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EXCRETION OF CYCLOPHOSPHAMIDE METABOLITES IN BILE

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INTRODUCTION

Cyclophosphamide (CP) is an oxazaphosphorine drug used in cancer chemotherapy. Its pharmacokinetics have been well studied, however its biliary excretion has received little attention. In a recent patient study (Dooley et al, 1982) unchanged CP and alkylating activity were shown to be present in bile following intravenous administration of CP; this suggests excretion of both CP and its alkylating metabolites by the biliary route.

In the present study radiolabelled CP was given to the bile duct-cannulated rat, bile was collected and analysed by liquid scintillation counting and radiochromatography. This enabled us to further investigate the biliary excretion of CP and its metabolites, and possibly suggest identities for the metabolites excreted.

MATERIALS AND METHODS

A duplicate experiment was carried out in a single bile duct-cannulated rat (cannulation method of Barrow & Griffiths, 1972). The rat was given [^{14}C-C4]-CP intravenously (50mg/kg body weight; approx. 5uCi ^{14}C) 3 hours after surgery and again 16.5 hours later (i.e. when negligible further radioactivity was excreted in the bile; % of dose in bile at 19.5 hours: ^{14}C = 0.005%). Bile was collected in half hour fractions, the volume of each fraction was measured and then the radioactivity of each determined. Selected fractions (see results) were analysed by thin layer chromatography (TLC) on silica gel plates developed in chloroform-methanol (9:1 v/v), followed by radiochromatographic scanning both before and after treatment with β-glucuronidase (method of Bolton and Griffiths, 1972).

RESULTS AND DISCUSSION

Approximately 6.3% (mean of two doses) of the ^{14}C radioactivity given to the animal was excreted in the bile within 6 hours. The major peak of excretion was at about 1 hour after the CP dose; several smaller peaks appeared later (fig. 1). This might represent excretion of different metabolites of CP at different times. The radiochromatogram of the second bile fraction following the first dose of CP is represented in fig. 2. One single major peak and two smaller peaks appeared on the chromatogram. The major peak did not migrate from the origin, this is consistent with the component(s) of that peak being polar.

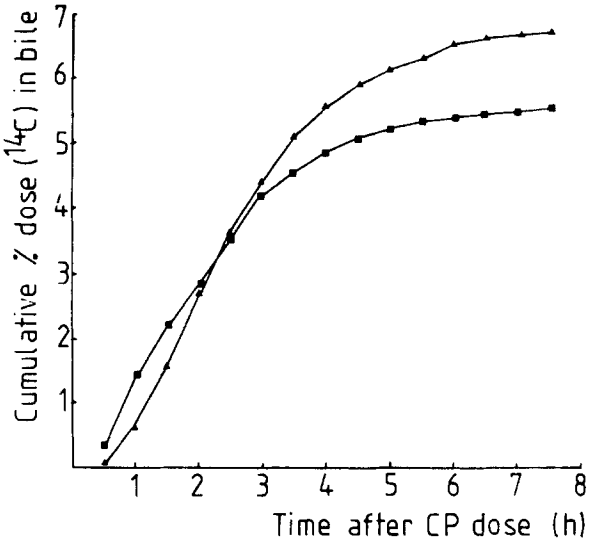

Fig.1 Cumulative plot of ^{14}C-radioactivity in bile fractions (expressed as % of ^{14}C given to the rat) vs. time after each CP dose.

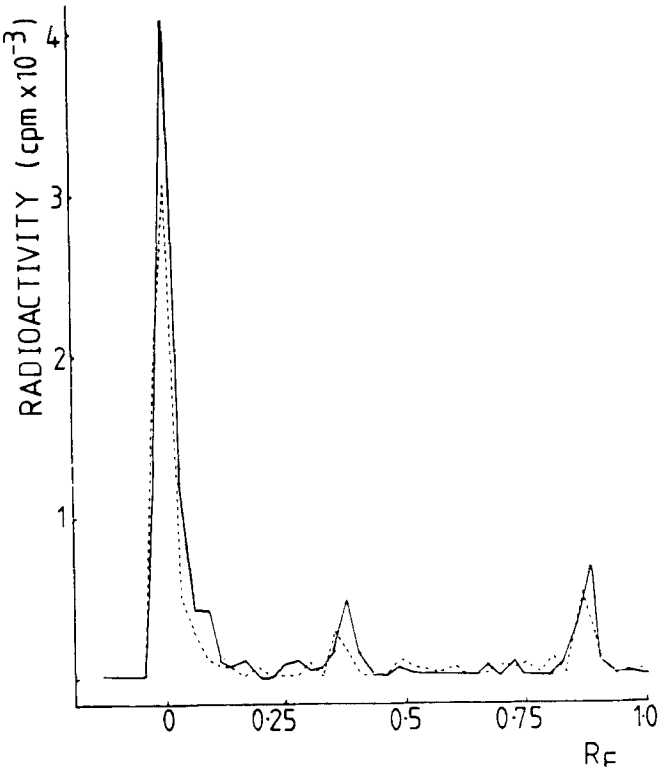

Fig.2 TLC* separation of ^{14}C metabolites of [^{14}C-C4]-CP excreted in bile (0.5 - 1.0h fraction following first dose).
= untreated bile
= bile treated with
- glucuronidase.

*Silica plates developed over 15cm in chloroform-methanol (9:1 v/v).

Peak A represents polar metabolite(s); peak B was not identified; peak C chromatographed with authentic CP and 4-keto CP.

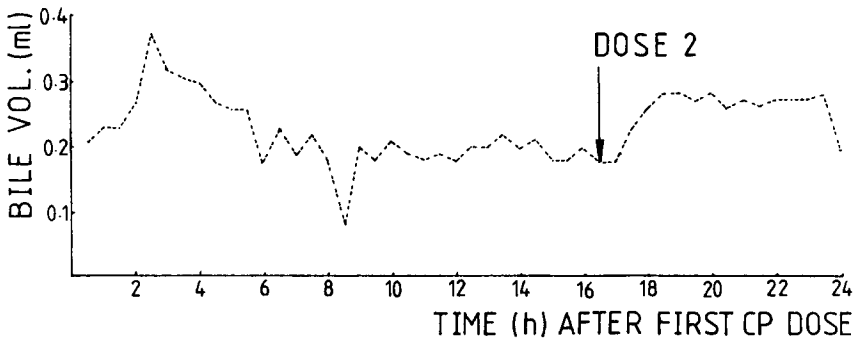

Fig. 3 Bile volumes following two iv CP doses (50mg/kg body weight) to a bile duct-cannulated rat.

Treatment of the bile sample with β -glucuronidase before chromatography did not change the Rf value of this peak. Rf values of authentic standards of CP metabolites in the same chromatography system are listed in table 1.

Bile volumes were measured (fig.3), and bile flow rates were calculated for the 0 - 6 hour period (excretion period for the first CP dose), the 6 - 16.5 hour period (during which time CP metabolites were not excreted) and the 16.5 - 24 hour period (excretion period for the second CP dose). The flow rates were 0.54ml/h (peak value 0.76ml/h), 0.38ml/h and 0.51ml/h (peak value 0.56ml/h) respectively. It therefore appears that CP and/or its metabolites stimulate bile flow.

From these experiments it may be concluded that one or more polar metabolites of CP, and possibly a trace of unchanged CP, are excreted in bile. Bearing in mind both the molecular weight threshold and preference for anionic charge for biliary excretion in the rat (Smith, 1973), it is most likely that carboxyphosphamide is the major biliary metabolite of CP. Furthermore authentic carboxyphosphamide behaves similarly to the unknown biliary metabolite in this TLC system.

METABOLITE	Rf	m.w.
Carboxyphosphamide	0†	293
4 - Hydroxy CP	-	277
Aldophosphamide	-	277
4 - Keto CP	0.91†	275
Phosphoramide mustard	0.09†*	221
Nornitrogen mustard	0.91†	142

* As cyclohexylamine salt

† Calculated from Domeyer & Sladek, 1980

Table 1 The major metabolites of CP arranged in order of molecular weight, showing their Rf values on silica plates developed in chloroform-methanol (9:1 v/v).

The recognised metabolites of CP all have molecular weights well below the probable excretion threshold for man (see Smith, 1973), and therefore would not be expected to be excreted in human bile (unless associated with molecules of higher molecular weight, e.g: proteins). However in cases of cholestasis, leakage of CP and its metabolites across the tight junctional complex into the bile might occur, in a manner analogous to other chemicals (Elias, 1980). This possibly explains the appearance of CP and its alkylating metabolites in the bile of an elderly patient presenting with jaundice and cholestasis (Dooley et al, 1982).

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