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STUDIES ON THE PHARMACOKINETICS OF CHLORAMBUCIL AND PREDNIMUSTINE IN MAN

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1 Chlorambucil (10 mg) and prednimustine (20 mg), the prednisolone ester of chlorambucil, were administered orally on separate occasions to six patients.

2 Chlorambucil was rapidly absorbed such that the parent compound was observed in the plasma 30 min after administration.

3 A preliminary comparison of chlorambucil levels following oral and intravenous administration, and after repeat oral dosage indicated that chlorambucil was well (> 70%) and consistently absorbed.

4 Following prednimustine no parent drug or alkylating metabolites (chlorambucil or phenyl acetic mustard) could be detected in the plasma.

5 In studies with intravenously administered chlorambucil plasma levels of the parent drug were described by a two-compartment open model with first-order kinetics. Significant levels of the cytotoxic metabolite phenyl acetic mustard were detected.

6 It is concluded that:

a. the bioavailability of orally administered prednimustine is much lower than that of chlorambucil. Thus the use of prednimustine in routine combination therapy is not recommended.

b. due to the lower therapeutic index of phenyl acetic mustard in experimental systems, the production of this metabolite in man may be disadvantageous. Thus research aimed at producing chlorambucil analogues, which cannot be metabolised, seems justified.

Introduction

Chlorambucil (4(4-bis(2-chloroethyl)aminophenyl)-butyric acid) (Figure 1a) is a bifunctional alkylating agent used in the treatment of both neoplastic and non-neoplastic disorders. Chlorambucil is administered by the oral route; however, the bioavailability of the drug given by this route has not been investigated. Further, the feasibility of using the intravenous route has not been studied.

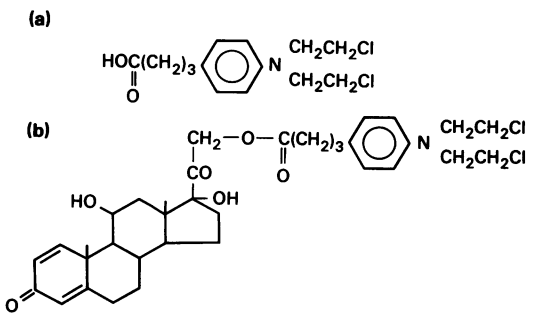

Figure 1 The structures of chlorambucil (a) and prednimustine (b).

Chlorambucil is metabolised in man by a β-oxidation process to phenyl acetic mustard (Figure 2) (Newell *et al.*, 1979; McLean *et al.*, 1979; Alberts *et al.*, 1980). Metabolism is extensive such that less than 1% of the administered dose is excreted as the unchanged drug in the urine (Alberts *et al.*, 1980), despite the presence of substantial quantities of drug-derived material (McLean *et al.*, 1979).

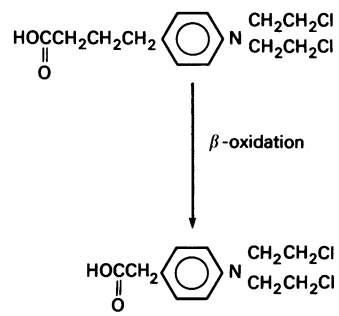

Figure 2 The metabolism of chlorambucil.

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Prednimustine (pregna-1,4-diene-11β,17α,21-triol-3,20-dione-21 (4(4-bis(2-chloroethyl)amino-phenyl) butyric acid)) (Figure 1b), the prednisolone ester of chlorambucil, was synthesised (Konyves & Ljekvist, 1976) in an attempt to improve the anti-tumour selectivity of chlorambucil by facilitating uptake into tumours possessing high concentrations of glucocorticoid receptors. Prednimustine has recently undergone a number of clinical trials where activity was reported against various tumour types. It is apparent that prednimustine is less toxic than chlorambucil, on an equimolar basis, since higher doses of prednimustine can be given before toxicity is encountered.

Definitive studies on the metabolism of prednimustine in man have not been reported. *In vitro* the ester linkage is subject to rapid hydrolysis by non-specific esterases from a number of tissues, including human plasma (Wilkinson *et al.*, 1978). *In vivo*, in the rat, the hydrolysis of the ester bond is also rapid such that, following subcutaneous administration, only the metabolites chlorambucil and phenyl acetic mustard are detected in the plasma (Newell *et al.*, 1981).

Recently it has been shown that the side effects of the MVPP (mustine, vinblastine, procarbazine and prednisone) schedule for the treatment of Hodgkin's disease can be reduced, without any loss of efficacy, if mustine is replaced by chlorambucil (Kaye *et al.*, 1979). It was postulated that the therapeutic effects of this schedule might be further improved, without any increase in toxicity, by replacing chlorambucil with prednimustine. To assist in evaluating this postulate, while ensuring satisfactory prednimustine bioavailability, a comparative study of the pharmacokinetics of orally administered chlorambucil and prednimustine in patients with Hodgkin's disease was carried out.

Due to the current interest in high-dose intravenous alkylating agent therapy (McElwain *et al.*, 1979) this study has been extended to cover a preliminary investigation of the pharmacokinetics of intravenously administered chlorambucil. Certain aspects of this study have been reported previously in a preliminary form (Newell *et al.*, 1979).

Methods

Treatment

Previously untreated patients with Hodgkin's disease requiring chemotherapy were studied immediately before the start of conventional therapy (Kaye *et al.*, 1979). Informed consent was obtained from all patients. Following the insertion of an indwelling intravenous cannula patients received 10 mg chlorambucil and 20 mg prednimustine orally (approximately equimolar doses), administered on separate occasions (>48 h interval). Ten milligrams of chlorambucil (6 mg m^{-2} day^{-1} > 10 mg/day) given orally is the standard dose used in this regimen. For intravenous therapy patients received 10–200 mg of chlorambucil dissolved in 10 ml of 10% ethanol—90% phosphate buffered propylene glycol pH 7.0 (v/v) (Burroughs Wellcome Foundation, Beckenham, Kent, England) given as a bolus injection, within 15 min of dissolution, into the arm opposite to that containing the indwelling cannula. Of the three patients who received high dose intravenous chlorambucil therapy (100–200 mg) two patients had carcinoma of the ovary and one carcinoma of the pancreas. Patients did not receive any other chemotherapy during the study period with the exception of the patients receiving high dose i.v. therapy who received antiemetics as required.

Blood sampling and plasma analysis

Blood samples (7 ml) were taken into heparinised tubes (10 iu/ml) at various times after drug administration and plasma immediately prepared by centrifugation at 600 *g* for 10 min. Plasma samples were frozen immediately and stored (−20°C) until analysis. Duplicate 1 ml plasma samples were analysed for chlorambucil, phenyl acetic mustard and prednimustine by high performance liquid chromatography (Newell *et al.*, 1979).

Data analysis

Following the intravenous administration of chlorambucil a two-compartment open model was fitted to the chlorambucil plasma levels by a non-linear least squares analysis (Sampson, 1970). The areas under the plasma concentration versus time curves (AUC) were calculated from the computer fit.

Following the oral administration of chlorambucil, insufficient samples were taken to allow the absorption phase to be described. Hence lines were fitted manually to the chlorambucil phasma levels using the mean of duplicate estimations. All phenyl acetic mustard levels were treated in a similar manner. The AUC values for these data were determined using the trapezoidal rule and the overall elimination phase half-lives determined by a method of residuals analysis using a linear least squares fit (Wagner, 1975). The AUC values of chlorambucil following oral administration and for phenyl acetic mustard were corrected for the time period from last sample point to infinity. Throughout the text data are given as the mean of *n* observations ± s.e. mean with the number of observations (*n*) in brackets.

Results

Pharmacokinetics of oral chlorambucil and prednimustine

Following the oral administration of chlorambucil (10 mg) to six patients chlorambucil was observed in the plasma 30 min after administration with peak plasma levels occurring within 2 h. The plasma chlorambucil concentrations in the six patients studied are shown in Figure 3. Chlorambucil had a mean elimination phase half-life in these patients of 109 ± 16 min (n = 5).

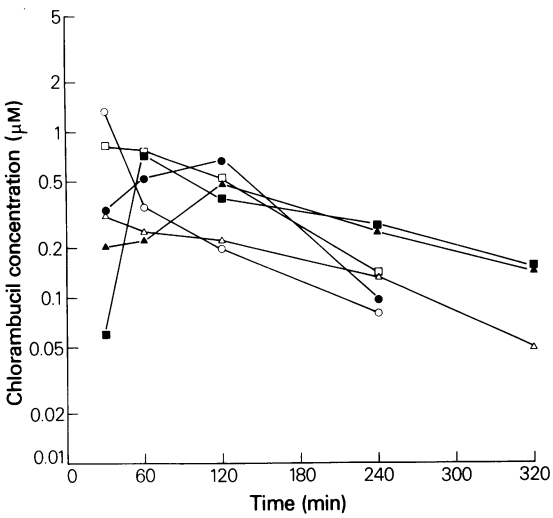

Figure 3 Plasma chlorambucil concentrations in man following a 10 mg oral dose at time = 0, CR (■), AC (▲), ACh (○), CH (□), ES (●) and HD (△). Values for ACh and CH are the mean of data from three doses.

Individual half-lives and the correlation data for these values are given in Table 1. Only values significant at the 95% level were used in computing the mean chlorambucil elimination phase half-life. The product of β-oxidation, phenyl acetic mustard, was also detected in the plasma of these patients. The limit of detection for this metabolite (0.25 μM) precluded a complete study of phenyl acetic mustard pharmacokinetics although the peak plasma levels (0.40 ± 0.04 μM, n = 6) did not exceed those of the parent compound. As previously reported (Newell *et al.*, 1979), the structures of chlorambucil and phenyl acetic mustard were confirmed by mass spectrometry.

The same six patients also received an equimolar dose of prednimustine (20 mg). At no time after administration (0–48 h) were prednimustine, chlorambucil or phenyl acetic mustard detectable in the plasma (limits of detection: prednimustine 0.05 μM,

Table 1 Linear least-squares analysis of chlorambucil plasma concentrations following 10 mg chlorambucil given orally

	Chlorambucil			
Patient	$t_{½,z}$	n*	r*	P*
CH	141	4	0.979	0.02
AC	140	3	0.997	0.05
ACh†	63	10	0.751	0.01
CH†	81	12	0.947	<0.01
ES	—	3	0.897	>0.1
HD	120	5	0.963	<0.01
± s.e. mean	109 ± 16 (n = 5)			

* n is the number of terms in the regression equation, r the correlation coefficient of the equation, and P the significance of the correlation.
† value for ACh and CH determined from the composite of data from three doses.

chlorambucil 0.05 μM, phenyl acetic mustard 0.25 μM). The absorption of chlorambucil was further investigated to examine the consistency of absorption and bioavailability of the orally administered drug. Two patients received a 10 mg oral dose of chlorambucil on three successive days, As shown in Table 2 the AUC for the chlorambucil concentration *vs* time curve was consistent, within each patients, on the three days studied. The bioavailability of orally administered chlorambucil was examined by comparing the AUC values in two patients who received two 10 mg doses of chlorambucil, one given orally and one intravenously, on separate occasion (48 h interval). An example of one patient is given in Figure 4. The ratios of the chlorambucil AUC following oral and i.v. administration were 0.73 and 1.02 indicating that chlorambucil was well absorbed in these two patients following oral administration.

Pharmacokinetics of i.v. chlorambucil

Five patients received chlorambucil given intravenously at various doses as shown in Table 3. Chlorambucil was rapidly distributed in all the patients $t_{½,\alpha}$ = 7.0 ± 1.3 min (n = 4)) while in one patient (CR) a distribution phase could not be detected. The elimination phase half-life for chlorambucil following

Table 2 Area under the plasma chlorambucil concentration *vs* time curves (AUC) following the oral administration of chlorambucil (10 mg) on three consecutive days

	AUC (μM × min)		
Patient	*Day 1*	*Day 2*	*Day 3*
CH	137	127	114
ACh	85	82	91

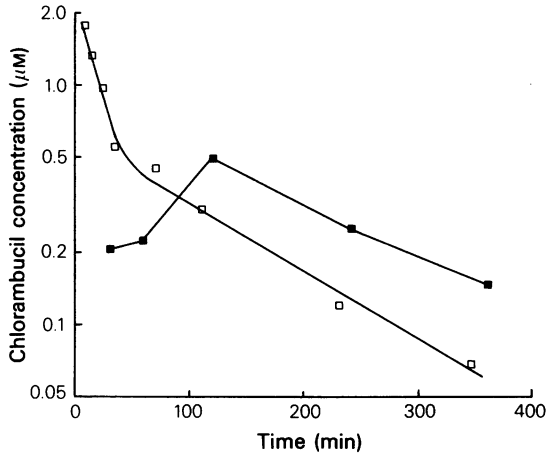

Figure 4 Plasma chlorambucil concentrations in one patient (AC) following a 10 mg dose of chlorambucil given orally (■) or intravenously (□). Levels following oral administration were plotted manually using the mean of duplicate estimations. Levels following i.v. administration are the computer fit.

intravenous administration ($t_{½,z}$ 80 ± 24 min (n = 5)) did not differ significantly from the elimination phase half-life following oral administration (109 ± 16 min (n = 5)) (t-test, P = 0.35). Two patients (VM and PS) received chlorambucil intravenously at two dose levels, i.e. 10 mg and 100 mg. The use of higher doses than 200 mg was not possible because of the occurrence of convulsions. As shown in Table 3, the chlorambucil distribution and elimination half-lives were similar within each patient, at the two dose levels, whilst the chlorambucil (Table 3) and phenyl acetic mustard (Table 4) AUC values were appropriately related. The metabolite phenyl acetic mustard was detected in the plasma of all the patients who received intravenous chlorambucil. In four patients the elimination phase half-life was longer than that of chlorambucil ($t_{½,z}$ = 14) ± 24 min (n = 4) (Table 4). In one patient (VM) phenyl acetic mustard kinetics were not linear. The pharmacokinetic data concerning intravenously administered chlorambucil are summarised in Tables 3 and 4, and an example of one patient (PS) shown in Figure 5.

Discussion

Following oral administration, chlorambucil is well absorbed such that in all of the patients studied chlorambucil was detected in the plasma 30 min after administration. In the same patients, following the administration of prednimustine, neither the parent drug nor either of its alkylating metabolites, chlorambucil and phenyl acetic mustard, could be detected. These data suggest that prednimustine is poorly absorbed from the gastrointestinal tract. In agreement with this suggestion is the observation that following the oral administration of radiolabelled prednimustine (20–100 mg) to mammary carcinoma patients, there is extensive and highly variable faecal excretion (0.3–55.2% dose administered) (Konyves *et al.*, 1975). This latter study estimated only the parent compound and hence further drug-derived material may have been present in the faeces, i.e. the products of chemical or enzymatic hydrolysis.

The failure to achieve cytotoxic plasma levels of alkylating agents following the administration of a 20 mg oral dose of prednimustine precluded the substitution of chlorambucil with prednimustine in the ChlVPP regimen for the treatment of Hodgkin's disease. Oral doses of prednimustine, greater than 20 mg, could have been used in an attempt to achieve adequate plasma levels of alkylating agents. However, the problem of erratic and unpredictable absorption would have remained. Thus the use of larger doses of prednimustine could not be justified due to the possibility of compromising the ChlVPP regimen, a combination of established efficacy in

Table 3 The pharmacokinetics of i.v. chlorambucil

		Chlorambucil		
Patient	*Dose (mg)*	$t_{½\alpha}$ *(min)*	$t_{½,z}$ *(min)*	*AUC (μM × min)*
AC	10	9.9	112	123
CR	10	ND	28	196
PS	10	2.8	29	208
	100	4.6	29	1884
VM	10	7.2	82	148
	100	9.1	72	1422
JB	200	6.4	155	7043
Mean ± s.e. mean		7.0 ± 1.3	80 ± 24	

ND = not detected

Table 4 The pharmacokinetics of phenyl acetic mustard following i.v. chlorambucil

Patient	*Chlorambucil dose (mg)*	$t_{½,z}$ *(min)*	*AUC (μM × min)*
AC	10	185	157
CR	10	113	235
PS	10	83	201
	100	92	4341
VM	10	ND	280
	100	ND	2277
JB	200	177	3309
Mean ± s.e. mean	—	141 ± 24	—

ND = not determined due to non-linear pharmacokinetics

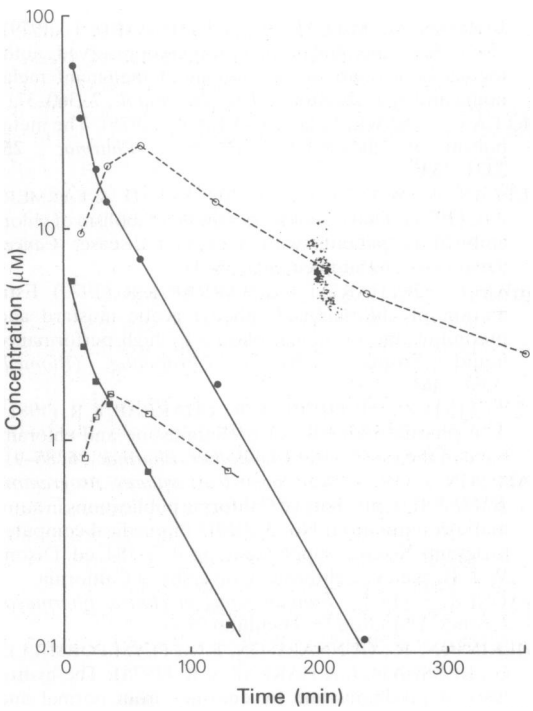

Figure 5 Plasma chlorambucil (closed symbols ● ■) and phenyl acetic mustard (open symbols ○ □) concentrations in one patient (PS) following 10 mg (square symbols ■ □) and 100 mg (circles ● ○) intravenous doses of chlorambucil. Chlorambucil levels are given as the computer fit and phenyl acetic mustard levels as the manual plot using the mean of duplicate estimations.

which chlorambucil absorption is efficient and reproducible. Although the intravenous administration of prednimustine was considered, it is not possible to give the drug by this route as a soluble preparation due to poor aqueous solubility. Kirdani *et al.* (1978) have in fact administered prednimustine intravenously as a suspension in ethanolic-saline, but this procedure is not to be recommended for routine clinical use.

If prednimustine is used at doses above 20 mg (100–200 mg) alkylating agents might be detectable in the plasma. However, the rapid hydrolysis of prednimustine in human plasma *in vitro*, and in the rat *in vivo*, suggest that the parent compound would not remain intact in man. Thus the selective uptake of chlorambucil into tumours containing high concentrations of glucocorticoid receptors is unlikely to occur.

The data concerning chlorambucil pharmacokinetics following oral administration determined in the present study are in general agreement with those previously reported. Alberts *et al.* (1980) observed elimination phase half-lives of 86 ± 22 min (n = 5) for chlorambucil and 162 ± 45 min (n = 5) for phenyl acetic mustard. Leff & Bardsley (1979) reported similar peak plasma levels (0.5–1 μM) to those observed in the present study following a 10 mg dose of chlorambucil given orally, although the elimination phase half-life was more rapid (28 ± 7 min (n = 5)). This latter study did not estimate phenyl acetic mustard. However, the work of Alberts *et al.* (1980), McLean *et al.* (1979) and the present study indicates that this bifunctional alkylating agent is present in sufficient quantities to exert a biological effect. The contribution of this metabolite to the antitumour activity and toxicity of chlorambucil in man remains to be determined although it is of interest that in both mice (Godeneche *et al.*, 1980) and rats (McLean *et al.*, 1976) phenyl acetic mustard has similar antitumour activity but is more toxic than the parent drug. Studies are currently under way with the aim of producing chlorambucil analogues which cannot be metabolised to phenyl acetic mustard (Farmer *et al.*, 1979). Such studies will allow the role of phenyl acetic mustard to be defined.

Following intravenous administration chlorambucil pharmacokinetics followed a two-compartment open model with first-order kinetics. Phenyl acetic mustard was again observed as a metabolite which persisted for longer than the parent compound.

In conclusion, this study has demonstrated that orally administered chlorambucil is well absorbed whilst prednimustine is poorly absorbed such that neither the parent drug or its metabolites could be detected in the plasma. Preliminary studies with intravenously administered chlorambucil suggest that this drug is suitable for intravenous use. In view of the predictable absorption of chlorambucil, as compared to prednimustine, it is recommended that the use of the former drug be continued in those clinical circumstances where its combination with prednisolone is indicated or where it is used as a single agent.

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