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Roles of bile acid conjugates and phospholipids in *in vitro* activation of pancreatic lipase by bear bile and cattle bile

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ABSTRACT

Ethnopharmacological relevance: Bear bile (BB) originally used as a traditional Chinese medicine has also been adopted in Japan as a traditional home remedy mainly for gastrointestinal problems due to impaired digestion. However, recently, efforts have been made to find alternatives to BB for ecological and ethical reasons.
Aims of the study: To find alternatives to BB for facilitating fat digestion, we compared the potency of cattle bile (CB) or synthetic mixtures of major bile components to activate pancreatic lipase with that of BB.
Materials and methods: The compositions of bile acid conjugates and phospholipids in BB and CB were determined by high-performance liquid chromatography and thin layer chromatography, respectively. The effects of BB and CB as well synthetic mixtures of bile acid conjugates and phospholipids on pancreatic lipase activity *in vitro* were examined.
Results: BB and CB contained markedly different types and quantities of bile acid conjugates and phospholipids, although the potencies of BB and CB to activate pancreatic lipase were not significantly different. The potency of BB to activate pancreatic lipase was reconstituted by the major bile acid conjugates and phospholipids found in BB. In contrast, only bile acid conjugates found in CB could reconstitute its potency to activate pancreatic lipase.
Conclusions: Our observations indicate that CB or the synthetic mixture of bile components can be used as an alternative to BB for facilitating fat digestion.

1. Introduction

Bear bile (BB) has been used in traditional Chinese medicine for over 1300 years and its extensive applications for treatment of various disease conditions have been recorded in many Chinese reports (Jeng and Cheng-Low, 1992; Gao, 2002). However, extensive consumption of BB made bears an endangered species, which is now listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). To obtain sustainable sources of BB, the People's Republic of China has developed bear farms to produce artificially drained bile juice from living bears. This artificially drained BB is proven to have the same chemical constituents as naturally derived BB (Liang et al., 1993; Zhang et al., 1993; Xu et al., 1999; Gong et al., 2002), however, the procedures to obtain artificial from living bears are highly invasive. As an international consensus, the establishment of alternatives to BB is recognized as an effective strategy for stopping the trading and use of BB.

In Japan, BB has been used mainly for gastrointestinal problems due to impaired digestive functions. Interestingly, Japan has a unique history of utilizing bile preparations harvested from other domestic animals such as cattle and pig similar to BB. However, the compositions of bile preparations harvested from different animal species differ markedly (Yokota et al., 2004). In particular, BB contains a unique bile acid conjugate, tauroursodeoxycholic acid (TUDCA), which is implicated in the pharmacological actions of BB (MacDonald and Williams, 1985). Ursodeoxycholic acid, an unconjugated form of TUDCA, is produced as a pharmacological agent for treating gallstones and other liver diseases (Bachrach and Hofmann, 1982a,b; Borum and Fromm, 1990; Ishizaki et al., 2005). In addition to bile acid conjugates, lipids such as phospholipids and cholesterol are also major components of animal bile preparations (Jones and Zollman, 1997), which may contribute to their pharmacological actions.

Bile acid conjugates as well as phospholipids contained in animal bile preparations facilitate emulsification and digestion of ingested triacylglycerols (TAGs) by pancreatic lipase (Lykidis et al.,

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1977). Therefore, bile acid conjugates and phospholipids in animal bile preparations may be implicated in their efficacies to improve digestive functions. The present study was carried out to compare the potencies of BB and CB to activate pancreatic lipase. In addition, the role of bile acid conjugates and phospholipids in the potencies of BB and CB to activate pancreatic lipase was investigated. We here demonstrate that CB or a synthetic mixture of major bile constituents is a possible alternative to BB in the facilitation of fat digestion through pancreatic lipase.

2. Materials and methods

2.1. Reagents

BB and CB were purchased from Mitsuhoshi Pharmaceutical Co., Ltd. (Lot C-083, Nara, Japan) and Hino Pharmaceutical Co., Ltd. (Lot 237K1119, Shiga, Japan), respectively. BB and CB were deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama as TMP No. 25675 and TMP No. 25676, respectively. Sodium salts of tauroursodeoxycholic acid (TUDCA), taurochenodeoxycholic acid (TCDCA), taurocholic acid (TCA), taurodeoxycholic acid (TDCA) and glycocholic acid (GCA) were purchased from Sigma–Aldrich (St. Louis, MO). Sodium glycodeoxycholate (GDCA) was purchased from Steraloids, Inc. (Newport, RI). Phosphatidylcholine purified from egg yolk was obtained from Dosan Sardary Research (Pusan, Korea). Porcine pancreatic lipase (147 units/mg protein) was purchased from Sigma–Aldrich. Other reagents and solvents were of analytical grade and obtained from Wako Pure Chem (Osaka, Japan).

2.2. Bile acid analysis

The contents of bile acid conjugates in BB and CB were determined by high-performance liquid chromatography (HPLC) coupled with evaporative light-scattering detection (ELSD) as described previously (Torchia et al., 2001). Known amounts of BB and CB were dissolved in the mobile phase of HPLC described below and the solutions were filtrated through membrane filters. Aliquots of the filtrates were subjected to HPLC-ELSD using an octadecylsilica (C18) reverse phase column (2.1 mm × 150 mm, Symmetry, Waters Milford, MA) and a mobile phase composed of methanol:acetonitrile:water (53:23:24, v/v/v) containing 5 mM ammonium, acidified with acetic acid to pH 5.6. The flow rate was set at 0.2 ml/min and ELSD was operated under air pressure at 3.5 bar and 85 °C of the drift chamber. Authentic standards of bile acid conjugates were analyzed as described above to establish calibration curves.

2.3. Phospholipid analysis

Known amounts of BB and CB were homogenized in chloroform/methanol/1% sodium chloride (1:2:0.8, v/v/v) and their total lipid fractions were extracted by the method of Bligh and Dyer (1959). The phosphorous content in the lipid fractions obtained from BB and CB was determined by the method of Rouser et al. (1966). Phospholipids in total lipid extracts were separated by silica gel thin-layer chromatography (TLC) using a mixture of chloroform/methanol/14% (w/v) ammonium hydroxide (65:35:8, v/v/v) as a developing solvent. Phospholipid spots on TLC plates were visualized using Dittmer reagent (Dittmer and Lester, 1964).

2.4. Pancreatic lipase activity

Pancreatic lipase activity was measured on the basis of the amounts of nonesterifed fatty acids (NEFAs) generated during the incubation with safflower oil emulsified with 5% Gum Arabic and porcine pancreatic lipase as described by Yoshizumi et al. (2006). BB and CB as well as pure bile acid conjugates were dissolved in distilled water. Phosphatidylcholine (PC) dissolve in chloroform was evaporated to dryness under nitrogen gas at the bottom of test tubes and dispersed in distilled water containing desirable amounts of bile acid conjugates by sonication. These aqueous samples were added to the reaction mixture 5 min before the addition of porcine pancreatic lipase. The effects of the test materials on pancreatic lipase activity were expressed as the percentages of the control values obtained in the absence of the test materials.

2.5. Data analysis

Analysis of variance (ANOVA) followed by Bonferroni's post hoc test was carried out for statistical analysis of data.

3. Results

3.1. Bile acid and phospholipid composition of BB and CB

HPLC was carried out to compare the composition of bile acids contained in BB and CB. It was shown that taurine conjugates of ursodeoxycholic acid (TUDCA) and chenodeoxycholic acid (TCDCA) were the major bile acid species of BB (Fig. 1A and Table 1). TCA was a minor bile acid species in BB but its content in CB was relatively higher. CB also contained glycine conjugates of cholic acid (GCA) and deoxycholate (GDCA) as well as taurine conjugate of dexoycholate (TDCA). The sum of the bile acid conjugates accounted for approximately 75 and 40% of the total weight of BB and CB, respectively. The total lipid phosphorous of CB (0.22 μmol/g) was much lower than that of BB (1.5 μmol/g). TLC revealed that PC was

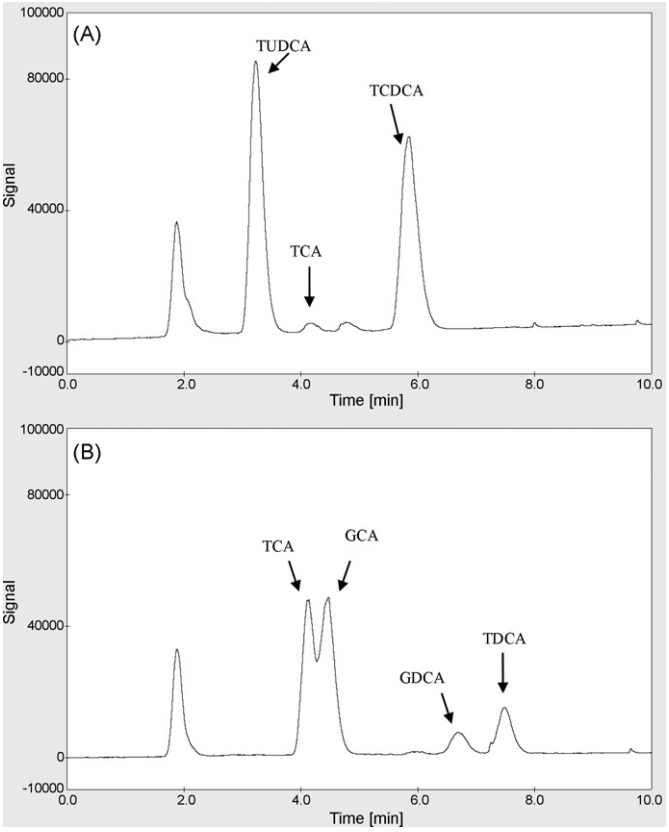

Fig. 1. Bile acid profiles of bear bile (A) and cattle bile (B) determined by high-performance liquid chromatography coupled with evaporative light-scattering detection.