Oral bioavailability and hypoglycaemic activity of tolbutamide/cyclodextrin inclusion complexes

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Abstract

The purpose of the present study was to evaluate the enhancement of tolbutamide (TBM) oral bioavailability and hypoglycaemic activity through complexation with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD). TBM and its freeze-dried inclusion complexes were administered to rabbits (New zealand breed; $n = 6$), in a dose of 20 mg/kg. TMB plasma levels were measured by HPLC and glucose levels were analysed according to Trinder (Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6, 24–28). The pure drug attained a maximum of plasma concentration (C_{max}) of 18.58 ± 3.27 μg/ml at 8.5 h (T_{max}), whereas with inclusion complexes, C_{max} increased about two times and appeared at ca. 4 h. AUC_{0-24} of complexes was about 1.6 times as much as that of the pure drug. Thus, the extent of oral absorption of TBM from inclusion complexes was significantly greater and faster when compared with drug alone. In addition, without cyclodextrins the maximum hypoglycaemic effect (CVG_{max}) of TBM (34.1%) was observed at 5.6 h (Tg_{max}). CVG_{max} of TBM/β-CD and TBM/HP-β-CD inclusion complexes were 34.1% (at 6.5 h) and 37.7% (at 5.1 h), respectively. AAC_{0-24} of inclusion complexes was 1.4 times larger than that of pure drug. Hence, the oral administration of complexed TBM not only improved the drug absorption, but also the TBM hypoglycaemic activity. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cyclodextrins; Tolbutamide; Inclusion complexes; Bioavailability; Hypoglycaemic activity

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, containing six (α-CD), seven (β-CD) or eight (γ-CD) α-1,4-linked D-glucose units, with a hydrophilic outer surface and a hydrophobic cavity, in which may be included a great variety of 'guest' molecules of suitable size and shape, resulting in a stable association without formation of covalent bonds (Szejtli, 1990). In the pharmaceutical field this phenomenon has been extensively applied to enhance the solubility, dissolution rate and bioavailability of slightly soluble drugs in gas-

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trointestinal fluids (Vila-Jato et al., 1988; Soliman et al., 1997; Ventura et al., 1997; Dhanaraju et al., 1998; Özdemir and Ordu, 1998; Mura et al., 1999).

Among the cyclodextrins, β-CD is the most useful compound for drug complexation. However, the relatively low aqueous solubility of β-CD (about 1.8% w/v, at 25°C) suggested the use of chemically-modified CDs with different physical properties and inclusion behaviour. In particular, hydroxypropyl-β-cyclodextrin (HP-β-CD) is widely used, because of its amorphous nature, high water solubility and solubilising power and low toxicity (Pitha and Pitha, 1985; Brewster, 1991).

Tolbutamide (TBM) is used as an oral hypoglycaemic agent. The drug is practically insoluble in water and its dissolution is supposed to be the rate-limiting step for its absorption from the gastrointestinal tract (Miralles et al., 1982). Several studies on commercial brands of tolbutamide dosage forms have shown marked variations in dissolution rate and bioavailability (Carminetsky, 1963; Varley, 1968; Ayres et al., 1984).

We previously reported (Veiga et al., 1996) that the solubility and dissolution rate of TBM is markedly increased due to complexation with β-CD and HP-β-CD. These preliminary studies suggested that the TBM oral bioavailability could be improved through complexation with these CDs.

The objective of the present study was to evaluate the influence of the complexation on the bioavailability and hypoglycaemic activity of tolbutamide after oral administration to rabbits of TBM/β-CD and TBM/HP-β-CD complexes when compared with pure drug.

2. Material and methods

2.1. Materials

Tolbutamide (Sigma), β-CD (Roquette Frères) and HP-β-CD with a molar substitution of 0.39 (Janssen) were used as received. All other reagents and solvents were of analytical grade.

2.2. Inclusion complex preparation

The inclusion complexes of TBM/β-CD (1:2) and TBM/HP-β-CD (1:1) were prepared by the freeze-drying method, previously described by Veiga et al. (1996). Stoichiometric quantities of TBM and CDs were dissolved in distilled water with a small amount of ammonia 25% to aid the active ingredient to dissolve. The solution was frozen by immersion in a shell freezer and freeze-dried over 24 h in a Lyph-lock 6 apparatus (Labconco). No trace of ammonium was detected in the resulting mixture with Nessler reagent.

2.3. In vivo studies

Male rabbits (New zealand breed) were used with an average weight of 3.5 kg. They were kept on a standard diet (702 GR, Rico Gado) and made to fast for 12 h prior to experiments. At least 15 days were allowed to elapse between the administrations.

The three formulations (commercial tolbutamide, TBM/β-CD and TBM/HP-β-CD inclusion complexes) with a dose of 20 mg/kg of tolbutamide or equivalent were previously suspended in 30 ml of water. The aqueous suspensions were given by intragastric tubing. The administration scheme is given in Table 1.

Table 1
Administration scheme of TBM alone, TBM/β-CD and TBM/HP-β-CD inclusion complexes

Animals	1st Set	2nd Set	3rd Set	4th Set
Group I	Control	TBM	TBM/β-CD	TBM/HP-β-CD
Group II	TBM	TBM/β-CD	TBM/HP-β-CD	Control
Group III	TBM/β-CD	TBM/HP-β-CD	Control	TBM

The blood samples (1 ml) were collected over a period of 24 h from the marginal ear vein. EDTA was added to the blood samples to prevent clotting and subsequently, plasma was obtained by centrifugation at 2800 rev./min for 15 min. The plasma samples were stored at $-30°C$ until analysis.

2.4. Determination of TBM plasma levels

The plasma levels of TBM were measured by high-performance liquid chromatography (HPLC).

2.4.1. HPLC conditions

HPLC equipment consisted of a Gilson 305 pump, Rheodyne injector (with a 20-μl loop), Gilson 117 UV spectrometric detector, Kipp and Zonen register and a Li-Chrospher RP 18 reversed-phase column (10 μm particle size, 250 × 4.6 mm i.d., Jones). A guard column (Jones), similarly packed, was used before the analytical column. The mobile phase was prepared by degassing and filtering (through 0.45-μm membranes, Millipore) a mixture of acetonitrile and 0.05% phosphoric acid (45:55). The chromatograph was operated at a flow rate of 1.5 ml/min and the eluent was monitored spectrophotometrically at the UV maximum of TBM (226 nm). The sample injection size was 20 μl. All the determinations were performed at room temperature ($20 \pm 2°C$).

2.4.2. HPLC method validation

Standard curves for TBM were developed in the mobile phase and in the rabbit plasma, for the TBM range of 1–60 μg/ml. For construction of standard curves in plasma, different volumes (5–300 μl) of a 100 μg/ml TBM methanolic solution were used. After methanol evaporation, 200 μl of rabbit plasma and 500 μl of acetonitrile were added. The mixtures were vortexed for 10 s and subsequently centrifuged at 2500 rev./min for 1 min.

TBM recovery from plasma was determined with two concentrations ($n = 3$) by comparing the peak areas obtained for TBM plasma samples and those of TBM phase mobile solutions. Recoveries of TBM from plasma samples with 20 and 40 μg/ml concentrations were 98.7 and 99.1%, respectively.

Validation of HPLC method was evaluated by linearity, precision (repeatability and intermediate precision), accuracy and limit of detection. The calibration curve of TBM was linear over the concentration range of 1–60 μg/ml ($r > 0.999$). The repeatability was evaluated by six replicate determinations of solutions with two different concentrations of TBM. The RSD obtained for 20 and 40 μg/ml TBM solutions were 1.9 and 1.63%, respectively. The intermediate precision was determined by analysing these solutions in five consecutive days. The RSD were 2.86% (20 μg/ml) and 2.27% (40 μg/ml). The accuracy, expressed as recovery percentage of TBM from plasma, was assessed by spiking plasma samples with TBM in 20 μg/ml concentration. The mean recovery for six replicate plasma samples was compared with the absolute recovery (100%) and the statistical analysis, performed with Student's t-test, demonstrated that this difference was statistically not significant ($P < 0.05$). The detection limit for the assay was estimated at 1 μg/ml.

2.5. Determination of plasma glucose

The glucose levels were analysed according to Trinder (1969) using the glucose enzymatic reagent system (glucose oxidase/peroxidase method), in an auto-analyser (Pronto BPC Biosed).

Plasma glucose levels (Glyc) were transformed into the plasma glucose variation coefficient (GVC) according to the expression:

$$GVC = \frac{Glyc. \ at \ t - Glyc. \ at \ t = 0}{Glyc. \ at \ t = 0} \times 100$$

2.6. Pharmacokinetics and pharmacodynamics

The pharmacokinetic and pharmacodynamic parameters were calculated using a statistical and analytical computer programme (PKCALC, Software Arts).

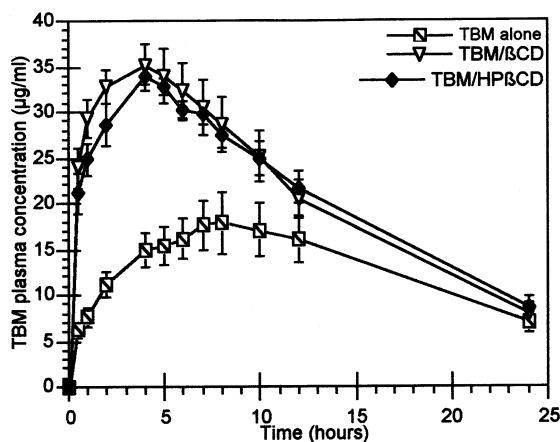The areas under TBM plasma concentration–time curves (AUC_{0-24} and $AUC_{0-\infty}$), calculated

Fig. 1. TBM plasma concentration–time profiles after administration of TBM, TBM/β-CD and TBM/HP-β-CD inclusion complexes (equivalent to TBM 20 mg/kg) to rabbits ($n = 6$). Each point represents the mean $\pm$ S.D.

by the linear trapezoidal method, the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (T_{max}) and TBM elimination half-life ($t_{1/2}$) were chosen as parameters for pharmacokinetic evaluation. The relative bioavailability between the inclusion complexes and commercial drug was calculated from the ratio of their AUC values.

The area above the curve of glucose variation coefficient (GVC) versus time (AAC_{0-24}), calculated by linear trapezoidal rule, the percent maximum of GVC depression (GVC_{max}) and the time to reach GVC_{max} (Tg_{max}) were selected as parameters for pharmacodynamic evaluation.

Statistical analysis of results was performed using one-way analysis of variance (ANOVA) and the least-significant difference test (LSD) at 95% of confidence ($P < 0.05$).

3. Results and discussion

Plasma concentration–time profiles for TBM after oral administration of drug alone to rabbits, TBM/β-CD and TBM/HP-β-CD inclusion complexes (equivalent dose of TBM 20 mg/kg) are illustrated in Fig. 1. The pharmacokinetic parameters derived from the plasma data are presented in Table 2. The plasma levels of TBM after administration of inclusion complexes were clearly faster and higher than those achieved with an equal TBM dose given alone. In particular, the C_{max} after administration of TBM alone was observed at 8.5 h; on the other hand, TBM complexes resulted in the rapid appearance of TBM in plasma, attaining the C_{max} after ca. 4 h. In addition, the value of C_{max} for the complexes (36.26 ± 2.17 and 34.99 ± 1.36 µg/ml to β-CD and HP-β-CD complexes, respectively) was about two times greater that of TMB alone (18.58 ± 3.27 µg/ml). The differences in the mean of C_{max} and T_{max} between the TBM and the complexes were statistically significant ($P < 0.05$), but no significant differences in these plasma parameters were observed between the two types of complexes. A similar trend was observed with the $t_{1/2}$, in which there was a significant difference ($P < 0.05$) only between TBM alone and the inclusion complexes. The high $t_{1/2}$ values can be attributed to the strong and extensive binding of TBM to plasma proteins, in animals and humans (about 96%) (Tillement, 1983).

The AUC_{0-24} of complexes was found to be about 1.6-fold greater than the TBM alone. Similarly, $AUC_{0-\infty}$ for the complexes was 1.4 times as much as that from reference TBM. The statistical

Table 2
Pharmacokinetic parameters and relative bioavailability of TBM after oral administration of TBM, TBM/β-CD and TBM/HP-β-CD inclusion complexes (equivalent to TBM 20 mg/kg) to rabbits[a]

System	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-24} (µg h/ml)	$AUC_{0-\infty}$ (µg h/ml)	$T_{1/2}$ (h)	Rel. bioavailability (%)
TBM	18.58 ± 3.27	8.50 ± 1.15	312.95 ± 47.21	443.96 ± 63.33	12.48 ± 1.33	
β-CD complex	36.26 ± 2.17	3.83 ± 0.65	516.26 ± 45.05	621.62 ± 52.73	8.79 ± 0.47	165.00
HP-β-CD complex	34.99 ± 1.36	4.00 ± 0.45	510.76 ± 28.22	654.63 ± 62.42	10.22 ± 1.19	163.21

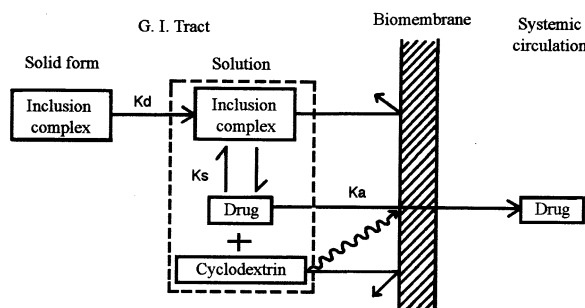[a] Each value represents the mean $\pm$ S.D. for six rabbits.

Fig. 2. Drug absorption mechanism involving cyclodextrin complexation, adapted from Uekama and Otagiri (1987).

study demonstrated that there were significant differences in these areas between TBM and the complexes ($P < 0.05$), while the differences of the two complexes were statistically insignificant. This enhancement in TBM AUCs can be attributed to the increase in TBM solubility and dissolution rate upon complexation with CDs. However, this improvement in TBM bioavailability was clearly independent of the cyclodextrin employed in complexation, although the solubility of TMB was 2.5-fold higher in HP-β-CD complexes than in the β-CD complexes, as previously reported (Veiga et al., 1996).

Since only the free form of the drug can pass through the lipid barrier of the gastrointestinal tract, after oral administration of cyclodextrin complex, the drug absorption is conditioned by the equilibrium between the free and complexed drug (Fromming, 1987; Uekama and Otagiri, 1987). Therefore, the effect of cyclodextrin complexation on drug absorption is largely dependent upon the magnitude of the stability constant (K_s) as well as the solubility and dissolution rate of the inclusion complex. This overall process can be described by the scheme shown in Fig. 2 proposed by Uekama and Otagiri (1987). In this scheme, the rate constant for the dissolution of the solid complex and stability constant of the complex in the fluids at the absorption side are represented by K_d and K_c, respectively; K_a is the rate constant for the passage of the free form of the drug across the biomembrane. Obviously, the free drug concentration that may be available for absorption is

greater when the complex has a smaller stability constant. The complex with a very small stability constant is diluted in the biological fluids after administration, which results in a quick dissociation of the complex with little or no effect on its overall absorption properties. On the contrary, if the complex is very stable or the cyclodextrin concentration is sufficiently high, the equilibrium is shifted greatly toward complexation and the absorption is considered hindered. In the present study, since the K_s values of complexes are relatively low and of the same magnitude (195.7 and 144.8 M^{-1} to TBM/β-CD and TBM/HP-β-CD complexes, respectively) (Veiga et al., 1996), it seems very unlikely that they have some negative influence in complex dissociation. Then, we can conclude that the solubility, dissolution rate and K_s magnitude of the complexes are not the only limiting factors of TMB absorption.

Simultaneously with the determination of TBM plasma levels, the corresponding hypoglycaemic effects were monitored up to 24 h. The hypoglycaemic response, expressed as glucose variation coefficient (GVC), and the pharmacodynamic parameters are presented in Fig. 3 and Table 3, respectively.
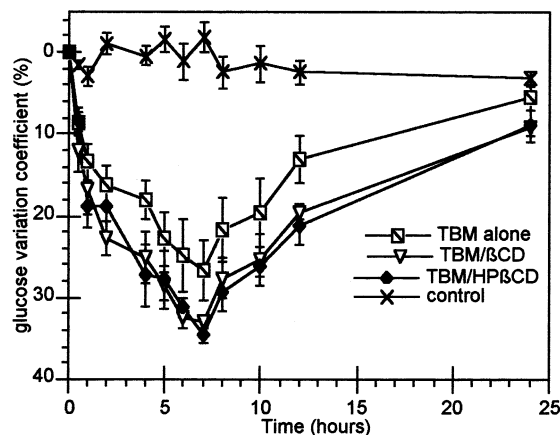

Fig. 3. Glucose variation coefficient–time profiles after administration of TBM, TBM/β-CD and TBM/HP-β-CD inclusion complexes (equivalent to TBM 20 mg/kg) to rabbits ($n = 6$). Each point represents the mean ± S.D.

The GVC profiles, like TBM plasma profiles, were practically coincident for β-CD and HP-β-CD inclusion complexes. In the absence of CDs, the TBM hypoglycaemic effect was observed with a maximum of 30.55% (CVG_{max}) at 5.7 h (Tg_{max}), after administration of TBM alone. Although the CVG_{max} was increased upon CD complexation (34.07 ± 1.01 and $37.67 \pm 1.48\%$ to β-CD and HP-β-CD inclusion complexes, respectively), only the enhancement caused by HP-β-CD was statistically significant. The analysis of variance of the areas above the glucose variation coefficient versus time (AAC_{0-24}) demonstrated that there was a significant difference between the AAC_{0-24} of TBM alone and the inclusion complexes. However, the AAC_{0-24} ratio of the hypoglycaemic effect between the complexes and TBM (about 1.4) was smaller than the corresponding ratio (AUC_{0-24}) of TMB plasma levels (about 1.6).

The mean values of Tg_{max} were greater for TBM/β-CD inclusion complexes than for pure drug and TBM/HP-β-CD, but statistical analysis showed that this difference was not significant. Since Tg_{max} values were similar in the three systems, the pharmacodynamic response was not dependent on TBM absorption rate.

The enhanced hypoglycaemic effect displayed by the improvement of TBM bioavailability revealed that there was a correlation between the TBM plasma levels and the hypoglycaemic response. This correlation is clearly evident by comparing Fig. 2 and Fig. 4, in which one seems to be the mirror image of the other. The complexation with cyclodextrins promoted an enhanced and faster TBM oral absorption, but only increased the hypoglycaemic effect, without any influence on its rate. The absence of proportional relation between TMB plasma concentration and pharmacodynamic effect was also observed by Balant (1981). For this reason, it is common that in TBM and other sulphonylurea studies, the pharmacodynamic effect is evaluated instead of the determination of drug plasma concentrations (Vila-Jato et al., 1987; Kedzierewicz et al., 1993; Torres-Labandeira et al., 1994; Babu and Pankit, 1995).

4. Conclusion

Reference TBM demonstrated its poor pharmacokinetic and bioavailability performance following oral administration. Administration of the TBM inclusion complexes was characterised by an oral absorption pattern that was faster and more efficient than that of TBM alone. A correlation between TBM plasma levels and hypoglycaemic effect was achieved. The pharmacodynamic evaluation clearly indicated that TBM hypoglycaemic activity was promoted when complexed with CDs, although without affecting the time for reaching the maximum effect.

The enhanced bioavailability and corresponding potentiation of hypoglycaemic activity by CD complexation of TBM suggests a dose decrease in oral sulphonylurea therapy with reduction of side effects. With the growing acceptance of CDs as excipients and the presence of CD in formulations rapidly approaching regulatory approval, these pharmacokinetic and pharmacodynamic studies might be beneficial in generating dosage forms not only for TBM but also for a variety of other drugs (Brewster et al., 1997).

Table 3
Pharmacodynamic parameters of TBM after oral administration of TBM, TBM/β-CD and TBM/HP-β-CD inclusion complexes (equivalent to TBM 20 mg/kg) to rabbits[a]

System	GVC_{max} (%)	Tg_{max} (h)	AAC_{0-24} (% h)
TBM	30.55 ± 2.94	5.67 ± 0.80	336.70 ± 54.16
β-CD complex	34.07 ± 1.01	6.50 ± 0.81	460.02 ± 20.10
HP-β-CD complex	37.67 ± 1.48	5.12 ± 0.48	471.03 ± 22.70

[a] Each value represents the mean $\pm$ S.D. for six rabbits.

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