Br J clin Pharmac 1995; **39**: 289–295

Pharmacokinetics and adverse reactions after a single dose of pentamidine in patients with *Trypanosoma gambiense* sleeping sickness

U. BRONNER[1], L. L. GUSTAFSSON[1], F. DOUA[2], Ö. ERICSSON[1,3], T. MIÉZAN[2], M. RAIS[1] & L. ROMBO[1]
[1]Unit of Tropical Pharmacology, Divisions of Clinical Pharmacology and Infectious Diseases, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden, [2]Projet de Recherches Cliniques sur la Trypanosomiase (P.R.C.T.), Daloa, Côte d'Ivoire and [3]Hospital Pharmacy, Huddinge University Hospital, Huddinge, Sweden

1 Plasma concentrations of pentamidine were measured up to 1–8 months after a single 2 h i.v. infusion of 3.0 to 4.8 mg kg^{-1} pentamidine isethionate in 11 patients with late stage *Trypanosoma gambiense* sleeping sickness.

2 Maximum plasma drug concentrations varied between 713 and 2461 nmol l^{-1}. After termination of infusion, a rapid distribution phase over 10 min was followed by a slower distribution phase and an elimination phase prolonged over weeks to months.

3 The 'terminal' elimination rate constant could be determined in six patients and subsequent kinetic calculations showed a three to fourfold variation in plasma clearance and 'terminal' half-life (median 1126 (range 553–2036) ml min^{-1} and 265 (107–446) h, respectively). The median apparent volume of distribution (V_{ss}) was 11 850 l. Renal clearance accounted for a median of 11% of total plasma clearance, indicating that metabolism is a major route of pentamidine elimination in man.

4 Side effects were few and mild and a slight or moderate decrease in blood pressure was the most common registered adverse reaction observed in four subjects.

5 The prolonged elimination of pentamidine seems inconsistent with the present recommended dosage regimen of pentamidine for treatment of trypanosomiasis of 7 to 10 parenteral doses given once daily or every second day.

Keywords pentamidine pharmacokinetics adverse reactions *Trypanosoma gambiense* sleeping sickness

Introduction

The pharmacokinetics of pentamidine are poorly characterized despite its use over many years for the treatment of early stage sleeping sickness caused by *Trypanosoma gambiense*. The recommended dosage schedule is 7 to 10 intramuscular injections or intravenous infusions given daily or every second day [1]. Adverse effects, such as nephrotoxicity and diabetes mellitus [2], cause problems when treating patients in tropical Africa. A recent *in vitro* study indicates that pentamidine has a concentration-dependent parasiticidal effect on *T. gambiense* parasites [3], but the minimal parasiticidal concentration *in vivo* is not known and proper dose-finding studies have not been conducted.

H.p.l.c. assays [4, 5] are now being used for pharmacokinetic studies of pentamidine in AIDS patients with *Pneumocystis carinii* pneumonia (PCP) [6–8] and in patients with trypanosomiasis in West Africa [9]. These studies indicate that the drug accumulates during repeated dosing [6–9] and that the 'terminal' elimination half-life is in the order of 12 days [7]. There is considerable interindividual variation in plasma drug concentrations which, in part, has been explained by variation in muscular blood flow affect-

Correspondence: Dr Ulf Bronner, Division of Infectious Diseases, I73, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

ing absorption from the injection site after intramuscular administration [9]. Renal clearance is reported to account for only 2% of plasma clearance [7] which implies that metabolism is an important route of elimination of the compound.

In order to be able to conduct dose-finding clinical trials, detailed data on the pharmacokinetics of pentamidine in patients with sleeping sickness are needed. Such information is not available as the studies of Conte [6, 7] were performed in heterogenous groups of AIDS patients and our own previous study on trypanosomiasis patients only included blood sampling for 2 days after intramuscular administration of the drug [9]. We have now characterized the single-dose pharmacokinetics of pentamidine and its variability in patients with *Trypanosoma gambiense* sleeping sickness by collecting blood samples for up to 8 months after drug administration as well as urine samples. Adverse effects were also investigated by clinical and laboratory monitoring.

Methods

Patients

Between November 1990 and July 1992, 181 patients with late stage *Trypanosoma gambiense* sleeping sickness were treated at Projet de Recherches Cliniques sur la Trypanosomiase (P.R.C.T.) in Daloa, Côte d'Ivoire. Only patients living close to the Centre could take part in the investigation as they had to visit repeatedly for follow-up. Twelve of the 181 patients were included in the study. One was excluded from data analysis owing to a malfunction of the infusion pump.

Before therapy, all patients had a positive plasma card agglutination test for trypanosomiasis (Testryp CATT®, Institute of Tropical Medicine Prince Léopold, Department of Serology, Antwerp, Belgium), and trypanosomes were detected in the peripheral blood by the mini-anion exchange centrifugation technique (mAECT) [10]. Using the double centrifugation technique [11], parasites were also found in the cerebrospinal fluid (CSF) in all but one individual (7), and every patient had an increased CSF white cell count (median 354, range 34–1390 $\times$ 10^6 l^{-1}) (Neubauer haemacytometer chamber). Thus, the 11 subjects were considered to suffer from late stage disease according to the WHO criteria [1]. The routine therapy for late stage trypanosomiasis at the Centre was followed; i.e. a single dose of pentamidine before the administration of three series of intravenous melarsoprol injections, treatment with phenobarbitone and corticosteroids to avoid encephalopathy and oral chloroquine for therapy of a possible malaria infection.

All patients complained of headache and fever with a median duration of 12 (range 3–24) months for both symptoms. Nine patients also suffered from tiredness (median duration 6 (2–12) months) and all reported itching. Other symptoms at admission were impotence and abdominal pain in five and two patients, respectively. Physical examination revealed cervical lymphadenopathy and the presence of archaic reflexes in nine patients, hyperpathia in eight subjects, tremor in five and unsteady gait in four patients. One individual had generalized lymphadenopathy. Two patients were euphoric and another three behaved indifferently. One patient was in stupor. The median age was 26 (range 19–42) years and the median weight was 63 (50–84) kg. Two patients were females. Sera from all patients were tested for the presence of HIV-antibodies by ELISA. Positive results in patients 4 and 5 (both males, aged 25 and 32, respectively) were confirmed by subsequent Western Blot analyses.

Study design

The contents of 300 mg ampoules of pentamidine isethionate (Pentacarinat®, May & Baker, Rhône-Poulenc Rorer, Dagenham, England), were dissolved in 3 ml sterile water and added to 500 ml 5% w/v glucose. A single dose of the pentamidine solution was given as a 2 h constant rate intravenous (i.v.) infusion using a pump (IMED 960R, San Diego, California, USA). The exact administered dose of pentamidine for each patient was assessed by taking two samples from the i.v. catheter for analysis of the pentamidine concentration in the glucose solutions; before and immediately after the infusion. The dose varied between 3.0 and 4.8 mg^{-1} kg pentamidine isethionate in the 11 patients (Table 1).

Venous blood samples for assay of plasma pentamidine were drawn through an indwelling i.v. catheter before infusion, and at 0.25, 0.50, 1.0, 2.0 h, 2 h and 5 min, 2 h and 10 min, 2 h and 20 min, 2.5, 3 and 8 h after start of the pentamidine infusion. Additional blood samples were obtained by separate venepunctures at 24 and 48 h, and at 4, 7, 10, 14 and 29–34 days after pentamidine infusion. In patients 1–6 and 8–10, blood samples were also collected at 4 to 8 months after dose.

Urine was collected from 0–24 and 24–48 h after the start of the pentamidine infusion.

The study design was approved by the Ministry of Health, Abidjan, Côte d'Ivoire and by the Ethics Committee of the Karolinska Insititute, Stockholm, Sweden. All subjects or their accompanying relatives (if the mental status of the patient was deranged) gave verbal consent to the study after explanation of the protocol.

Concomitant medication

Pentamidine was given 3–8 days before treatment with melarsoprol (Arsobal®, Spécia, Paris) started. The latter drug was given in three series of daily i.v. injections for 4 days separated by an interval of 10 days. To reduce the risk of melarsoprol-related encephalopathy the patients were also given 100 mg phenobarbitone (Gardenal®, Spécia, Paris) orally once daily during the melarsoprol-treatment period. However, the plasma concentration of phenobarbitone, assayed by h.p.l.c. [12], in blood samples obtained at 1, 2, 4, 7, 14 and 29–34 days after pen-

tamidine administration, showed that only three patients (2, 3 and 10) had uniformly detectable concentrations. The plasma phenobarbitone concentrations varied between 20 and 86 μmol l^{-1}, except in patient 10 whose levels were in the range 7–22 μmol l^{-1} (the therapeutic range for treatment of convulsive disorders is 40–135 μmol l^{-1}). A majority of the subjects were also given a single intramuscular injection of betamethasone (Diprostene®, Schering-Plough, Levallois-Perret, France). Five patients were prescribed chloroquine orally (Nivaquine®, Spécia, Paris) for therapy of possible malaria infection.

Adverse reactions

Adverse reactions were registered by open enquiry, as well as from 19 specific questions, before infusion, every 30 min during the infusion, at 3, 8, 24 and 48 h and at 4 days after the beginning of the infusion of pentamidine. The specific questions related to symptoms previously reported during use of the compound. If any symptom was present, the patient was asked to rate its severity on a five-graded scale (insignificant, mild, moderate, severe, very severe). Blood pressure, measured in the supine position with a stethoscope and blood pressure cuff, was monitored at the same times up to 48 h after start of drug administration.

Collection of blood samples for determination of haemoglobin, white blood cell count and platelets as well as serum analyses of glucose, creatinine, urea, calcium, alkaline phosphatase, ASAT, ALAT, γ-glutamyltransferase and lactate dehydrogenase were performed before infusion and 1, 4, 14 and 30 days later. Glucose, albumin, leukocytes and erythrocytes were tested by urine strips on the same occasions as the serum samples were taken and also at 7 days after the infusion.

Handling of samples

Blood was collected in 4.5 ml Vacutainer® tubes containing ethylene diaminetetraacetic acid (EDTA) and centrifuged within 15 min in a Beckman refrigerated centrifuge (Beckman Instruments, Palo Alto, USA) at 1000 *g* for 10 min. The plasma was transferred to a NUNC® tube (InterMED, Roskilde, Denmark) and frozen at −20° C within a few minutes.

Urine was collected in 2.5 l plastic (high density polyethylene) containers (LAGAN-plast, Ljungby, Sweden), the volume was measured and 10 ml aliquots were transferred to two NUNC® tubes and frozen as above. Both plasma and urine were kept frozen at −20° C pending assay.

Blood for clinical chemistry was obtained in 10 ml Vacutainer® tubes. After coagulation at room temperature (25° C), the sera were transferred to polyethylene tubes (Sarstedt, Nymbrecht, Germany) and then kept frozen at −20° C until analysis in Sweden. The haematological tests as well as the urine strip tests were performed at the Research Centre on the sampling day.

Drug analyses

Drug concentrations in 100–500 μl of plasma were analysed in duplicate using an h.p.l.c. assay previously described by us [5] with a few modifications. In order to increase the sensitivity, a Waters 470 fluorescence detector (Waters Associates, Milford, USA) was used. The eluent was a mixture of 130 ml acetronitrile, 650 ml water, 10 ml tetrahydrofuran, 3 ml triethylamine and 2 ml phosphoric acid (pH 2.8). A Gilson 231 autoinjector (Gilson Medical Electronics Inc., Middleton, USA) was used. These changes made it possible to determine drug concentrations in plasma down to 3 nmol l^{-1} with a coefficient of variation of 4.3%. Below 3 nmol l^{-1} the CV exceeded 10% and this concentration was therefore defined as the lower limit of determination in plasma.

Urine aliquots of 250–1000 μl were analysed in duplicate. The urine was diluted 1:10 with water before analysis. The coefficient of variation of the urine assay was 6% at 28 nmol l^{-1}.

Seven oxidized metabolites of pentamidine have previously been detected in experiments with isolated rat livers [13]. None of them interfered with the assay, nor did melarsoprol, phenobarbitone, betamethasone, chloroquine, doxycycline, ampicillin, sulphamethoxazole, paracetamol or acetylsalicylic acid. Penicillin V and trimethoprime caused base-line irregularities but such irregularities did not occur during the analysis of the samples.

Pharmacokinetic analysis

Non-compartmental methods were used for estimation of the pharmacokinetic parameters. The elimination rate constant (λ_z) and the half-life ($t^{1}/_{2,z}$) during the last observed period of monophasic decline were assessed by visual inspection of the log plasma concentration *vs* time curve and calculated by log-linear regression using 4–6 sample points. AUC values were calculated using the linear trapezoidal rule. The area to infinite time was obtained by dividing the last determined concentration by λ_z. AUMC, the area under the first moment of the concentration *vs* time curve, was determined by the linear trapezoidal rule with extrapolation to infinity using AUMC = $t_z C_z/\lambda_z + C_z/(\lambda_z)^2$, where C_z was the concentration at the last sampling time t_z. Plasma clearance was calculated from Dose/AUC and V_{ss} from [(Dose × AUMC)/AUC^2] − [(Dose × T)/2 × AUC], where T was the duration of infusion. MRT, the mean residence time, was calculated from AUMC/AUC. Renal clearance was calculated from *Ae*/AUC, where *Ae* was the amount of pentamidine base extracted unchanged in the urine per collection period.

Results

Adverse reactions

Systolic and diastolic blood pressures decreased 10–30 mm Hg in four patients (2, 3, 7 and 9). The

decrease in blood pressure occurred within 0.5 to 8 h after the beginning of the intravenous infusion and pressure remained low at 48 h in patients 2, 3 and 7. In patient 9 blood pressure returned to normal at 24 h. The lowest recorded blood pressure was 90/50. None of the patients had any symptoms considered to be due to the lowered blood pressure, except for patient 2 who had mild vertigo. In one patient (1), the blood pressure increased from an initial value of 120/70 mm Hg to 150/90 at the end of 2 h infusion. The same patient complained of slight pain in the arm used for infusion until the infusion stopped. His blood pressure returned to normal at 48 h after drug administration. Patient 7 spontaneously reported hypersalivation at 3 h after beginning of infusion but did not have any symptoms later.

Two patients (7 and 8) showed signs of a liver reaction 30 days after drug administration, with elevated ASAT, ALAT, alkaline phosphatase, γ-glutamyltransferase and lactate dehydrogenase (2–15 × pretreatment values), However, at that time they had received two series of i.v. melarsoprol injections. All other haematological measurements and serum analyses were unchanged during the study period. Another two subjects (9 and 10) developed erythrocyturia on the 4th day after pentamidine administration. Erythrocytes were still detected 26 days later.

Drug disposition

Plasma The pharmacokinetic data of all patients are summarized in Table 1. Maximum plasma drug concentrations were generally attained by the end of the 2 h infusion period (median value 923 nmol l^{-1}) (Figure 1a, Table 1). The plasma concentration decreased temporarily during i.v. infusion in three patients. In another three the concentration had already started to fall by the end of infusion (Figure 1a). After termination of the infusion, there was a rapid distribution phase resulting in a sharp decline in plasma drug concentrations to a median of 32 (range 23–52)% of initial values within 10 min. During the following hours, a slower distribution followed (Figure 1a). The 'terminal' elimination phase occurred over a prolonged period of time as shown in Figure 1b. In most patients, the plasma drug concentrations at the sampling points beyond 14 days after dose were below the lower limit of determination (Figure 1b). Seven plasma samples obtained after approximately 4 months and another seven samples taken 7–8 months after drug administration, were available for analysis. In the sample drawn from patient 3 at 7 months, pentamidine was detected in plasma but the concentration was too low to be determined accurately. In the other samples, pentamidine was not detected.

The 'terminal' elimination rate constant (λ_z) and the other kinetic parameters could not be determined in five of the subjects. Patients 1 and 7 showed an increase in plasma drug concentrations during the later part of the sampling period and it was, therefore, not possible to define λ_z. In patients 2 and 5, plasma drug concentrations were already below the lower limit of determination after 4 days and 48 h, respectively, which made estimation of the elimination phase impossible. In patient 3, the *r* value for the log-linear regression over 7 to 14 days was –0.55 and the residual AUC was estimated at 53% of the total AUC. The plasma drug concentrations in the five patients excluded from the pharmacokinetic analysis are shown in Figure 1c. The median elimination half-life in the remaining six patients was 265 h (first data point at 24–168 h and last data point 168–816 h). In these subjects, values of CL and V_{ss} varied threefold; median values were 1126 ml min^{-1} and 11 850 l, respectively. Differences in MRT were more pronounced (range 69–354 h).

Table 1 Doses and pharmacokinetic parameters in 11 patients with *T. gambiense* sleeping sickness administered a single i.v. infusion of pentamidine isethionate

Patient number	*Dose (mg kg^{-1})*	C_{max} *(nmol l^{-1})*	*Last time point used for calculations (h)*	r *value for terminal slope*	$t_{1/2,z}$ *(h)*	*AUC (nmol l^{-1} h)*	*Extrapolated area as % of AUC*	*CL (ml min^{-1})*	V_{ss} *(l)*	*MRT (h)*	*AUC (0,168 h) (nmol l^{-1} h)*
1	3.2	1231	—	—	—	—	—	—	—	—	3914
2	4.7	857	—	—	—	—	—	—	—	—	—
3	4.8	1377	—	0.55	—	—	53	—	—	—	4695
4	4.0	1409	240	0.90	107	6000	8	1454	5900	69	5140
5	3.9	881	—	—	—	—	—	—	—	—	—
6	3.9	1173	336	0.73	296	7791	33	943	16900	299	4171
7	3.8	821	—	—	—	—	—	—	—	—	3297
8	4.4	923	336	0.85	234	5736	18	1195	12000	168	4014
9	3.1	713	336	0.92	311	4772	28	1057	16700	265	2886
10	3.8	2461	816	0.92	446	16160	16	553	11700	354	9578
11	3.0	865	168	0.99	148	3504	18	1502	7700	87	2862
Median	3.9	923	336	0.91*	265	5868	18*	1126	11850	217	4014

* Patient 3 excluded.

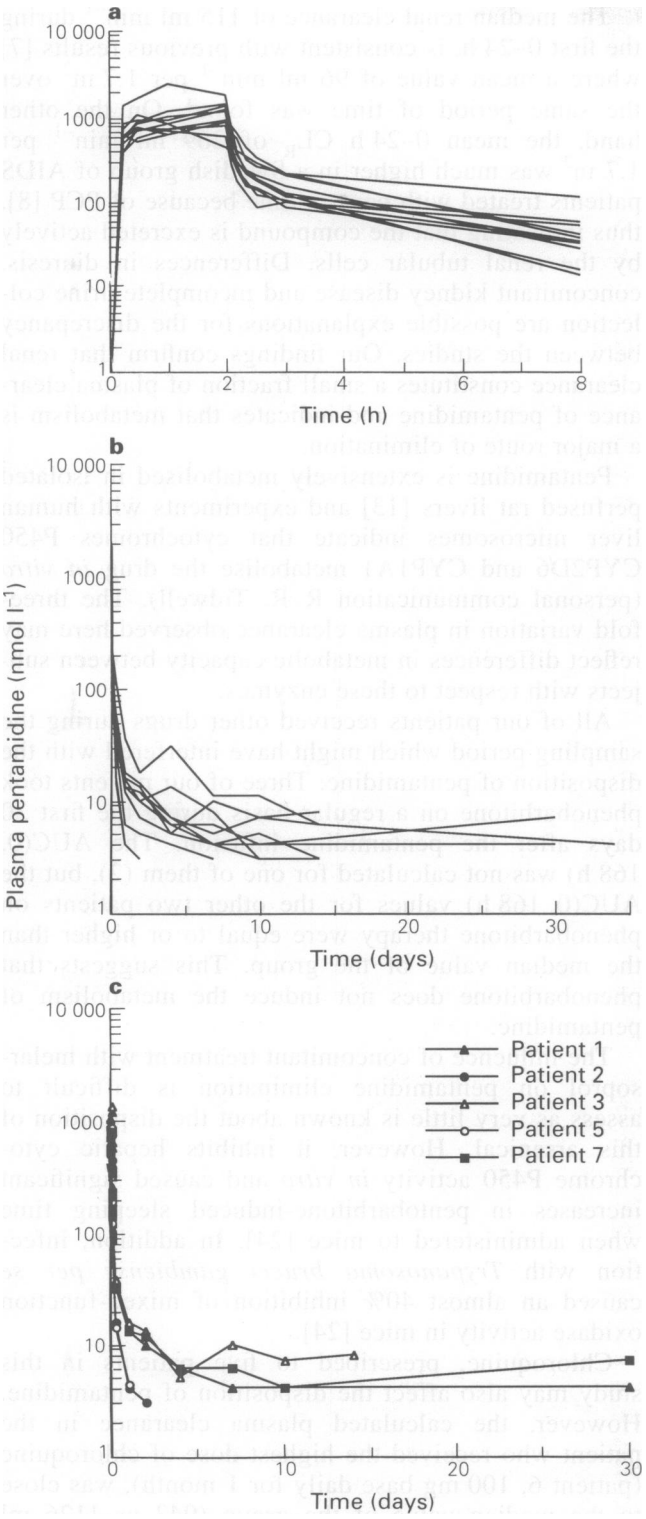

Figure 1 Plasma concentrations of pentamidine in 11 patients 0–8 h (a) and 0–34 days (b) after a single i.v. infusion of 3.0–4.8 mg kg^{-1} pentamidine isethionate. (c) Plasma concentrations of pentamidine 0–30 days after the infusion in five patients excluded from the calculation of kinetic parameters.

Urine The median 0–24 h urinary excretion of pentamidine was 19.5 (range 8.3–33.6) μmol which corresponds to a median of 5 (1–8)% of the administered dose. The median renal clearance during the first 24 h was 115 (61–206) ml min^{-1} and constituted 11 (4–17)% of the total plasma clearance. During the 24–48 h collection period, the median CL_R was 104 (61–256) ml min^{-1}.

Discussion

Pentamidine is usually administered intramuscularly for therapy of sleeping sickness but the drug is irritant to tissues and sterile abscesses with pronounced local pain are common in patients given pentamidine in this way [14]. This complication can be avoided with i.v. infusion. On the other hand, i.v. administration requires more equipment and trained personnel which are often lacking in the endemic areas in rural Africa. The use of intramuscular injections can cause a delayed and sometimes erratic absorption with ensuing late plasma drug peaks, as indicated in our previous study [9]. For proper evaluation of the pharmacokinetics, i.v. administration was therefore preferred in this study.

Two of the 11 patients in this study were infected with HIV, which corresponds to the 10–12% HIV-prevalence in the area. Plasma drug concentrations and renal clearances of pentamidine were not exceptional in these patients. The weights of the patients were considered normal, 77 and 65 kg, respectively, indicating that they had not developed AIDS.

Adverse reactions

Clinical and laboratory monitoring showed that the single i.v. infusion of pentamidine was not associated with significant untoward effects. However, its lack of major adverse reactions is not surprising as the development of side effects, especially renal toxicity, occurs on repeated dosing [15, 16]. Haematuria might have been related to the use of pentamidine in two of our patients but seems to be a rare manifestation of pentamidine toxicity as there are few reports in the literature [17, 18]. We cannot exclude other reasons for haematuria in our patients, e.g. melarsoprol toxicity or a concomitant *Schistosoma haematobium* infection.

Low blood pressure is a well-known side effect of pentamidine and has been reported to occur more frequently after intravenous than after intramuscular administration [19]. However, two more recent retrospective studies showed that when pentamidine was infused i.v. for at least 60 min, hypotension was equally or more common in patients who received pentamidine intramuscularly [14, 20]. The asymptomatic decreases in blood pressures observed in a few of our subjects are consistent with the latter data and show that slow i.v. administration of pentamidine has a marginal effect on the blood pressure.

Pharmacokinetic evaluation

The pronounced decrease in plasma drug concentrations within a short time after stopping the infusion indicates that pentamidine is distributed extremely rapidly from plasma. A transient reduction of the flow of the i.v. infusion (e.g. caused by flexing the

infusion arm) could, therefore, explain the temporary decreases in plasma drug concentration during infusion observed in a few patients. Similarly, a 1 min delay when taking the blood sample at the end of infusion could explain the observation that the plasma drug concentrations had started to fall already by the end of infusion. In a previous study [8], similar fluctuations in plasma drug concentration curve during i.v. infusion were observed, and in another study [21] there were substantial deviations of mean concentrations during infusion which may reflect the same phenomenon as observed in this study.

During the later part of the sampling period (i.e. 4–30 days), plasma drug concentrations fluctuated and even showed small increases in a few subjects. In another study, the mean concentration in plasma after a first i.v. dose increased slightly at 12 h after administration [7]. Animal experiments [22] and data from deceased patients with AIDS [23] show that pentamidine has a very high tissue affinity, as the drug was detected in various tissues weeks to months after the last dose. The increased concentrations in plasma some time after administration may be explained by redistribution of the drug to plasma from deeper compartments, such as the muscles, due to increased blood flow induced by physical activity.

The 'terminal' elimination half-life, assessed in six patients, was very long (median 11 days) and in accordance with the findings of Conte [7], who followed plasma drug concentrations in five patients after 12 to 21 i.v. doses of pentamidine and reported a mean elimination half-life of 12 days. This indicates that the elimination rate constant does not change during multiple dosing and, therefore, contradicts a previous suggestion of capacity-limited kinetics [7]. The large volume of distribution and long mean residence time also reflect the pronounced tissue affinity of the drug. Approximately 70% of pentamidine is bound to proteins in plasma (unpublished data obtained from our group).

We found a median plasma clearance of 1100 ml min^{-1} but a much higher estimate of approximately 4500 ml min^{-1} has been reported by Conte [7]. The same author calculated that renal clearance constituted only 2% of the total plasma clearance. His estimate of CL greatly exceeds normal liver blood flow (1500 ml min^{-1}), which implies that the drug is extensively metabolized outside the liver. However, the calculations were based on plasma sampling during the first 24 h after drug administration and extrapolation to infinity beyond 24 h. Therefore, the subsequent AUC estimates only reflected the distribution phase and the very long elimination phase, amounting to a substantial part of total AUC, was not included. Thus, AUC was underestimated and the estimate of CL became too high.

Our median estimate of AUC(0, 24 h) of 2730 nmol l^{-1} h (data not shown), is similar to the median AUC(0, 28 h) of 2680 nmol l^{-1} h found in Swedish AIDS patients with PCP after the first i.v. dose of the same dose of pentamidine isethionate [8]. This indicates that the initial disposition of the drug does not differ between patients with trypanosomiasis and HIV-infected patients with PCP.

The median renal clearance of 115 ml min^{-1} during the first 0–24 h, is consistent with previous results [7] where a mean value of 96 ml min^{-1} per 1.7 m^2 over the same period of time was found. On the other hand, the mean 0–24 h CL_R of 269 ml min^{-1} per 1.7 m^2 was much higher in a Swedish group of AIDS patients treated with pentamidine because of PCP [8], thus indicating that the compound is excreted actively by the renal tubular cells. Differences in diuresis, concomitant kidney disease and incomplete urine collection are possible explanations for the discrepancy between the studies. Our findings confirm that renal clearance constitutes a small fraction of plasma clearance of pentamidine and indicates that metabolism is a major route of elimination.

Pentamidine is extensively metabolised in isolated perfused rat livers [13] and experiments with human liver microsomes indicate that cytochromes P450 CYP2D6 and CYP1A1 metabolise the drug *in vitro* (personal communication R. R. Tidwell). The threefold variation in plasma clearance observed here may reflect differences in metabolic capacity between subjects with respect to these enzymes.

All of our patients received other drugs during the sampling period which might have interfered with the disposition of pentamidine. Three of our patients took phenobarbitone on a regular basis during the first 30 days after the pentamidine infusion. The AUC(0, 168 h) was not calculated for one of them (2), but the AUC(0, 168 h) values for the other two patients on phenobarbitone therapy were equal to or higher than the median value of the group. This suggests that phenobarbitone does not induce the metabolism of pentamidine.

The influence of concomitant treatment with melarsoprol on pentamidine elimination is difficult to assess as very little is known about the disposition of this arsenical. However, it inhibits hepatic cytochrome P450 activity *in vitro* and caused significant increases in pentobarbitone-induced sleeping time when administered to mice [24]. In addition, infection with *Trypanosoma brucei gambiense per se* caused an almost 40% inhibition of mixed-function oxidase activity in mice [24].

Chloroquine, prescribed to five patients in this study may also affect the disposition of pentamidine. However, the calculated plasma clearance in the patient who received the highest dose of chloroquine (patient 6, 100 mg base daily for 1 month), was close to the median value of the group (943 *vs* 1126 ml min^{-1}), suggesting that chloroquine does not markedly affect the disposition of pentamidine *in vivo*.

Since the 'terminal' elimination half-life of pentamidine is about 11 days, steady-state will not be achieved when treating trypanosomiasis with the currently recommended dosage schedule, i.e. a total of 7 to 10 doses given once daily or once every second day. The concentrations needed to kill trypanosomes *in vivo* are not known, but a recent *in vitro* study [3] showed that sustained concentrations over a prolonged period of time seem to be more important than rapid attainment of high concentrations. Exposure to as little as 3.4 nmol pentamidine l^{-1} over 8 days was parasiticidal while exposure to 1700 nmol l^{-1}

required 3 days to kill all parasites [3]. In the present study, the median plasma drug concentration was 4 nmol l^{-1} at 7 days after a single i.v. infusion (Figure 1b). Since the binding of pentamidine to plasma proteins is around 70%, and the *in vitro* medium contains considerably less protein than human plasma, the figures obtained *in vitro* and *in vivo* are not comparable. Assuming a protein-free *in vitro* medium, the total concentration in plasma would have to be approximately 12 nmol l^{-1} to correspond to 3.4 nmol l^{-1} *in vitro*. Nevertheless, a revised dosage schedule may be justified. As plasma drug concentrations are quite low at 8 h after an i.v. dose, more frequent infusions might be appropriate.

The authors thank Mr Jean René Sanon for excellent clinical assistance. Pentamidine isethionate was provided by Dr Björn Rubin, Rhône-Poulenc Rorer, Helsingborg, Sweden. The study was supported by the Swedish Agency for Cooperation with Developing Countries (grants no. SWE-90-133 and SWE-93-053), by funds of the Karolinska Institute and by a grant from 'Förenade Liv' Mutual Group Life Insurance Company, Stockholm, Sweden.

References

1 WHO. *Epidemiology and control of African trypanosomiasis.* Geneva: World Health Organization 1986, Technical Reports Series no. 739.
2 Sands M, Kron MA, Brown RB. Pentamidine: a review. *Rev Infect Dis* 1985; **7**: 625–634.
3 Miézan TW, Bronner U, Doua F, Cattand P, Rombo L. Long term exposure of *Trypanosoma brucei gambiense* to pentamidine *in vitro*. *Trans R Soc Trop Med Hyg* 1994; **88**: 332–333.
4 Lin JM-H, Shi RJ, Lin ET. High performance liquid chromatographic determination of pentamidine in plasma. *J liquid Chromatogr* 1986; **9**: 2035–2046.
5 Ericsson Ö, Rais M. Determination of pentamidine in whole blood, plasma and urine by high-performance liquid chromatography. *Ther Drug Monit* 1990; **12**: 362–365.
6 Conte JE Jr, Upton RA, Lin ET. Pentamidine pharmacokinetics in patients with AIDS with impaired renal function. *J infect Dis* 1987; **156**: 885–890.
7 Conte JE Jr. Pharmacokinetics of intravenous pentamidine in patients with normal renal function or receiving hemodialysis. *J infect Dis* 1991; **163**: 169–175.
8 Lidman C, Bronner U, Gustafsson LL, Rombo L. Plasma pentamidine concentrations vary between individuals with *Pneumocystis carinii* pneumonia and the drug is actively secreted by the kidney. *J Antimicrob Chemother* 1994; **33**: 803–810.
9 Bronner U, Doua F, Ericsson Ö, *et al.* Pentamidine concentrations in plasma, whole blood and cerebrospinal fluid during treatment of *Trypanosoma gambiense* infection in Côte d'Ivoire. *Trans R Soc Trop Med Hyg* 1991; **85**: 608–611.
10 Lumsden WHR, Kimber CD, Evans DA, Doig J. *Trypanosoma brucei:* miniature anion exchange centrifugation technique for detection of low parasitemias: adaptation for field use. *Trans R Soc Trop Med Hyg* 1979; **73**: 312–317.
11 Cattand P, Miézan BT, De Raadt P. Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bull World Health Organ* 1988; **66**: 83–86.
12 Tomson T, Svensson, JO, Hilton-Brown P. Relationship of intraindividual dose to plasma concentration of carbamazepine: indication of dose-dependent induction of metabolism. *Ther Drug Monit* 1989; **11**: 533–539.
13 Berger BJ, Naiman NA, Hall JE, Peggins J, Brewer TG, Tidwell RR. Primary and secondary metabolism of pentamidine by rats. *Antimicrob Agents Chemother* 1992; **36**: 1825–1831.
14 Navin TR, Fontaine RE. Intravenous *versus* intramuscular administration of pentamidine. *New Engl J Med* 1984; **311**: 1701–1702.
15 Laachal M, Venuto RC, Nephrotoxicity and hyperkalemia in patients with acquired immunodeficiency syndrome treated with pentamidine. *Am J Med* 1989; **87**: 260–263.
16 Comtois R, Pouliot J, Vinet B, Gervais A, Lemieux C. Higher pentamidine levels in AIDS patients with hypoglycemia and azotemia during treatment of *Pneumocystis carinii* pneumonia. *Am Rev resp Dis* 1992; **146**: 740–745.
17 Shuster M, Dunn M. Pentamidine and hematuria. *Ann intern Med* 1986; **105**: 146.
18 Pattullo ALS, Meynard M, Bow EJ. Hematuria associated with pentamidine therapy for *Pneumocystis carinii* pneumonia. *AIDS* 1992; **6**: 595–597.
19 Western KA, Perera DR, Schultz MG. Pentamidine isethionate in the treatment of *Pneumocystis carinii* pneumonia. *Ann intern Med* 1970; **73**: 695–702.
20 Helmick CG, Green JK. Pentamidine-associated hypotension and route of administration. *Ann intern Med* 1985; **103**: 480.
21 Conte JE Jr, Upton RA, Phelps RT, Wofsy CB, Zurlinden E, Lin ET. Use of a specific and sensitive assay to determine pentamidine pharmacokinetics in patients with AIDS. *J infect Dis* 1986; **154**: 923–929.
22 Waalkes TP, Denham C, DeVita VT. Pentamidine: clinical pharmacological correlations in man and mice. *Clin Pharmac Ther* 1970; **11**: 505–512.
23 Donnelly H, Bernard EM, Rothkotter H, Gold JWM, Armstrong D. Distribution of pentamidine in patients with AIDS. *J infect Dis* 1988; **157**: 985–989.
24 Shertzer HG, Hall JE, Seed JR. Hepatic mixed-function oxidase activity in mice infected with *Trypanosoma brucei gambiense* or treated with trypanocides. *Mol Biochem Parasitol* 1981; **3**: 199–204.

(Received 7 March 1994, accepted 19 October 1994)