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Biliary Elimination of Cyclophosphamide in Man

J. S. Dooley[1], C. A. James[2], H. J. Rogers[2], and R. Stuart-Harris[3]

[1] Department of Medicine, Royal Free Hospital School of Medicine, London NW3 2QG
[2] Department of Clinical Pharmacology, Guy's Hospital, London SE1 9RT
[3] Department of Medicine, Royal Marsden Hospital, London SW3 6JJ, Great Britain

Summary. *A 72-year-old male with a lymphoma and obstructive jaundice received 900 mg cyclophosphamide IV as a part of a chemotherapeutic regimen whilst external biliary drainage was in progress. Plasma, urinary, and biliary pharmacokinetics of cyclophosphamide and nitrobenzylpyridine (NBP)-alkylating metabolites were studied. In 32 h 891 ml bile was collected, and this contained unchanged cyclophosphamide and NBP-alkylating material. Despite fluctuations in biliary flow, estimates of the half-life of cyclophosphamide from plasma, urine, and bile were similar. Good correlation existed between plasma and biliary cyclophosphamide concentrations after the initial plasma had been completed. The ratio of bile to plasma concentrations was 0.7 and showed no time dependence, as evidenced by a lack of hysteresis in the correlation curve. Of the administered dose, 3.5% was excreted as unchanged cyclophosphamide in the bile over 32 h. NBP-alkylating activity was found in bile up to 25 h but not after this time, despite the presence of unchanged cyclophosphamide in plasma. NBP-alkylating material was not found in the bile when it could not be detected in plasma, and vice versa.*

Introduction

Cyclophosphamide has been widely used for cancer chemotherapy and immunosuppression for over 20 years, but many details of its disposition in man are obscure. Cyclophosphamide is the inactive precursor of several highly reactive metabolites produced mainly by hepatic activation. Biliary excretion of drug allows enterohepatic circulation, metabolism within the intestine, or elimination in the faeces, and may significantly determine the pharmacokinetic profile of a drug. Due to the large species variations in biliary excretion it is impossible to extrapolate animal data to man. We were therefore fortunate to be able to study a patient with external bile drainage, which allowed a pharmacokinetic study of the biliary elimination of cyclophosphamide. The secretion of this drug into the bile has not hitherto been studied quantitatively.

Materials and Methods

Patient Details. A 72-year-old male presented with cachexia, jaundice, pale stools, and dark urine. He had previously been well, apart from pain in the left hip treated with phenylbutazone for 8 months. On examination he was jaundiced and weighed 46 kg. He had 4 cm hepatomegaly, and enlarged right submandibular and left inguinal lymph nodes. Investigation showed cholestatic liver function tests, and ultrasound examination demonstrated dilated intrahepatic bile ducts and a dilated common bile duct. A percutaneous cholangiogram confirmed bile duct obstruction due to a lesion at the lower end of the common bile duct. Lymph node biopsy was reported to show malignant histiocytic, nodular, and diffuse lymphoma, but lymphography demonstrated no abnormal abdominal lymph nodes. An intravenous pyelogram revealed a non-functioning left kidney.

The cause of the bile duct obstruction was still in doubt and therefore a further percutaneous cholangiogram was done and fine-needle aspiration cytology was performed under screening control. The tissue obtained contained malignant cells, but neither cytological nor immunological studies could identify these definitely as lymphoma or carcinoma. Liver biopsy showed changes consistent with large bile duct obstruction, and no tumour or evidence of lymphoma was seen.

A percutaneous transhepatic drainage catheter was inserted at the time of cholangiography and external bile drainage [3] was established to improve liver function before subsequent cytotoxic therapy. This technique allows complete collection of the bile output; radiography showed the catheter to be correctly sited and the daily flow was 900–1,000 ml.

Investigations at this stage revealed normal plasma electrocytes and urea; a haemoglobin of 9.8 g dl^{-1}; white cell count 10×10^9 l^{-1}; platelets 430×10^9 l^{-1}; erythrocyte sedimentation rate 107 mm in first hour; serum bilirubin 72 μmol l^{-1} (normal 5–17); serum aspartate transaminase 13 IU l^{-1} (normal 5–15); serum alkaline phosphatase 21 KA units (normal 3–13); serum gamma glutamyl transpeptidase 64 IU l^{-1} (normal 7–30); total protein 56 g $^{-1}$ (normal 62–80); serum albumin 38 g l^{-1} (normal 35–50), and normal prothrombin and partial thromboplastin times.

Since the patient was febrile, treatment comprised ampicillin, gentamicin and metronidazole for presumed cholangitis, although subsequent culture of the bile was sterile.

Ater 48 h of biliary drainage, cytotoxic therapy for the lymphoma was begun with 900 mg cyclophosphamide IV, 2 mg vincristine IV, 40 mg prednisolone PO, and 12.5 prochlorperazine IV. With the patient's consent, bile and plasma samples were taken before treatment. All bile was collected in 30-min aliquots for the first 24 h after the start of this therapy, and in hourly aliquots for the next 8 h. Venous blood samples were taken at 0.03, 0.32, 0.5, 1.05, 2, 3, 4, 8, 16, 24, and 32 h,

Reprint requests should be addressed to H. J. Rogers

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and plasma was rapidly separated by centrifugation. Complete urine collections were made at approximately 2-hourly intervals over the 32-h study period. Bile, plasma, and urine samples were stored at −20° C pending analysis. Despite this and further therapy the patient died $2^1/_2$ weeks later, the immediate cause being peritonitis due to a perforated gastric ulcer. At autopsy the gallbladder was distended and contained white bile and the cystic duct was distended with a solitary mixed stone occluding its lumen. The pancreas was grossly normal and enlarged lymph nodes were present in the porta hepatis.

Analytical Methods

Cyclophosphamide was estimated in plasma, urine, and bile as its trifluacetyl derivative, using gas-liquid chromatography with alkali flame ionisation detection [6]. Total nitrobenzylpyridine (NBP)-alkylating activity was determined in plasma and bile spectrophotometrically, using the technique described by Juma et al. [7], and expressed in terms of equivalents of the standard nor-nitrogen mustard concentration.

Data Analysis. Plasma concentration by time data were fitted by a digital computer program employing a simplex non-linear optimisation algorithm [10]. The apparent first-order rate constant of elimination of plasma alkylating activity was determined from the slope of the linear regression of ln (plasma concentration) versus time. The areas under the plasma concentration time curve (AUC) were estimated using the linear trapezoidal rule with appropriate extrapolation for the infinite portion of the curve. Total body clearance was determined from Dose/AUC, and renal clearance from (amount eliminated in urine)/AUC. The sigma minus plot employs the relationship

$$\log (D_u^\infty - D_u) = - Kt/2.3 + \log D_u^\infty$$

where D_u^∞ is the total amount of drug eliminated in the urine; D_u is the cumulative amount eliminated in the urine at time t; and K is the first-order elimination rate constant [9].

Results

The plasma concentration by time data for unchanged cyclophosphamide (Cp) were adequately fitted by a bi-exponential function $Cp = Ae^{-\alpha t} + Be^{-\beta t}$, where α and β are apparent first-order distribution and disposition rate constants, respectively, A and B are coefficients. The rate parameters of the model were $\alpha = 4.50\ h^{-1}, \beta = 0.129\ h^{-1}$, which gives a $t_{1/2}\beta$ of 5.37 h. The data and computer-fitted model are shown in Fig. 1. The distribution phase comprised only 1.2% of the total AUC, suggesting that the errors involved in using a one-compartment model for sigma minus plots will not introduce much inaccuracy. The AUC was 1,146.5 μg ml^{-1} h, yielding a total body or systemic clearance of 795 ml h^{-1} (17.1 ml h^{-1} kg^{-1}). The plasma NBP-alkylating activity is also shown in Fig. 1. Alkylating activity was undetectable (i.e., < 0.6 nmol nor-nitrogen mustard equivalents ml^{-1}) in a plasma sample taken at 32 h, despite the presence of unchanged cyclophosphamide in this sample. The apparent first-order rate constant of elimination of alkylating activity was 0.086 h^{-1}, giving a $t_{1/2}$ of 8.1 h.

The total urinary elimination of unchanged cyclophosphamide over 32 h was 320 mg. This gives a renal clearance of 6.1 ml h^{-1} kg^{-1}, the fraction of the dose excreted unchanged being 0.36. A sigma minus plot of the urinary excretion rate of unchanged cyclophosphamide gave an elimination rate constant of 0.1136 h^{-1}, indicating a plasma half-life of 6.1 h (Fig. 2).

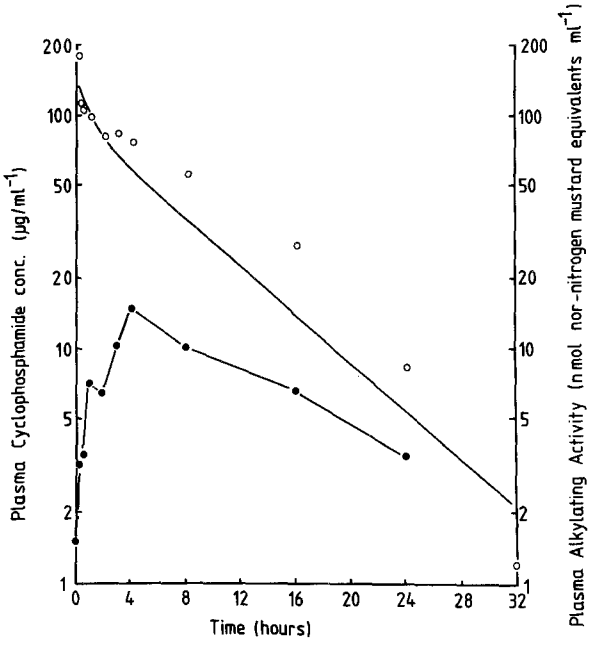

Fig. 1 Plasma concentration: time profiles for unchanged cyclophosphamide (○) and NBP-alkylating activity (●)

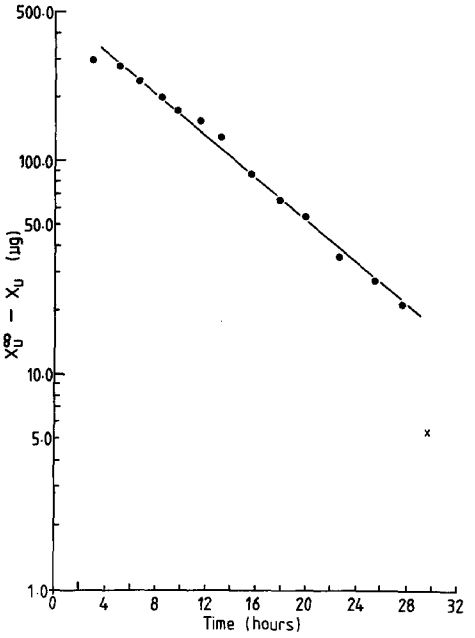

Fig. 2. Sigma minus plot of urinary excretion of unchanged cyclophosphamide. Point X excluded from regression. Amount of drug remaining to be excreted is plotted along the *ordinate*

Bile flow rates varied throughout the study period and the mean flow rate (± SD) was 0.48 (± 0.29) ml min^{-1}. The cumulative volume of bile produced over 32 h was 891 ml. A sigma minus plot of cyclophosphamide elimination gave an elimination rate constant of 0.135 h^{-1} and a plasma half-life of 5.13 h (Fig. 3). The mean biliary cyclophosphamide concen-

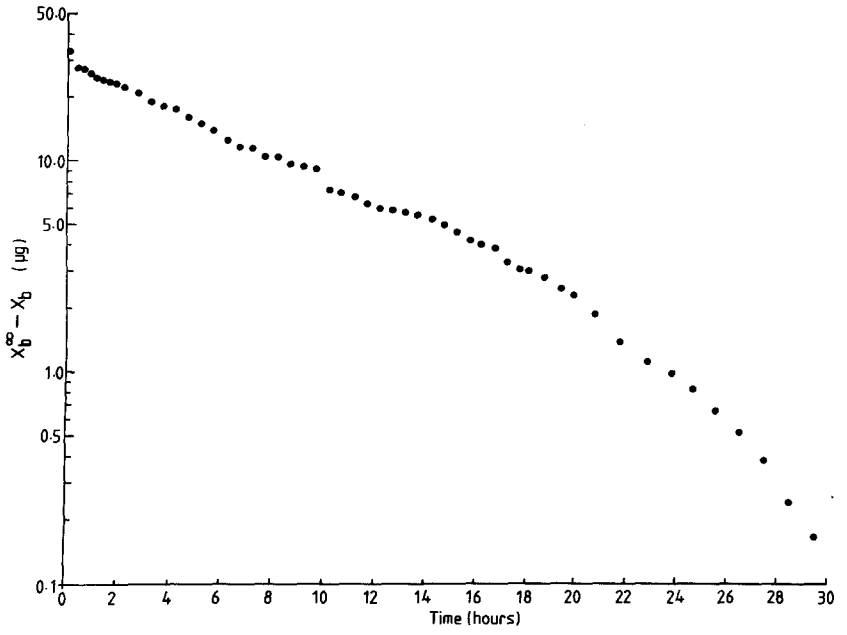

Fig. 3. Sigma minus plot of biliary excretion of unchanged cyclophosphamide. Amount of drug remaining to be excreted is plotted along the *ordinate*

tration over the time period of which a plasma cyclophosphamide concentration estimate was the mid-point was calculated, and these were plotted to show their correlation (Fig. 4). Excluding the two highest plasma cyclophosphamide concentrations which fall in the distribution phase of the plasma concentration by time profile it was found that a good correlation was obtained ($r = 0.950$; $P < 0.001$) with a slope of 0.70. Plotting the data in time order by the method suggested by Galeazzi et al. [4] produced a hysteresis loop reflecting only the scatter of the data. The total biliary elimination of unchanged cyclophosphamide in 32 h was 31.2 mg (0.035 of the total dose), giving a biliary clearance of 0.59 ml h^{-1} kg^{-1}. The metabolic clearance [= systemic clearance −(urinary clearance + biliary clearance)] of unchanged cyclophosphamide was 10.4 ml h^{-1} kg^{-1}. NBP-alkylating activity was also detected in the bile up to 25 h but was not found after this time. This resembled the plasma alkylating activity results. The relationship between plasma and bile alkylating activities was less well correlated ($r = 0.72$; $0.05 > P > 0.02$) than the plasma and bile concentrations of unchanged drug. The rate of elimination of alkylating activity into the bile fluctuated widely. The total NBP-alkylating found in the bile over 32 h was 5,265 nmol nor-nitrogen mustard equivalents.

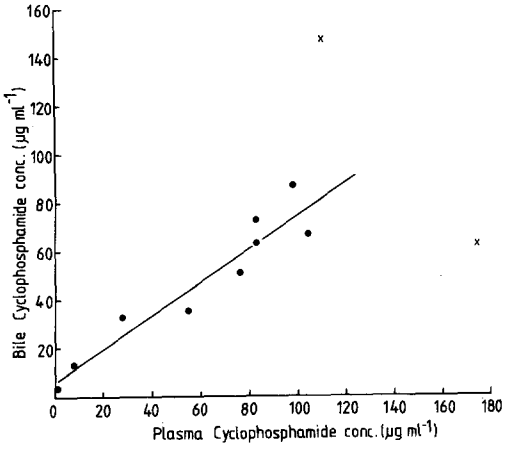

Fig. 4. Relationship between plasma and biliary concentrations of unchanged cyclophosphamide. Points X were excluded from the regression since they fell within the plasma distribution (α-)phase

Discussion

The bi-exponential parameters for elimination of cyclophosphamide from the plasma and the half-life are similar to those reported elsewhere. The urinary cyclophosphamide elimination data corroborate the half-life determined from the plasma data. The total body clearance of cyclophosphamide is only 25%−33% of that determined in previous studies [2, 6, 7]. The half-life of plasma NBP-alkylating activity is similar to that previously reported [7]. The fractional urinary elimination of unchanged cyclophosphamide was, by contrast, somewhat higher than reported previously in a group of lymphoma patients [7], but was closer to although still greater than the values reported in some other studies [1, 2, 5]. This might indicate that the hepatic clearance of cyclophosphamide in this patient was reduced and possibly accompanied by a compensatory increase in renal elimination.

A further estimate of the plasma half-life of cyclophosphamide determined by the sigma minus method was consistent with that determined from direct plasma concentration measurements. A good correlation existed between bile and plasma cyclophosphamide concentrations observed (Fig. 4) once the distribution phase had been completed. The ratio of bile/plasma concentration was 0.70 and showed no time dependence, as evidenced by the lack of hysteresis in the correlation curve. This suggests that the plasma and bile may be considered to occupy the same compartment (in a pharmakokinetic sense) after drug distribution. This distribution ratio between bile and plasma is similar to the value of 0.77 ± 0.24 (SD) found for the saliva/plasma ratio in man [8]. Cyclophosphamide is only weakly bound to albumin, and estimates of its binding to human plasma proteins include 13.4% ± 5.3% (SD) [8], 12%−14% [1], and 24% [5]. These predict free cyclophosphamide fractions sufficienctly close to

the observed bile/plasma ratio to prompt the suggestion that the diffusion of the free fraction into bile accounts for its presence in that fluid. Cyclophosphamide is lipid-soluble and could penetrate both hepatocytes and the canalicular membrane, so that postulation of a transport mechanism is unnecessary. It is possible that some of the cyclophosphamide entering the gut via the bile is reabsorbed, thereby completing on enterohepatic cycle, since Bagley et al [1] found that in six patients given ^{14}C-labelled cyclophosphamide IV only 0.92%–2.5% of the label was found in the stools over 4 days.

Activated cyclophosphamide metabolites were also detected in the bile. NBP alkylation is not necessarily an indication of cytotoxicity, but probably indicates the presence of antimitotic substances in the bile. The secretion of this material was erratic, perhaps because NBP-alkylating activity represents a mixture of a number of active species and no formal kinetic modelling was possible. There is no evidence that the mixture of metabolites in bile contains the same components in the same proportions as that detected in the plasma. Despite the presumptive formation of NBP-alkylating substances in the liver, they were not detected in bile when they were absent from the plasma, despite the presence of small concentrations of unchanged cyclophosphamide in both fluids.

It must be considered whether this patient was representative of younger, fitter patients without hepatic impairment: this assurance cannot be given in the present absence of data on the effect of hepatic impairment on cyclophosphamide disposition. This patient had a history of several weeks of cholestasis and its affect cannot be assessed. The interruption of the normal enterohepatic circulation of bile and the effects of a biliary drainage tube in situ are also unknown factors. However, bile collection was complete in our patient and this new technique of bile recovery [3] is superior to that via a T-tube, which is known to be incomplete. Thus the recovery of common duct bile by T-tube drainage determined by sulphobromophthalein and Rose Bengal ^{131}I, was found to be only 66% and 57%, respectively [11]. Cyclophosphamide was also given in combination with other drugs in this patient, and therefore extrapolation to treatment with cyclophosphamide as a single agent cannot be made. Despite these cautions it is clear that cyclophosphamide can be eliminated in the bile as both unchanged and activated drug.

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