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Lactobacillus brevis G101 Inhibits the Absorption of Monosodium Glutamate in Mice

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To evaluate the effect of *Lactobacillus brevis* G-101 on absorption of monosodium glutamate (MSG), we orally administered MSG with or without G-101 in mice and measured the maximum concentration (Cmax) and blood concentration curve (AUC) of MSG and γ-aminobutyric acid (GABA). Oral administration of G-101 (1×10^9 CFU/mouse) potently inhibited Cmax and AUC of MSG by 97.8% and 94.3%, respectively ($p < 0.05$), but increased those of GABA by 32.1% and 67.7%, respectively ($p < 0.05$). G-101 inhibited the absorption of MSG. These results suggest that G-101 may reduce the side effect of MSG by inhibiting the absorption of MSG.

Keywords: *Lactobacillus brevis* G-101, monosodium glutamate, γ-aminobutyric acid, absorption

Monosodium glutamate (MSG), a common flavor enhancer frequently used in various canned and Chinese restaurant foods [9, 12, 18], has been claimed to have various side effects, including headache and dizziness [11, 13, 19]. A double-blind, placebo-controlled, randomized study has confirmed that MSG usage may be related to these various symptoms [13]. Oral administration of MSG to humans induces mechanical sensitization in the masseter muscle and adverse effects such as headache and short-lasting blood pressure elevation for which tolerance did not develop over 5 days of MSG intake [14].

Lactic acid bacteria (LAB) are a heterogeneous group of gram-positive facultative anaerobic microorganisms that produce large amounts of lactic acid [7]. LAB are found in yogurt, cheese, *kimchi*, and intestinal microbiota [1, 6]. LAB are safe microorganisms that improve disturbances of the indigenous microbiota [1, 10], have antidiabetic [15], and anticolitic effects [1], inhibit carcinogenesis [10], and induce nonspecific activation of the host's immune system [9]. Some LAB, such as *Lactobacillus buchneri*, convert MSG to gamma-aminobutyric acid (GABA) *via* the decarboxylation of glutamic acid by glutamate decarboxylase, which is a pyridoxal phosphate-dependent enzyme [3, 16]. GABA has been reported to possess various physiological functions, such as neurotransmission, tranquilization, antihypertension, prevention of diabetic conditions, and diuretic effects [2]. Therefore, LAB have been used as starters to produce GABA-rich foods. However, studies on the ability of LAB to reduce the absorption of orally administered MSG into the blood have not been performed.

Therefore, we isolated *Lactobacillus brevis* G-101, which exhibits anticolitic effect in mice [4], potently converting MSG to GABA, from *kimchi*-derived LAB and investigated whether G-101 could reduce the blood concentration of MSG in mice orally administered MSG.

Fifty LAB were isolated from *kimchi* and anaerobically cultured in MRS containing 5% MSG (St Louis, MO, USA) at 30°C for 24 h: 25 *Lactobacillus sakei* strains, 8 *Leuconostoc mesenteroides* strains, 12 *Lactobacillus plantarum* strains, 2 *Lactobacillus curvatus* strains, 2 *Lactobacillus brevis* strains, and 1 *Lactobacillus pentosus* strain. The culture broth was centrifuged at 1,500 ×*g* for 15 min, and the supernatant was analyzed for determination of GABA by thin-layer chromatography (TLC). The isolated GABA-producing strain was identified by morphological observations under a microscope, by Gram staining, from its biochemical

properties by using an API kit (50CHL; bioMeriux Co., France), and by 16S rDNA sequence determination [5].

For experiment in mice, *L. brevis* G-101 was grown to an optical density between 3 and 4 at 600 nm (early stationary phase), harvested by centrifugation (10,000 ×g for 30 min), and washed with PBS. The collected cells (1×10^8 and 1×10^9 CFU) were orally administered to mice as a suspension in 50 mM $NaHCO_3$ buffer containing 1% glucose [8].

For the determination of MSG and GABA by HPLC, the culture broth was centrifuged at 1,500 ×*g* for 15 min, and MSG and GABA in the supernatant were determined. TLC was conducted using an acetic acid:1-butanol:distilled water (1:4:5) solvent mixture, and plates were subsequently immerged into 0.5% (w/v) ninhydrin solution, and then heated.

To assay MSG and GABA by HPLC, 1 ml of culture broth was collected and centrifuged at 1,500 ×*g* for 15 min. The supernatant was derivatized with phenylisothiocyanate, filtered through a 0.45 μm filter, and then analyzed by HPLC (Hewlett Packard 1100 series) equipped with a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA), a HP 1100 series binary pump, a HP 1100 series autosampler, column oven (30°C), and UV detector (254 nm). The elution solvent system consisted of 6% CH_3CN (pH 6.1) containing 1.4 mM NaHAc and 0.1% TEA (A) and 30% CH_3CN containing 1.4 mM NaHAc and 0.1% TEA (B). The column was eluted for 25 min with a linear gradient of 0–100% B in A. The flow rate was 1.0 ml/min. The retention times of MSG, GABA, and acetaminophen were 5.32, 9.41, and 12.21 min, respectively.

Male ICR mice (19–22 g, 5 weeks old) were supplied by Orient Experimental Animal Breeding Center (Sungnam, Korea). All animals were housed in wire cages (5 mice per cage) at 20–22°C and 50 ± 10% humidity, fed standard laboratory chow (Samyang Co., Seoul, Korea), and allowed water *ad libitum*. All experiments were performed in accordance with the NIH and Kyung Hee University Guides for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University.

The mice were divided into four groups (treated with vehicle alone (normal control), with MSG (100 mg/kg) in the absence or presence of G-101 (1×10^9 CFU/mouse or 1×10^{10} CFU/mouse). Each group consisted of 30 mice. G-101 was administered once a day for 3 days. MSG was orally treated 1 h after the final administration of G-101. Blood was taken (0.5 ml) from the portal vein of six mice at 0, 0.25, 0.5, 1, and 2 h after oral administration of MSG. The obtained serum was deproteinized by MeOH and then analyzed for MSG and GABA by HPLC.

The maximum concentration (Cmax), maximum concentration time (Tmax), and the area under the blood concentration curve (AUC) for the MSG and GABA concentrations in plasma are expressed as the mean ± standard deviation. Statistical analyses were done using SPSS for Windows ver. 12.0, and statistical significance was set to a *p*-value less than 0.05.

To search LAB that could transform MSG to GABA, we incubated LAB strains (isolated from *kimchi*) with MSG, and then measured the transforming activity of MSG to GABA, which is the glutamate decarboxylase activity in *Lactobacillus* spp. [20]. Of the isolated LAB, G-101 transformed MSG to GABA most efficiently (Fig. 1). Its glutamate decarboxylase activity transforming MSG to GABA was 0.691 mmol/h·g wet weight. The most potent glutamate decarboxylase activity-producing G-101 was identified to be a *Lactobacillus brevis* by gram staining, 16S rRNA sequencing, and the API kit test.

Next, to confirm the ability of G-101 to transform orally administered MSG to GABA *in vivo*, we orally administered G-101 for 3 days in mice, treated with MSG 1 h after the final administration of G-101, and then MSG and GABA levels were periodically analyzed in samples taken from the portal vein of the mice. We could detect MSG and GABA in the blood. The Tmax of MSG was 15 min (Fig. 2). There was no significant difference between mice treated with or without G-101. However, treatment with G-101

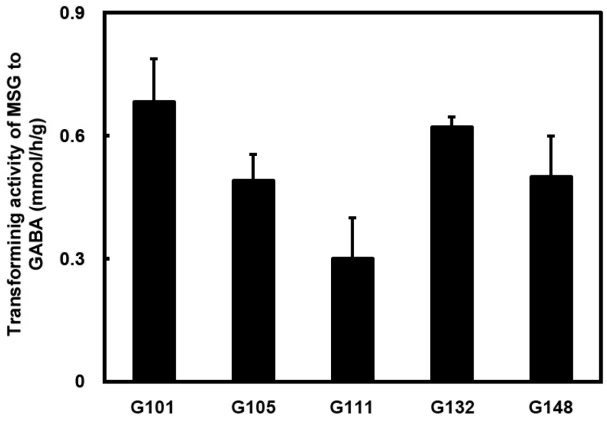

Fig. 1. Monosodium glutamate (MSG)-transforming activities of lactic acid bacteria isolated from Chinese cabbage *kimchi* on gamma-aminobutyric acid (GABA).
The activity transforming MSG to GABA, which is the glutamate decarboxylase activity, was assayed using MSG as a substrate. All values are indicated as the mean ± SD (n = 3).

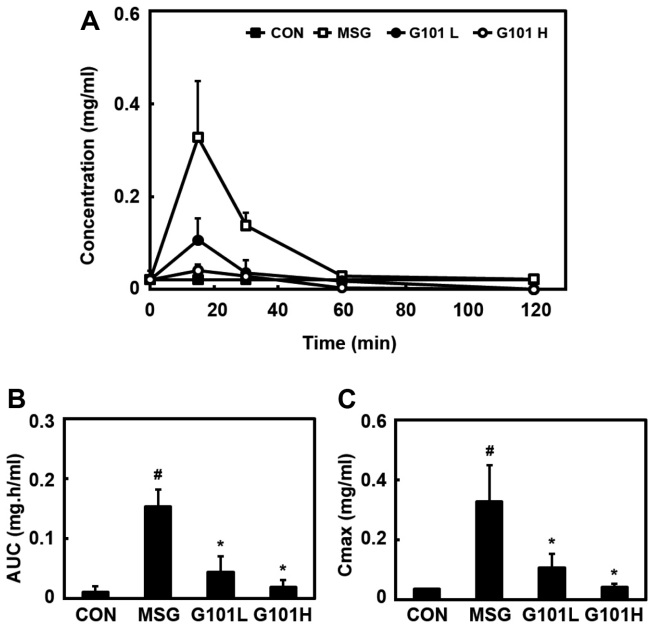

Fig. 2. Effect of *Lactobacillus brevis* G-101 on pharmacokinetic parameters of monosodium glutamate (MSG) in mice orally administered with MSG.

(**A**) Effect on the absorption of MSG. (**B**) Effect on AUC. (**C**) Effect on Cmax. *Lactobacillus brevis* G-101 (1×10^8 and 1×10^9 CFU/mouse) was orally administered once a day for 3 days. MSG (100 mg/kg) was orally administered 1 h after the final administration of G-101. Then the blood was taken from the portal vein 15 min, 30 min, 1 h, and 2 h after administration of MSG, and was then centrifuged. The obtained sera were deproteinized by MeOH and then analyzed for MSG by HPLC. All values are indicated as the mean ± SD ($n = 6$). #Significantly different from the normal control group ($p < 0.05$). *Significantly different from group treated with MSG alone ($p < 0.05$).

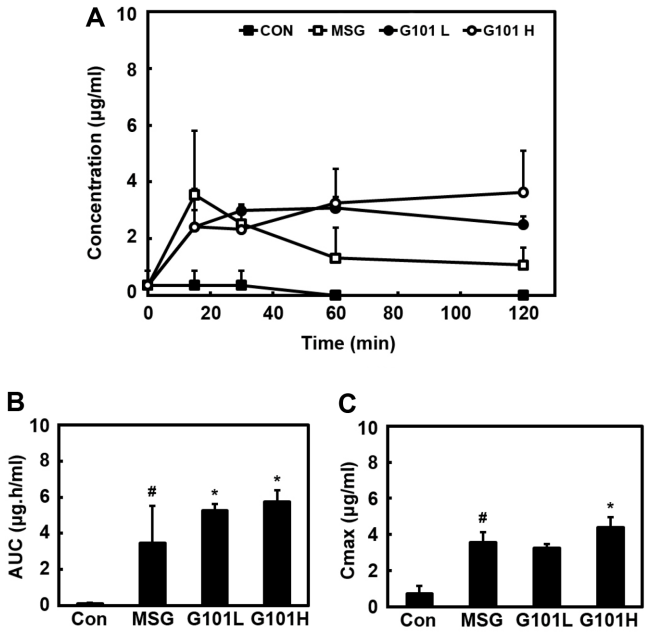

Fig. 3. Effect of *Lactobacillus brevis* G-101 on pharmacokinetic parameters of gamma-aminobutyric acid (GABA) in mice orally administered with MSG.

(**A**) Effect on the absorption of GABA conversed from MSG. (**B**) Effect on AUC of GABA. (**C**) Effect on Cmax of GABA. *Lactobacillus brevis* G-101 (1×10^8 and 1×10^9 CFU/mouse) was orally administered once a day for 3 days. MSG (100 mg/kg) was orally administered 1 h after the final administration of G-101. Then the blood was taken from the portal vein 15 min, 30 min, 1 h, and 2 h after administration of MSG, and was then centrifuged. The obtained sera were deproteinized by MeOH and then analyzed for GABA by HPLC. All values are indicated as the mean ± SD ($n = 6$). #Significantly different from the normal control group ($p < 0.05$). *Significantly different from group treated with MSG alone ($p < 0.05$).

significantly reduced the Cmax and AUC of MSG. When MSG was orally administered alone, the Cmax and AUC of MSG were 0.328 ± 0.121 mg/ml and 0.153 ± 0.030 mg.h/ml, respectively. However, when MSG was simultaneously treated with 1×10^9 CFU of G-101, the Cmax and AUC of MSG were reduced by 97.8% and 94.3%, respectively. Nevertheless, the comparisons of body weight, clinical signs, and gross finding of necropsy showed no significant differences between mice treated with or without C29 in the presence of MSG.

Next, the pharmacokinetic parameters of GABA were measured (Fig. 3). When MSG was orally administered alone, the Cmax and AUC of GABA were 3.542 ± 0.583 mg/ml and 3.448 ± 2.068 μg.h/ml, respectively. When MSG was treated with 1×10^9 CFU of G-101, the Cmax and AUC of MSG were significantly increased by 32.1% and 67.7%, respectively. When MSG alone was treated, Tmax of GABA was 15 min. Its Tmax was extended by treatment with G-101. However, the reduction of MSG AUC by treatment with G-101 was not proportional to the increase of GABA AUC. Thus, the amount of blood GABA in G-101-treated mice was less than 10% of intestinal MSG content non-transformed by G-101.

Since the report of "Chinese Restaurant Syndrome" in 1968, various studies have confirmed that MSG exhibits side effects, including headaches, in humans [11, 19]. Although the proper MSG dose has no side effect, oral and intravenous doses of MSG of >50 mg and >3 g, respectively, also produce these side effects [18]. Although the MSG concentration in the blood is difficult to predict with oral doses of MSG, a direct intravenous injection of 50 mg MSG

is expected to yield a blood concentration of 53 μM (based on the total blood volume of 5 L). Although the blood brain barrier (BBB) has low permeability to MSG, the presence of high-affinity glutamate transporters located at the BBB capillary luminal membrane could facilitate the uptake of MSG into the brain [18]. The toxicity of MSG against neuron cells was not reduced by heating, including the boiling of food in Chinese restaurants [18]. Therefore, to reduce the side effects of MSG, the absorption of MSG in food into the blood should be minimized. In the present study, pretreatment with G-101, which potently converted MSG to GABA, significantly reduced the blood level of MSG in mice. G-101 not only inhibited the absorption of MSG, but also increased the absorption of GABA into the blood. These results suggest that G101 may potently express glutamate decarboxylase, which transforms MSG to GABA, *in vitro* and *in vivo*. This was supported by the previous report that *Lactobacillus plantarum* potently converts MSG to GABA [17]. However, the absorption of GABA was not proportional to intestinal MSG content unabsorbed into the blood. The absorption of GABA was less than 10% of the intestinal MSG content unabsorbed into the blood. Nevertheless, the amount of the absorbed GABA was time-dependently increased after treatment with MSG. Thus, G-101 did not influence Tmax of MSG, whereas it extended Tmax of GABA. Furthermore, we could not find other transformant(s) of MSG in the blood. These results suggest that, although G-101 can convert MSG to GABA, G-101 could not transform GABA to other compounds. Furthermore, G-101 may inhibit the absorption of MSG into the blood, rather than convert MSG to GABA. However, MSG unabsorbed by G-101 may be slowly transformed to GABA, which is continuously absorbed from the intestine to the blood. These results suggest that G-101 may directly inhibit the absorption of MSG into the blood, unabsorbed MSG may be gradually transformed to GABA by the decarboxylation of glutamate decarboxylase, and then the transformed GABA may be slowly absorbed. Based on these findings, G-101 may reduce the side effects of MSG with maintaining its taste *in vivo* by inhibiting the absorption of MSG rather than transforming MSG to GABA *in vivo*.

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