EUROPEAN JOURNAL OF DRUG METABOLISM AND PHARMACOKINETICS, 1990, Vol. 15, No. 4, pp. 333-338

Pharmacokinetic studies on IdB 1016, a silybin–phosphatidylcholine complex, in healthy human subjects

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Received for publication : May 8, 1990

Keywords : IdB 1016, silymarin, silybin, pharmacokinetics, bioavailability, humans

SUMMARY

IdB 1016 is a complex of silybin (the main active component of silymarin) and phosphatidylcholine, which in animal models shows greater oral bioavailability and therefore greater pharmacological activity compared with pure silybin and silymarin. In order to assess its pharmacokinetic profile in man, plasma silybin levels were determined after administration of single oral doses of IdB 1016 and silymarin (equivalent to 360 mg silybin) to 9 healthy volunteers. Although absorption was rapid with both preparations, the bioavailability of IdB 1016 was much greater than that of silymarin, as indicated by higher plasma silybin levels at all sampling times after intake of the complex. Regardless of the preparation used, the terminal half-life was relatively short (generally less than 4 h). In a subsequent study, 9 healthy volunteers received IdB 1016 (120 mg b.i.d., expressed as silybin equivalents) for 8 consecutive days. The plasma silybin level profiles and kinetic parameters on day 1 were similar to those determined on day 8. Most of the silybin present in the systemic circulation was in conjugated form. Less than 3% of the administered dose was accounted for by urinary recovery of free plus conjugated silybin, a significant proportion of the dose probably being excreted in the bile. It is concluded that complexation with phosphatidylcholine in IdB 1016 greatly increases the oral bioavailability of silybin, probably by facilitating its passage across the gastrointestinal mucosa.

INTRODUCTION

Silybin is the main active component of silymarin, the extract of the seeds of milk thistle (*Silibum marianum*) which is widely used in Europe for the treatment of a variety of hepatic disorders. In animal models, the antihepatotoxic activity of silybin is much greater after parenteral than after oral administration (1, 2), suggesting that the oral bioavailability of this compound is low (3).

Based on the hypothesis that formation of complexes between poorly absorbed flavanolignans and phospholipids may result in increased bioavailability, a complex of silybin and phosphatidylcholine has been developed (4) and tested in animal models. Compared with underivatized silybin and silymarin, this compound, known as IdB 1016 (5), shows similar biochemical properties in vitro but markedly increased pharmacodynamic activity in vivo (2), due to a remarkable enhancement of oral bioavailability (3).

Prior to initiation of clinical trials, it was considered important to evaluate the pharmacokinetic profile of IdB 1016 in man. The studies described in this article were designed to investigate the comparative pharmacokinetics of silybin following single-dose administration of IdB 1016 and silymarin in normal volunteers and to assess whether any

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Table I : Pharmacokinetic parameters (means ± SEM) derived from plasma silybin concentration curves following single oral doses (360 mg expressed as silybin equivalents) of IdB 1016 and silymarin in 8 subjects. Data from subject 9 were excluded from the calculation (*see* text and Fig. 1)

	IdB 1016.	*Silymarin*
Peak concentration (C_{max}), ng/ml	298 ± 96	102 ± 22*
Time of peak (T_{max}), h	1.6 ± 0.3	1.4 ± 0.3
Mean residence time (MRT), h	3.6 ± 0.4	3.5 ± 0.4
Area under curve (AUC_{0-12h}), ng/ml.h	881 ± 207	257 ± 66**
Oral availability (relative to silymarin)	4.6 ± 1.5	—

* $P = 0.05$, ** $P < 0.01$

significant change in IdB 1016 disposition occurs after multiple dosing.

MATERIALS AND METHODS

Subjects and study protocols

Single-dose study : Nine healthy male volunteers aged 21 to 26 years took, after an overnight fast and on two randomized occasions separated by an interval of at least one week, single oral doses of IdB 1016 (3 x 120 mg capsules, expressed as silybin equivalents, Inverni della Beffa S.p.A., Milan, Italy) and silymarin (3 sachets, each containing 120 mg silybin). All subjects remained fasting for 4 h after dosing. Plasma samples were collected at times 0 (before dosing) 0.5, 1.0, 1.5, 2, 3, 4, 6, 8 and 12 h after administration and stored at –20°C until assay.

Multiple-dose study : Nine healthy male volunteers aged 20 to 32 years received IdB 1016 at a dosage of 120 mg (expressed as silybin equivalents) every 12 h away from meal times for 8 consecutive days. Plasma samples were collected at times 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after the morning dose on days 1 and 8 and 2 h after the morning dose on days 4 and 7. Urine samples during the dosing interval were also collected on days 1 and 8. All samples were stored at –20°C until assay.

Drug assay

The concentration of unchanged silybin in plasma was determined by a specific HPLC method (6) with sensitivity limits of 5 ng/ml and a precision better than 7% within the working range of the assay. In the multiple dose study, some plasma samples and all urine samples were re-assayed by the same method after enzymatic hydrolysis with glucuronidase/arylsulphatase (Sigma) at 37°C for 48 h in order to evaluate the total (free + conjugated) silybin concentration. The sensitivity for conjugated drug in both plasma and urine was 25 ng/ml.

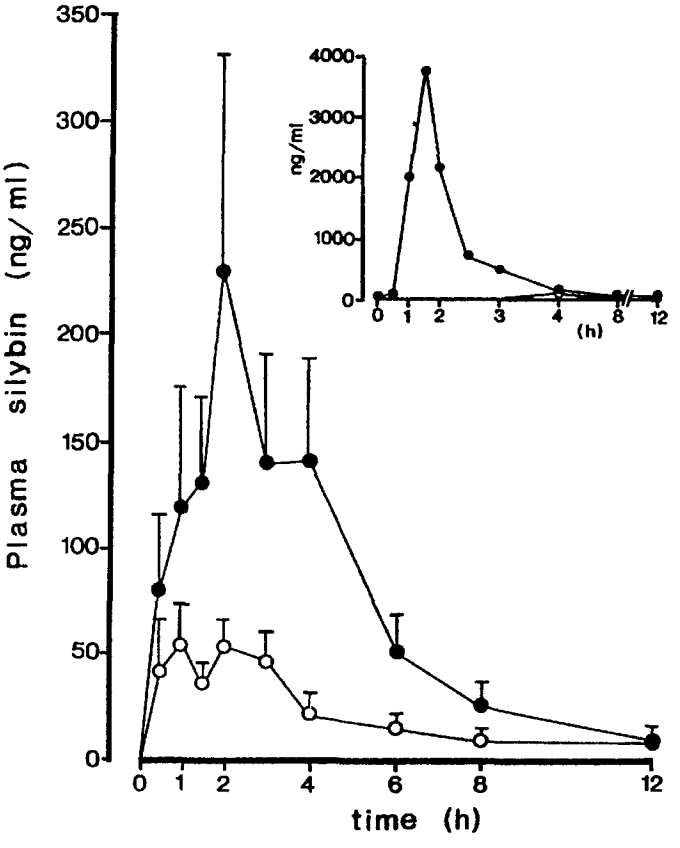

Fig. 1 : Plasma silybin levels (means ± SEM) in 8 subjects following single oral doses of IdB 1016 (●) and silymarin (O), each equivalent to 360 mg of silybin. Inset : Individual profiles in an additional subject, who showed extremely high plasma silybin levels after IdB 1016 intake

Table II : Pharmacokinetic parameters (means ± SEM) derived from plasma silybin concentration curves on day 1 and day 8 of an 8-day treatment with IdB 1016 (120 mg b.i.d., expressed as silybin equivalents) in 9 subjects. Rate constants of the elimination phase and half-life could be calculated in only 6 subjects

	Day 1	*Day 8*
Peak concentration (C_{max}), ng/ml	240 ± 54	183 ± 50
Time of peak (T_{max}), h	1.9 ± 0.4	0.9 ± 0.1*
Rate constant of the elimination phase (λ), h^{-1}	0.45 ± 0.13	0.34 ± 0.09
Terminal half-life, h	2.6 ± 1.0	3.4 ± 1.2
Mean residence time (MRT), h	3.9 ± 0.5	3.7 ± 0.4
Area under curve (AUC_{0-12h}), ng/ml.h	691 ± 106	564 ± 108
Percentage of dose recovered in urine as free + conjugated silybin	2.8 ± 0.6	2.7 ± 0.6

* $P < 0.05$

Pharmacokinetic and statistical analysis

Peak concentrations (C_{max}) and peak times (T_{max}) were derived directly from the experimental points. The rate constants of the terminal phase (λ) and terminal half-lives ($t_{1/2}$) were calculated by linear regression from the log concentration-time curves. Areas under the curve (AUC) and areas under the first moment curve (AUMC) were determined by the trapezoidal rule without extrapolation to infinity. Mean residence times (MRT) were calculated as AUMC/AUC.

Calculated values reported in the text are means ± SEM. Statistical comparisons were made by using the Student's t-test for paired data.

RESULTS

Single-dose study

The time course of plasma silybin levels (means ± SEM) following administration of equimolar doses (360 mg, in terms of silybin equivalents) of IdB 1016 and silymarin in 8 subjects are shown in Figure 1. The curves for the ninth subject are shown separately in the inset of Figure 1, since the extremely high plasma drug levels achieved in this case after intake of IdB 1016 were clearly incompatible with a normal distribution of data. Pharmacokinetic parameters derived from these curves are reported in Table I.

Although gastrointestinal absorption was relatively rapid in the two study occasions, there was a striking difference between the two formulations in that plasma levels were invariably very much higher after intake of IdB 1016. Based on the comparison of AUC values, the bioavailability of IdB 1016 was on average 4.6 times as high as that of silymarin, without considering subject 9 in whom the bioavailability difference in favour of IdB 1016 was much higher (Fig. 1 inset).

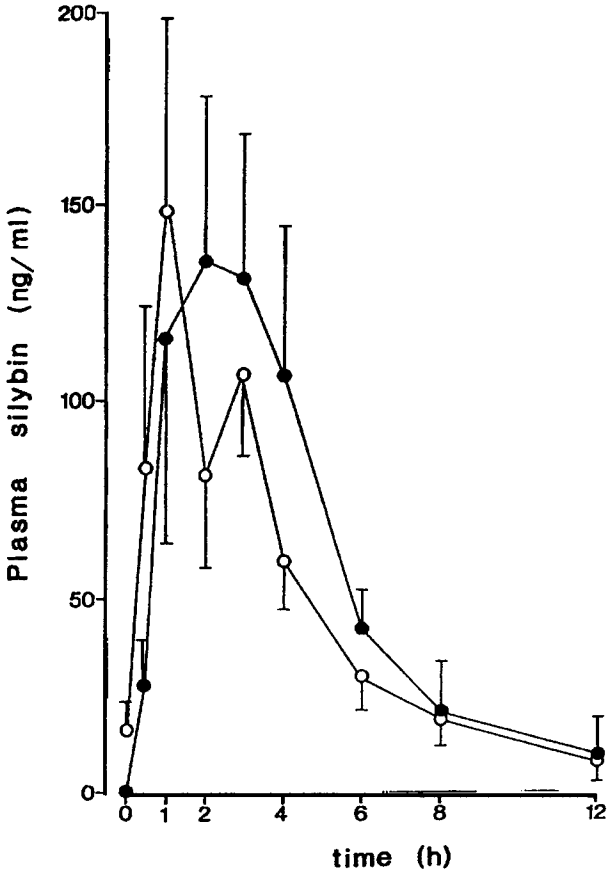

Fig. 2 : Plasma silybin levels (means ± SEM) in 9 subjects on the first (●) and the last (O) day of treatment with IdB 1016, 120 mg (expressed as silybin) b.i.d. for 8 consecutive days

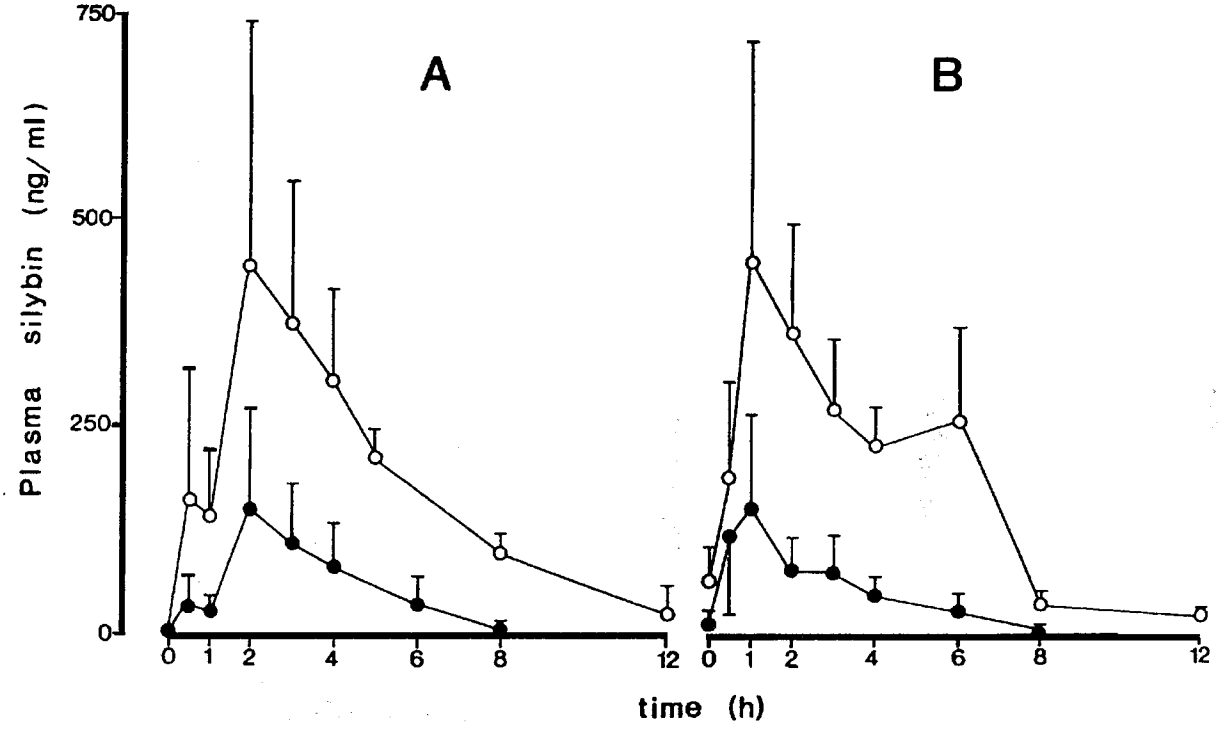

Fig. 3 : Plasma levels (means ± SEM) of unconjugated (●) and total (O) silybin in 3 subjects on the first (**A**) and the last (**B**) day of treatment with IdB 1016, 120 mg (expressed as silybin) b.i.d. for 8 consecutive days

Irrespective of the preparation used, secondary peaks were frequently observed within the first 6 h, with a relatively rapid decline of plasma silybin levels thereafter. Mean residence times ranged between 1.8 and 5.5 h after IdB 1016 and between 1.7 and 5.0 h after silymarin. Due to the limited number of samples and to frequent irregularities occurring during the elimination phase, terminal half-lives could be calculated only in 4 subjects after IdB 1016 (with values of 0.6, 1.3, 2.2 and 3.2 h respectively) and in 2 subjects after silymarin (with values of 2.0 and 4.4 respectively).

Multiple dose study

The plasma level profiles of silybin (means ± SEM) after the first dose of IdB 1016 (day 1) and during the dosing interval on the 8th day of treatment (120 mg b.i.d.) in 9 subjects are shown in Figure 2. A summary of pharmacokinetic parameters on the two occasions are shown in Table II.

As observed in the previously described single-dose study, the drug was absorbed rapidly, peak plasma levels being already observed within 0.5 to 4 h on day 1 and within 1 h on day 8. Apart from a slightly more rapid absorption on day 8, the curves and the kinetic parameters obtained on the two occasions were virtually superimposable, indicating the lack of substantial changes in IdB 1016 pharmacokinetics during multiple dosing. Plasma silybin levels measured 2 h after the morning dose on day 4 (124 ± 44 ng/ml) and day 7 (144 ± 69 ng/ml) were not significantly different from those observed at the same time on day 1 (135 ± 43 ng/ml) and on day 8 (81 ± 23 ng/ml), suggesting that steady-state conditions were already present after 4 days of treatment.

In three subjects, plasma silybin levels on day 1 and day 8 were re-assayed after enzymatic hydrolysis of the samples. The curves of free (without hydrolysis) and total (free + conjugated) plasma silybin in these subjects are shown in Figure 3. On average, total levels were approximately 4-fold higher than the free levels, indicating that most of the silybin present in the systemic circulation is in conjugated form. The proportion of conjugated drug tended to increase slightly during the sampling period.

The fraction of the administered dose recovered in urine as total (free + conjugated) silybin during the dosing interval on days 1 and 8 averaged 2.8 ± 0.6% and 2.7 ± 0.6% respectively.

DISCUSSION

Despite the fact that silymarin is widely prescribed in clinical practice, little or no information has been available on the plasma level kinetics of its main component silybin in man. In the only study in which an attempt was made to measure silybin in plasma, the samples were hydrolyzed before the TLC determination and therefore the values reported do not represent the concentration of unchanged drug (7). Characterization of unchanged silybin kinetics in our study was made possible by the development of a highly sensitive and specific HPLC method, which allowed the detection of the low levels found in plasma after therapeutic doses of silymarin.

After intake of single silymarin doses (equivalent to 360 mg silybin), silybin was found to be rapidly absorbed and to produce peak plasma levels ranging from 24 to 201 ng/ml. Although the interindividual variability in the levels was high, silybin could be measured in the plasma of all silymarin-treated subjects, a remarkable finding when it is considered that, by using the same assay and sampling schedule, no detectable levels of the drug were found after administration of equivalent doses of pure silybin (8). Presumably, the additional compounds which are present in silymarin (a multi-component natural extract) exert a favourable influence on the gastrointestinal absorption of silybin, even though the bioavailability of the latter is likely to remain low, as indicated by the marked increase in plasma drug levels which was observed when the IdB 1016 preparation was used.

IdB 1016 is a complex between silybin and soybean phosphatidylcholine. The rationale behind its development stems from the hypothesis that the lipophilic character of the complex, which facilitates diffusion across biological membranes in the gastrointestinal tract (9), could result in improved absorption. This mechanism is likely to be responsible for the remarkable increase in plasma silybin levels which was observed after administration of IdB 1016 both in animal experiments (3) and in our subjects in the present study. Alternative explanations, such as interference of the phospholipid with silybin metabolism, are very unlikely, since there is no evidence for an inhibitory effect of phosphatidylcholine (a normal dietary constituent) on drug biotransformation.

Characterization of pharmacokinetic parameters of Idb 1016-derived silybin indicated that absorption is rapid, that most of the drug present in the general circulation is in conjugated form, that elimination is relatively rapid and that no important changes in kinetic pattern occur after multiple dosing compared with single dose administration. In the only previous study on plasma silybin kinetics, Lorenz et al. (7) reported peak values of 340 ng/ml, a mean half-life of 6.3 h and a mean residence time of 4.9 h in 6 subjects given a silymarin dose containing 240 mg silybin. These data, however, are based on total (free + conjugated) plasma silybin levels determined by a TLC method and therefore cannot be meaningfully compared with the free (unconjugated) levels measured after intake of silymarin in our subjects.

Irrespective of the formulation used, silybin pharmacokinetic parameters showed a wide intersubject variability, especially as far as peak concentrations are concerned. Since silybin appears to have a high therapeutic ratio (10, 11), this variability is most unlikely to result in a toxicological hazard, a consideration underlined by the lack of any detectable side effects in an atypical subject who showed very high peak silybin levels after IdB 1016 intake. The high intersubject variability in peak concentrations, the relatively low plasma levels of unchanged drug coupled with its relatively rapid elimination half-life, the finding of considerable levels of conjugated drug in plasma at all sampling times, and the low urinary recovery are all consistent with a significant pre-systemic elimination of the compound. This interpretation is further supported by literature data indicating that silybin undergoes extensive biliary excretion (12, 13) and an appreciable entero-hepatic circulation (7), which could have accounted for the secondary peaks and curve irregularities seen in our subjects.

In conclusion, the present study demonstrates that, compared with silymarin, IdB 1016 ensures markedly higher silybin levels in plasma without significant differences in pharmacokinetic pattern between single and multiple dose administration. In animal models, the increased bioavailability of IdB 1016 resulted in marked potentiation of pharmacological activity after oral intake (4). Clinical studies are in progress to determine whether a similar effect would also ensure clinically important therapeutic advantages in man.

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