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Some pharmacokinetic characteristics of furafylline, a new 1,3,8-trisubstituted xanthine

JORDI SEGURA*, INOCENCIO GARCIA, EMILI TARRUS, *Division of Biochemistry and Biopharmaceutics, Institute of Research, Laboratorios Almirall, Cardoner, 68–74, Barcelona 08024, Spain*

After administration of furafylline, a, 1,3,8-trisubstituted xanthine, to beagle dogs at 0·5 and 10 mg kg^{-1} an elimination half-life 2–10 times longer than that of theophylline was observed. The kinetics were dose-dependent but no phase I metabolites were detected. When the compound was given orally to humans at a dose of 1·3–1·9 mg kg^{-1} a mean β elimination half-life of 48·1 ± 10·8 h was obtained after an initial distribution phase. Evidence of non-linear kinetics for plasma levels below 0·6 μg ml^{-1} was also obtained. Other 1,3,8-trisubstituted xanthines, may also have a lower elimination rate than theophylline.

Although oral theophylline is widely used for the long-term treatment of asthma (Ogilvie 1980) and chronic obstructive airways diseases (Arnold et al 1981), it has some important drawbacks. Thus differences in metabolism of genetic, environmental or physiological origin (Lesko 1979) give rise to large interindividual variations in blood levels and the need for plasma monitoring (Ogilvie 1978; Richens & Warrington 1979; Morselli et al 1980; Williams & Mamelok 1980) to ensure maintenance within the therapeutic range (8–20 μg ml^{-1}). Furthermore the high metabolic clearance of theophylline (Jusko et al 1979) results in a short elimination half-life and the consequent need for frequent doses or the use of sustained release formulation.

In an attempt to circumvent some of these drawbacks, N^7-substituted derivatives of theophylline were synthesized (Zuidema & Merckus 1979) and, more recently, the use of 1,3,8-trisubstituted xanthines has been described (Grassi et al 1980, 1981; Cho et al 1981; Griffith et al 1981; Wells et al 1981). Following this latter lead, a large series of 1,3,8-substituted xanthines has been recently studied and furafylline (1,8-dimethyl-3-(2′-furfuryl)-1*H*-purine-2,6-dione) was selected as one of the most interesting compounds examined (Vega et al 1985). However, in spite of the potent bronchodilator activity of 1,3,8-substituted xanthines, compared with theophylline, little is known about their pharmacokinetic properties. We now report studies undertaken with furafylline administered to dogs and man.

Materials and methods

Animal studies. Nine fasted beagle dogs of either sex (8·5–14·5 kg) were used. Both drugs were given at 0·5 or 10 mg kg^{-1} by oral intubation to 2 or 3 dogs. Theophylline was administered as an aqueous solution containing 0·05 or 1 mg ml^{-1}. Furafylline, being poorly soluble, was administered as a finely precipitated aqueous suspension containing 0·15 or 0·5 mg ml^{-1} of the compound and 20 mg ml^{-1} methylcellulose. Blood (5 ml) from the cephalic veins of the thoracic limbs was collected into heparinized tube before and at 0·25, 0·5, 0·75, 1, 1·5, 2, 3, 4, 5, 6, 7, 8 and 24 h after drug administration. The plasma was separated after centrifugation (3000*g*, 10 min) and stored at −20 °C.

Human studies. Four healthy male volunteers (Table 1) gave written informed consent to participate in the study. Furafylline (125 mg finely suspended in 15 ml water containing 20 mg ml^{-1} methylcellulose) was administered orally after an overnight fast. A standard breakfast (cheese sandwich and 300 ml orange juice) was allowed 2 h post dose. Ingestion of alcohol and xanthine-containing foods or beverages was prohibited during the study. No other restrictions were imposed. Smoking was monitored by the analysis of blood thiocyanate by the colorimetric method of Pettigrew & Fell (1972). Blood (5 ml) from a forearm vein was collected into heparinized tubes before and at 0·25, 0·5, 0·75, 1, 1·5, 2, 3, 4, 8, 24, 48, 72 and 96 h after administration of furafylline. The plasma was separated after centrifugation (3000*g*, 10 min) and stored at −20 °C.

Analytical method. Aliquots (1 ml) of plasma were extracted with chloroform (7 ml) for 35 min in a tilt-shaker. The organic phase was aspirated and evaporated to dryness under a stream of nitrogen. The residue was taken up in 100–200 μl methanol and 20 μl were assayed using an HPLC system (Waters Assoc.), with a compressed Radial Pak C_{18} column. The mobile phases used were 0·01 M sodium acetate–acetonitrile (86 : 14) for theophylline (retention time 3 min), and 0·02 M sodium acetate–methanol (6 : 4) for furafylline (retention time 5 min), both adjusted to pH 4·0 with acetic acid (1 M). The effluent was monitored at 280 nm. Standard curves obtained using spiked plasma samples were rectilinear for both theophylline (50 ng to 20 μg ml^{-1}) and furafylline (50 ng to 10 μg ml^{-1}). In the latter case the correlation coefficient was 0·9998 and the reproducibility, measured by the variation coefficient at the concentrations of 100 and 2000 ng ml^{-1} was 3·2 and 1·6%, respectively (n = 6). The overall yield of the process, including extraction, was 93%, the sensitivity limit was some 20 ng ml^{-1} and the interassay variability

* Correspondence.

was 5·1% calculated on the basis of assays of samples of 1000 ng ml^{-1}.

Results

After oral administration of theophylline, 0·5 and 10 mg kg^{-1}, to 2 and 3 beagle dogs, respectively, rapid absorption and an early peak concentration (at 1 h) of 0·45–0·55 μg ml^{-1} with the low dose and 8·5–10 μg ml^{-1} with the high dose were obtained. From 2 h post dose an elimination phase with a half life of 1·7–3·1 h was observed (Fig. 1A). The calculated values for the area under the concentration time curve (AUC) in the period 0–8 h were 1·79–1·98 and 30·2–34·0 μg h ml^{-1} for the low and high dose, respectively.

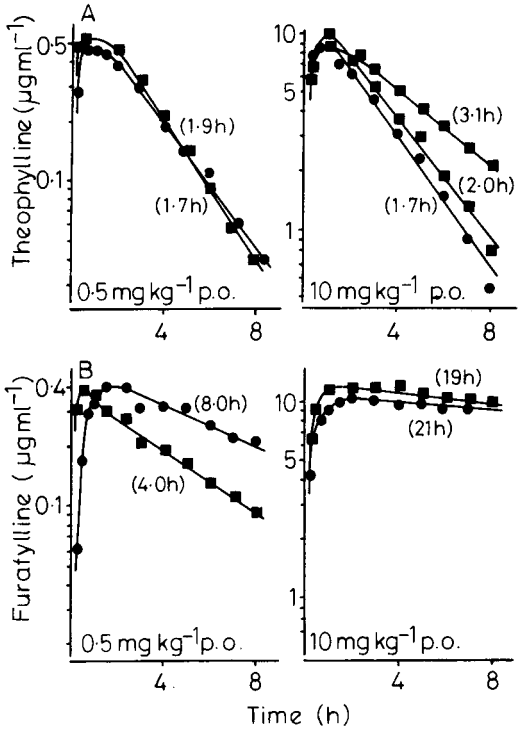

FIG. 1. Plasma levels of (A) theophylline and (B) furafylline in dogs (■, male; ●, female) after a single dose at 0·5 or 10 mg kg^{-1}.

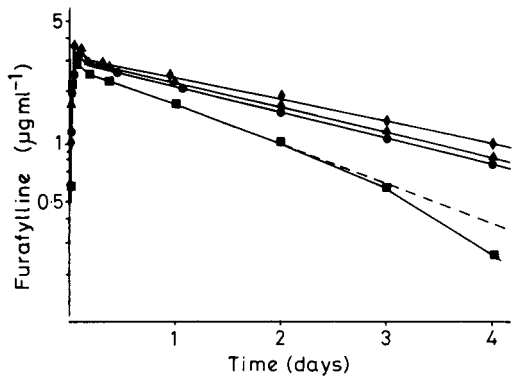

FIG. 2. Plasma levels (corrected to 70 kg body weight) of furafylline in four volunteers (▲, I; ■, II; ◆, III; ●, IV) after oral administration of 125 mg to each subject.

When equivalent experiments were carried out with furafylline in two pairs of dogs, the peak plasma concentration (0·4 μg ml^{-1}, low dose; 10–12 μg ml^{-1}, high dose) was reached 1–2 h after dosing (Fig. 1B). The elimination half-life (4·0–8·0 h low dose; 19–21 h high dose) was much higher than that observed after theophylline, the effect being most notable at 10 mg kg^{-1}. The value for the AUC in the period 0–8 h was 1·6–2·3 and 73·8–82·8 μg h ml^{-1}, respectively.

When furafylline was administered to four healthy male volunteers (Table 1) at a dose of 1·3–1·9 mg kg^{-1}, a peak plasma concentration (corrected to 70 kg body weight) of 2·90–3·42 μg ml^{-1} was observed at 2 h followed by a distribution phase up to 4 h post dose (Fig. 2). Beyond this time a monoexponential elimination phase with a mean half-life of 53·1 ± (s.d.) h was found for volunteers I, III, IV, the main pharmacokinetic data being recorded in Table 1. Subject II showed a shorter half-life and the kinetic characteristics of a saturable process (Fig. 2, from 2 to 4 days). Nevertheless no metabolites were detected in the HPLC chromatograms of plasma extracts of either dog or man.

Discussion

The data obtained after administration of 0·5 or 10 mg kg^{-1} of theophylline or furafylline to beagle dogs shows that although the times of appearance of each

Table 1. Subject characteristics and kinetic parameters after furafylline dosage (125 mg p.o.).

Volunteer	Age (years)	Height (cm)	Body weight (kg)	Plasma SCN$^-$ (nmol ml^{-1})[a]	C max (μg ml^{-1})	t max (h)	$t_{\frac{1}{2}}\beta$ (h)	$AUC_{0-96\,h}$ (μg h ml^{-1})
I	35	179	94	99	2·51	2	51·2	124·9
II	33	177	73	176	2·78	1·5	32·8[b]	107·3
III	23	180	78	21	3·07	2	58·4	165·1
IV	33	168	66	46	3·36	2	49·8	167·4
Mean	31	176	77·8	85·5	2·93	1·88	48·1	141·2
s.d.	5·4	5·4	11·9	68·5	0·37	0·25	10·8	29·8

[a] Normal range for smokers is 100–150 nmol ml^{-1} and for non-smokers 20–50 nmol ml^{-1} (Pettigrew & Fell 1972).
[b] After 48 h the $t_{\frac{1}{2}}$ decreased, see Fig. 2 and text.

compound in the plasma and the peak plasma concentrations are similar, the elimination of furafylline from the body is markedly prolonged and its elimination half-life is 2–10 times longer than theophylline, depending on the dose administered.

Theophylline is known to be eliminated mainly by metabolism, although its kinetics in dogs in the range studied in this report follows a linear relation since the AUC_{0-8h} is 3–4 μg h kg mg^{-1} ml^{-1} for both the 0·5 and the 10 mg kg^{-1} dose. When these parameters are calculated for furafylline a value of 3·87 μg h kg mg^{-1} ml^{-1} is found at the low dose level but a higher value of 7·83 μg h kg mg^{-1} ml^{-1} is found at the high dose level and this value would be much higher for $AUC_{0-\infty}$. An increase in the AUC and half life with increase in dose frequently reflects the saturation of an elimination process that is usually metabolism. However, if furafylline metabolites are formed they are not detectable by the analytical method used to quantify the unchanged compound and the relevance of metabolism in the disposition of furafylline remains to be clarified.

The estimated volumes of distribution of theophylline and furafylline in dogs obtained by simple extrapolation of the elimination phase on the 10 mg kg^{-1} dose plasma concentration versus time graph is similar for both drugs. Nevertheless the octanol/water partition coefficient of furafylline is 300 times higher than that of theophylline as a consequence of its stronger lipophilic character. Thus the storage of furafylline in deep compartments within the body should give rise to a larger distribution volume and lower blood levels than those of an equivalent dose of theophylline. However, the rapid elimination of the latter, even during the early absorption and distribution phases, probably precludes it from reaching higher blood levels after oral administration.

A low elimination rate (mean half-life of 48·1 h, with a range from 32·8 to 58·4 h) was also found when furafylline was given to humans and these values are 3–10 times greater than those reported for theophylline (range 3–15 h; Blaive et al 1981). A distribution phase is clearly visible up to 4 h after oral dosing (Fig. 2) whereas, when theophylline is given to humans the distribution phase is short and is only detected after intravenous administration (Buchanan 1979). Based on their lipophilic properties an extensive body distribution is envisaged for other 1,3,8-substituted xanthines, and is probably responsible, at least in part, for their high therapeutic potency as compared with theophylline (Grassi et al 1980, 1981; Cho et al 1981). The β phase of furafylline shows a monoexponential decline except for the volunteer with the more rapid elimination, the effect being detectable below 0·6 μg ml^{-1}. As observed in dogs, the elimination half-life of furafylline decreases when plasma levels become lower suggesting that a saturable step is present. However no metabolites are detectable in these samples. Since the therapeutic range in man as extrapolated from animal pharmacological data and a human pilot study appears to be between 1–3 μg ml^{-1} the therapeutic implication of the variable elimination rate of furafylline below 0·6 μg ml^{-1} is probably irrelevant.

The long half-life of furafylline within the proposed therapeutic range suggests that once daily administration would be adequate, a considerable improvement over the usual dosage regimen for theophylline. The comparison of blood thiocyanate levels with furafylline elimination half-lives (Table 1) shows that smoking will not greatly affect the disposition of the drug, another advantage over theophylline where *N*-demethylation is much faster in smokers than in non-smokers (Grygiel & Birkett 1981). The presence of an N^3-furfuryl group in furafylline seems to interfere with *N*-dealkylation. The presence of the C^8-methyl group makes C^8-oxidation to uric acid derivatives impossible and also results in a lower metabolic clearance of furafylline. Similarly a lower clearance and a longer elimination half-life compared with theophylline might be expected for other 3,8-substituted xanthines. The twice daily dosage established in humans for verofylline (3,7-dihydro-1,8-dimethyl-3-(2-methylbuthyl)-1*H*-purine-2,6-dione; Grassi et al 1981), the long lasting presence in the body of some metabolites of bamifylline (7-(2-(ethyl(2-hydroxy ethyl)amino)-ethyl)-3,7-dihydro-1,3-dimethyl-8-(phenylmethyl)-1*H*-purine-2,6-dione; Dodion & Aylward 1978) and the relatively long half-life (13·2 h) of mexafylline (3,7-dihydro-1,8-dimethyl-3-(3-cyclohexenyl methyl)-1*H*-purine-2,6-dione) in humans (Moragues et al 1982) are some data available to support this assumption.

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Antitumour effect of fibrinogen microspheres containing doxorubicin on Ehrlich ascites carcinoma

SHOZO MIYAZAKI*, NORIO HASHIGUCHI, CHIZUKO YOKOUCHI, MASAHIKO TAKADA, WEI-MIN HOU†, *Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tohbetsu, Hokkaido 061-02, Japan and † Department of Dosage Form, Shanghai Institute of Pharmaceutical Industry, 1320 Beijing Road West, Shanghai, China*

The antitumour activity of fibrinogen microspheres containing doxorubicin has been evaluated against Ehrlich ascites carcinoma in mice in terms of changes in body weight and survival. Tumour cell injections were made on day 0 and microsphere injections on day 1, both intraperitoneally. The suppressive effect of the drug-containing microspheres on increase in body weight was higher than that of the free drug, and tumour-bearing mice given the microspheres lived longer than those given the free drug.

Attempts have been made to deliver antitumour drugs to target sites within the tumour by means of drug delivery systems. The use of microspheres as sustained release targeting agents for antitumour drugs has received much attention. Albumin (Kramer 1974), gelatin (Yoshioka et al 1981), and polylactic acid (Juni et al 1985) have been investigated for use in drug delivery systems for antitumour drugs.

The possible use of fibrin film, a bioplastic prepared from human plasma, as a biodegradable carrier for drug delivery systems has been examined (Miyazaki & Nadai 1980; Miyazaki et al 1982). In addition to fibrin, the drug-carrier properties of fibrinogen in chemotherapy also appeared to be of interest and its potential use as microspheres forming an injectable, biodegradable system for the sustained release of drugs has been investigated. Fibrinogen, the precursor of fibrin, is a large, highly elongated, asymmetric molecule with a weight of approximately 340 000; it is usually used as a coagulant and rarely as a carrier of antitumour drugs (Szekerke et al 1972). Injectable microspheres prepared from fibrinogen should yield a novel biodegradable device from drug delivery.

This preliminary report describes the preparation, release characteristics in-vitro, and evaluation of antitumour activities of fibrinogen microspheres containing doxorubicin.

* Correspondence.

Materials and methods

Materials. Doxorubicin hydrochloride (Adriamycin) was generously supplied by Kyowa Hakko Kogyo Co., Tokyo. Fibrinogen from bovine blood (Type I-S) was purchased from Sigma Chemical Co., St Louis and used without further purification.

Preparation of the fibrinogen microspheres. Fibrinogen microspheres containing doxorubicin were prepared on the same principle as albumin microspheres (Scheffil et al 1972). Doxorubicin hydrochloride (30 mg) and bovine blood fibrinogen (100 mg) were each dissolved in 1 ml of distilled water and the solutions combined and mixed with 100 ml of 10% Span 85 in cottonseed oil, and homogenized (Nihonseiki Seisakusho, Type HB) for 10 min at 4500 rev min^{-1}. The emulsion was added to an additional 100 ml of cottonseed oil preheated to a desired temperature (140 °C), allowed to stand for 30 min with constant stirring, and then cooled to room temperature (20 °C). The microspheres were washed free of oil by adding 200 ml of ether, centrifuging for 10 min at 4500 rev min^{-1}, and decanting the supernatant. After the third wash, the microspheres were allowed to dry in a desiccator.

Measurement of release rate. Drug release from the fibrinogen microspheres was determined as described by Miyazaki & Nadai (1980), plastic dialysis cells with a cellulose membrane (Visking Co., Type 36/32) being used. The capacity of each half cell was 4 ml and the surface area of the membrane was 3·14 cm^2. Fibrinogen microspheres containing 1 mg of doxorubicin were suspended in 4 ml of 0·9% NaCl and the suspension placed in the donor compartment. An equal volume of 0·9% NaCl was placed in the receptor compartment. The assembled cell was shaken horizontally at the rate of 100 strokes min^{-1} in an incubator maintained at 37 °C. The total volume of the receptor solution was