International Journal of Pharmaceutics, 94 (1993) 69–74

IJP 03066

Bioavailability study of tolbutamide β-cyclodextrin inclusion compounds, solid dispersions and bulk powder

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(Received 12 July 1992)
(Accepted 29 September 1992)

Key words: Tolbutamide; β-Cyclodextrin; Complexation; Solid dispersion; PEG 6000; Bioavailability; In vivo study

Summary

Tolbutamide PEG 6000 solid dispersions as well as tolbutamide β-cyclodextrin complexes were prepared with a view to increasing the bioavailability of this poorly soluble drug. Absolute and relative bioavailabilities were determined by comparison with the administration of a commercial solution of the drug. The study was carried out in rabbits ($n = 5$ per dosage form). The aqueous solution of tolbutamide (Dolipol®) was administered either intravenously (10 mg/kg) or orally (20 mg/kg). Bulk powder, comelt, coprecipitate and solid complex of tolbutamide were administered orally at a dose of 20 mg/kg. Plasma tolbutamide concentrations were measured by an HPLC method. Our results indicate that the absorption of tolbutamide is not increased in comparison with either bulk powder or a solution of the drug. However, there are obvious differences in the kinetics of absorption: indeed, tolbutamide is absorbed rapidly from the complex and the bulk powder. The process of absorption is much slower for the other dosage forms. Finally, even if the quantitative part of bioavailability is not modified, complexation with cyclodextrins could be interesting in order to increase the kinetic process of absorption of poorly soluble drugs.

Introduction

Solid dispersions of drugs in water-soluble carriers such as urea, PEG 6000, PEG 4000, PEG 20 000, dextrose and mannitol (alone or combined in various proportions) have been studied and reported with a view to increasing the dissolution rate, often supposed to be the rate-limiting step for absorption, and hence bioavailability (Miralles et al., 1982; McGinity et al., 1984; Duchene et al., 1985; Alonso et al., 1988). On the other hand, complexation with cyclodextrins has been extensively applied to improve the solubility, stability and bioavailability of various drugs (Frijlink et al., 1990; Abdel Rahman et al., 1991; Otero-Espinar et al., 1991; Puglisi et al., 1991). Using tolbutamide, a sulfonyl urea compound employed as hypoglycemic agent, we described in a previous paper the preparation of an inclusion complex between β-cyclodextrin and this drug (Kedzierewicz et al., 1990). In the same paper we compared

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the physico-chemical characteristics as well as the dissolution profiles of this complex and fresh solid dispersions prepared by either the comelt or the coprecipitate method. We showed that the increase in dissolution rate was more significant with β-cyclodextrin than when PEG 6000 was used as carrier. After 20 min, all the drug present in the β-cyclodextrin complex was dissolved. Furthermore, we observed that the solubility of tolbutamide was, at this time, 5.5-fold greater for β-cyclodextrin complex, whereas the increase was only 2-fold for the solid dispersion. Since there is still a majority of published work dealing with in vitro studies, the objective of the present work was to extend our earlier study, i.e., to verify if the results obtained in vitro could be correlated with in vivo results. Therefore, we measured the bioavailability parameters of the tolbutamide complex and solid dispersions as well as tolbutamide presented in powder and in aqueous solution by comparison to the intravenous administration of tolbutamide in solution.

Materials and Methods

Materials

Tolbutamide powder (< 115 mesh) (m.p. 127°C) (Sigma, St. Louis, MO), polyethylene glycol 6000 (m.p. 55–62°C) (Merck, Hohenbrunn, Germany) and β-cyclodextrin (Aldrich, Steinheim, Germany) were used as received. All other reagents and solvents were of analytical grade. Dolipol®, an aqueous solution of 5% tolbutamide (P/V), kindly provided by Hœchst (Neuilly, France), was used as the reference dosage form for oral and intravenous administrations. Heparin (Heparin Choay®) was kindly supplied by Choay (Paris, France).

Solid dispersions and complex preparations

Comelts, coprecipitates and solid complexes of tolbutamide were prepared according to previously described methods (McGinity et al., 1984; Alonso et al., 1988; Kedzierewicz et al., 1990). Briefly, in the case of the tolbutamide comelt, tolbutamide was dissolved in molten PEG 6000 in a ratio of 1:2, respectively. The dispersion was flash-cooled by immersion in a bath of dry ice plus acetone. For the tolbutamide coprecipitate, tolbutamide and PEG 6000 (1:2, w/w) were dissolved in chloroform and evaporation was carried out in vacuo at 25°C. As for the solid complex it was prepared by gently stirring, at room temperature for 2 weeks, tolbutamide (1 g) and β-cyclodextrin (15.89 g) in 1000 ml of distilled water. The complex, which precipitated as a white powder, was then filtered. The three dosage forms (comelt, coprecipitate and solid complex), were dried at room temperature in vacuo and the 28–48 mesh fraction was retained for in vivo administration.

Animals

Male rabbits (New Zealand breed) with an average weight of 2.75 ± 0.43 kg were used. They were kept on a standard diet and made to fast for 24 h prior to experiments. At least 7 days were allowed to elapse between the administrations.

In vivo administration

Intravenous administration After being treated with heparin (5000 IU/kg), rabbits ($n = 5$) were rapidly injected (bolus) via the marginal ear vein with an aqueous solution of tolbutamide (Dolipol®) at a dose of 10 mg/kg.

Oral administration The oral dose for tolbutamide was fixed at 20 mg/kg, this being based on preliminary results. The three prepared dosage forms as well as the pure powder (used as reference) were previously filled in hard capsules (size 1) in order to facilitate the administration. The aqueous solution was given by intragastric tubing. Five rabbits received each dosage form.

Sampling

After intravenous administration, blood samples (1 ml) were collected over a period of 10 h from each rabbit from the marginal ear vein. In the case of oral administration, blood was sampled from 0 to 24 h. After centrifugation, the plasma was kept at −20°C until analysis.

Determination of plasma tolbutamide concentrations

Plasma tolbutamide concentrations were measured by an HPLC method. This method was

specific and sensitive enough for kinetic studies (Nation et al., 1978). Briefly 200 μl of plasma experimental samples were added to 500 μl of acetonitrile. After vortex-mixing for a few seconds, the organic phase was separated by centrifugation. 20 μl of this solution were then injected into the chromatograph for analysis. A calibration line was prepared by adding blank rabbit plasma with known concentrations of tolbutamide. This was achieved by placing aliquots of a methanolic solution of tolbutamide in tubes. The methanol was allowed to evaporate before adding the 200 μl of rabbit plasma. The HPLC system consisted of a Spectra Physics liquid chromatograph (SP 8700 solvent delivery system, SP 8750 organizer, SP 8400 detector, SP 4270 recorder-integrator) fitted with Merck® column (LiChrospher RP 18, end-capped 10 μm, 250 mm long, 4.6 mm i.d.). A 20 μl sample loop was used to inject the sample into the analytical column. The samples were chromatographed with a mobile phase containing acetonitrile (45%) and 0.05% phosphoric acid solution (55%). After stirring, this mobile phase was filtered on a Millipore membrane (0.47 μm). The flow rate was 1.5 ml/min, the detector wavelength was set at 226 nm, the sensitivity was 0.08 AUFS and the chart speed was 0.5 cm/min. All experiments were performed at room temperature.

Pharmacokinetic calculations and statistical analysis

Compartmental analysis of tolbutamide obtained after i.v. administration was performed on an IBM-AT microcomputer using the Siphar modeling program (Version 3.3, 1989, Simed, Creteil, France). The disposition of drug was fitted to a two-compartment pharmacokinetic model with elimination from the central compartment according to Eqn 1:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where α and β are first-order rate constants, and A and B denote concentration constants. The plasma concentration-time data of each rabbit were fitted to Eqn 1 using the reciprocal of the observation squared as the weighing function (based on Akaike's Information Criteria) (Yamaoka et al., 1978). Non-compartmental analysis was utilised to calculate clearance, Vdss, MRT (mean residence time) and MAT (mean absorption time).

Areas under the plasma-concentration time curves were calculated using the trapezoidal rule and extrapolated to infinity $(AUC)^{\infty}$. MRT was obtained from the ratio of the area under the first moment concentration time curve $(AUC)^{\infty}$. The clearance was calculated from dose/$(AUC)^{\infty}$. The absolute and relative bioavailability were calculated according to:

$$\text{Absolute bioavailability} = \frac{\text{AUC (oral)} \times \text{dose (i.v.)}}{\text{AUC (i.v.)} \times \text{dose (oral)}}$$

$$\text{Relative bioavailability} = \frac{\text{AUC (p)} \times \text{dose (s)}}{\text{AUC (s)} \times \text{dose (p)}}$$

where AUC is the total area under the curve after either oral or intravenous (i.v.) administration of the solution (s) or the different tolbutamide dosage forms (p) i.e. powder, comelt, coprecipitate and complex. The rate of absorption of tolbutamide after oral administration was estimated by the mean absorption time (MAT) based on differences in MRT after oral and intravenous administration (Gibaldi, 1984). All data were tested for statistical significance using Anova test. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

Fig. 1 shows the mean plasma levels of tolbutamide after the intravenous administration of the aqueous solution of tolbutamide (Dolipol®). Plasma concentrations decline biexponentially according to a two-compartment open model.

Mean pharmacokinetic parameters are summarized in Table 1. The global elimination process, as reflected by the clearance and the half-life of elimination after intravenous administration (Table 1), is very slow. The distribution process is very short ($T_{1/2\alpha}$ 7 min) and the distribution volumes are also rather low. The mean residence

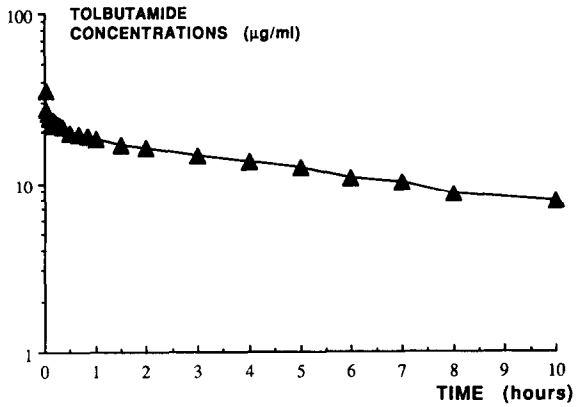

Fig. 1. Cronological evolution of mean plasma tolbutamide levels ($n = 5$) after intravenous administration of 10 mg tolbutamide/kg to rabbits.

time is considerable due to the very long elimination half-life. It is difficult to compare our pharmacokinetic results in rabbits with other studies since, to our knowledge, previous works with tolbutamide in rabbits were only concerned with blood glucose levels. However, when comparing our pharmacokinetic results with those obtained in rats (St Hilaire and Belanger, 1989) a common behaviour is evident: indeed, the total clearance is also slow but slower in rat (21.8 ml h^{-1} kg^{-1}) than in rabbit (48.8 ml h^{-1} kg^{-1}) and the elimination half-life is a little shorter in rat (5.1 h) than in rabbit (6.8 h). This result could be due to a relatively larger distribution in rabbit than in rat. The low plasma clearance in rabbits is consistent with extensive binding of tolbutamide to plasma proteins. Indeed, it is well known that, in animals, as in man, tolbutamide is highly and

TABLE 1

Disposition kinetic parameters of tolbutamide after intravenous administration (10 mg / kg)

Parameters	Non-compartmental analysis	Compartmental analysis
Cl_T (ml h^{-1} kg^{-1})	48.8	
V_C (ml kg^{-1})		284.5
Vd_{ss} (ml kg^{-1})	493	
$T_{1/2}$ (α) (min)		6.7
$T_{1/2}$ (β) (h)		7.1
MRT (h)	10.3	

strongly bound to plasma proteins (Tillement et al., 1984) which may explain the very long elimination half-life and the slow clearance.

The mean plasma levels produced by the five different tolbutamide dosage forms after administration of equivalent doses (20 mg/kg) are shown in Fig. 2. Tolbutamide concentrations are the lowest after the administration of the comelt and the solution. The maximal concentration (15.4 μg/ml) obtained with the comelt solid dispersion at $t = 8$ h is very close to that of the solution (14.6 μg/ml), but the T_{max} is shorter with the solution (7 h). The tolbutamide coprecipitate displays behaviour intermediate between the two latter dosage forms and both the complex and the powder. The coprecipitate solid dispersion attains a maximum plasma level of 20.9 μg/ml with a T_{max} identical to that of the solution. With the tolbutamide powder, the T_{max} was also delayed from 1 h, but the C_{max} was the highest (26.9 μg/ml). The tolbutamide-β-cyclodextrin complex leads to a high C_{max} (25.1 μg/ml) and the shortest T_{max} (5 h).

TABLE 2

Bioavailability parameters after oral administration of a dose of 20 mg / kg in the case of solution, powder, comelt, coprecipitate and solid complex of tolbutamide

Parameters	Solution	Powder	Comelt	Coprecipitate	Complex
C_{max} (μg ml^{-1})	14.6 ± 1.4	26.9 ± 1.4	15.4 ± 3.0	20.9 ± 2.3	25.1 ± 4.2
T_{max} (h)	7 ± 0.7	8 ± 0.2	8 ± 1.1	7 ± 1.1	5 ± 0.6
Absolute bioavailability (%)	89 ± 6	110 ± 13	72 ± 8	103 ± 6	92 ± 13
Relative bioavailability (%)	100	121 ± 19	80 ± 11	113 ± 3	102 ± 19
$T_{1/2}$ (β) (h)	15.9 ± 1.7	7.8 ± 0.9	11.0 ± 1.5	10.4 ± 1.7	7.7 ± 0.8
MRT (h)	20.8 ± 3.0	12.7 ± 0.6	16.3 ± 2.0	16.4 ± 1.7	12.5 ± 0.9
MAT (h)	10.5	2.4	6.0	6.1	2.2

Mean values ± S.E. ($n = 5$).

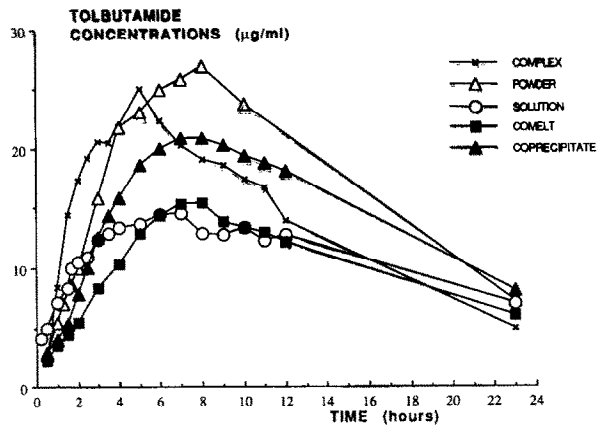

Fig. 2. Cronological evolution of mean plasma tolbutamide levels after oral administration of complex, solid dispersions and powder at a dose of 20 mg tolbutamide/kg to rabbits, $n = 5$ for each dosage form.

The bioavailability data are presented in Table 2. The results of absolute and relative bioavailabilities show that bioavailabilities are total for each dosage form except the comelt and the solution. However, the absolute and relative bioavailability results, obtained with the powder and the coprecipitate, were only statistically different from the comelt solid dispersions.

The absorption rate of tolbutamide, as reflected by peak time values and MAT (Table 2), is the fastest with the tolbutamide complex. During the apparent elimination process, the profile of tolbutamide concentrations obtained with the powder is practically similar to that of the complex. For the three other dosage forms, absorption is much slower: there is not really a peak but rather a plateau (absorption rate = elimination rate) between 240 and 720 min when the elimination process gets predominant. At the exception of the comelt, the absolute figures of bioavailabilities do not display major differences as reflected by the statistical analysis. Differences are only evident in the kinetic part of bioavailability. Indeed, it appears that the complex gives the earlier T_{max} with the second largest C_{max} (Table 2). This could be related to the in vitro results where we showed that the complex dosage form had the highest dissolution rate (Kedzierewicz et al., 1990). As already mentioned there is a plateau around C_{max} in the case of all dosage forms, at the exception of the complex and the powder. In the case of the complex there is immediately after C_{max} a fall in the tolbutamide concentrations. This phenomenon, although less intensive, is also evident with the powder. This is confirmed by the half-lives of eliminations. The mean half-life in the case of the complex and the powder is similar to that obtained after intravenous administration: the global elimination process is about the same as that after intravenous administration. This means that after the peak, the absorption process is already over and plasmatic concentrations display only the apparent elimination of tolbutamide. For the other three dosage forms (coprecipitate, comelt, aqueous solution) the half-lives range between 10.3 and 15.9 h. This emphasizes that absorption occurs during a longer period of time. Therefore, according to this latter hypothesis and considering the total bioavailabilities, tolbutamide would be adsorbed in the first part of the gastrointestinal tract with the powder and the complex dosage form. In the case of the complex dosage form, the previous hypothesis would also mean that tolbutamide available for absorption is released very quickly (which would give the shortest T_{max}).

One possible hypothesis to explain the interesting results obtained with the powder consists in the granulometry difference of the various dosage forms. Indeed, the complex and solid dispersion dosage forms have a similar granulometry (28–48 mesh), but tolbutamide powder was used as received, i.e., with a granulometry inferior to 115 mesh. It is also well known that hydrophobic interactions (due to considerable size reduction) could involve agglomeration and therefore a decrease in bioavailability. However, it seems that, due to the smaller powder size, the larger contact area between the gastrointestinal tract and the powder could counterbalance the influence of the three dosage forms (solid dispersions, complex) on the kinetic part of the absorption process. However, the absolute as well as the relative bioavailabilities are not statistically different between the powder, the coprecipitate and the complex.

It is surprising that the solution and the comelt present lower bioavailabilities. In general aque-

ous solutions of drugs are used as references for oral absorption and present the highest bioavailability due to the presentation of the drug under its molecular species. In our case the aqueous reference solution was the marketed dosage form Dolipol®. The lower bioavailability of the tolbutamide administered as a solution can be explained by its poor solubility at intestinal pH, leading to tolbutamide precipitation. Indeed, Dolipol® is presented as a solution at pH 9.8 which begins to precipitate at pH 7.8 (determined in vitro). This is why, in order to achieve dissolution of the drug, the pH of the solution is maintained between 9 and 10 in the marketed product. Therefore, when the aqueous tolbutamide solution is in the stomach (pH 1–3) there occurs precipitation of the drug that slows down the kinetic part of bioavailability and decreases the overall absorbed amount of tolbutamide.

Conclusion

We prepared three dosage forms known to increase the bioavailabilities of drugs (Uekama et al., 1979; Sekikawa et al., 1983; Puglisi et al., 1991), namely complex with β-cyclodextrin and solid dispersions prepared by either the comelt or the coprecipitate method. Our results indicate that the absorption of tolbutamide is not increased in comparison with either bulk powder or a solution of the drug. However, there are obvious differences in the kinetics of absorption: indeed, tolbutamide is absorbed rapidly from the complex and the bulk powder when the process of absorption is much slower for the other dosage forms. Finally, even if the quantitative part of bioavailability is not modified, complexation with cyclodextrins could be interesting to increase the kinetic process of absorption of poorly soluble drugs.

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