Pharmacokinetics of \textit{dipyridamole-\textbeta-cyclodextrin complex} in healthy volunteers after single and multiple doses

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SUMMARY

Dipyridamole is a well known anti-aggregating agent characterized by poor water solubility as well as scant and variable bioavailability. Recently, the compound was complexed with \textbeta-cyclodextrin forming a molecular encapsulation resulting in better oral absorption and stronger biological activities in animals. In the present study, a randomized double blind cross-over comparison between dipyridamole-\textbeta-cyclodextrin complex (dip-\textbeta-CD) and dipyridamole was performed in 12 healthy subjects after single (75mg) and multiple oral treatments (75mg TID). Dip-\textbeta-CD showed better bioavailability and less interindividual variability than dipyridamole either after single or multiple doses. In particular, dip-\textbeta-CD had a greater AUC and \textit{C_{max}}, and a smaller \textit{T_{max}} even at the steady state. In addition, 100\% of the subjects receiving a single dose of dip-\textbeta-CD, as compared to 66.7\% of those treated with dipyridamole, had plasma levels superior to 1 \textmu g/ml (which is the supposed anti-aggregating threshold level). In contrast, 0 and 33.03\% of the subjects showed plasma levels superior to 2.5 \textmu g/ml (which might cause the appearance of side-effects) on the 7th day of the multiple treatment with dip-\textbeta-CD and dipyridamole, respectively. In fact, the subjects presenting higher levels after uncomplexed dipyridamole also complained of headache and/or dizziness on occasion. No adverse side effects were reported for dip-\textbeta-CD.

INTRODUCTION

The complexation of active drugs with cyclodextrins, may change their physico-chemical properties. Oral bioavailability, for example, is generally increased in animals and men when poorly water-soluble drugs are complexed. As a consequence, lag time and \textit{T_{max}} are shortened, \textit{C_{max}} is increased and prompter and stronger activity is observed. Occasionally, longer-lasting blood levels with more durable therapeutic effects are obtained. In the present study, the pharmacokinetic profile of dipyridamole-\textbeta-cyclodextrin complex (dip-\textbeta-CD) was determined in healthy volunteers after single and multiple oral treatments. Dipyridamole is a well known anti-aggregating agent widely used in the therapy of thrombo-embolic disorders. Unfortunately, the compound is poorly and variably absorbed after oral treatment (36--67\%) (1) and also presents great interindivial variability (2, 3). Furthermore, its therapeutic index is very narrow in humans, not showing evident platelet anti-aggregating activity with plasma levels below 0.5 \textmu g/ml and evoking side-effects above 2.5 \textmu g/ml (4). Previous studies have indicated that dipyridamole was able to form inclusion complexes with \textbeta-cyclodextrin which are
more stable, more water soluble, more active and more bioavailable in animals (5-7). The main objective of the present investigation was to compare dip-β-CD and uncomplexed dipyridamole bioavailability in the same subjects, according to a randomized crossover design.

MATERIALS AND METHODS

Subjects

12 healthy volunteers, 7 males and 5 females, 24 to 49 years old, 53 to 87 kg of body weight, took part in the study. Subjects had normal physical examination results and clinical laboratory profiles. Concomitant medications were excluded from one week prior to the study until its completion.

Trial design and treatment scheme

A double blind cross over study was performed, with the subjects divided into two treatment groups (dip-β-CD and dipyridamole), according to a Latin square design, each of which received one or the other drug on the same day. After a two-week wash-out period the volunteers were assigned to the other treatment so as to make up a total of 12 individuals for each drug. The subjects were uninterruptedly treated for 7 days with each substance. On the 1st and 7th day the drugs were administered only once at 08.00 in the morning, while from the 2nd to 6th day they were administered thrice a day as close as possible to 08.00, 16.00 and 24.00. The morning dose was always taken with a glass of water after an overnight fast; light breakfast (cup of coffee plus a brioche) and lunch were given 2 and 5 h later. Each dose contained 75 mg of active principle, so that on the 1st and 7th day only 75 mg/day were administered and from the 2nd to 6th day 225 mg/day (75 mg TID) were given.

Sample collection

Two millilitres of blood were drawn into heparinized tubes just before dosing and 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h postdose on the first and seventh mornings. Blood samples were also collected before and 2 h after each morning dose from the 2nd to 6th day of treatment. Samples were immediately centrifuged and the separated plasma frozen at −20°C until assayed for dipyridamole. Urine was also collected immediately before, 0-6, 6-12 and 12-24 h after the morning dose on days 1 and 7. For each collection period, total urine voided was measured and 100 ml aliquots were lyophilized and stored at −20°C until assayed.

Sample analysis

After 1–2 weeks the biological samples were thawed. Plasma samples (0.2 ml) were placed in a 15 ml tube and 50 µg (in 0.010 ml) of benzocaine, 1 ml of sodium hydroxide 1 N and 4 ml of terbutylmethylether were added. The samples were immediately extracted using a vortex mixer at high speed for 1 min, followed by centrifugation at 1000 g for 5 min to separate the aqueous and organic phases. The lower aqueous phase was frozen by immersing the tube in a dry ice-acetone bath and the upper organic phase decanted into another tube. The terbutylmethylether was evaporated under purified nitrogen at 40–50°C. The residue was dissolved in 100 µl of the mobile phase and all or part of this volume was injected into a Beckman model 110A HPLC equipped with an injector and fitted with a Merck column C18 (150 x 3 mm i.d., particle size 5 µm). The mobile phase was a mixture of acetonitrile/monobasic potassium phosphate 0.01 M at pH 7 with addition of NaOH 0.1 N (45:55). The flow-rate was 0.6 ml/min. Fluorescence was measured with an Hitachi model F-1000 fluorometer. An excitation wavelength of 285 nm was selected in conjunction with a 470 nm emission filter. An aliquot (1–10 g) of lyophilized urine was brought to room temperature, 50 µg of internal standard were added, mixed with appropriate volumes of sodium acetate 0.5 M at pH 4.7 and of β-glucuronidase (5000 units/ml). The samples were incubated at 37°C for 12 h and extracted with adequate volumes of sodium hydroxide 1 M at pH 8.6 and of terbutylmethyl ether. Separation, exsiccation and chromatography of the organic phase were then performed as described for the plasma. Standard calibration curves were obtained by adding 1, 3, 20, 30, 100, 300 and 600 ng of dipyridamole and 50 µg of internal standard (benzocaine) to 0.2 ml of control biological samples, which were assayed concurrently with the unknowns. Linear regression analysis was performed on the results obtained from the standard samples. The equation of the best fit for the standard curve was used to calculate the concentration of an unknown sample from the peak height ratio measured. Under the described conditions