet diet) and water ad libitum. The entire study was conducted in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification No. 1999-61) and the Good Laboratory Practice Regulations for Non-clinical Laboratory Studies (Notification No. 2000-63) issued by the Korea Food and Drug Administration.

HCl/EtOH-induced mucosal membrane lesions

After 24 hours fasting with free access to water prior to the

Table 1. Colonization inhibiting effect of 4-guanidinobutyric acid for H. pylori

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (μM)</th>
<th>Colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>4GBA</td>
<td>50</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

- : none, + : colonies (0-2×10⁴ CFU), ++ : colonies (2-4×10⁴ CFU), +++ : colonies (>4×10⁴ CFU), µg/ml}