MAGNESIUM LITHOSPERMATE B IS EXCRETED RAPIDLY INTO RAT BILE MOSTLY AS METHYLATED METABOLITES, WHICH ARE POTENT ANTIOXIDANTS

Ying Zhang, Teruaki Akao, Norio Nakamura, Masao Hattori, Xiu-Wei Yang, Chang-Ling Duan, and Jian-Xun Liu

Faculty of Pharmaceutical Sciences and Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan (N.N., M.H.); and Xiyuan Hospital, China Academy of Traditional Chinese Medicine, School of Pharmaceutical Science, Beijing University, Beijing, China (Y.Z., X.-W.Y., C.-L.D., J.-X.L.)

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ABSTRACT:

To elucidate the in vivo pharmacological activities of magnesium lithospermate B (MLB), an active constituent of Radix Salviae Miltiorrhizae, in the rat, its metabolic fate both in vivo and in vitro was investigated. High-performance liquid chromatography revealed that four major metabolites with lower polarity were excreted into bile after intravenous and oral administration of MLB. The metabolites present in combined samples of bile from rats after intravenous injection were isolated and purified by column chromatography and identified as four meta-O-methylated products, namely 3-monomethyl- (M1), 3,3’-dimethyl- (M2), 3,3’-dimethyl-, and 3,3’,3’-trimethyl-lithospermic acid B according to their spectroscopic characteristics (1H, 13C NMR, 1H-1H correlation spectroscopy, 1H-detected multiple quantum coherence, and heteronuclear multiple bond coherence combined with positive ion fast atom bombardment-mass spectroscopy). After administration of MLB at an intravenous dose of 4 mg/kg or an oral dose of 100 mg/kg, the total biliary recovery of the four metabolites after 30 h reached 95.5 ± 2.4% (with approximately 90% recovered within 2 h) or 5.5 ± 0.7%, respectively. The metabolic pathway was proposed to involve sequential formation of the four methylated metabolites. Incubation of MLB, M1, M2, or M4 in rat hepatic cytosol in the presence of S-adenosyl-L-methionine demonstrated the formation of all four metabolites, which indicated that the enzyme responsible for the biotransformation is catechol O-methyltransferase. MLB and its main metabolites M1 and M2 showed potent 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activities, the activity of M1 being stronger than those of caffeic acid (the monomer form of MLB) and α-tocopherol (a representative antioxidant) but weaker than that of MLB. The rapid and high biliary excretion levels of these metabolites suggested that they could undergo enterohepatic circulation in rats and that they might thereby be largely responsible for the pharmacological effects of MLB.

The dry roots of Salvia miltiorrhiza (danshen) are a representative oriental medicine used for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrhea, and neurasthenic insomnia (Li, 1998). The active constituents had been considered to be certain liposoluble substances, such as tanshinones (Chang et al., 1990; Zhang et al., 1990); however, in recent years, the water-soluble components in the dried root decoction used in traditional Chinese medicines have been examined for their pharmacological activities (Zhang and Liu, 1996). Among caffeic acid and its oligomer condensates, which have been isolated from Radix Salviae Miltiorrhizae, lithospermic acid B (LSB, the tetramer) is the most common component in Salvia species and the most abundant in their aqueous extracts (Kasimu et al., 1998; Li, 1998). LSB and magnesium lithospermate B (MLB), the most general form present in the decoction, have antioxidative (Huang and Zhang, 1992; Chen et al., 1999), antifibrotic (Shigematsu et al., 1994), and myocardial salvage (Fung et al., 1993) effects; prevent hepatitis (Hase et al., 1997) and uremia (Tanaka et al., 1989); and improve blood circulation and renal function (Yokozawa et al., 1988, 1989, 1991, 1994, 1995). In some studies, MLB has shown significant activity after oral administration; however, our previous study of the pharmacokinetics of MLB revealed extremely low bioavailability after oral dosing (Zhang et al., 2004), suggesting that MLB itself does not act in vivo after oral administration. In addition, our pharmacokinetic data suggested that LSB was largely metabolized. We therefore hypothesized that, in addition to the parent compound, some metabolic products of LSB may show biological activities, thereby contributing to the pharmacological efficacy of MLB (LSB) in vivo.

Although the precise mechanism of action of MLB (LSB) in vivo is unclear, it is considered that its strong antioxidant and free radical-scavenging activity could be clinically important for repairing damage to vascular endothelial cells, which are responsible for regulating vessel tone, thus reducing pathological changes in target organs and restoring vascular and tissue functions (Chen et al., 1999). Therefore, the objective of the present study was to investigate the metabolic fate of LSB both in vivo and in vitro and to assess the antioxidative activities of the methylated metabolites obtained.

ABBREVIATIONS: LSB, lithospermic acid B; MLB, magnesium lithospermate B; IR, infrared; FAB-MS, fast atom bombardment-mass spectroscopy; SAM, S-adenosyl-L-methionine; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHT, butylated hydroxytoluene; HMBC, heteronuclear multiple bond coherence; COMT, catechol O-methyltransferase.
Materials and Methods

General. Optical rotations and UV spectra were measured on a Jasco DIP-360 digital polarimeter (Jasco, Tokyo, Japan) and Shimadzu UV-160A (Shimadzu, Kyoto, Japan), respectively. IR spectra were recorded on a Jasco FT/IR-230 Fourier transform infrared spectrometer (Jasco). NMR spectra, including 2D-NMR spectra (1H-1H correlation spectroscopy, 1H-detected multiple quantum coherence, and HMBC), were obtained on a Varian Unity Plus 500 (Varian Inc., Palo Alto, CA) spectrometer operating at 500 MHz for protons and 125 MHz for carbon in methanol-d₄ at 25°C. Chemical shifts were expressed as δ values (parts per million) relative to the tetramethylsilane used as an internal standard. Fast atom bombardment-mass spectroscopy (FAB-MS) data were determined on a JEOL JMS-AX505HAD mass spectrometer (JEOL, Tokyo, Japan) using 3-nitrobenzyl alcohol as a matrix.

Chemicals. MLB was isolated and prepared from Radix Salviae Miltiorrhizae (Shanxi Province, China) according to the method reported previously (Zhang et al., 2004). Diaion HP-20 was obtained from Mitsubishi Chemical Co. (Tokyo, Japan). (+)-α-Tocopherol was obtained from Sigma-Aldrich (St. Louis, MO). Wakogel 100Cl8 (63–212 μm), α-adenosyl-L-methionine (SAM) chloride, and all other regents of analytical or HPLC grade were purchased from Wako Pure Chemicals (Osaka, Japan). Methanol-d₄ was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Animals. Male Wistar rats (7 weeks old, weighing about 220 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were provided with standard laboratory chow and water and maintained on a 12:light dark cycle at 22 ± 2°C in the Laboratory Animal Center of Toyama Medical and Pharmaceutical University (Toyama, Japan). The animals were starved overnight with free access to water before any experiments. The animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Toyama Medical and Pharmaceutical University.

Bile and Urine Collection. Under light anesthesia with diethyl ether, bile from 16 rats with biliary fistulization was collected into successive vials on ice in 2 ml of water. All bile and urine samples were stored at −30°C until analysis.

Isolation of Biliary Metabolites. Bile from 16 rats with biliary fistulization for 10 h after bolus intravenous injection at a dose of 25 mg/kg was combined, diluted with water to 200 ml, and applied to a Diaion HP-20 (porous polymer resin, aromatic type absorbents based on crosslinked polystyrene matrix) column. After washing with 800 ml of water, the column was eluted with MeOH-H₂O (700 ml with 1:4 and 1000 ml with 3:2, respectively). Further repeated column chromatography using Wakogel 100Cl8, which was eluted with 0.1% TFA-MeOH, afforded M1 (80 mg), M2 (40 mg), M3 (11 mg), and M4 (2 mg) as free acid forms. The purity of each metabolite was 97, 98, 90%, and 90%, respectively, based on HPLC analyses.

M1. A pale yellow powder, [α]D₂⁰ +62.5° (c = 0.2, MeOH). UV(MeOH) λmax (log ε): 287 (4.16), 309 nm (4.11). IR(KBr) vmax: 3421, 1702, 1612, 1529, 1469, 1265, 1180 cm⁻¹. 1H NMR, Table 1. 13C NMR, Table 2. FAB-MS (positive ion mode) m/z: 755 [M + Na]⁺, 733 [M + H]⁺.

M2. A pale yellow powder, [α]D₂⁰ +68.3° (c = 0.1, MeOH). UV(MeOH) λmax (log ε): 287 (4.15), 309.5 nm (4.10). IR(KBr) vmax: 3421, 1702, 1608, 1516, 1469, 1273, 1173, 1030 cm⁻¹. 1H NMR, Table 1. 13C NMR, Table 2. FAB-MS (positive ion mode) m/z: 769 [M + Na]⁺, 747 [M + H]⁺.

M3. A pale yellow powder, [α]D₂⁰ +62.5° (c = 0.1, MeOH). UV(MeOH) λmax (log ε): 287 (4.09), 309 nm (4.15). IR(KBr) vmax: 3444, 1702, 1628, 1516, 1469, 1273, 1180, 1034 cm⁻¹. 1H NMR, Table 1. 13C NMR, Table 2. FAB-MS (positive ion mode) m/z: 783 [M + Na]⁺.

M4. A pale yellow powder, [α]D₂⁰ +62.5° (c = 0.05, MeOH). UV(MeOH) λmax (log ε): 287.5 (3.89), 309 nm (3.97). IR(KBr) vmax: 3425, 1702, 1608, 1520, 1469, 1273, 1034 cm⁻¹. 1H NMR, Table 1. FAB-MS (positive ion mode) m/z: 769 [M + Na]⁺.

Quantification of Biliary Metabolites. Bile samples were mixed with 4 volumes of MeOH, shaken vigorously for 2 min, and centrifuged at 7000g for 5 min. The supernatant (20 μl), which was filtered through a 0.45-μm membrane filter, was applied to an HPLC-UV system [Shimadzu LC-6A liquid chromatograph equipped with a Shimadzu SPD-6A UV detector and a reverse-phase ODS column, 4.6 mm i.d. × 150 mm, packed with CHEMSORB 7-ODS-H (Chemco Scientific Co., Ltd., Osaka, Japan)]. The analytical conditions were as follows: mobile phase, 0.1% TFA/MeOH; flow rate, 1.0 ml/min; and wavelength, 288 nm. The metabolites were quantified by measuring their peak areas. Calibration plots of the peak areas of each of the metabolites in drug-free bile were linear over a concentration range of 0.002 to 1 mM for M1, M2, and M3 and 0.002 to 0.5 mM for M4. The recoveries of M1 through M4 in bile were evaluated as 97.2, 95.9, 102.9, and 93.2%, respectively, by determination of standard samples added to drug-free bile. LSB in

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The supernatant was filtered through a 0.45-μm filter. The assay mixture in ethanol was solved in ethanol, and the experiments were performed on freshly prepared butylated hydroxytoluene (BHT), 60 to 1200 μM. After a 10-min incubation, the reaction was terminated by adding MgCl₂, 1 mM SAM, and 60 μM KMgPO₄ buffer (pH 7.4). The mixture was preincubated at 37 °C. The radical concentration at zero time, and the second-order rate constants (k₂) were calculated from the slope of this plot to determine the radical-scavenging activity of the compounds tested.

**Preparation of Rat Liver Cytosol.** Livers from five rats were homogenized with 9 volumes of 1.15% KCl and then centrifuged at 12000 g for 15 min at 4 °C. The supernatant was further centrifuged at 100,000 g for 90 min to obtain microsomal and cytosol fractions, and these were stored at −80 °C. Protein determination was carried out according to Lowry et al. (1951).

**Incubation with Rat Liver Cytosol.** The incubation mixture contained 5 mM MgCl₂, 1 mM SAM, and 60 μl of rat liver cytosol (680 μg protein) in 50 mM K₂HPO₄/KH₂PO₄ buffer (pH 7.4). The mixture was preincubated at 37 °C for 5 min before the addition of the MLB or metabolites at a final concentration of 0.25 mM. After a 10-min incubation, the reaction was terminated by adding methanol and 0.1% H₂PO₄. After centrifugation at 7000 g for 5 min, the supernatant was filtered through a 0.45-μm membrane-filter and then applied to the HPLC-UV assay system described above for quantification.

**Determination of DPPH Radical-Scavenging Activity.** DPPH was dissolved in ethanol, and the experiments were performed on freshly prepared solution according to Espin et al. (2000). The assay mixture in ethanol contained 60 μM DPPH, and each compound was tested as follows: MLB, 4 to 60 μM; M1, 12 to 40 μM; M2, 20 to 100 μM; M3, 40 to 160 μM; caffeic acid, 40 to 120 μM; ferulic acid, 160 to 800 μM; α-tocopherol, 60 to 120 μM; and butylated hydroxytoluene (BHT), 60 to 1200 μM. Absorbance was recorded at 515 nm during an interval from 6 s to 30 min of reaction in a UV-visible spectrophotometer (Shimadzu UV-2100) at room temperature.

Second-order rate constants were calculated to evaluate the radical-scavenging activity of antioxidants. The decay of [DPPH] was expressed as micromoles. The pseudo-first-order rate constant kobs was calculated from the first equation. This kobs was linearly dependent on the concentration of antioxidants, and the second-order rate constants (k₂) were calculated from the slope of this plot to determine the radical-scavenging activity of the compounds tested.

**Results**

**Characterization of MLB Metabolites in Bile.** HPLC chromatography of bile samples from rats administered MLB intravenously or orally revealed four new large peaks, named M1 through M4, in addition to a small peak of LSB (Fig. 1). These peaks showed longer retention than LSB.

Using Diaion HP-20 and repeated Wakogel C18 column chromatography, the four metabolites were purified from the combined bile of rats injected intravenously with MLB. The UV and IR spectra of the four metabolites were similar to those of LSB, suggesting that they possessed the same functional groups. FAB-MS showed that M1, M2, and M3 had molecular ion peaks at m/z 755, 769, and 783 [M + Na]⁺, respectively, which were 14, 28, and 42 mass units higher than LSB, respectively. The 1H NMR spectra of M1 through M4 showed signals of 1, 2, and 3 methoxyl groups, respectively. Detailed analysis including the 2D-NMR spectra showed that these metabolites had the same structure apart from differences in their methoxy groups. As shown in Fig. 2, M3 showed long-range correlations between methoxy protons (δ 3.81, 3.79, and 3.60) and phenolic carbons at δ 149.2 (C-3), 148.6 (C-3'), and 148.6 (C-3'''), respectively, on the HMBC spectra. Therefore, the structure of M3 was determined as 3,3',3''-O-trimethyl-LSB. Similarly, the structures of M1 and M2 were identified as 3-O-monomethyl-LSB and 3,3'-O-dimethyl-LSB, respectively, from the long-range correlations between methoxy pro-

![FIG. 1. HPLC chromatogram of bile after oral administration of MLB. Bile was collected from rats during 0 to 2 h after oral administration of MLB at a dose of 100 mg/kg. The identified metabolites are labeled M1 through M4 in the order of peak size, and the parent compound is labeled as LSB.](https://doi.org/10.1097/01041346-200710000-00001)
tions and phenolic carbons (M1: δH 3.80 and δC 149.1; M2: δH 3.80 and δC 149.2, δC 3.77 and δC 148.8) on the HMBC spectra. 1H NMR and MS data suggested that metabolite M4 was the dimethyl ether of LSB, and the methyl positions were determined to be 3-O and 3″-O by comparing the chemical shift with those of other metabolites (Table 1). This structure was supported by the experiment described below that investigated the in vitro transformation of MLB with rat liver cytosol.

**Excretion of the Metabolites into Bile.** The cumulative biliary excretion profiles of the four metabolites after intravenous injection or oral administration of MLB are shown in Figs. 3 and 4. The total cumulative recovery of the four metabolites in bile was 95.5 ± 2.4%, comprising 31.2 ± 8.8, 38.3 ± 1.1, 22.1 ± 8.1, and 5.3 ± 0.4% for M1 through M4, respectively, over 30 h after a 4-mg/kg intravenous dose of MLB, although only 5.10 ± 2.36% of unchanged LSB was recovered. Approximately 90% of the dose was excreted as the four metabolites into bile within 2 h after injection, suggesting rapid uptake of MLB (LSB) into hepatic cells, its rapid methylation, and coordinated excretion of the methylated metabolites into bile. On the other hand, only 0.70 ± 0.26% of LSB with trace amounts of the methylated metabolites (detectable but immeasurable) was excreted into urine over a 30-h period.

After oral administration of 100 mg/kg MLB, percentages of mean excretion into bile over 30 h for LSB, M1, M2, M3, and M4 were 0.12 ± 0.04, 2.0 ± 0.3, 2.4 ± 0.3, 0.77 ± 0.05, and 0.37 ± 0.06%, respectively, accompanied by urinary excretion of 0.010 ± 0.001% of LSB alone. Total cumulative recovery in bile after oral administration was only 5.6 ± 0.7%, apparently reflecting the rate of MLB absorption from the rat alimentary tract. The excretion profiles including M1 and M2 as the main metabolites after oral administration were similar to those after intravenous injection, despite the slower biliary excretion.

**In Vitro Metabolism of MLB and Its Biliary Metabolites with Rat Liver Cytosol.** When MLB was incubated with rat liver cytosol in the presence of SAM for a prolonged period (more than 60 min), most of the MLB disappeared, and four peaks corresponding to M1 through M4 in bile were obtained on HPLC. Three major metabolites isolated by large-scale incubation were also identified as M1 (3-O-monomethyl-LSB), M2 (3,3″-O-dimethyl-LSB), and M3 (3,3″,3″-O-trimethyl-LSB) by spectroscopic analysis. When LSB, M1, M2, and M4 were incubated separately under the same conditions, except for the use of a shorter incubation period (10 min), only one product was obtained, as shown in the HPLC profiles in Fig. 5A–D (M1 from LSB, M2 from M1, M3 from M2, and M4 from M3, respectively). These results reveal the transformation pathway shown in Fig. 6. In this pathway, the first methylation proceeds at the C-3 meta-hydroxyl group of catechol to give M1, the second at C-3″ overwhelmingly, or the third at C-3″ rarely to give M2 and M4, respectively, and finally to give M3. The observation that both M2 and M4 were transformed to M3 confirmed that M4 was 3,3″-O-dimethyl-LSB. Accordingly, effectiveness as substrates for the enzyme, probably catechol O-methyltransferase (COMT), seemed to be in the decreasing order LSB > M4 > M1 > M2.

**Antioxidative Activities of the Metabolites.** The radical-scavenging activities of the three major methylated metabolites were compared with those of MLB, caffeic acid (the monomer form of MLB), ferulic acid (caffeic acid 3-methylester), α-tocopherol (representative natural antioxidant), and BHT (synthetic antioxidant) by calculating the second-order rate constant (k2) for the reaction with DPPH+ (Table 3). All the metabolites tested showed potent DPPH radical-scavenging effects. M1 showed the most potent activity and was stronger than α-tocopherol and caffeic acid but less potent than MLB. M2 and M3 were less potent than α-tocopherol and caffeic acid but stronger than ferulic acid and BHT. Following methylation of the meta-hydroxyl group, the rate constant decreased significantly in turn.

**Discussion**

In our previous pharmacokinetic study, MLB was found to have extremely low bioavailability in the rat after intravenous or oral administration (Zhang et al., 2004). This extremely low bioavailability was mainly due to poor absorption of MLB through the gastrointestinal tract. Accordingly, only 0.010 and 0.12% of the dose was excreted as unchanged LSB into urine and bile, respectively, after oral
administration of MLB; however, even after intravenous injection, only 0.07 and 5.1% was excreted. In addition, wide distribution and extensive metabolism of MLB (LSB) was suggested from the pharmacokinetic data. Accordingly, we attempted to clarify the metabolites excreted into urine and bile. We successfully isolated four metabolites from rat bile, and their structures were deduced by spectroscopic analysis, as shown in Fig. 6.

The excretion of these metabolites was found to be appreciable, and almost all of the four methylated metabolites were excreted into bile, and not into urine, of rats injected intravenously with MLB. Surprisingly, almost 100% of the dose—95% as the methylated metabolites and 5% as LSB—was recovered in the bile within 30 h after injection. Moreover, over 90% of the dose was excreted into bile within the first 2 h. These results suggested that MLB (LSB) was rapidly and completely taken up by hepatocytes, that most of the MLB (LSB) was methylated there, and that the methylated metabolites were excreted into bile in a coordinated manner, probably through a transporter-dependent mechanism. After oral administration of MLB, the same methylated metabolites, as well as LSB, were also recovered from bile in only small amounts. Accordingly, the percentage of total biliary excretion (5.6%) after oral administration seemed to reflect the ratio of MLB absorption from the rat gastrointestinal tract; the absolute bioavailability of MLB was 0.02% in our previous pharmacokinetic study (Zhang et al., 2004). Thus, the difference between 5.6 and 0.02% seems to be due to the first-pass effect, mainly metabolism in the liver.

Compared with the dispositions of caffeic acid and rosmarinic acid, a dimer of caffeic acid, in rats, some characteristic features of MLB, which is a tetramer of caffeic acid, could be deduced. After oral or intraperitoneal administration of caffeic acid to rats, some metabolites derived from dehydroxylation and methylation of phenolic hydroxyl groups and β-oxidation of a phenolic side chain double bond are excreted mainly into urine (Booth et al., 1957). Moreover, in isolated rat liver, over 90% of caffeic acid seems unchanged in the perfusate after a single passage, indicating no major first-pass effect for caffeic acid (Gumbinger et al., 1993). Even after oral administration of rosmarinic acid, similar metabolites have been determined with a total recovery of 31.8% in urine rather than in bile, indicating selective para-dehydroxylation, methylation, and sulfate conjugation following cleavage of the ester bond to give caffeic acid (Nakazawa and
Ohnaw, 1998). Thus, MLB (LSB) seems to have a unique metabolic fate involving only methylation of the phenolic hydroxyl groups that is quite different from that of dihydroxycinnamic acids such as caffeic acid and rosmarinic acid. It is possible that the large molecular volume of MLB (LSB) impedes exposure of the two ester bonds to esterase(s), compared with the easy hydrolysis of the ester bond of rosmarinic acid. In fact, no MLB was hydrolyzed upon incubation with rat hepatic microsomes containing abundant esterases (data not shown). The absence of sulfate or glucuronide conjugates of LSB in bile may also be due to its poor accessibility to conjugation enzymes.

Additionally, O-methylation of MLB (LSB) both in vivo and in vitro resulted exclusively in the selective formation of meta-O-methylated derivatives, and in vitro O-methylation with liver cytosol required SAM, suggesting that the reaction was catalyzed by COMT. COMT catalyzes the transfer of the methyl group from SAM to the meta-hydroxyl group of phenolic compounds with a catechol structure (Axelrod and Tomchick, 1958). The highest activities and protein contents have been found in rat liver and kidney (Guldberg and Marsden, 1975; Karhunen et al., 1994), but the hepatic enzyme may mainly contribute to the in vivo meta-O-methylation of MLB (LSB). Since MLB and its methylated intermediates M1 and M4 were good substrates for COMT (Fig. 5), they may act as competitive inhibitors of the enzyme. Further studies will be needed to characterize the inhibitory ability and relevant pharmacological response of these metabolites for the treatment of diseases such as Parkinson’s disease, depression, and heart failure.

Meanwhile, the catechol moieties of caffeic acid analogs are known to be very important for scavenging of free radicals (Chen et al., 1999), M1, M2, M4, and MLB (LSB), which contain catechol moieties, are considered to have antioxidant properties. In fact, the major metabolites M1 and M2 had strong antioxidative activities (Table 3), with M1 showing higher radical-scavenging activity than caffeic acid (a well known potent antioxidant and monomer of LSB) and α-tocopherol (a representative natural antioxidant) but lower activity than MLB. In addition, M2 and M3 also showed stronger effects than ferulic acid, caffeic acid 3-methylester, and BHT, a representative synthetic antioxidant. Because MLB (LSB) seemed to enter liver cells rapidly after oral or intravenous dosing, and methylated metabolites were produced there, these compounds may exert some pharmacological activities—including antioxidant action—in the liver. Furthermore, the possibility of enterohepatic circulation of the methylated metabolites should not be neglected, because not only are they excreted from the intestinal tract via bile, but their lower polarity could also result in easier absorption from the gastrointestinal tract compared with MLB (LSB).

In summary, four major metabolites have been isolated from bile collected from rats after oral or intravenous administration of MLB, and all of them were characterized as meta-O-methylated products. Within 2 h after intravenous injection, over 90% of the dose was excreted into bile as the four methylated metabolites and not into urine. COMT seemed to be responsible for the metabolic transformation of MLB, as suggested by in vitro formation of the same metabolites upon incubation with rat hepatic cytosol in the presence of SAM. The unique metabolic fate of MLB, which is rapidly incorporated into liver cells and exhaustively methylated, may be related to its pharmacological activities in vivo. To fully elucidate the biological fate of MLB, further studies will be necessary to investigate the bioavailability of M1 through M4 as well as their characteristic inhibitory activity on COMT and the relevant pharmacological response following metabolism in liver cells.

References

Address correspondence to: Dr. Teruaki Akao, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan. E-mail: ta0113@ms.toyama-mpu.ac.jp