The Role of Intestinal Microflora in Anti-Inflammatory Effect of Baicalin in Mice

Myung-Ah Jung¹, Se-Eun Jang², Sung-Woon Hong³, Myung Joo Hana and Dong-Hyun Kim²*  
¹Department of Food and Nutrition, ²Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130-701, Republic of Korea

Abstract
Baicalin, a main constituent of the rhizome of Scutellaria baicalensis, is metabolized to baicalein and oroxylin A in the intestine before its absorption. To understand the role of intestinal microflora in the pharmacological activities of baicalin, we investigated its anti-inflammatory effect in mice treated with and without antibiotics. Orally administered baicalin showed the anti-inflammatory effect in mice than intraperitoneally treated one, apart from intraperitoneally administered its metabolites, baicalein and oroxylin A, which potently inhibited LPS-induced inflammation. Of these metabolites, oroxylin A showed more potent anti-inflammatory effect. However, treatment with the mixture of cefadroxil, oxytetracycline and erythromycin (COE) significantly attenuated the anti-inflammatory effect of orally administered baicalin in mice. Treatment with COE also reduced intestinal bacterial fecal β-glucuronidase activity. The metabolic activity of human stools is significantly different between individuals, but neither between ages nor between male and female. Baicalin was metabolized to baicalein and oroxylin A, with metabolic activities of 1.427 ± 0.818 and 1.025 ± 0.603 pmol/min/mg wet weight, respectively. Baicalin and its metabolites also inhibited the expression of pro-inflammatory cytokines, TNF-α and IL-1β, and the activation of NF-κB in LPS-stimulated peritoneal macrophages. Of them, oroxylin A showed the most potent inhibition. Based on these findings, baicalin may be metabolized to baicalein and oroxylin A by intestinal microflora, which enhance its anti-inflammatory effect by inhibiting NF-κB activation.

Key Words: Baicalin, Baicalein, Oroxylin A, Scutellaria baicalensis, Metabolism, Inflammation

INTRODUCTION
Most herbal medicines are orally administered to human. Their components are inevitably contacted with intestinal microflora in gastrointestinal tract and may be metabolized by intestinal microflora, before absorption from the gastrointestinal tract to the blood (Kobashi and Akao 1997; Kim 2002). Therefore, to express the pharmacological effects of herbal medicines, the metabolic activities of intestinal microflora for the constituents of herbal medicines may be of a great importance.

The rhizome of Scutellaria baicalensis (SB), which contains baicalin as a main constituent, has been used in China, Japan and Korea as a traditional medicine and functional food for inflammation, fever, hepatitis, allergic disease, hypertension, etc (Lin and Shieh, 1996; Zu, 1998; Wu et al., 2005). Baicalin exhibits anti-inflammatory, anti-allergic, anti-oxidant, hepatoprotective, and anti-tumor effects (Chou et al., 2003; Jang et al., 2003; Kim et al., 2005; Xing 2005a). It is generally assumed that baicalin is poorly absorbed from the gastrointestinal tract in its native form and must be hydrolyzed by intestinal microflora in the intestine to their aglycones in human and rats (Akao et al., 2000; Yim et al., 2004; Trinh et al., 2010). The metabolite, baicalein, is subsequently metabolized to baicalin, baicalein 6-0-β-glucuronide-7-O-sulfate and baicalein 6,7-diglucuronide in the gut mucosae, liver and blood (Abe et al., 1990; Akao et al., 2004; Lu et al., 2007). However, Abe et al. found two baicalein conjugates and one oroxylin A conjugate as main metabolites in the bile of rats orally treated with baicalin (Abe et al., 1990). We also found that human fecal microflora could transform baicalin to baicalein and oroxylin A, which exhibit more potent anti-scratching behavioral effect in histamine-treated mice than a parental compound baicalin (Trinh et al., 2010). Nevertheless, the role of intestinal microflora in the pharmacological effect of baicalin has not been clarified.

Therefore, to understand the role of intestinal microflora in the pharmacological effect of baicalin, we isolated baicalin from SB and its metabolites, and investigated their anti-inflammatory effects in antibiotics-treated mice and in lipopolysac-
MATERIALS AND METHODS

Materials
p-Nitrophenyl-β-D-glucuronide, LPS were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Baicalin (purity, >95%), baikalein (purity, >93%) and oroxylin A (purity, >95%) was purified from the rhizome of Scutellaria baicalensis according to the previously reported methods of Trinh et al. (2010).

Subjects
The subjects were 100 healthy Korean persons (average, 40.74 ± 13.87 years; range, 20-72 years; 54 males, 46 females). Exclusion criteria included smoking and current medication, especially regular or current use of antibiotics. The recruitment of subjects and collection of their stools were approved by the Committee for the Care and Use of Clinical Study in the Medical School, Kyung Hee University.

Fecal specimen preparation
The human fecal specimens (1 g) were collected in plastic cups, and then carefully mixed with a spatula and suspended with cold 9 ml saline according to a previous method (Choi et al., 2011). Fecal bacterial suspension was centrifuged at 500 × g for 5 min. The resulting supernatant was sonicated for 10 min and then centrifuged at 10,000 × g for 20 min. The resulting supernatant was used for the assay of enzyme activity.

Animals
Male ICR mice (24-28 g) were supplied by Orient Experimental Animal Breeding Center (Sungnam, Korea). All animals were housed in wire cages 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 Guidelines 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.

Assay of fecal of β-D-glucuronidase and β-D-glucosidase activity
For the assay of β-D-glucuronidase and β-D-glucosidase activity in mouse stools, the reaction mixture (total volume of 1 ml) contained 0.4 ml of 1 mM p-nitrophenyl-β-D-glucuronide or p-nitrophenyl-β-D-glucopyranoside, 0.4 ml of 0.1 M phosphate buffer, pH 7.0, and 0.2 ml of the fecal enzyme fraction. The reaction mixture was incubated at 37°C for 20 min. The reaction was stopped by the addition of 1 ml of 0.5 N NaOH, centrifuged at 3,000 × g for 10 min and measured the absorbance at 405 nm (UV-vis spectrophotometer, JASCO V-530, Tokyo, Japan).

Assay of fecal baikalin-metabolizing activity
For the fecal baikalin-metabolizing activity, the reaction mixture (2 ml) containing 0.2 ml of the human fecal suspension 50 mg of fresh feces and 0.2 ml of 1 mM baikalin was incubated at 37°C for 6 h, and 2 ml of MeOH was added to stop the reaction. The reaction mixture was centrifuged at 3,000 × g for 10 min and the levels of baikalin and its metabolites baikaline and baikaldehyde (LPS)-stimulated peritoneal macrophages.

Fig. 1. Anti-inflammatory effect of baikalin in LPS-stimulated mice, treated with or without antibiotics. Male ICR mice were intraperitoneally injected with LPS (4 mg/kg) in the absence or presence of test agents (20 mg/kg). Test agents were orally (o) or intraperitoneally (i) administered either 6 h before treatment with LPS in mice treated with or without antibiotics. The antibiotics-treated groups were treated with COE once a day for 3 day and then test agents were treated 48 h before the LPS injection. Mice were sacrificed 4 h after LPS injection, and whole blood was obtained by cardiac puncture. The levels of IL-6, TNF-α, and IL-1β in the serum were determined using ELISA kit. *Significantly different vs. each group treated with LPS alone (p<0.05). #Significantly different vs. each LPS-non-treated group (p<0.05).