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In vitro characterization of some biopharmaceutical properties of praziquantel

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Abstract

In several studies of patients with neurocysticercosis under treatment with praziquantel (PZQ), the pharmacokinetic data were difficult to interpret probably because of the low solubility as well as its variable oral bioavailability. Because there is limited information available regarding the biopharmaceutical properties of PZQ, the aim of this work was to evaluate the absorption characteristics of the drug and its dissolution behaviour in simulated media. Additionally, its in vitro protein binding and displacement by highly bound drugs was evaluated. Permeability evaluation was carried out by using Caco-2 cells. Dissolution release profiles were evaluated using the USP apparatus and the following dissolution media: HCl containing 2 mg of sodium laurylsulfate per milliliter, milk, FeSSIF and FaSSIF. Protein binding of PZQ was carried out by equilibrium dialysis.

Results showed that praziquantel was absorbed by passive diffusion. The apparent permeability constant value was 4.4×10^{-5} cm/s. Binding was not influenced by the addition of highly bound drugs. Dissolution from a tablet formulation showed that the rate of praziquantel was dependent on the components of the media. Although the simulated media could explain the influence of the lipids on praziquantel absorption, they were not able to forecast the influence of carbohydrates. Further refinements are required to explain the in vivo data.

Keywords: Praziquantel; Dissolution; Physiological media; Milk; Official media; Caco-2 cells; Permeability; Protein binding

1. Introduction

Praziquantel (PZQ, 2-cyclohexylcarbonyl [1,2,3,6,7,11b] hexahydro-4H-pyrazin [2,1a] isoquinolin-4-one), is a broadly effective trematocide and cestocide

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drug widely used in developing countries for the treatment of schistosomiasis and brain cysticercosis (Sotelo et al., 1990). In man, after oral administration, PZQ undergoes an extensive first pass-effect metabolism (Patzschke et al., 1979). Also great pharmacokinetic and therapeutic variability after its oral administration has been found (Jung et al., 1990). Despite its wide use in México, little information is available on the biopharmaceutics characteristics of the drug. Studies performed in adults have shown a significant increase in plasma levels of PZQ, when it was co-administered with food, mainly carbohydrates (Castro et al., 2000). Also it has been observed that plasma levels decrease significantly, when it is administered with carbamazepine or phenytoin (Bittencourt et al., 1992). Because plausible explanations are that food increases the dissolution of the drug and that carbamazepine and phenytoin displace PZQ from protein binding sites the main objectives of this study were to determine its permeability across Caco-2 cells, to characterize its dissolution performance in different dissolution media, as well as to determine its possible displacement by the antiepileptic drugs carbamazepine and phenytoin and the anti-inflammatory drugs naproxen and ibuprofen.

2. Material and methods

2.1. Chemicals

2.1.1. Permeability

The Caco-2 cell line was donated by Dr. I. Hidalgo (Absorption Systems, USA). Dubelcco's Modified Eagle Medium (DMEM/high) and sodium pyruvate (200 mM) were purchased from Gibco (Life Technologies, USA); L-glutamine, HEPES, D-glucose, praziquantel, atenolol and propranolol were purchased from Sigma–Aldrich Co. (USA). Hank's balanced salt solution (HBSS), non-essential amino acids (NEAA, 100×), trypsin 0.05% versene (0.5%) and antibiotic mixture (10,000 U/ml penicillin G, 10,000 μg/ml streptomicyn) were purchased from in vitro, S.A (México). Fetal bovine serum (FBS) fetalclone III was purchased from Hyclone Laboratories (USA). Colagen rat tail type I (10 mg/ml) was obtained from Boehringer Mannheim (Germany) and lucifer yellow was purchased from Molecular Probes (Oregon, USA). Transwell polycarbonate membrane was acquired from Corning Costar Corp. (Cambridge, MA, USA).

2.1.2. Protein binding

Carbamazepine, phenytoin, ibuprofen, naproxen and bovine serum albumin (crystallized, essentially fatty acid-free) were obtained from Sigma Chemical Co. The dialysis cell and the Spectra/Por membranes (molecular weight cut-off, 12,000–14,000) were obtained from Spectrum Medical Industries. Solutions of serum albumin (45 g l^{-1}) were prepared in phosphate buffer pH 7.4.

2.1.3. Dissolution

Praziquantel tablets (Cisticid®, 600 mg) were obtained from Merck México S.A. Diazepam (assay 99%) was a gift from Bayer de México S.A. De C.V. Praziquantel standard as well as sodium taurocholate and lecithin were purchased from Sigma–Aldrich Chemical Co. (USA).

Methanol and acetonitrile were HPLC grade and all other reagents were of highest purity.

Potassium dihydrogen phosphate, potassium chloride, hydroclorhic acid, acetic acid, diisopropyl ether and ethyl acetate, all analytical grade were purchased from J.T. Baker-Mallincrodt (México). The source of milk, 2.8% fat, was Alpura® de México.

2.2. Experimental procedure

2.2.1. Permeability

2.2.1.1. Preparation of Caco-2 monolayer. Caco-2 cells were maintained at 37 °C in DMEM/high supplemented with 10% FBS, 1% L-glutamine, 1% NEAA, 1% sodium pyruvate and 100 U/ml penicillin and 100 μg/ml streptomycin in atmosphere of 5% CO_2 and 90% relative humidity. Cells were grown and routinely seeded in 75 cm^2-flasks and harvested by regular trypsinization. For transport experiments, 60,000 cells/cm^2 were seeded onto each 12-mm collagen-coated (rat tail collagen type I) polycarbonate Transwell filters (area of 1.13 cm^2 and mean pore size of 3 μm). The medium was changed every 48 h during 6 days and every 24 h thereafter. The monolayers were used between passages 58 and 65, after days 26 and 30 of growth. Before starting the experiment monolayer integrity was verified via transepithelial electrical

resistance (TEER) measurements showing a value between 250 and 300 Ω cm^2.

2.2.1.2. Bidirectional transport studies. Praziquantel, atenolol and propranolol stock solutions (10 mM) each drug were dissolved in 2 ml ethanol and diluted with water. Test solutions 100 μM of each drug were prepared from stock solution dissolving with HSBB for transport experiments. Transport medium containing the drug was added either to the apical (AP) or basolateral (BL) compartment. The cells monolayers were incubated at 37 °C with 500 μl in the apical compartment and 1500 μl in the basolateral compartment. For AP–BL transport experiments, each insert was changed at a new well with fresh transport medium. A 1000 μl of medium from the basolateral side was removed at 15, 30, 45, 60 and 90 min, whereas for BL–AP transport, 200 μl of medium from the apical side was removed. The receiver medium was totally replaced with fresh medium.

Samples (100 μl) were injected directly into a high performance liquid chromatograph system (Hewlett-Packard series 1050) equipped with a variable wavelength detector at 217 nm using a Spherisorb ODS2 column (250 mm × 4.6 mm i.d.; particle size 5 μm). Flow was maintained at 1.5 ml/min.

2.2.1.3. Apparent permeability calculation. The accumulated amount of drug appearing in the receptor compartment over time, dQ/dt, was used to calculate the apparent permeability (Papp) using the following equation (Grass and Sweetana, 1989).

$$P_{\text{app}} = \frac{\mathrm{d}Q}{\mathrm{d}t} \times \frac{1}{C_{\text{O}}} \times \frac{1}{A}$$

where, C_{O} is the initial concentration of the drug in the donor compartment and A is the area of the insert (1.13 cm^2).

2.2.2. PZQ protein binding

Plasma or bovine serum albumine samples (1 ml) containing PZQ at two concentration ranges, between 0.187 and 3.00 μg/ml and from 10 to 100 μg/ml, were dialysed against a phosphate buffer pH 7.4, 0.064 M (1 ml). After 3 h of incubation, at 37 °C and 30 rpm, samples (0.5 ml) were removed from both compartments and analyzed by an HPLC method. Each concentration was carried out by quintuplicate. Total protein concentration was measured by Biuret method (Lowry et al., 1951).

Interaction studies between PZQ and other drugs highly bound to albumin were carried out by using the same procedure as above. To plasma samples containing PZQ at concentrations of 0.37, 0.75 and 1.5 μg/ml, carbamazepine (7 μg/ml), phenytoin (15 μg/ml), ibuprofen (10 μg/ml) or naproxen (50 μg/ml) were added.

The extent of binding (Eb) was calculated using the equation,

$$\text{Eb}(\%) = \left[\frac{C_{\text{p}} - C_{\text{f}}}{C_{\text{p}}}\right] \times 100$$

where C_{p} and C_{f} are the drug concentrations in the protein and buffer compartments after dialysis, respectively.

2.2.2.1. Praziquantel assay. Samples were analyzed by using an HPLC method previously reported (González-Esquivel et al., 1993). Briefly, to 0.5 ml of plasma, 50 μl of a solution containing the internal standard, diazepam (10 μg/ml) plus 1 ml of 0.2 M sodium hydroxide were added, shaken on a vortex mixer for 15 s and extracted by passage through a Sep Pack C_{18} cartridge. The sample was washed with 20 ml of phosphate buffer. The compounds were eluted with 6 ml of ethyl acetate–diisopropyl ether (70:30, v/v) The sample was then evaporated to dryness under a nitrogen steam at 25 °C. The residue was dissolved in 100 μl of the mobile phase of acetonitrile–water (45:55). Aliquots of 50 μl were injected on the HPLC system as described previously.

Sensitivity was 15.6 ng/ml and the maximum coefficient of variation was 7%. The recovery ranged between 95 and 100%.

2.2.3. Dissolution

Dissolution release profiles of Praziquantel tablets were evaluated using a USP (Vankel-VK 700) apparatus 2 (paddle method), and employing 500 or 900 ml of dissolution medium at a temperature of 37 ± 0.5 °C. All experiments were run in triplicate.

2.2.3.1. Composition of the dissolution media.

USP medium: Nine hundred mililiters of HCL 0.1N containing 2 mg of sodium laurylsulfate per milliliter at 50 rpm.

Milk: Bovine milk with 2.8% of fat (Alpura, México). This is a homogenized milk treated with ultra high temperature to extend the shelf-life and containing no preservatives. Content 48 g carbohydrates, proteins 31 g, sodium 0.5 g and water qs 1 l, pH was 6.64. The volumes used were 500 and 900 ml at 100 rpm.

Media simulating conditions in the fasted and fed states were prepared as previously reported (Galia et al., 1996). Briefly fasted state simulated intestinal fluid (FaSSIF) contained 3 mM sodium taurocholate, 0.75 mM lecithin and had a pH 6.5. The volume used was 500 ml at 100 rpm. The fed state medium FeSSIF simulated intestinal fluid contained 15 mM sodium taurocholate, 3.75 mM lecithin and had a pH 5.0. The volume used was 900 ml at 100 rpm.

2.2.3.2. Sampling and assay. When USP media was used, 3 ml samples were taken with replacement at 5, 10, 15, 20, 30, 40, 50, 60, 90 and 120 min and assayed spectrophotometrically at 263 nm.

When FaSSIF, FeSSIF and milk media were used, 2 ml samples were taken with replacement at 10, 20, 30, 45, 60, 90 and 120 min of medium. In the case of FaSSIF and FeSSIF, dissolution samples were filtered and diluted 1:10 with acetonitrile–water (60:40) and analyzed directly by HPLC using the same chromatographic conditions previously described in permeability studies.

When milk was used as dissolution medium, samples were extracted with ethyl acetate–diisopropyl ether (70:30). Organic layer was separated by centrifugation (3000 rpm/15 min) and evaporated to dryness under nitrogen stream and the residue was dissolved in 1 ml of mobile phase. Chromatographic conditions were the same as described previously. The mean recovery was 97%.

Standard curves were constructed for each media. Linearity was confirmed in the range of 0.13–0.8 mg/ml for USP medium and from 1 to 70 μg/ml, in the case of FeSSIF, FaSSIF and milk. Coefficients of determination were at least 0.99 and coefficient of variation were not higher than 3%. No chromatographic interferences with media compounds were found. Samples were stable during 24 h.

2.3. Statistical analysis

Student–Newman–Keuls test and analysis of variance were used to compare data from dissolution profiles; p-values < 0.05 were considered indicative of significance.

In order to describe the PZQ release kinetics dissolution, data were fitted to different dissolution kinetic functions: zero-order, first-order, cubic root law (Hixson–Crowell) square root of time equation (Higuchi) and Weibull's equation (Sathe et al., 1996; Yuksel et al., 2000). Non-linear regression to fit the data was used; standard criteria, i.e., higher determination coefficient ($r^2 > 0.99$), and minimum residual mean square (RMS), were the criteria used to select the best model.

3. Results

Apparent permeability coefficients for propranolol and atenolol were: 3.12×10^{-5} and 1.5×10^{-6} cm/s. These values are similar to those obtained in other studies (Rinaki et al., 2003; Yazdanian et al., 1998). The apparent permeability coefficient for PZQ was 4.4×10^{-5} cm/s, which indicates that it is a highly permeable drug. When basolateral to apical direction was evaluated, we found a mean value of 3.79×10^{-6} cm/s. The efflux ratio for PZQ was almost 1.0, which shows that this drug is not actively transported under the applied experimental conditions. Considering that the drug is highly permeable our data suggest that transcellullar transport may be the main absorption process.

Binding of PZQ to plasma proteins varied from 79 to 80.4% in the range of 0.187–3.0 μg/ml. When the same experiment was performed in albumin, the percentage bound ranged from 78 to 81%. These data show that PZQ is bound exclusively to BSA. When higher PZQ concentrations were evaluated (10–100 μg/ml), binding decreased from 80.4 to 49.7%. The Scatchard plot of the data showed the presence of one class of binding site with association constant equal to K_a of $4.059 \times 10^4\ M^{-1}$. No displacement of PZQ was found when carbamazepine, phenytoin or ibuprofen were added, however a slight displacement of PZQ (7%) was found in the concentration range studied (0.37–1.5 μg/ml) when naproxen was used.

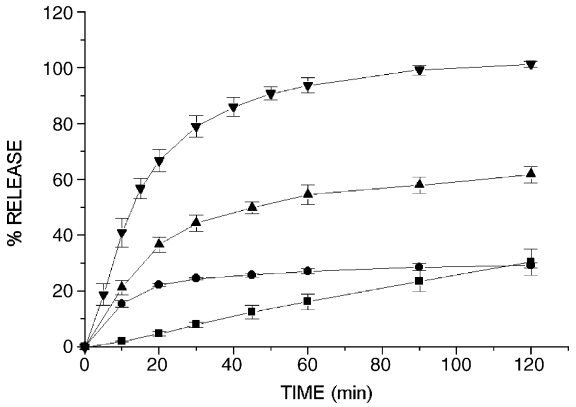

Fig. 1. Percentage of PZQ release in the different dissolution media used: milk (■), FaSSIF (●), FeSSIF (▲) and USP (▼).

Fig. 1 shows the differences in the dissolution profiles of PZQ from commercial product Cisticid in the different media. When USP method was used, dissolution was high and complete. At 60 min, the mean percentage dissolved was 91%. When fasted/fed simulated media were used, we found that in both media dissolution was not complete. In FaSSIF, we found that at 60 min 27.1% was dissolved. In FeSSIF medium, dissolution was improved with a 55% dissolved at 60 min. When the release of PZQ in milk was evaluated, we found that dissolution was slower. At 60 min, only the 16.1% was dissolved however at 120 min dissolution was similar with those obtained in FaSSIF. In this case, no plateau was reached within the study interval.

Table 1 shows the results of the fit of the data to the various kinetic models. As can be seen, Weibull's function fitted best to the dissolution data for USP, FaSSIF and Fessif media (higher determination coefficient, $r^2 > 0.99$ and minimum residual mean square) and zero-order kinetics was the best kinetic fit for the release of PZQ in milk.

4. Discussion

From the obtained results, it can be seen that PZQ is a highly permeable substance. This drug did not show a potential for interacting with cellular efflux pumps at the concentration range studied. Previous clinical studies have shown that after a dose of 1800 mg, plasma levels were very low (0.2–3 μg/ml). Results obtained from the current study show that the main causes for low bioavailability are not related to the permeability and that solubility. Intestinal and hepatic metabolism may be the limiting factors in the disposition of this drug.

In vitro binding studies showed that the unbound fraction of PZQ is dependent on drug concentration. The unbound fraction of the drug increased progressively when the PZQ concentrations increased from 10 to 100 μg/ml, however binding was relatively constant over the PZQ range found in man (0.18–3.0 μg/ml) with a mean unbound fraction of 0.2. Due to its intermediate binding, no interaction was found with highly bound drugs. Bittencourt et al. (1992) showed that bioavailability of PZQ was markedly reduced, when it was administered concomitantly with phenytoin or CBZ. The authors suggested that this effect could be due to the displacement of PZQ from protein binding sites by the antiepileptic drugs. The results of the present study show that the main interaction process between PZQ and antiepileptic drugs is not related with

Table 1
Criteria used for the selection of the best kinetic model

Dissolution media	Zero-order	First-order	Higuchi	Hixon Crowell	Weibull
Determination coefficient r^2					
USP	0.6565	0.9965	0.8528	0.9628	0.994
FaSSIF	0.5528	0.5930	0.7140	0.5528	0.996
FeSSIF	0.7120	0.7191	0.9045	0.6005	0.992
Milk	0.9972	0.9968	0.8504	0.9980	0.973
Residual mean square					
USP	3986.75	40.01	1707.66	430.79	39.39
FaSSIF	299.13	692.92	191.29	752.59	1.86
FeSSIF	888.40	866.57	294.47	1232.07	8.85
Milk	2.20	2.51	120.14	4.59	17.34

competition in protein binding. Metabolic induction play a more important role in the bioavailability reduction of PZQ.

Considering that for Class II substances dissolution is the rate limiting step and depends on a wide variety of factors such as surfactants, pH and volume available for dissolution, we selected the official USP test, milk, FaSSIF and FeSSIF in order to evaluate the in vitro behaviour of the drug and to explain the previous in vivo results.

When USP method was used, dissolution was complete, however the use of sodium laurylsulfate could overpredict the contribution of surfactants in the in vivo absorption process. Although it has been proposed that medium containing surfactants can better simulate the environment of the gastrointestinal tract, up to date it is no clear if sodium lauryl sulfate is a good surrogate for bile salts.

Nicolaides et al. (1999) showed that milk can have a large effect on the dissolution characteristics of low solubility drugs. Unexpectedly we found that dissolution in milk was very slow and incomplete, which could be due to its low solubility in dietary compounds or that milk had a lower percentage of fat than those used in previous studies. Considering that milk does not always reflects the gastric dissolution of drugs at fed state, and in order to have a closer simulation of gastric dissolution it would be interesting to evaluate the release of the drug by adding pepsin and lipase to this medium.

The model dependent method used to fit dissolution data in USP, FaSSIF and FeSSIF media showed that used the kinetic model was independent of the media used however milk influenced the release mechanism of PZQ presumably by the nature of the milk components.

It is well known that PZQ is a poorly water soluble (0.4 mg/ml) drug, which exhibits variable oral bioavailability in fasted state. Considering that the highest strength is 600 mg then the dose solubility ratio is 6.0. From these properties, it is expected that the drug exhibit dose solubility limited absorption.

It has been proposed that for low solubility neutral compounds the concentration of solubilizing compounds in bile salts or in meals is the prime determinant of solubility and hence, dissolution behaviour (Galia et al., 1996). From the dissolution experiments (Fig. 1), we found that the dissolution of PZQ in FeSSIF was faster than FaSSIF therefore one would predict an increase in the absorption when it was administered with food. In a previous study, we evaluated the influence of food on the bioavailability of PZQ (Castro et al., 2000). The results showed that when the drug was administered with a lipid diet or after a high carbohydrate diet, plasma levels increased two- and five-fold, respectively. The results of the present study show that the percent released was two-fold, when FeSSIF rather than FaSSIF was used which is consistent with the in vivo data when the lipid diet was administered, however the media were not able to simulate the higher plasma levels observed after the high carbohydrate diet. These findings show that although solubilization of PZQ by bile is one of the several factors that increase the bioavailability of this lipophilic compound there are other issues, which could not be explained by this media.

The mechanism by which carbohydrates increase the bioavailability of PZQ remains to be demonstrated. The effect could be due to an increase of splanchnic blood flow (McLean et al., 1981), to changes in luminal metabolism or to the ability of carbohydrates to inhibit the synthesis of certain enzymes. More work has to be done in order to explain our results, however if the effect is related with the inhibition of drug metabolism we consider the possibility that a dissolution test would not be able to simulate the in vivo behaviour of this drug.

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