J. Pharm. Pharmacol. 1989, 41: 418–420
Communicated December 20, 1988

© 1989 J. Pharm. Pharmacol.

Pharmacokinetics of 8-phenyltheophylline in the rat

A. WORMALD, C. J. BOWMER, M. S. YATES, *M. G. COLLIS, *Department of Pharmacology, Medical & Dental Building, The University of Leeds, Leeds LS2 9JT and *Bioscience II, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

Abstract—The pharmacokinetics of the adenosine antagonist 8-phenyltheophylline (8-PT) have been investigated in the rat. After intravenous administration to male rats with normal renal function, 8-PT was rapidly cleared from plasma with a $t_{\frac{1}{2}}$ of about 14 min. Its apparent volume of distribution was some 76 mL/100 g and plasma clearance was 3·5 mL min^{-1}/100 g. Injection via the carotid artery did not alter the kinetics of 8-PT, but when given through the portal vein a first-pass effect was observed. Moreover, 8-PT could not be detected in plasma following intraperitoneal injection. The kinetics of 8-PT were not altered in male rats with acute renal failure and no difference was noted between male and female animals with respect to the kinetics of 8-PT following intravenous injection. It is concluded that the pharmacokinetic properties of 8-PT are likely to complicate its use in-vivo.

8-Phenyltheophylline (8-PT) is an alkylxanthine which acts as a competitive adenosine antagonist in a variety of tissues (Smellie et al 1979; Bruns 1981; Collis et al 1984). This drug differs from the classical alkylxanthines, such as theophylline, in that it has little activity against cyclic adenosine monophosphate phosphodiesterases (Smellie et al 1979; Scotini et al 1983) and a greater potency as an adenosine receptor antagonist (Collis et al 1984). Recently we have reported that 8-PT is effective in attenuating the development of glycerol-induced acute renal failure in the rat (Bowmer et al 1986; Yates et al 1987). Consequently, adenosine antagonists like 8-PT might prove useful in defining the role of adenosine in the pathogenesis of acute renal failure.

Whereas the pharmacodynamics of 8-PT have been investigated in some detail (Collis et al 1984, 1986), little is known about its pharmacokinetic properties. The purpose of this communication is, therefore, to report the kinetic properties of 8-PT in anaesthetized rats. A knowledge of such properties should be of value in defining the factors that govern the efficacy of 8-PT in the whole animal. In addition, details are given of a high pressure liquid chromatography (HPLC) method developed to assay 8-PT in rat plasma.

Materials and methods

Materials. 8-PT, propyl paraben (n-propyl p-hydroxybenzoate), polyethylene glycol 400 and urethane were purchased from Sigma. Glycerol and HPLC grade acetonitrile and methanol were obtained from BDH Chemicals, UK. Heparin sodium (mucous) was supplied by Weddel Pharmaceuticals, UK.

Experimental protocol. Male and female Wistar rats (University of Leeds Laboratory Animal Service) were used and their mean body weights were 264±6 g (n = 25) and 266±10 g (n = 3), respectively. Acute renal failure was induced in one group of male animals (n = 3) by an intramuscular injection of 50% v/v glycerol (10 mL kg^{-1}) after dehydration for 24h (Thiel et al 1967). These rats were studied 48h after the injection of glycerol when their plasma urea levels were greater than 200 mg dL^{-1}. All rats were anaesthetized with an intraperitoneal injection (i.p.) of urethane (1·5g kg^{-1}) and a cannula was placed in the right carotid artery. This was used for the collection of blood samples and also for dosing in the intra-arterial (i.a.) studies. In some rats an additional cannula was inserted into the left jugular vein for intravenous (i.v.) administration of 8-PT. In a third group, a mid-line abdominal incision was made and the portal vein was exposed. These animals were dosed with 8-PT through a 23 gauge needle, attached to a short length of cannula, which was inserted into the portal vein and left in place for the duration of the experiment. A fourth group of rats received 8-PT by the i.p. route.

8-PT was dissolved in polythylene glycol 400:0·1 M NaOH (50:50 v/v) and administered at a dose of 10 mg kg^{-1} (10 mg mL^{-1}). Injections were made over 4 min and where appropriate, cannulae were flushed with a volume of 0·9% NaCl (saline) equal to twice the injected dose. Heparinized saline (500 units mL^{-1}) was placed in the arterial cannula to prevent blockage. Blood samples (200 μL) were taken before dosage (0h) and in most experiments at 10, 20, 30, 40, 50 and 60 min after the mid-point of injection. When 8-PT was given via the portal vein, blood samples were removed at 0, 10, 15, 20, 25 and 30 min. Samples were not taken after 30 min because of difficulty in maintaining animal survival beyond this time. Following i.p. injection, blood was taken at either 2·5, 5, 10, 15 and 30 min or at 30 min intervals for 4h. Blood was replaced with an equal volume of saline and the packed cell volume was about 8 per cent lower at the end of the sampling periods than at 0h.

HPLC assay. (i) Extraction. All plasma samples were assayed in duplicate. A 50 μL sample of plasma was diluted with 10 μL of 10 μg mL^{-1} propyl paraben (internal standard) dissolved in 0·01 M sodium phosphate-NaOH buffer, pH11. To this mixture a further 40 μL of buffer were added and 8-PT plus propyl paraben were extracted using ethylsilane bonded-silica columns (Bond-Elute C_2, 1 mL capacity, Analytichem). Columns were prepared for use by washing with two column volumes of acetonitrile followed by the same volume of water. An aliquot (40 μL) of sample and 200 μL of buffer were loaded onto each column which was then allowed to stand for 1 min before the mixture was drawn into the stationary phase by vacuum. Two minutes were allowed for equilibration and the columns were washed with 1 mL of water and dried under vacuum for 5 min. 8-PT and internal standard were eluted with 200 μL of methanol. The eluant was evaporated to dryness under N_2 at room temperature (20°C) and the residue was reconstituted in 200 μL of buffer.

(ii) Chromatography. Samples (50 μL) of reconstituted residue were chromatographed at room temperature with a 100 × 5 mm i.d. Radial-Pak cartridge packed with 4 μm octadecylsilane (C_{18}) bonded-silica (Waters Assoc.). The mobile phase was methanol–0·01 M sodium phosphate buffer, pH6·0 (50:50 v/v) with a flow rate of 0·8 mL min^{-1}. The chromatograph system consisted of a Model 510 constant volume pump; a U6K injector and a Model 440 ultraviolet detector (Waters Assoc.). Ultraviolet absorption was measured at 254 nm.

Correspondence to: C. J. Bowmer, Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9JT, UK.

Table 1. Pharmacokinetic parameters for 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) following: (a) intravenous (i.v.), intra-arterial (i.a.) and portal vein administration to male rats and (b) i.v. injection to male rats with acute renal failure and female rats with normal renal function.

(a) Route	AUC (μg min mL^{-1})	k (min^{-1})	$t\frac{1}{2}$ (min)	Clp (mL min^{-1}/100g)	V (mL/100g)
i.v. (n = 6)	297 ± 26†	0·0494 ± 0·0035	14·4 ± 1·2	3·50 ± 0·34	75·5 ± 13·8
i.a. (n = 3)	262 ± 26	0·0630 ± 0·0110	11·7 ± 2·0	3·86 ± 0·40	64·2 ± 10·0
portal (n = 6)	162 ± 19**	0·0389 ± 0·0076	21·2 ± 3·6	6·72 ± 0·99*	185 ± 16**
(b) Group					
Male rats with acute renal failure (n = 3)	266 ± 61	0·0475 ± 0·0055	15·0 ± 1·6	4·23 ± 1·02	90·6 ± 23·7
Female rats (n = 3)	277 ± 25	0·0566 ± 0·0030	12·3 ± 0·6	3·67 ± 0·31	64·8 ± 3·3

† mean ± s.e. mean.
* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$ compared to male rats injected i.v.

Analysis of results. Plasma concentration-time data were fitted to a mono-exponential equation by non-linear least squares regression analysis (Ralston 1983). This procedure provided estimates of k, the rate constant for elimination. The area under the concentration-time curve from t = 0 to t = ∞ after i.v. ($AUC_{i.v.}$) or portal (AUC_{portal}) injection; apparent volume of distribution (V); half-life ($t\frac{1}{2}$); hepatic extraction ratio and plasma clearance (Clp) were calculated from standard equations (Rowland & Tozer 1980). Results are given as mean ± s.e. mean and statistical analyses were done using the Mann-Whitney test.

Results

Fig. 1 shows a typical chromatogram of rat plasma in the absence and presence of 8-PT and propyl paraben. The retention times for these compounds were 11·2 and 14·3 min, respectively. No interfering peaks were noted in plasma from rats which had not received 8-PT, but in animals dosed with 8-PT an unidentified component was present with a retention time of 5·3 min (Fig. 1). It seems likely that this peak was due to the presence of a more hydrophilic metabolite of 8-PT. Optical density was a linear function of concentration over the range of 1 to 12 μg mL^{-1} in plasma (correlation coefficient = 0·99; n = 6) and the limit of sensitivity was about 250 ng mL^{-1}. Using peak-height ratios, the intra-assay coefficient of variation was 3·6% at 2 μg mL^{-1} (n = 10) and 5·3% at 10 μg mL^{-1} (n = 10) whereas the inter-assay variation was 3·4 and 7·9% (n = 5), respectively at these concentrations. The recovery of 8-PT from plasma samples was 96·9 ± 1·2% (n = 10) and 90·6 ± 2·8% (n = 10) at 2 and 10 μg mL.

The log concentration-time profiles for all the groups studied were linear over the times at which blood samples were collected and the data obtained following i.v., i.a. and portal vein injection of 8-PT (10 mg kg^{-1}) are shown in Fig. 2. In male rats, there were no statistical differences ($P > 0{\cdot}05$) between mean plasma concentrations after i.v. and i.a. injections and so, the kinetic parameters associated with these different routes of administration were similar (Table 1). When given via the portal vein, however, plasma levels of 8-PT were substantially less than those noted after i.v. injection (Fig. 2). Thus the mean AUC_{portal} was some 45% less than the mean $AUC_{i.v.}$ and the hepatic extraction ratio was about 0·45. In addition, both Clp and V were significantly greater ($P < 0{\cdot}05$) than the corresponding values obtained after i.v. injection (Table 1). No 8-PT could be detected in plasma from male rats following i.p. dosage from as early as 2·5 min to 4 h post-dose.

For rats with acute renal failure, the concentration-time data were superimposable upon those of male rats with normal renal function. Table 1 shows that V was about 30% larger in animals with renal failure, although this increase was not statistically significant. There were no significant changes in either Clp or $t\frac{1}{2}$ in these rats (Table 1). No difference in any of the calculated kinetic parameters was found between male and female rats after i.v. administration (Table 1). Therefore in the rat it is unlikely that there is any sex difference in the way in which 8-PT is handled after i.v. administration.

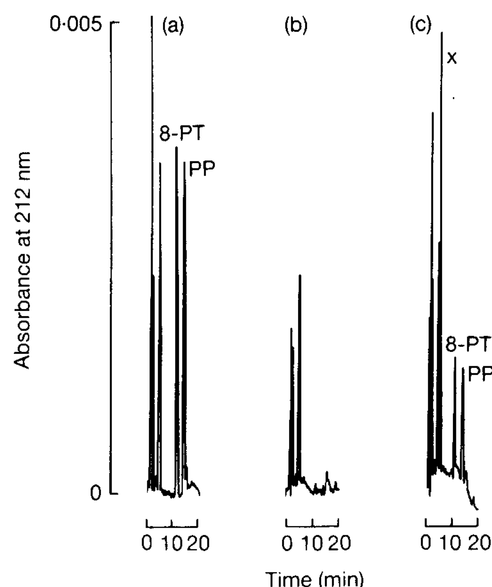

FIG. 1. Typical chromatograms of: (a) 8-phenyltheophylline (8-PT) and internal standard, propyl paraben (PP), when 60 and 20 ng, respectively were injected in 0·01 M sodium phosphate—NaOH buffer, pH 11; (b) rat plasma with no internal standard or 8-PT present and (c) plasma from a male rat that had received 8-PT (10 mg kg^{-1}; i.v.). In (c) an unidentified component is present and labelled x.

Discussion

In male rats the $t\frac{1}{2}$ of 8-PT was about 14 min and this is short by comparison with the related alkylxanthine theophylline which is reported to have a $t\frac{1}{2}$ of between 2 to 6 h (Williams et al 1979; Fruncillo & Digregorio 1984). It was not possible to monitor plasma levels beyond 60 min because at this time concentrations of 8-PT were close to the limit of sensitivity. As a result the duration of sampling might not have been long enough to allow detection of the terminal elimination phase and therefore, the value of $t\frac{1}{2}$ could have been underestimated. The rapid elimination of 8-PT was not the result of clearance by the lung as there was no difference in Clp between i.a. and i.v. routes of delivery. In addition, there would seem to be little clearance of drug by the kidneys because acute renal failure did not significantly affect Clp. If renal clearance were a major determinant of Clp, then after 48h of renal failure some perturbation of Clp should have

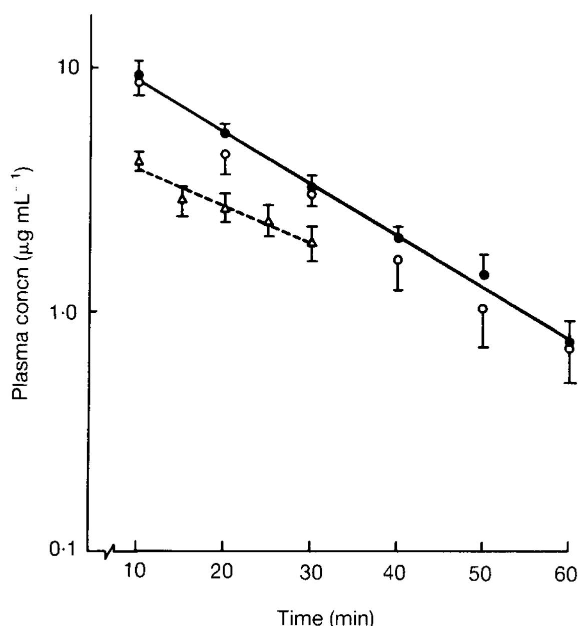

FIG. 2. Plasma concentration-time data following intravenous (●), intra-arterial (○) and portal vein (△) administration of 8-phenyltheophylline (8-PT; 10 mg kg^{-1}) to male rats. Data for female rats and animals with acute renal failure are not shown because they were not significantly different from data obtained for male rats after intravenous injection. Each point is mean ± s.e. mean of 3 to 6 observations.

been apparent since at this time renal function is severely impaired (Bowmer et al 1986). The lack of any detectable effect of renal failure on the kinetics of 8-PT together with negligible clearance by the lung, suggests that hepatic clearance makes the major contribution to Clp. This is supported by the substantial reduction in AUC following portal vein administration. The hepatic extraction ratio was estimated to be 0·45 and this is about four times greater than that reported for theophylline (Miller & Oliver 1986).

We could not detect 8-PT in plasma after i.p. administration. This is interesting because 8-PT when given i.p. at the same dose as used here is beneficial in reducing the severity of acute renal failure and antagonizes adenosine-induced bradycardia (Bowmer et al 1986; Yates et al 1987). 8-PT is poorly soluble in aqueous media (Daly et al 1985) and on examining the contents of the abdominal cavity, at the end of blood collection, it was found that 8-PT had precipitated at the site of injection. Consequently, a depot had been formed which would slowly release 8-PT into the peritoneal fluid and hence the systemic circulation. It is likely that dissolution rate-limited absorption coupled to first-pass clearance by the liver would have resulted in plasma levels which were not detectable using the present method of assay.

In a previous study of the pharmacodynamics of 8-PT we showed that a single dose of 10 mg kg^{-1} (i.v. or i.p.) antagonized adenosine-induced bradycardia in conscious rats for 3 to 5 h (Bowmer et al 1986). Recently we have found that this dose of 8-PT will block adenosine-induced reductions in renal blood flow for 2 h (Bowmer et al unpublished results). Thus there appears to be a discrepancy between the duration of 8-PT's effect and its half-life of some 14 min. There are several possible explanations for this. 8-PT could be sequestered in the tissues where it acts; an active metabolite may be produced or 8-PT is effective at concentrations in plasma which are much less than those that can be measured by the present assay. Clearly further work is needed to distinguish between these possibilities.

In sum, 8-PT is rapidly cleared from rat plasma; it undergoes first-pass removal by the liver and its apparent volume of distribution (76 mL/100 g) is similar to the total body water of the rat (69 mL/100 g; Spector 1956). When not injected directly into the systemic circulation, it is likely that 8-PT's bioavailability will be poor because of its limited water solubility and, for some routes of administration, first-pass hepatic clearance. Overall the physical and pharmacokinetic properties of 8-PT are likely to complicate interpretation of in-vivo studies that use 8-PT as an adenosine antagonist.

References

Bowmer, C. J., Collis, M.G., Yates, M. S. (1986) Effect of the adenosine antagonist 8-phenyltheophylline on glycerol-induced acute renal failure in the rat. Br. J. Pharmacol. 88: 205–212

Bruns, R. F. (1981) Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. Biochem. Pharmacol. 30: 325–333

Collis, M.G., Keddie, J. R., Torr, S. R. (1984) Evidence that the positive inotropic effects of alkylxanthines are not due to adenosine receptor blockade. Br. J. Pharmacol. 81: 401–407

Collis, M. G., Baxter, G. S., Keddie, J. R. (1986) The adenosine receptor antagonist, 8-phenyltheophylline, causes diuresis and saliuresis in the rat. J. Pharm. Pharmacol. 38: 850–852

Daly, J. W., Padgett, W., Shamin, M. T., Butts-Lamb, P., Waters, J. (1985) 1, 3-Dialkyl-8-(p-sulfophenyl) xanthines: Potent water-soluble antagonists for A_1- and A_2- adenosine receptors. J. Med. Chem. 28: 487–492

Fruncillo, R. J., Digregorio, G. J. (1984) Pharmacokinetics of pentobarbital, quinidine, lidocaine, and theophylline in the thermally injured rat. J. Pharm. Sci. 73: 1117–1121

Miller, R., Oliver, I. F. (1986) The influence of oxygen tension on theophylline clearance in the isolated perfused liver. J. Pharm. Pharmacol. 38: 236–238

Ralston, M. (1983) Derivative-free nonlinear regression. In: Dixon, W. J., Brown, M. B., Engelman, L., Frane, J. W., Hill, M. A., Jennrich, R. I., Toporek, J. D. (eds). BMDP Statistical Software. University of California Press, Berkeley, pp 305–314

Rowland, M., Tozer, T. N. (1980) Clinical Pharmacokinetics. Lea & Febiger, Philadelphia pp 82–85

Scotini, E., Carpendo, F., Fassina, G. (1983) New derivatives of methyl-xanthines: effect of thiocaffeine, thiotheophylline and 8-phenyltheophylline on lipolysis and phosphodiesterase activities. Pharmacol. Res. Commun. 15: 131–143

Smellie, F. W., Davis, C. W., Daly, J. W., Wells. J. N. (1979) Alkylxanthines: inhibition of adenosine—ellicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. Life Sci. 24: 2475—2482

Spector, W.S. (1956) Handbook of Biological Data. W. B. Saunders Company, Philadelphia and London, p 340

Thiel, G., Wilson, D. R., Arce, M. L., Oken, D. E. (1967) Glycerol induced hemogloburinuric acute renal failure in the rat. II. The experimental model, predisposing factors, and pathophysiologic features. Nephron 4: 276–297

Williams, J. F., Lowitt, S., Szentivanyi, A. (1979) Effects of phenobarbital and 3-methylcholanthrene pretreatment on the plasma half-life and urinary excretion profile of theophylline and its metabolites in rats. Biochem. Pharmacol. 28: 2935–2940

Yates, M. S., Bowmer, C. J., Kellett, R., Collis, M. G. (1987) Effect of 8-phenyltheophylline, enprofylline and hydrochlorothiazide on glycerol-induced acute renal failure in the rat. J. Pharm. Pharmacol. 39: 803–808