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Can food affect the bioavailability of chlorambucil in patients with haematological malignancies?

C. G. Adair, J. M. Bridges, and Z. R. Desai

Department of Haematology, The Queen's University of Belfast, N. Ireland

Summary. Pharmacokinetic studies in ten patients with haematological disorders were undertaken on the first and second days of one course of chemotherapy. Patients received chlorambucil under fasting and non-fasting conditions. Plasma concentrations of chlorambucil were determined by a reversed-phase high-performance liquid chromatography assay. Statistical analysis by the Wilcoxon signed rank test for non-parametric data indicated that food caused a significant reduction in peak plasma levels ($P<0.01$), elimination rate constants ($P<0.01$) and area under the plasma chlorambucil/time curve ($P=0.01$). Food was also found to prolong the time taken to attain peak plasma levels ($P<0.01$). Regression analysis of renal function with elimination rate constants showed that chlorambucil elimination was independent of renal function ($n=8$; $r=-0.007$; $P=0.72$). In view of these results we suggest that chlorambucil is given on an empty stomach.

Introduction

The effect of food and fluid volume on the rate and extent to which drugs administered orally are absorbed has received much attention in recent years [15]. Studies have shown that the concurrent ingestion of food with medication can greatly affect the pharmacokinetics of many drugs. The effect is mainly to reduce absorption efficiency and slow absorption, although the clinical significance is largely determined by the therapeutic index and dose-response curve of the drug [14].

Recent work has investigated the effect of food on the pharmacokinetics of chlorambucil [8] and the structurally related antineoplastic agent, melphalan [4]. Chlorambucil (Leukeran) is an alkylating agent which has been in clinical use for more than two decades. Currently it is used in the treatment of chronic lymphocytic leukaemia [13], Hodgkin's and non-Hodgkin's lymphoma and ovarian carcinoma [9]. Ehrsson and co-workers noted that, unlike that of melphalan [4], chlorambucil bioavailability was not affected by the concurrent ingestion of food (5 patients). However, the authors were unable to determine the elimination rate constants under non-fasting conditions. Consequently, it was impossible to determine whether an impaired bioavailability was offset by reduced drug elimination. In this larger study we show that concurrent ingestion of food reduces the bioavailability of chlorambucil.

Offprint requests to: C. G. Adair, Department of Haematology, The Queen's University of Belfast, Institute of Pathology, Grosvenor Road, BELFAST, BT12 6BL, N. Ireland

Patients, materials and methods

Chlorambucil was assayed in plasma using an isocratic reversed-phase high-performance liquid chromatography (HPLC) procedure, which permits detection of drug in plasma to 10 ng/ml, using UV absorption at 260 nm [1]. Separation of chlorambucil from plasma components was performed on a Spherisorb ODS 5 μm column (250 × 4.6 mm), which was maintained at 40 °C using a column oven. The mobile phase was a mixture of 80% methanol and 20% water (v/v). Drug extraction necessitates the precipitation of the macromolecular components in 3 ml plasma using 132 μl concentrated perchloric acid. The resulting supernatant was passed through a C_{18} reversed-phase Sep Pak and the polar components removed by washing the cartridge with 10 ml 15% methanol in water (v/v). Chlorambucil was eluted from the Sep Pak with 2 ml cold (−20 °C) methanol, 200 μl of which was injected onto the column. Drug retention was in the order of 4.2 min. No interference from plasma was observed in any of the patients at the retention time of chlorambucil.

Details of the ten patients studied are given in Table 1. Pharmacokinetic studies were carried out on patients who received chlorambucil chemotherapy on a routine basis and with their informed consent. The dose ranged from 11 to 40 mg daily. Drug plasma levels were measured on consecutive days of chemotherapy, with patients being randomly assigned to fasting on the first or second days of treatment. Details of the standard breakfast are given in Table 2.

After an overnight fast from 8.00 p. m., subjects were given breakfast at 9.30 a. m. the following morning, followed by immediate administration of chlorambucil. Peripheral blood samples (6 ml), taken via an indwelling cannula in a forearm vein, were stored in heparinised tubes (2 °C). Samples were taken before drug administration and 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300 and 360 min after the dose. Patients remained seated throughout the study and were not permitted food or drink until 3 h after drug administration. After separation (−6 °C), plasma samples were kept on ice (6 h at 2 °C) prior to their analysis by HPLC the same day. Stability studies for chlorambucil indicate that it did not undergo hydrolysis during

Table 1. Patients studied

Patient	Diagnosis	Age (years)	Sex	GFR (ml min^{-1})	Daily dose (mg)	Surface area	mg/m^2
WN	CLL	62	M	94	40	2.00	20
JS	NHL	77	M	72	30	2.04	15
DM	CLL	58	F	50	30	1.60	19
RS	NHL	73	M	70	25	1.73	14
NL	CLL	49	M	97	30	2.10	15
ML	NHL	71	F	*	11	1.49	7.5
RL	NHL	73	M	68	30	1.84	16
GL	NHL	35	M	94	30	1.70	19
RH	CLL	65	F	54	30	1.49	21
JF	NHL	63	M	92	15	1.82	8.5

CLL, chronic lymphocytic leukaemia; NHL, non-Hodgkin's lymphoma

the conditions reported here for sample collection and extraction. The rate of spontaneous hydrolysis of chlorambucil at 37 °C was determined in normal donor plasma by incubating plasma samples spiked with chlorambucil for 8 h. Aliquots (1 ml) were removed at hourly intervals and assayed for parent compound.

Serum creatinine levels were measured by routine procedure at the Biochemistry Laboratory of the Royal Group of Hospitals. Glomerular filtration rates (GFR) were then calculated according to a formula adapted from that of Cockcroft and Gault [6]:

$$\text{GFR} = \frac{k\,[140 - \text{Age (years)}] \times \text{wt (kg)}}{\text{Serum creatinine } (\mu\text{M})}, \quad \text{Eq. 1}$$

where k = 1.23 for males and 1.04 for females.

Elimination rate constants for chlorambucil were calculated by regression analysis of the terminal data points on log plasma chlorambucil/time profiles. The area under the chlorambucil/time curve (AUC) was calculated using the trapezoidal rule and extrapolated to infinity.

Table 2. Details of the standardised breakfast

	Quantity	Weight of components (g)		
		Carbohydrate	Protein	Fat
Orange juice	200 ml	19	1	Trace
Cornflakes	20 g	17	2	–
Milk	230 ml	11	8	9
Sugar	15 g	16	–	–
Egg	60 g	–	7	7
Whole bread	40 g	17	4	–
Butter	10 g	–	–	8
Marmalade	20 g	14	–	–
Tea	–	–	–	–
		94	22	25

Results

The pharmacokinetic parameters obtained for each patient are given in Table 3. Statistical analysis of data was performed using the Wilcoxon signed rank test for non-parametric data (two-tail test). There was a statistically significant difference in all pharmacokinetic data between fasting (F) and nonfasting (NF) conditions. Results (mean ± standard error) were variable, with peak plasma levels occurring 44 ± 10 min (F) and 102 ± 25 min (NF) after administration ($P < 0.01$). Peak plasma levels attained were 1130 ± 185 ng/ml (F) and 505 ± 82 ng/ml (NF) and the dif-

Table 3. Summary of pharmacokinetic parameters following administration of oral chlorambucil

	Time to peak plasma conc. (min)		Peak plasma conc. (ng/ml)		Area under curve (μg min ml^{-1})				Terminal rate constant (min^{-1} × 10^{-3})		Terminal half-life elimination (min)	
					t = 0–360		t = 0–∞					
Patient	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
WN	36	38	1249	821	103.6	105.6	106.4	112	10.46	9.15	66	76
JS	70	70	703	391	76.0	58.4	77.7	60.8	12.58	10.45	55	66
DM	35	118	798	323	66.9	70.4	71.0	78.3	9.81	8.61	71	80
RS	125	230	983	384	232.0	74.7	291.5	172.5	*	*	*	*
NL	35	72	742	409	75.7	55.4	77.6	60.0	10.70	8.6	65	80
ML	33	245	738	131	56.1	23.8	57.6	32.2	12.75	7.02	54	99
RL	30	118	2134	631	134.5	94.3	137.6	97.9	11.32	9.38	61	74
GL	29	42	2222	1025	150.7	121.7	155.8	125.3	9.67	7.88	72	88
RH	28	57	1072	468	145.7	84.9	152.2	92.6	10.76	10.23	64	68
JF	15	29	655	458	48.0	27.3	49.1	28.7	10.42	7.65	67	91

F, fasting; NF, non-fasting

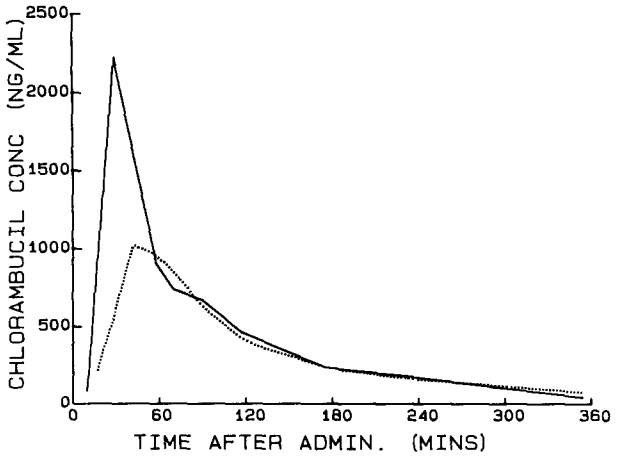

Fig. 1. Representative plasma chlorambucil/time profile for patient GL, receiving 30 mg daily for 3 days: ———— Fasting; ..... non-fasting

ference was significant at $P<0.01$. $AUC_{0-\infty}$ fasting values (118 ± 23 μg min ml^{-1}) were significantly greater ($P=0.01$) than $AUC_{0-\infty}$ non fasting (86 ± 14 μg min ml^{-1}). Similarly, values for AUC_{0-360} were greater ($P<0.01$) under fasting conditions (109 ± 18 μg min ml^{-1}) than under non-fasting conditions (72 ± 10 μg min ml^{-1}. The rate constants for elimination were lower in value ($P<0.01$) under non fasting conditions (8.78 ± 0.38 $min^{-1}\times10^{-3}$; $t\frac{1}{2}=80\pm4$ min) than when patients fasted (10.93 ± 0.37 $min^{-1}\times10^{-3}$; $t\frac{1}{2}=64\pm2$ min).

The relationship between chlorambucil elimination and renal function was investigated by regression analysis of GFR (77 ± 6 ml min^{-1}) with the rate constant for drug elimination. The results were not significant ($N=8$; $r=-0.007$ $P=0.72$).

The hydrolysis rate constant for chlorambucil in normal donor plasma at 37 °C was 1.4×10^{-3} min^{-1} ($t\frac{1}{2}=497$ min), which is in close agreement with the data of Alberts and co-workers [2]. At 2 °C no significant hydrolysis of chlorambucil occurred in plasma over a period of 8 h.

Discussion

The results of this study indicate that AUC and peak plasma levels are -educed when chlorambucil is given with food (Fig. 1). This differs from the conclusions of Ehrsson et al. [8], who suggested that food did not affect AUC. However, the smaller breakfast used in their study with five patients may not have affected bioavailability significantly.

Recent work with melphalan [7] suggests that peak plasma levels may be the major determining factor for tumour cytoxicity. If this is also true for chlorambucil, then the effect of food is more significant because of the greater degree of significance obtained with peak plasma levels ($P<0.01$), compared to AUC ($P=0.01$). The effect of food on chlorambucil to AUC is less than that observed for melphalan [4], which perhaps reflects the better absorption characteristics of the former from the gut.

The delay in peak plasma levels under non-fasting conditions compared with fasting suggests that absorption is slowed by food. The probable cause of this is a reduction in the rate of gastric emptying. It has been noted previously that the predominant effect of food has been to delay gastric emptying and increase drug residence time in the stomach [14]. This may be caused by food of high viscosity [10], fat and, to a lesser extent, protein and carbohydrate [13]. The increase in gastric residence time of chlorambucil may, therefore, reduce absorption efficiency by hydrolysis. Chatterji [5] has shown that chlorambucil undergoes rapid spontaneous hydrolysis in aqueous media. In addition, it has been suggested that food, by stimulating intestinal motility, increases transit of the drug through the intestine, thereby reducing absorption.

The rate constant for chlorambucil elimination was reduced under non-fasting conditions. Only in one patient could this not be determined because the terminal data points represented the absorption rate constant rather than the elimination rate constant.

Although chlorambucil undergoes extensive metabolism, it is unlikely that food interferes with this to reduce elimination. To date, the only direct effects of food on drug metabolism have been inductive and concern drugs which are subject to high first-pass extraction [11]. In view of these considerations, a simplistic but probable explanation is that residual drug absorption continues into the elimination phase, thus increasing drug half-life under non-fasting conditions.

The long half-life of hydrolysis of chlorambucil in plasma (497 min) indicates systemic spontaneous hydrolysis to be a minor route of elimination. Regression analysis of renal function (GFR) with elimination rate constants suggests that chlorambucil is not dependent on renal function for its elimination. This supports earlier animal studies [12], which found renal function to be an important mechanism in the excretion of chlorambucil metabolites but of minor significance for the parent compound.

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