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Effects of 2-hydroxypropyl-β-cyclodextrin on pharmacokinetics of digoxin in rabbits and humans

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Considering the narrow therapeutic index of digoxin and the low range between the safe and toxic serum concentrations of this drug, to evaluate the relative bioavailability of tablets and oral solution is necessary. The pharmacokinetic properties of digoxin after oral administration of its hydroxypropyl-β-cyclodextrin (HPCD) inclusion complex to rabbits and human volunteers were investigated in comparison with those of commercially available tablets. The aqueous solubility of digoxin was enhanced by HPCD for about 2000 times at HPCD concentration of 50% (w/v). But in a human bioavailability study no significant difference was observed in the extent of absorption (AUC_{0-t}) and C_{max} between the two formulations. Time to reach peak was significantly shorter for the solution than for the tablets ($p < 0.01$). The pharmacokinetic results from the rabbit study were similar to human studies and no significant difference was observed for AUC, C_{max} and T_{max}. As the bioavailability of both tablets and solution is equivalent HPCD based oral digoxin solution could serve as an alternative to tablets.

1. Introduction

Digoxin, a drug used for the treatment of congestive heart failure, exhibits a narrow therapeutic index and is usually administered orally (Lisalo 1977). Studies have indicated that the bioavailability of digoxin from commercial tablets varies significantly (Mooradian 1988). The main cause of this variability appears to be related to such factors as low water solubility and chemical instability in acidic media (Uekama et al. 1982). As digoxin has a narrow therapeutic window, it is essential to adjust the dose individually. Liquid formulations, as no dissolution process is involved, usually have a high and stable bioavailability. An elixir is the only liquid formulation of digoxin available in the market until nowadays and a dry elixir was formulated to improve the drug dissolution rate (Kim et al. 1995). But either elixir or dry elixir contain alcohol or surfactants. It was reported that the bioavailability of digoxin could be improved by forming inclusion complexes with γ-cyclodextrin (Uekama et al. 1983). β-Cyclodextrin, unfortunately, has low water solubility, and is difficult to incorporate in liquid formulations. Thus, attempts to improve cyclodextrins by chemical derivatization have been made and among these derivatives 2-hydroxypropyl-β-cyclodextrin (HPCD) was well evaluated for its safety and pharmacokinetics and could be largely produced in pharmaceutical industry (Zhao et al. 2001; Wu et al. 2002). The objective of this study was to study the bioequivalence or possible bioavailability improvement of a HPCD based oral digoxin solution compared to a commercially available tablet.

2. Investigations, results and discussion

2.1. Phase solubility study

It was already shown that β- and γ-cyclodextrin were able to form inclusion complexes with digoxin and that they were able to increase its aqueous solubility (Uekama et al. 1983). Fig. 1 shows that the aqueous solubility of digoxin increases as a function of the concentration of HPCD in the HPCD concentration range studied. Since the digoxin molecule is too large to be included within the β-cyclodextrin cavity, it is reasonable to assume that at least one complex with a stoichiometric host-to-guest mo-

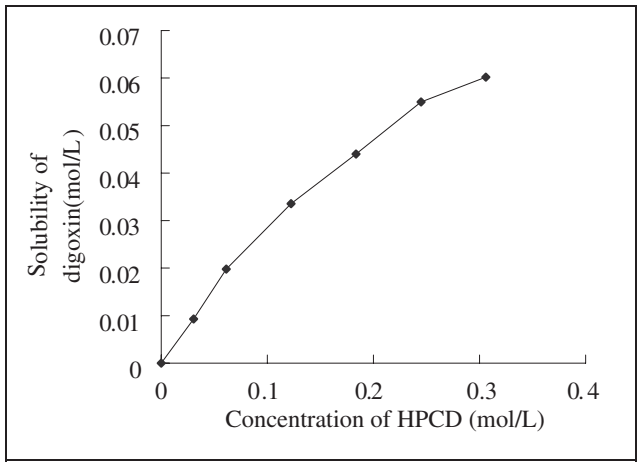

Fig. 1. Phase solubility of digoxin with HPCD in water at 25 °C

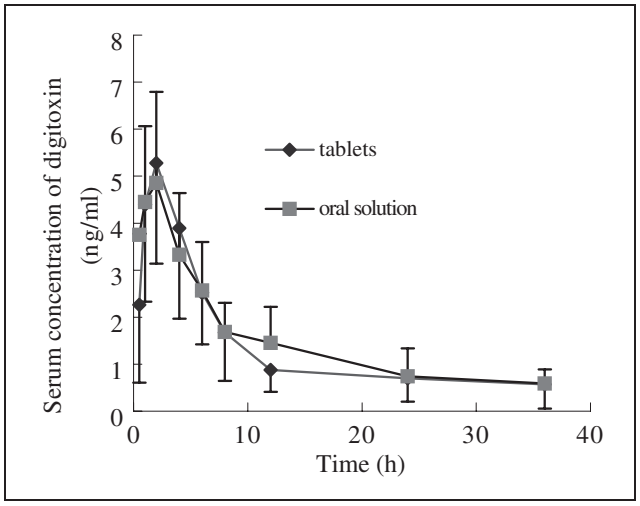

Fig. 2: Concentration time profile of digoxin after its oral administration as a tablet (0.25 mg) and solution (0.25 mg) to rabbits (n = 10, mean ± SD)

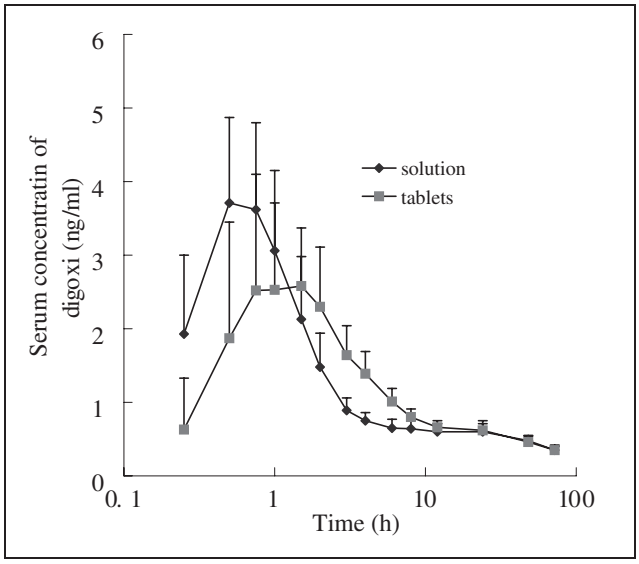

Fig. 3: Concentration time profile of digoxin after oral administration of 0.75 mg tablets and solution to humans (n = 20, mean ± SD)

lecular ratio greater than one may be formed, in particular for higher concentrations of cyclodextrin. Although the shape of solubility curve in Fig. 1 is of A_N type, and this kind of diagram cannot be completely explained in terms of a stoichiometric relationship, an apparent stability constant as a tentative measure of inclusion complexation, was estimated from the equation based on the assumption that a 1 : 1 complex is initially formed (Frömming et al. 1994). The apparent stability constant (Kc) was calculated according to the literature (Frömming et al. 1994) as 9372 M^{-1}. The apparent stability constants of digoxin with β- and γ-cyclodextrin were 11200 and 12200 M^{-1}, respectively (Uekama et al. 1983)

2.2. Bioavailability studies

Fig. 2 presents the serum concentration-time profile of digoxin after oral administration in rabbits and the pharmacokinetic parameters are shown in Table 1. No significant difference is noticed between C_{max}, T_{max} and $AUC_{0-36\,h}$ values when the digoxin was administered to rabbits either in HPCD inclusion solution or in commercially available tablets. Though the T_{max} for HPCD based solution is shorter than that for tablets and the peak concentration for the solution is higher than that of tablet, a significant difference was not observed for the big deviation among each rabbit.

Fig. 3 shows the serum concentration-time profile of digoxin after oral administration in human volunteers and the pharmacokinetic parameters are shown in Table 2. There were no significant differences of $AUC_{0-72\,h}$ values when the drug was administered either in tablets or in HPCD based oral solution. The mean T_{max} was shorter and the serum concentration peak value was higher in humans receiving solutions but only T_{max} differences were statistically significant ($p < 0.01$). This is reasonable because tablets need time to disintegrate and release drugs. From the apparent stability constants of digoxin with HPCD, β- and γ-cyclodextrin (9372, 11200 and 12200 M^{-1}, respectively), the inclusion ability of HPCD for digoxin is similar to that of β- and γ-cyclodextrin. But the digoxin-γ- cyclodextrin inclusion increased the bioavailability of digoxin for 3 times (Uekama et al. 1983) whereas a digoxin-HPCD complex solution could not increase bioavailability. There are serval reasons for this observation. First, dogs were used in the digoxin-γ-cyclodextrin inclusion bioavailability studies but rabbits and humans were used in our studies. Another possible reason is that the dissolution of digoxin tablets tested in earlier studies was not well determined according to USP and the Chinese Pharmacopoeia. The tablets in our experiment were tested according to both the USP and Chinese Pharmacopoeia standard for dissolution. It was reported that digoxin tablets with good dissolution properties and liquid filled capsules have the same absolute bioavailability of about 70% (Brandenburg 1993). So it is reasonable that HPCD complexed digoxin solution and tablets have the same bioavailability. Our results also show that digoxin bioavailability determined in rabbits corresponds to that in humans and can be used as secondary model for bioequivalence testing of digoxin formulations (Bansinath et al. 1986). Though the absorption extent for digoxin tablets and oral solution is equal, the solution has some advantage in biopharmaceutic characteristics as the drug in solution is absorbed more rapidly than from tablets and exerts actions more quickly. Other advantages for the digoxin oral solution are that it is easy to adjust the dose for individual patients and that it is easy for children and aged people to take the medicine.

Table 1: Pharmacokinetic parameters of digoxin after its oral administration of as tablet or HPCD based solution (0.25 mg) to rabbits (n = 10, mean ± SD)

Parameter	$AUC_{0-36\,h}$ (ng/ml · h)	T_{max} (h)	C_{max} (ng/ml)
Tablets	49.0 ± 17.5	2.5 ± 2.1	5.6 ± 2.0
Solution	54.0 ± 16.1[a]	1.6 ± 1.0[a]	6.0 ± 2.1[a]

[a] $p > 0.05$

Table 2: Pharmacokinetic parameters of digoxin after its oral administration as digoxin tablet or HPCD based solution (0.75 mg) to healthy volunteers (n = 20, mean ± SD)

Parameter	$AUC_{0-72\,h}$ (ng/ml · h)	T_{max} (h)	C_{max} (ng/ml)
Tablet	44.9 ± 6.0	1.4 ± 0.8	3.5 ± 1.0
Solution	42.0 ± 6.2[a]	0.6 ± 0.2[b]	4.0 ± 1.2[a]

[a] $p > 0.05$
[b] $p < 0.01$

3. Experimental

3.1. Phase solubility studies

The stability constants (K_c) for inclusion complex formation between digoxin and HPCD were determined using the phase solubility method (Okimoto et al. 1996). Digoxin with a content of 99.0% was obtained from Minsheng Pharmaceutical Company (Hangzhou, China). HPCD (with a degree of substitution of 4.5 and β-cyclodextrin content less than 1%) was supplied by Shijiazhuang Pharmaceutical company. All other chemicals and solvents used were of analytical reagent grade or pharmaceutical grade. An excess of digoxin was added to water. The concentration of HPCD varied from 0–0.05 mol/L. The suspensions were agitated at 25 °C for 7 days (equilibrium was confirmed in all cases in preliminary studies). After equilibration, the solutions were filtered through a 0.45 μm cellulose membrane filter. The filtrate was isolated and diluted with HPLC mobile phase and analyzed by HPLC method. The HPLC system included a Jasco PU-980 solvent pump, a Jasco UV-975 detector and Chrom King chromatographic integrator. HPLC was performed using a Spherisorb C_{18} column (250 × 3.9 mm); water-acetonitrile (70–30) as the mobile phase; flow rate 1.2 ml/min and detection wave length 220 nm. All assays were performed at room temperature.

3.2. Preparation of digoxin oral solution

HPCD 20 g was dissolved in 100 ml pH 7.0 phosphate buffer and then 50 mg digoxin was added and agitated to disslove digoxin. Phosphate buffer was added to make the total solution volume to 1000 ml.

3.3. Animal studies

Ten male rabbits weighing 3.5 ± 0.5 kg were acclimatized and conditioned in the core facility at Shenyang Pharmaceutical University's Department of Animals. Rabbits were fasted overnight and provided water *ad libitum* the night before the study. 5 ml of digoxin oral solution (0.25 mg) or 1 tablet of digoxin (0.25 mg) with 20 ml water was administered to rabbits at intervals of at least 1 week. The administration sequence was based on a crossover matrix designed to minimize any residual or cumulative effects of the preceding dose. Digoxin tablets (0.25 mg/tablet) were obtained from Minsheng Pharmaceutical Company (Hangzhou, China) and digoxin oral solution was prepared as mentioned above. Rabbit blood samples were obtained from the ear marginal vein at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 h post dose. Rabbits were sacrificed after the experiment by an overdose of oxyflurane. Serum was stored at −20 °C until analysis. The serum concentration of digoxin was determined by a fluorescence polarization immunoassay using TDx analyzer (Abbott, North Chicago, USA). The intra-and inter-assay variability were 2.65% and 1.93% at a concentration of 1.5 ng/ml. The linear range for digoxin serum concentration was 0.2–5.0 ng/ml. Higher concentrations than 5 ng/ml were diluted with blank rabbit serum.

3.4. Human study

Twenty healthy male volunteers were recruited from a panel at Fuwai Hospital. The volunteers were aged between 18 and 30 years. They were nonsmokers and had not taken any other medication within 30 days before or during the study period. The study was approved by the State Drug Administration of China and Ethics Committee of Fuwai Hospital. After fasting overnight each volunteer randomly received a 0.75 mg dose of digoxin tablet or oral solution with 200 ml water. The study was conducted at crossover design and with a washout period of at least 10 days. On each study day blood samples were taken via an indwelling cannula pre-dose and then at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 48.00, 72.00 h post dose. Four hours post dose the volumteers were provided with a standard lunch. Blood samples taken during the study were placed in glass tubes and allowed to stand for 30 min at room temperature prior to centrifugation. Serum was separated from each sample and stored at −20 °C prior to analysis. The serum concentration of digoxin was determined by a fluorescence polarization immunoassay using a TDx analyzer (Abbott, North Chicago, USA). The intra-and inter-assay variability were 88% and 1.63% at concentration of 1.5 ng/ml. The linear range for digoxin serum concentrations was 0.2–5.0 ng/ml. Higher concentration than 5 ng/ml were diluted with blank human serum

3.5. Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method. The maximum digoxin concentration (C_{max}) and corresponding peak times (T_{max}) were determined by the inspection of the individual drug plasma concentration-time profiles. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule without extrapolation to infinity. The pharmacokinetic parameters for different formulations were compared using the Student's t-test. The drug concentration-time profiles between each formulation were compared using one-way analysis of variance. Significance level was considered to be $P < 0.05$.

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