BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **19**: 97–100 (1998)

No Effect of Chloroquine on Theophylline Pharmacokinetics in the Rat

Bedassa A. Motti[a,b,1] and Michael Ashton[a,*]

[a] *Department of Biopharmaceutics and Pharmacokinetics, Uppsala University, BMC, Box 580, S-751 23, Uppsala, Sweden*

[b] *Department of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia*

ABSTRACT: The immediate and delayed effects of chloroquine on theophylline kinetics were investigated in rats pretreated with chloroquine diphosphate (45 mg kg^{-1}) or saline intraperitoneally. One hour or 4 days after chloroquine, theophylline (10 mg kg^{-1}) was administered intravenously. Compared with the control animals pretreated with saline, the disposition parameters of theophylline was not altered after pretreatment with chloroquine. Chloroquine did not affect the *in vivo* metabolism of theophylline in the laboratory rat. A possible decrease in theophylline's volume of distribution at 4 days, but not immediately, after administration of chloroquine was suggested, although this just failed to achieve statistical significance ($p = 0.055$). Being marginal, it is unlikely to be of clinical concern. It is concluded that, judged from these animal data, there is no evidence of a drug–drug pharmacokinetic interaction for the combination of chloroquine and theophylline. © 1998 John Wiley & Sons, Ltd.

Key words: chloroquine; theophylline; pharmacokinetics; rat

Introduction

In spite of a challenge posed to it by resistant strains of malaria parasites, chloroquine is still the most widely used antimalarial drug. Because it is cheap, widely available, and well tolerated, it is still the drug of choice in areas where occurrence of resistant strains of malaria parasites has not been reported [1]. Chloroquine in rats [2] and in man [3] has been shown to have a long elimination half-life of at least 1 month due to extensive tissue binding. Although the drug, because of its long elimination half-life, has a high potential for causing drug–drug interactions, this aspect of chloroquine's pharmacokinetics, like that of many other antimalarial drugs, has received meagre attention [3]. Acute pretreatment of rats with chloroquine had no effect on pentobarbitone sleeping time or zoxazolamine paralysis time, but decreased antipyrine clearance significantly [4]. Chloroquine inhibited aminopyrine N-demethylase activity *in vitro*. There was no evidence of induction following chronic administration of chloroquine (50 mg kg^{-1}) over 4 days, but there was an apparent decrease in P-450 content and aminopyrine N-demethylase activity [4]. In the present study the immediate and delayed effect of chloroquine on theophylline pharmacokinetics in the rat was investigated.

Materials and Methods

Materials

Chloroquine diphosphate, theophylline, and the internal standard 7-(2-hydroxyethyl)-theophylline were purchased from Sigma (St. Louis, MO, USA). All other chemicals were obtained from usual commercial sources and were of analytical grade.

Animals

Outbred male Sprague–Dawley rats weighing 250–360 g (A-Lab, Sollentuna, Sweden) were housed in groups of two in polycarbonate (Macrolone) cages (Koge, Denmark), with a commercial wood shaving as bedding (B&K Universal AB, Sollentuna, Sweden), at the animal stables of the Biomedical Centre, Uppsala University. The animals were kept in a room with a 12 h light–dark cycle (lights from 07:00 to 19:00) and controlled temperature and humidity of 22.2°C and $55 \pm 5\%$ respectively. The animals were acclimatized for a period of at least 1 week during which they were provided a commercial food (Lactamin R 36® pellets, Vadstena, Sweden) and normal tap water, before starting the experiment. One day before theophylline administration, the right jugular vein and the right carotid artery of the animals were catheterized (PE 50 polyethylene

* Correspondence to: Michael Ashton, Department of Biopharmaceutics and Pharmacokinetics, Uppsala University, BMC, Box 580, S-751 23, Uppsala, Sweden.

[1] Deceased.

Contract grant sponsor: United Nations Development Agency

CCC 0142–2782/98/020097–04$17.50

Received 11 April 1994
Accepted 3 March 1997

Table 1. Theophylline pharmacokinetic parameters after an intravenous bolus dose of 10 mg kg^{-1} in rats who had received an single, intraperitoneal dose of 45 mg kg^{-1} chloroquine diphosphate, or saline, 1 h or 4 days prior to theophylline (mean ± S.D.)

Treatment	$t_{1/2}$ (min)	Cl (mL min^{-1} kg^{-1})	V^1_{SS} (L kg^{-1})	Cl_r (mL min^{-1} kg^{-1})	Cl_{nr} (mL min^{-1} kg^{-1})	Body weight (kg)
1 h after administration of						
chloroquine ($n = 9$)	332 ± 88	1.20 ± 0.25	0.54 ± 0.06	0.22 ± 0.12	0.98 ± 0.20	0.316 ± 0.054
saline ($n = 7$	333 ± 10	1.34 ± 0.54	0.57 ± 0.06	0.45 ± 0.47	0.89 ± 0.35	0.287 ± 0.047
4 days after administration of						
chloroquine ($n = 6$)	348 ± 88	1.36 ± 0.36	0.45 ± 0.07	0.24 ± 0.13	1.12 ± 0.29	0.293 ± 0.024
saline ($n = 6$)	326 ± 54	1.22 ± 0.20	0.56 ± 0.01	0.19 ± 0.11	1.03 ± 0.25	0.297 ± 0.016

catheter, OD 0.965 m, Clay Adams, Parsippany, NJ, USA) under light ether anaesthesia. The catheters were passed under the skin and exteriorized at the back of the neck. After surgery, the animals were individually placed in their cages and allowed to recover overnight. The animal protocol was approved by the Experimental Animal Ethics Committee of Uppsala.

Drug Administration

At 24 h after surgery, the animals involved in the immediate effect experiment were given 45 mg kg^{-1} of chloroquine diphosphate (20 mg L^{-1} in saline) intraperitoneally ($n = 9$) while control animals received an equal volume of saline ($n = 7$). One hour after chloroquine or saline administration, the animals were placed in metabolic cages, and 10 mg kg^{-1} theophylline (4 mg mL^{-1} in saline) was administered through the jugular catheter over a period of 1 min. The delayed effect experiment was performed in a manner identical to the previous one, except that chloroquine ($n = 6$) or saline ($n = 6$) was administered 3 days prior to surgery. Theophylline amounts and concentrations are given as anhydrous base.

Urine and Plasma Sample Collection

Blood samples (0.25 mL) were collected into heparinized plastic tubes at 5, 15, 30, 45, 60, 120, 124, and 480 min after the start of the theophylline administration. The blood loss incurred at each collection time was replaced by an equivolume of saline. The plasma, obtained by centrifugation of the blood at $13000 \times g$ for 5 min, was stored at −20°C pending analysis. Each animal was left in a metabolism cage overnight, and, after 24 h, the cage was rinsed with distilled water to quantitatively obtain the urine with the sample kept at −20°C until analysed. To prevent loss of urine into food material, the animals were provided with water only starting from the time of theophylline administration to the completion of urine collection.

Pretreatment of Urine and Plasma Samples for HPLC Analysis

Urine and plasma samples, thawed at room temperature, were centrifuged, the latter after precipitation of plasma proteins with methanol [5]. A 20 μL aliquot was mixed with 200 μL methanolic solution of the internal standard (4 mg mL^{-1}). After centrifugation at $13\,000 \times g$ for 5 min, 20 μL of the supernatant was injected onto the HPLC system.

HPLC Analysis of Theophylline in Plasma and Urine

The chromatographic system consisted of a LDC ConstatMetric model III pump, a Rheodyne 7125 injector, and LDC SpectroMonitor III UV detector set at 280 nm and connected to a Hewlett Packard 3390A integrator. Separation was performed on a 200 mm stainless steel column (ID 4.6 mm) packed with 5 μm C_{18} ODS-Hypersil. The mobile phase was acetonitrile–acetate buffer (0.01 M, pH 4.0), 1:15 v/v, maintained at a flow rate of 1.5 mL min^{-1}.

Blank rat plasma or, for urine samples, distilled water was spiked with aqueous solutions of theophylline to obtain standard samples with final concentrations of 2, 10, 50, and 100 μg mL^{-1}. The intra-day coefficient of variation of the analysis of eight replicates of spiked plasma sample at a concentration of 2 μg mL^{-1} was 5.6%.

Pharmacokinetic and Statistical Data Analysis

The pharmacokinetic parameters of theophylline for individual rats were determined by noncompartmental methods. The elimination rate constant (k) was calculated by log–linear regression of the last three to five plasma concentration–time points. Half-life ($t_{1/2}$) was calculated as $0.693/k$. The area under the plasma concentration time curve (AUC) was calculated by the log trapezoidal rule with extrapolation to infinity by dividing the estimated plasma concentration at the last time-point (from the log–linear regression) by the elimination rate constant. Total plasma clearance (Cl) was determined as Cl = dose/AUC . Renal clearance (Cl_r) was calculated as $Cl_r = f_e \times Cl$, where f_e is the fraction of

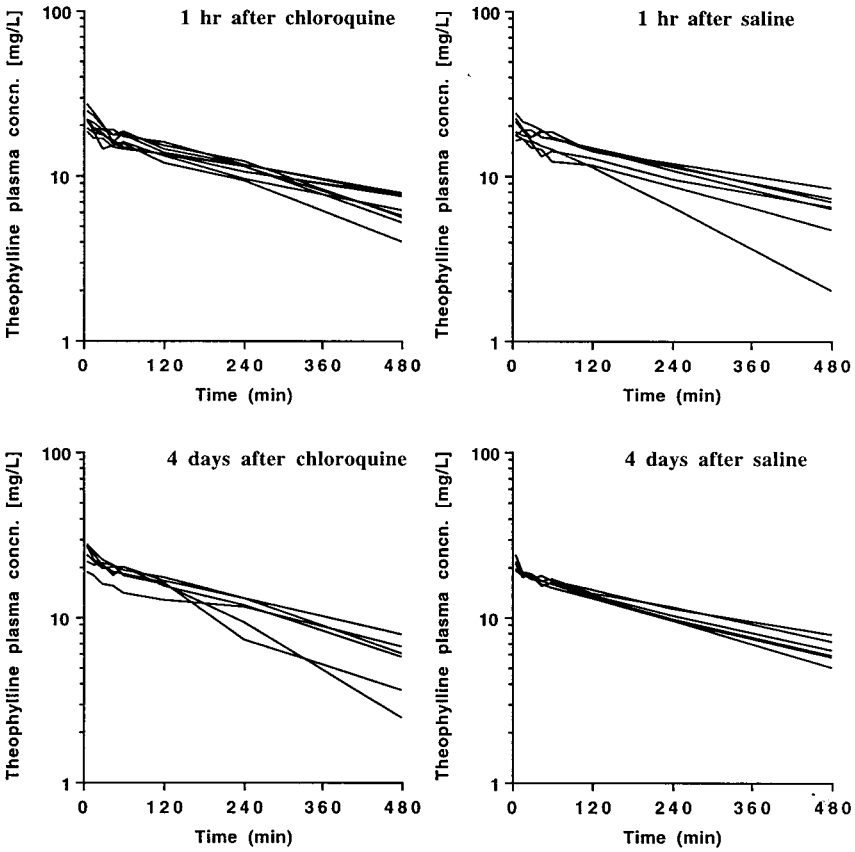

Figure 1. Theophylline plasma concentrations in individual rats ($n = 6$–9) pretreated with 45 mg kg^{-1} chloroquine diphosphate or saline 1 h or 4 days prior to an intravenous bolus dose of 10 mg kg^{-1} theophylline (anhydrous base)

theophylline excreted unchanged renally, determined by dividing the total amount of theophylline recovered in urine by the administered intravenous dose. Non-renal clearance (Cl_{nr}) was estimated as $Cl_{nr} = Cl - Cl_r$. The steady state volume of distribution (V_{SS}) was estimated as $V_{SS} = \text{dose} \times \text{AUMC}/\text{AUC}^2 - \text{infusion duration}/2$ where AUMC refers to the area under the first moment curve [6]. Different treatment groups were compared by the Mann–Whitney U-test. The minimum level of significance was set at $p < 0.05$.

Results

Administration of chloroquine did not alter theophylline's disposition parameters (Table 1). Importantly, there was no effect on theophylline clearance values. Plasma concentrations of theophylline in rats pretreated with chloroquine 4 days before theophylline administration (Figure 1) were slightly elevated. The difference in theophylline's distribution volume (L kg^{-1}) did not reach statistical significance when compared with saline on day 4 ($p = 0.055$) but was lower compared with values 1 h after both chloroquine ($p = 0.013$) and saline ($p = 0.022$). No other effect was indicated between groups for any pharmacokinetic parameter or for the fractions of total areas extrapolated to infinity for AUCs (overall mean 33%) or AUMCs (68%).

Discussion

The present study shows a lack of a metabolic interaction between chloroquine and theophylline in the laboratory rat. Since the drug exhibited a low extraction ratio these results are not confounded by organ blood flow restrictions. However, due to species differences, caution is necessary when extrapolating the lack of *in vivo* metabolic interaction from the rat to humans. Interestingly, a possible effect of chloroquine on theophylline's tissue binding was indicated. Studies conducted both in the rat [2] and in man [7] have shown chloroquine to undergo an extensive tissue binding. The rate of chloroquine distribution into and out of tissues appears to be very slow with times to peak tissue concentrations in the rat of approximately 22 h [2]. A decrease in the distribution volumes of theophylline in animals pretreated with chloroquine 4 days before theophylline administration could be a result of chloroquine's ability to decrease the tissue binding of theophylline. Administration of 8-methoxysoralen has been associated with a decreased steady state distribution volume of theophylline [8]. In our

study, there was no indication of a change in distribution volume in animals pretreated with chloroquine 1 h before theophylline administration, compatible with the fact that chloroquine, with its slow tissue distribution, would not yet have reached tissue concentrations high enough to cause a displacement. Once occurring, the interaction would be expected to linger. A decrease in distribution volumes due to increased plasma protein binding is not a likely explanation for theophylline and moreover would be expected to have been evident also within 1 h after chloroquine administration. Because of the similarity in tissue binding of chloroquine in man [7] and in the rat [2], it is possible that chloroquine may also decrease theophylline's tissue binding in humans. However, the effect of chloroquine on theophylline's distribution volume, if present, is altogether too marginal to raise concern for dose adjustment, even for theophylline with its narrow therapeutic window.

Acknowledgements

The United Nations Development Agency (UNDP) is acknowledged for support of B.A. Motti during his studies at Uppsala University.

References

1. L.T. Webster, Drugs used in the chemotherapy of protozoaol infections. Malaria. In *The Pharmacological Basis of Therapeutics*, 7th edn, A.G.L.S. Goodman, T.W. Rall, and F. Murad (Eds), Macmillan, New York, 1985, pp. 1029–1048.
2. S.A. Adelusi and A.L. Salako, Kinetics of the distribution and elimination of chloroquine in the rat. *Gen. Pharm.*, **13,** 433–437 (1982).
3. N.J. White, Clinical pharmacokinetics of antimalarial drugs. *Clin. Pharmacokinet.*, **10,** 187–215 (1985).
4. J.D. Back, Inhibition of drug metabolism by the antimalarial drugs chloroquine and primaquine in the rat. *Biochem. Pharmacol.* **32,** 257–263 (1983).
5. G.P. Butrimovitz and V.A. Raisys, An improved micromethod for theophylline determination by reversed-phase liquid chromatography. *Clin. Chem.*, **25,** 1461–1464 (1979).
6. D. Perrier and M. Mayerson, Noncompartmental determination of the steady-state volume of distribution for any mode of administration. *J. Pharm. Sci.*, **71,** 372–373 (1982).
7. L.L. Gustafsson, O. Walker, Alvan, Disposition of chloroquine in man after single intravenous and oral doses. *Br. J. Clin. Pharmacol.* **15,** 471–479 (1983).
8. G. Apseloff, D.R. Shephard, M.A. Chambers, S. Nawoot, D.C. Mays and N. Gerber, Inhibition and induction of theophylline metabolism by 8-methoxysoralen *in vivo* study in rats and humans. *Drug Metab. Disposit.*, **18,** 298–303 (1990).