Analysis of the Components of Guibitang and Fermented Guibi-tang and their Ability to Inhibit Angiotensin-converting Enzyme

Chun Liang¹, Na Young Yun¹, Sang Won Jung¹, Dong Seon Kim¹, Young Jae Lee², and Jin Yeul Ma¹∗

¹TKM Converging Research Division, Korea Institute of Oriental Medicine, 483 Expo-ro,Yuseong-gu, Daejeon 305-811, Korea
²College of Veterinary Medicine, Jeju National University, 66 Jejudaehakno, Jeju 690-756, Korea

Abstract – Guibi-tang is a traditional medicine used for the treatment of colds. We investigated the levels of several compounds in Guibi-tang before and after fermentation with Lactobacillus and tested their ability to inhibit angiotensin-converting enzyme. Six known compounds (decursin, decursinol angelate, nodakenin, liquiritin, formononetin, and 6-gingerol) and 2 unidentified compounds were detected in Guibi-tang (GB) and fermented Guibi-tang (FGB) by an established HPLC-DAD method. The levels of the 6 known compounds were decreased after fermentation. FGB showed more potent inhibition of angiotensin-converting enzyme activity than GB. In conclusion, fermentation with Lactobacillus affects the content of several compounds in GB and improves its angiotensin-converting enzyme inhibitory activity.

Keywords – Guibi-tang, fermentation, angiotensin-converting enzyme

Introduction

Hypertension is a multifactorial process and is a risk factor or complication in many diseases, including cardiovascular disease, renal disease, and diabetes. Angiotensin-converting enzyme (ACE; peptidyl-dipeptide hydrolase EC 3.4.15.1) is a zinc-containing enzyme that plays an important physiological role in regulating blood pressure. This enzyme increases blood pressure by hydrolyzing the decapeptide angiotensin I to angiotensin II. The latter is a potent vasoconstrictor that stimulates the secretion of aldosterone. In turn, aldosterone promotes sodium and water retention in the kidneys and thus increases arterial pressure (Erdos, 1975; Hernandez-Ledesma et al, 2003; Skeggs et al, 1956). Thus, inhibition of ACE activity has an overall anti-hypertensive effect.

Guibi-tang (GB) is a multi-herbal traditional Korean medicine that has been used for several hundred years to treat amnesia, poor memory or forgetfulness, fatigue, insomnia, anemia, palpitations, and neurosis. GB is composed of 12 herbs: Angelica gigas Nakai, Dimocarpus longan Lour, Zizyphus jujuba Miller, Polygala temuifolia Willdenow, Panax ginseng C. A. Meyer, Astragallus membranaceus Bunge, Atractyloides macrocephala Koidzumi, Pachyma hoelen Rumph, Aucklandia lappa Decne, Poria cocos Wolf, Glycyrrhiza uralensis Fischer, and Zingiber officinale Roscoe.

Bioconversion such as fermentation can maximize absorption of the active components from herbs as well as increase their bioactivity. Research on the effect of fermentation with microorganisms on the quality and efficacy of medicinal herbs was conducted recently (Kim et al, 2009; Doh et al, 2010; Hyon et al, 2009).

In this study, we fermented GB with Lactobacillus, which is widely used as a food material. Lactobacillus is known to inhibit the growth of some harmful bacteria by the production of lactic acid, and it has therapeutic effects, including anti-inflammatory and anti-cancer activities (Chen et al, 2009; Goldin, 1998). To determine the changes in levels of compounds in Guibi-tang after fermentation, 6 marker compounds, decursin (Angelica gigas Nakai), decursinol angelate (Angelica gigas Nakai), nodakenin (Angelica gigas Nakai), formononetin (Glycyrrhiza uralensis Fischer), 6-gingerol (Zingiber officinale Roscoe), and liquiritin (Glycyrrhiza uralensis Fischer) were studied (Fig. 1). Amounts of the 6 marker compounds in Guibi-tang (GB) and fermented Guibi-tang (FGB) were measured by an established HPLC-DAD method. In addition, the effect of GB and FGB on ACE activity was evaluated.

*Author for correspondence
Tel: +82-42-868-9466; E-mail: jyma@kiom.re.kr
Experimental

Materials and reagents – Samples of GB powder (3.0 g) were obtained from the Korea Institute of Oriental Medicine. HPLC grade solvents (water and acetonitrile) were purchased from J.T. Baker (USA). The compounds decursin, decursinol angelate, nodakenin and 6-gingerol were purchased from the Korea Food & Drug Administration. Liquiritin was purchased from Wako (Japan), and formononetin was purchased from Sigma-Aldrich (USA). The purities of the 6 standard compounds were greater than 98%. ACE (1 U/ml, rabbit lung) and N-Hippuryl-His-Leu (8.33 mM) were purchased from Sigma-Aldrich (USA).

Fermentation of Guibi-tang – The bacterial strain, Lactobacillus curvatus KFRI 166 was obtained from the Korea Food Research Institute (KFRI, Korea). The test organism was transferred into MRS broth for Lactobacillus spp. and grown at 37 °C for 24 h. The activated culture was then inoculated into the broth under the same conditions. The culture was diluted to obtain an initial population of 1 – 5 × 10^7 CFU/ml and was designated as the inoculum. A GB water extract was used as the culture media for fermentation after adjusting the pH to 7.0 using 1 M NaOH and autoclaving for 15 min at 121 °C. After cooling, 750 ml of GB was combined with 7.5 ml of the Lactobacillus inoculum described above. This was incubated at 37 °C for 48 h. A powder of the fermented GB culture was prepared by freeze-drying.

Preparation of samples – Powders of GB (50 mg) and FGB (50 mg) were weighed accurately and dissolved in 1 ml of water. The samples were stored at 4 °C and filtered through a 0.45 µm membrane filter before analysis by HPLC or by bioassay.

Analysis of compounds in GB and FGB – Our HPLC system was an Elite Lachrom HPLC system (Hitachi High-Technologies Co., Tokyo, Japan) equipped with a pump (L-2130), an auto sampler (L-2200), a column oven (L-2350) and a diode array UV/VIS detector (L-2455). System control and data analyses were executed by EZchrom Elite software (version 3.3.1a). The analysis of compounds in the GB and FGB samples was conducted using a HECTOR C18 column (5 µm, 4.60 mm I.D. × 250 mm) at 40 °C. The mobile phase consisted of acetonitrile (A) and water (B) at a flow rate of 1 ml/min. The mobile phase was a gradient of solvent A and solvent B as follows; 0 - 10 min, 1% A; 10 - 70 min, 50% A; 70 - 80 min, 50 - 100% A; 80 - 90 min, 100% A. The DAD detector UV wavelength was set at 203 nm according to the maximal UV absorption of 6 compounds: decursin, decursinol angelate, nodakenin, 6-gingerol, liquiritin and formononetin. The sample injection volume was 20 ml.

Assay for inhibition of ACE activity – ACE activity was assayed by the method of Cushman and Cheung (Cushman and Cheung, 1971) with minor modifications. Briefly, solutions of ACE (8 mU), test sample (0 - 5.0 mg extract/ml) and the ACE substrate, N-Hippuryl-His-Leu were prepared in a borate buffer (100 mM, pH 8.3) containing 0.3 M NaCl. A 50 µl aliquot of ACE solution was pre-incubated with various quantities of GB or FGB in a final volume of 100 µl at 37 °C for 10 min. The mixture was then added to 150 µl of N-Hippuryl-His-Leu