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Determination of pentoxifylline in serum by high-performance thin-layer chromatography

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Abstract: An analytical method to study the bioavailability of newly developed pentoxifylline sustained-release tablets has been developed and assessed in experiments on dogs. For the isolation of pentoxifylline and its metabolites from serum solid–liquid extraction was applied by involving the internal standard probe. HPTLC plates with a preconcentration zone were used for separation of the analysed substances, using chloroform–methanol (95:5, v/v). Quantification was by densitometric detection. The detector response was linear in the concentration range investigated for pentoxifylline: 0.02–1.5 μg ml^{-1} of serum.

Keywords: *Pentoxifylline; high-performance thin-layer chromatography; sustained-release dosage form; bioavailability.*

Introduction

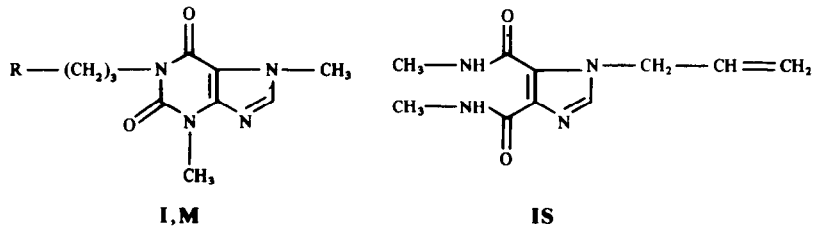
Pentoxifylline [1-(5′-oxohexyl)-3,7-dimethylxanthine] (**I**; Fig. 1) is a xanthine derivative. This drug is extensively metabolized in the body and the pharmacological action of metabolites, e.g. the monohydroxy metabolite [1-(5′-hydroxyhexyl)-3,7-dimethylxanthine] (**M**; Fig. 1), is considered to be involved along with the parent drug. Since after ingestion of the pure drug substance the pharmacological effects are relatively short, due to processes such as rapid and extensive drug metabolism, short elimination half-life of the drug and metabolites, high values of their clearance, etc. [1, 2], different sustained-release tablets are preferred at present for long-term oral treatment [3].

The aim of our work was to establish a simple analytical method to study the serum concentrations of pentoxifylline. The final goal was to exploit the method developed at the optimization study of the sustained-release pentoxifylline tablet, based on the addition of the most suitable synthetic polymer as the prolonged-release agent. This formulation should be optimal for the treatment of at-home patients with one or two tablets a day.

Experimental

Drugs and related compounds

Pure pentoxifylline substance (Slovakofarma, Hlohovec, Czechoslovakia), 300 mg, was packed manually into gelatine capsules at the Department of Galenic Pharmacy, Faculty of Pharmacy, Comenius University (Bratislava, Czechoslovakia) [4, 5] and introduced into the sustained-release tablet along with the synthetic polymer — a butylacrylate–hydroxy-



I, M **IS**

Figure 1
Chemical structure of pentoxifylline (R=CH_3COCH_2—), **I**; monohydroxy metabolite of pentoxifylline (R=$CH_3C(OH)CH_2$—), **M**; and the internal standard allylnorantiffeine, **IS**.

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ethylmethacrylate copolymer (Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia).

The monohydroxy metabolite was synthesized by Dr I. Jendrichovský, Drug Research Institute (Modra, Czechoslovakia) Allylnorantiffeine (**IS**, Fig. 1) was kindly provided by the Institute of Experimental Medicine, Academy of Medical Sciences (Leningrad, USSR).

Drug administration

Pentoxifylline was administered to four mongrel dogs (12–16 kg) in a dose of 300 mg as intravenous bolus in 0.9% NaCl or orally (after overnight fasting) in one gelatine capsule or as the studied sustained-release dosage form. One week was allowed to elapse between the administration of the different drug formulations. Blood was withdrawn from the cubital vein at selected time intervals after administration (Fig. 2) and centrifuged (1500*g*; 10 min at room temperature). The serum obtained was frozen until further processing.

Procedure

An appropriate volume (0.5–6 ml) of dog serum was diluted by addition of 1.0 ml of the aqueous internal standard solution (2.5 μg ml^{-1}). The samples were applied into Separcol SI C 18 minicolumns (Institute of Polymers, Slovak Academy of Sciences, Bratislava, Czechoslovakia), preconditioned by passing 2 ml of methanol followed by 2 ml of water through them. The compounds of interest, retained by the minicolumn sorbent, were further separated from the major part of retained endogenous compounds by washing with 1 ml of water. The residue of the water was blown out by pressurized nitrogen and 2.5 ml of acetonitrile was added to elute the retained compounds. Acetonitrile was evaporated under a stream of nitrogen at 50°C. The solid residue containing mostly the analysed substances (**I**, **M**, **IS**) was dissolved by using 25 μl of chloroform. The chloroform solution was applied onto the preconcentration zone of the HPTLC plate.

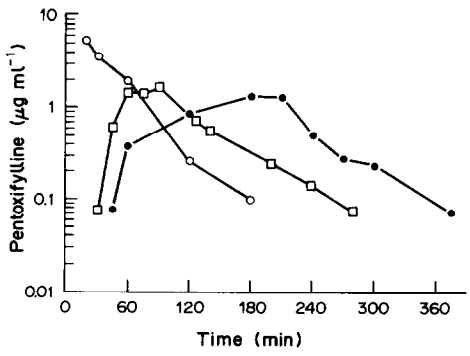

Figure 2
Serum concentration–time courses of pentoxifylline after intravenous administration of the drug solution (○), following oral ingestion of the reference (□) and sustained-release dosage forms (●) in a dog.

Chromatography

The HPTLC plates (No. 13728, Merck, Darmstadt, FRG) were used with chloroform–methanol (95:5, v/v) a binary solvent mixture similar to the mixture used by Smith *et al.* [6], in the ascending development mode.

At the given conditions (eluent migration up to 5 mm from the upper plate edge) the R_f values for **I** and **IS** were 0.53 and 0.81, respectively.

Calibration standards were randomly spaced on the HPTLC plates amongst the serum samples of interest. Quantification was carried out using a dual-wavelength flying-spot-scanner CS 9000 (Shimadzu, Japan). At the wavelength of 274 nm, the reflection measured as detector response was linear ($r^2 = 0.99$) in the concentration range investigated: 0.02–1.5 μg ml^{-1} of serum. For samples exceeding these ranges, the analysis was repeated using an appropriately reduced sample volume. The results of analysis were considered valid only when the ratio of the compound signal to noise was higher than 3.

Pharmacokinetic analysis

A first-order kinetic process governed the decline of pentoxifylline serum concentration after intravenous administration in the whole observation period and in the post-absorption interval following oral dosing. These data therefore were approximated with a mono-exponential equation. Non-linear least-squares regression analysis was applied for estimation of the equation parameters [7]. The area under the serum-concentration curves was approximated by trapezoidal rule. Extrapolation of the area from the last sampling time to infinity was made by dividing the last serum concentration by the first-order rate constant of the mono-exponential decline.

Results

Figure 3 shows the HPTLC chromatograms of (A) a spiked serum sample containing 500 ng of pentoxifylline and (B) a serum sample from a dog dosed with pentoxifylline by the above procedure. The peak having R_f value of 0.25 was found to be the monohydroxy metabolite of pentoxifylline, **M**. The other two peaks [Fig. 3 (B)] with R_f values of 0.18 and 0.32, respectively originated most probably from further pentoxifylline metabolites. Figure 2 illustrates the dog serum concentration–time courses of pentoxifylline after intravenous administration of the drug solution and following oral ingestion of the reference and sustained-release dosage forms. The pharmacokinetic parameters estimated are listed in Table 1. The elimination half-life ($t_{½}$) was prolonged following oral administration, in comparison with the intravenous data. This finding is generally explained as the influence of the prolonged drug absorption into circulation. The absolute bioavailability (F_{abs}) of pentoxifylline from the reference and sustained-release oral dosage formulations was 51 and 57%, respectively. The peak serum concentrations (C_{max}) were also comparable, namely 1.5 and 1.1 μg ml^{-1}, respectively. The t_{max} (time to reach the peak concentration) was attained with the interval of 60–90 min following administration of the reference dosage form and was prolonged to 180–240 min after sustained-release dosage form administration representing an increase of ×2.8.

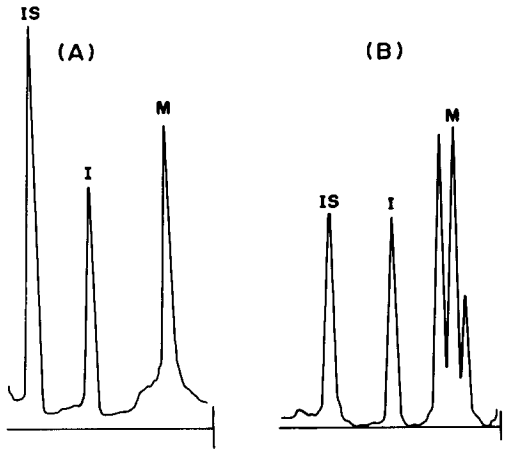

Figure 3
HPTLC chromatograms of (A) a spiked serum sample containing 500 ng of pentoxifylline and (B) serum sample from a dog dosed with pentoxifylline.

Discussion

Using solid–liquid extraction with an internal standard the recovery of pentoxifylline in the assayed concentration range was near quantitative (95 ± 5%). Elution of the retained compounds by acetonitrile provided considerably clearer eluates than those obtained with methanol, which is commonly used for elution. Since acetonitrile with the residual water yields an azeotropic mixture, after evaporation of the liquids the solid residue can be efficiently dissolved with a very small (25 μl) volume of chloroform.

Smith *et al.* [6] found R_f values of 0.70 and 0.41 (**I, M**) by using the chloroform–methanol ratio of 90:10 (v/v), whilst the composition of 95:5 (v/v) used in the present study resulted in R_f = 0.53 and 0.25. The lower R_f values provide better resolution of **I** (near the optimal value of 0.5) as well as of the internal standard from spots of endogenous compounds which run with the solvent front at HPTLC. However, exact quantification of the pentoxifylline monohydroxy metabolite, **M**, by using the mobile phase 95:5 (v/v) was omitted since the other two compounds (metabolites) with R_f = 0.18 and 0.32, might significantly influence the obtained results. Thus, at this stage of our investigation only the results of pentoxifylline

Table 1
Pharmacokinetic parameters of pentoxifylline administered intravenously and orally to dogs in a dose of 300 mg (the values are arithmetic means)

Parameter	Intravenous administration	Oral administration	
		Reference dosage form	Sustained-release dosage form
$t_{½}$ (min)	27	67	39
AUC (μg min ml^{-1})	318	162	180
C_{max} (μg ml^{-1})		1.5	1.1
t_{max} (min)	—	75	210
F_{abs} (%)	—	51	57

HPTLC analysis were considered in studying its pharmacokinetics, with the main focus on screening the reliability of the sustained-release dosage form studied, concerning the time profile of pentoxifylline serum concentration.

The HPTLC method of pentoxifylline analysis in serum approved the suitability of the butylacrylate–hydroxyethylmethacrylate copolymer for retardation of drug liberation from the tablet formulation to be observed. This property is expressed by the ×2.8 increase in the value of t_{max} when compared with the reference dosage form, at almost identical C_{max} values. No comparable studies on sustained-release dosage forms of pentoxifylline in animals are known to the Authors. The usage of a commercially available sustained-release dosage form in man has been reported [8] with retardation characteristics similar to our results [9].

In conclusion, an HPTLC method was developed for pentoxifylline determination in dog serum. This procedure is convenient and reproducible and allows the pharmacokinetics of this drug to be investigated when administered in sustained-release dosage forms.

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