Comparative bioavailability of Silipide, a new flavanolignan complex, in rats

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SUMMARY

The comparative pharmacokinetics of Silipide (IdB 1016, a silybin-phosphatidylcholine complex) and silybin were investigated by measuring unconjugated and total plasma silybin levels as well as total biliary and urinary silybin excretion in rats following administration of a single oral dose (200 mg/kg as silybin). Mean peak levels of unconjugated and total silybin after IdB 1016 were 8.17 and 74.23 µg/ml respectively. Mean AUC (0–6 h) values were 9.78 and 232.15 h·µg.ml⁻¹ indicating that about 94% of the plasma silybin is present in a conjugated form. After administration of silybin, plasma levels of both unconjugated and total compound were under the analytical detection limit. Cumulative biliary (0–24 h) and urinary (0–72 h) excretion values after administration of IdB 1016 accounted for 3.73% and 3.26% of the administered dose, respectively. After silybin administration, the biliary and urinary excretion accounted for only 0.001% and 0.032% of the dose respectively. Our results indicate a superior bioavailability of silybin administered orally as IdB 1016. This was due mainly to an impressive increase in gastrointestinal absorption.

INTRODUCTION

Silybin is the main flavanolignan constituent of silymarin, a standardized extract from the fruits of Silybum marianum, widely used as an antihepatotoxic agent and endowed with anti-lipoperoxidant activity (1, 2). To date, studies using the labelled compound and concerning the oral pharmacokinetic of pure silybin are few and incomplete and indicate that the oral bioavailability of the flavanolignan is very low (3–6). A preliminary study (in humans) by our laboratories, employing a specific HPLC method, supports this finding (7). In an attempt to increase the bioavailability of silybin, IdB 1016, a lipophilic complex with phosphatidylcholine, has been synthesized (8). In order to verify that the bioavailability has been improved, the comparative pharmacokinetics of IdB 1016 and silybin have been investigated by measuring unconjugated and total (after enzymatic hydrolysis with β-glucuronidase/arylsulfatase) plasma silybin levels and total biliary and urinary silybin excretion in rats after oral administration. Silybin concentrations in biological fluids have been determined by an adaptation of a previously described sensitive and specific HPLC method (9).

MATERIALS AND METHODS

Chemicals

IdB 1016 (silybin-phosphatidylcholine complex), sily-
bin, silycristin and (+)-catechin were from Inverni della Beffa Research and Development Laboratories. β-Glucuronidase/arylsulfatase and Helix pomatia juice were from Boehringer Mannheim (Mannheim, Germany). Chloral hydrate, acetate buffer, citrate buffer, n-hexane, methanol, ethanol, tert-butylmethylether and Extrelut 3 and 1 were supplied by E. Merck (Darmstadt, Germany).

Animals and treatment

Male Sprague-Dawley rats (Charles River, Calco, Italy; n = 24) weighing 200-350 g were used. The animals were housed at a constant temperature (21 ± 1 °C) and relative humidity (60 ± 5%) with free access to standard food and water.

IdB 1016 (200 mg/kg as silybin) and silybin (200 mg/kg) were administered by gavage as aqueous suspensions (10 ml/kg).

Plasma

The animals (n=12) were anaesthetized with chloral hydrate (330 mg/kg i.p.); a heparinized catheter was then inserted into the atrium through the jugular vein. The distal part of the catheter was sealed, subcutaneously inserted and fixed at the back side of the neck. The animals were then fasted overnight. Blood was withdrawn (350 μl on heparin): 0.25, 0.5, 1, 2, 4 and 6 h after treatment (a blank sample was also obtained before the administration). The plasma obtained after centrifugation was stored at -20°C until analyzed.

Bile

Two groups of 3 rats, fasted overnight, were cannulated under ether anaesthesia at the bile duct and placed in individual restraining cages. After treatment, bile was collected during the following intervals: 0–2, 2–4, 4–6, 6–8 and 8–24 h (a blank sample was also obtained before the administration). After collection, all samples were stored at -20°C until analyzed.

Urine

Two groups of rats, fasted overnight, were used. After treatment the animals were placed in individual metabolic cages and urine was collected during the following intervals: 0–4, 4–8, 8–24, 24–48 and 48–72 h (a blank sample was also obtained before administration). After collection all samples were stored at -20°C until analyzed.

HPLC analysis

Silybin was detected in biological fluids by HPLC using (+)-catechin and silycristin as internal standards. Analyses were carried out on a Waters System equipped with a WISP 700 Automatic Autosampler, a Waters 600 E System Control and a Waters 600 Multidelivery System; the detection at 214 nm was accomplished by a Model L-4200 UV-VIS (Hitachi).

A LiChrosorb Diol, 5 μm column (150 mm x 3 mm; E. Merck) and a LiChrosorb Diol, 7 μm pre-column (2.5 cm x 4.6 mm; E. Merck, only for bile and urine) were used. The mobile phase was n-hexane/ethanol (70:30 v/v), acidified with phosphoric acid 85% (120 μl/l). A flow rate of 1.5 ml/min (0.8 ml/min for plasma) was applied (9).

Plasma

Unconjugated silybin: to 50 μl of plasma, 2.9 ml of citrate buffer (pH 4) were added; samples were then placed on Extrelut 3 and eluted twice with 10 ml of tert-butylmethylether.

Total silybin: to 50 μl of plasma, 150 μl of acetate buffer (pH 5) and 10 μl of β-glucuronidase/arylsulfatase (about 0.055 U and 0.026 U of glucuronidase and arylsulfatase activity, respectively) were added. After incubation at 37°C for 48 h, 2.7 ml of citrate buffer (pH 4) were added. Samples were then placed on Extrelut 3 and eluted as above.

The eluates were evaporated under nitrogen and the residue resuspended in 150 μl of mobile phase. After addition of 5–10 μl of (+)-catechin methanol solution (internal standard; 0.1 mg/ml), the samples were analyzed for silybin content.

Standards: to 50 μl of plasma, 10 μl of methanol containing increasing amounts of silybin were added; samples were then processed as above. The range of concentrations was: 0.2–6.4 and 6.25–200 μg/ml for unconjugated silybin and 6.25–200 μg/ml for total silybin.

Bile and urine

0.5 ml of urine or bile were mixed with 0.8 ml of acetate buffer (pH 5.2), 220 mg of sodium sulphate and 200 μl of Helix pomatia juice. The samples were hydrolyzed at 37°C for 72 h. To 0.85 ml of hydrolyzed mixture, 0.25 ml of citrate buffer (pH 4) and 25 μl of silycristin methanol solution (internal standard; 40 μg/ml) were added. The samples were then eluted on Extrelut 1. After 15 min, the column was washed...