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A pharmacokinetic study of prednimustine as compared with prednisolone plus chlorambucil in cancer patients

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Summary. The pharmacokinetic characteristics of prednisolone and of chlorambucil and its β-oxidized metabolite, phenylacetic mustard (PAM) were studied in plasma after the oral administration of 200 mg prednimustine (Sterecyt) and a regimen consisting of 20 mg prednisolone plus 20 mg chlorambucil, respectively. A total of 12 cancer patients completed this trial. The drugs were given in a cross-over study as single doses, and serial plasma samples were collected for 32 h. Chlorambucil and PAM were assayed by a gas chromatographic/mass spectrometry method and prednisolone, by radioimmunoassay. The median relative availability of the prednisolone and chlorambucil moiety in prednimustine was 19% and 16%, respectively. Prednisolone, as well as chlorambucil and PAM, appeared later and at a significantly lower concentration in plasma after treatment with prednimustine as compared with the mixture of chlorambucil and prednisolone. We also found that the elimination phase of chlorambucil and PAM in plasma is prolonged after the administration of prednimustine as compared with chlorambucil per se. In contrast, the elimination of the prednisolone moiety of prednimustine and that following the administration of a plain prednisolone tablet did not seem to differ. The modified plasma profile of the alkylating components following prednimustine administration may be important for the clinical efficacy of prednimustine.

Introduction

The cytotoxic agent prednimustine (Sterecyt, LEO 1031, NSC 13487) is the 21-chlorambucil ester of prednisolone. Previous pharmacokinetic studies have shown that intact prednimustine cannot be detected in plasma following its oral administration [5, 13]. The drug is rapidly metabolized to its components prednisolone, chlorambucil and the β-oxidized chlorambucil metabolite, phenylacetic mustard (PAM), which are detected in plasma (Fig. 1).

For optimal drug therapy and for a contribution to clarification of the mechanism of drug action, basic information about the pharmacokinetics of prednimustine is needed. The kinetic properties of prednisolone and chlorambucil derived from prednimustine, however, have thus far been studied only in small groups of patients [2, 14, 15]. These studies indicate that the kinetic profile of prednimustine is different from that of a mixture of its components. The elimination phase of the cytotoxic metabolites of prednimustine, however, can be characterized in detail only using an extended sampling period, as in the present study. The study was designed as a randomized cross-over investigation comparing the plasma kinetics following a single 200-mg dose of prednimustine and that seen after the administration of a combination of 20 mg prednisolone and 20 mg chlorambucil. The selected dose of prednimustine is commonly used in intermittent schedules over 5 consecutive days, and preliminary studies have indicated that the doses selected for the two study regimens give similar systemic concentrations of the alkylating agents.

Patients and methods

Patients. A total of 13 cancer patients (6 men and 7 women) with a median age of 59 years (range, 41–74 years) entered the study after giving their informed consent to participate. The study was approved by the local ethics committee and was carried out according to the Helsinki declaration. All patients had malignant metastatic disease that did not involve the gastrointestinal tract. The median performance status was 1 (range, 0–2) and the expected survival was ≥2 months. Nine patients had previously received chemotherapy. Pretreatment leucocyte and platelet counts were within the ranges of 4.2–13.9 and 160–500 × 10^9/l, respectively. Among the clinical laboratory tests (sodium, potassium, calcium, creatinine, ALAT, bilirubin and normotest), serum calcium was below the lower limits in three patients, for one of whom the serum creatinine value was slightly elevated. All other laboratory tests were within normal limits.

Design of the study. The investigation was designed as a randomized two-way, cross-over study involving a minimum of 4 days between

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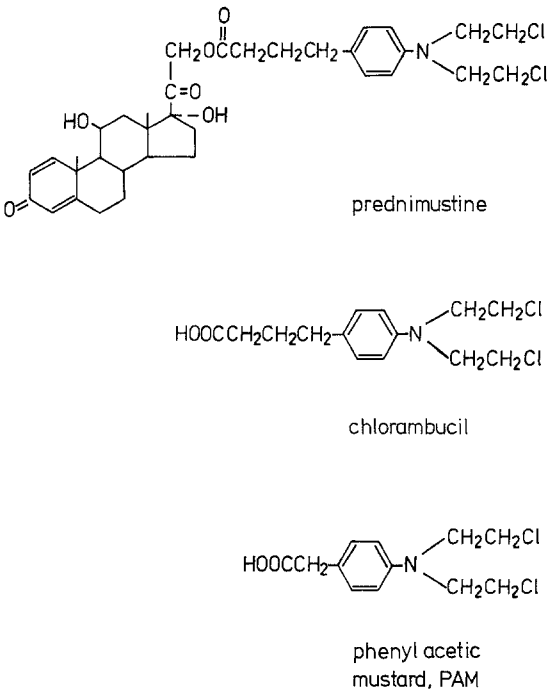

Fig. 1. Structural formulas of prednimustine, chlorambucil and phenylacetic mustard

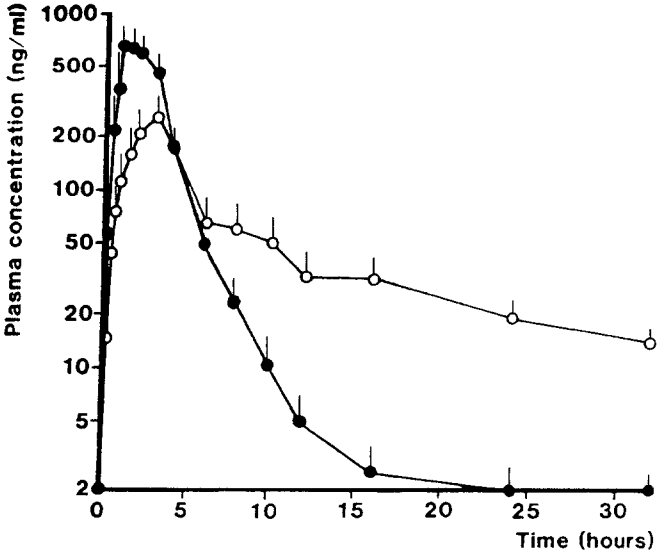

Fig. 2. Mean (± SEM) plasma levels of chlorambucil after administration of the free drug (●) and prednimustine (○). Patients (n = 12) were given 20 mg chlorambucil +20 mg prednisolone and 200 mg prednimustine, respectively

courses of drug administration. Each patient was to receive a single dose of 200 mg prednimustine given as tablets (Sterecyt) and a single oral dose of 20 mg prednisolone (Prednisolon ACO) plus 20 mg chlorambucil (Leukeran), respectively. The subjects were hospitalized and fasted overnight. The drugs were given at 8.00 a.m., at 1 h after patients had eaten a standardized, light breakfast. Lunch was provided at about 4 h after drug administration. Blood samples were collected in heparinized tubes prior to drug administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, and 32 h after dosing. After collection, the samples were cooled and the plasma was separated immediately and stored at –20° C until analysis.

Analytical methods. Chlorambucil and PAM were analysed using a mass fragmentographic determination [10]. According to this method, chlorambucil and PAM are selectively (i.e. regarding prednimustine and other lipophilic esters of chlorambucil) back-extracted from the organic phase to an alkaline-buffered phase [10]. Using a mass spectrometer type TSQ70 (FinniganMAT), the limit of detection in the present study was found to be 1.5 and 1.9 ng/ml for chlorambucil and PAM, respectively.

The plasma concentration of prednisolone was determined in triplicate by radioimmunoassay. The antiserum was purchased from BioBol (Stockholm, Sweden, product 1291). The antiserum was diluted 1:32,000 (v/v) which gave 40%–60% binding of the radioligand [2,4,6,7-^{3}H]-prednisolone. The radiochemical purity was >98% and the specific radioactivity was 57 Ci/mmol. The specificity of the antiserum was characterized by the following cross-reactivities: 20-dihydroprednisolone, 21%; prednisone, 11%; cortisol, 4%; and cortisone, 3%. Other steroids tested showed cross-reactivity values of 2%. The cross-reactivity of chlorambucil was <0.1%.

Prednisolone was extracted from plasma with 9:1 (v/v) ethylacetate:hexane. The extraction yield was estimated to be 91% using spiked plasma samples. The sensitivity of the assay was about 15 ng/ml, and the mean intra-assay coefficient of variation, based on 29 identical samples of patient plasma, was 10%. For calculation of inter-assay precision, daily determinations of internal standard were evaluated. The mean inter-assay coefficient was 8.5%.

Data analysis. The c_{max} and t_{max} values were determined from the observed plasma concentration versus time curve and represented the maximal plasma concentration found in each subject and the corresponding time, respectively. The area under the plasma concentration-time curve from zero to time t, AUC_{0-t}, was calculated by the linear trapezoidal method. The elimination rate constant, β, was estimated by linear regression analysis of the logarithmic curve. The elimination half-life, $t_{1/2}$, was determined from its relationship to β ($t_{1/2} = 1n2/\beta$).

The influence of treatment order was evaluated using the Mann-Whitney U-test. The results showed no significant difference between sequences for any substance, and further analyses were done without any attention being paid to treatment order. The individual pharmacokinetic parameters t_{max}, c_{max}, AUC and $t_{1/2}$ were evaluated and compared between treatments using nonparametric statistical tests. The statistical level of significance used was 5%. Pharmacokinetic parameters are shown as median values, followed by the Wilcoxon interval, with the 95% confidence limits being indicated in parentheses.

Results

A total of 13 patients were randomized and 12 completed the study; 1 subject dropped out for reasons not related to the treatment. Only mildly adverse gastrointestinal reactions were observed during the study, with no significant difference being found between treatments. In all, five cases of WHO grade 1 nausea [16] were observed.

The individual and median t_{max} and c_{max} values for chlorambucil, PAM and prednisolone are shown in Tables 1, 2 and 3, respectively. As compared with those obtained after the combined chlorambucil/prednisolone treatment, peak values for all three substances occurred later and were found to be significantly lower following the prednimustine treatment (Figs. 2–4).

The concentration of each substance at 32 h after the administration of chlorambucil plus prednisolone did not significantly differ from the initial pre-dose concentration. After treatment with prednimustine, however, the concentrations of chlorambucil and PAM did not return to zero during the 32 h of plasma sampling; therefore, the corre-

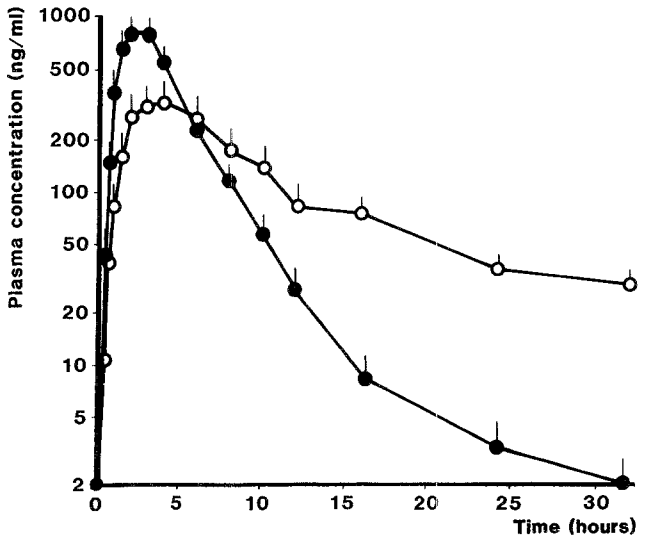

Fig. 3. Mean (± SEM) plasma levels of PAM after administration of the free drug (●) and prednimustine (○). Patients (n = 12) were given 20 mg chlorambucil +20 mg prednisolone and 200 mg prednimustine, respectively

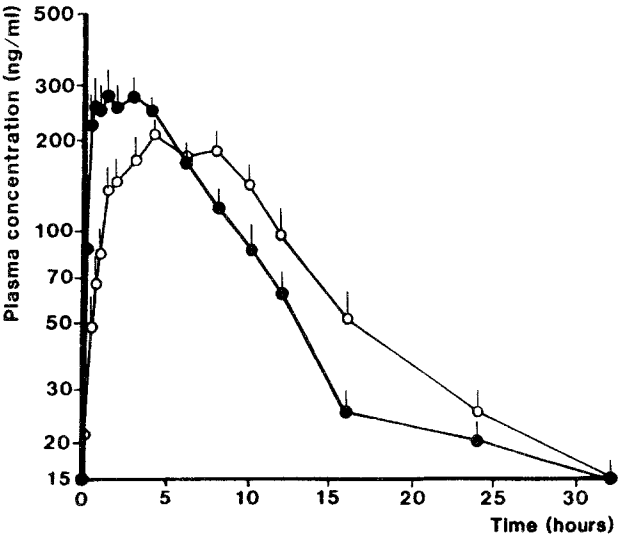

Fig. 4. Mean (± SEM) plasma levels of prednisolone after administration of the free drug (●) and prednimustine (○). Patients (n = 12) were given 20 mg chlorambucil +20 mg prednisolone and 200 mg prednimustine, respectively

Table 1. Pharmacokinetic parameters from plasma-concentration profiles of chlorambucil after treatment with 200 mg prednimustine and with 20 mg chlorambucil +20 mg prednisolone, respectively

Patient number	Treatment	AUC (ng h ml^{-1})	c_{max} (ng ml^{-1})	t_{max} (h)	$t_{1/2}$ (h)	$100 \times \frac{AUC_{0-10\,h}}{AUC_{0-32\,h}}$ (%)
1	I	1,010	170	2	5.8	70
	II	1,860	780	2.1	NE	90
2	I	1,850	670	3	NE	66
	II	2,430	2,010	0.8	1.2	99
3	I	3,570	1,390	2	NE	94
	II	1,760	1,040	0.9	1.2	98
4	I	2,320	570	3	7.4	75
	II	3,000	750	2	1.7	96
5	I	2,400	470	1	7.2	71
	II	3,830	1,840	0.8	1.2	99
6	I	2,450	330	3	NE	43
	II	5,980	2,150	2	3.3	93
7	I	1,250	230	2	NE	52
	II	1,810	1,040	0.7	1.2	99
8	I	2,880	290	3.1	5.5	71
	II	2,760	1,150	2.9	1.6	98
9	I	1,460	360	2	NE	79
	II	2,130	950	1	1.5	98
10	I	1,270	110	9.9	10.3	56
	II	2,030	730	1.5	1.9	97
11	II	2,000	750	2	1.5	98
12	I	2,140	340	4.3	16.4	68
	II	1,690	410	3.2	5.4	75
13	I	2,960	410	4.2	11.2	74
	II	1,470	460	3.1	2.1	97
Median	I	2,330	350	3	7.4	71
	II	2,080	1,000	1.8	1.6	98
95% confidence limits	I	(1,660; 2,640)	(260; 570)	(2; 4.2)	(5.8; 13.3)	(60; 75)
	II	(1,810; 3,290)	(740; 1,480)	(1; 2.5)	(1.4; 3.3)	(93; 98)

I, 200 mg prednimustine; II, 20 mg chlorambucil +20 mg prednisolone; NE, not evaluable

Table 2. Pharmacokinetic parameters from plasma-concentration profiles of phenylacetic mustard after treatment with 200 mg prednimustine and with 20 mg chlorambucil +20 mg prednisolone, respectively

Patient number	Treatment	AUC (ng h ml^{-1})	c_{max} (ng ml^{-1})	t_{max} (h)	$t_{1/2}$ (h)	$100 \times \frac{AUC_{0-10\,h}}{AUC_{0-32\,h}}$ (%)
1	I	3,600	480	3	9.3	67
	II	6,400	1,550	3	5	85
2	I	1,790	180	2	NE	43
	II	3,740	1,140	1.5	2.3	97
3	I	6,170	1,590	3	NE	86
	II	4,500	1,450	1.5	4.1	97
4	I	4,050	440	3.9	9.2	67
	II	5,090	830	3	4.1	88
5	I	4,290	500	2.1	5.2	69
	II	6,740	2,080	1	6.3	98
6	I	3,420	270	4.2	NE	36
	II	7,490	1,260	3	3.8	79
7	I	2,720	280	2.9	NE	51
	II	3,170	1,020	1.4	1.7	97
8	I	5,420	430	8	5.9	61
	II	5,910	1,220	2.9	5	91
9	I	2,630	490	2	NE	69
	II	3,790	960	1.9	2.5	95
10	I	2,170	220	9.9	10.6	48
	II	3,210	730	2.1	2.2	94
11	II	3,260	650	2	1.8	94
12	I	7,340	600	6.2	9.3	47
	II	5,500	750	4.1	5.9	68
13	I	4,440	490	4.2	9.5	71
	II	2,420	580	4	2.8	91
Median	I	3,830	460	3.4	9.3	64
	II	4,800	1,080	2.5	3.9	93
95% confidence limits	I	(2,920; 5,030)	(330; 550)	(2.5; 6)	(5.9; 10)	(49; 69)
	II	(3,750; 5,920)	(860; 1,400)	(1.7; 3)	(2.7; 5)	(84; 96)

I, 200 mg prednimustine; II, 20 mg chlorambucil +20 mg prednisolone; NE, not evaluable

sponding AUC values may be seen as minimal estimates of the total area.

The extent of bioavailability – defined as the plasma AUC from zero to 32 h after drug administration – is shown in Tables 1–3. As compared with the oral administration of its components, the relative bioavailability of prednisolone and chlorambucil following treatment with prednimustine was 19% (16%, 22%) and 16% (13%, 29%), respectively.

Analysis of the individual plasma concentration versus time curves for chlorambucil and PAM revealed a significantly longer half-life after the prednimustine treatment as compared with the administration of plain chlorambucil. Following treatment with chlorambucil, the half-lives for chlorambucil and PAM in plasma were 1.6 (1.4, 3.3) and 3.9 (2.7, 5) h, respectively. The corresponding half-lives after treatment with prednimustine were in the range of 6–12 h. Following dosing with prednimustine, however, a terminal half-life for its alkylating metabolites could not be calculated for some of the patients due to an irregular second phase. Therefore, the fraction of metabolites eliminated during the first 10 h was calculated and is presented in Tables 1 and 2. After the administration of chlorambucil per se, about 95% of the parent drug in plasma had been eliminated within the first 10 h. In contrast, following the administration of prednimustine, only about 70% of the chlorambucil metabolite was eliminated during this period. Furthermore, this result may be considered to be valid even after a lag-phase tendency following prednimustine treatment has been taken into account.

The same pattern of differences between treatment schedules was observed for the terminal phase of the plasma concentration versus time curve for PAM. During the first 10 h post-administration, 93% (84%, 96%) and 64% (49%, 69%) of this metabolite was eliminated after doses of plain chlorambucil and prednimustine, respectively. The terminal half-lives of plasma prednisolone are shown in Table 3. As judged from the observed half-lives of 3.9 (3.4, 6) and 3.8 (3.3, 4.9) h, respectively, the elimination of prednisolone was similar in both of the treatment modalities studied. Thus, after prednimustine treatment, elimination was highly prolonged for chlorambucil and PAM but not for prednisolone.

Discussion

Prednimustine is a prodrug of chlorambucil and prednisolone. No intact prednimustine has been detected in the

Table 3. Pharmacokinetic parameters from plasma-concentration profiles of prednisolone after treatment with 200 mg prednimustine and with 20 mg chlorambucil +20 mg prednisolone, respectively

Patient number	Treatment	AUC (ng h ml^{-1})	c_{max} (ng ml^{-1})	t_{max} (h)	$t_{1/2}$ (h)
1	I	2,480	210	3	3.5
	II	2,380	290	2.1	5.7
2	I	1,190	140	3	3.4
	II	1,920	450	0.5	2.8
3	I	3,390	440	3	3.9
	II	2,830	400	1.5	4.1
4	I	2,750	240	6.1	4.1
	II	2,010	270	4.2	3.4
5	I	3,040	290	1	3.7
	II	3,220	560	0.8	3.5
6	I	2,530	180	4.2	8.9
	II	3,360	420	1.5	5.6
7	I	1,290	160	2.9	2.7
	II	1,570	340	0.7	2.6
8	I	2,550	190	10.2	3
	II	2,030	270	2.9	3.4
9	I	1,940	230	1.5	3.8
	II	2,100	410	0.5	3.1
10	I	3,190	260	9.9	5.4
	II	2,890	290	3	4.3
11	II	2,200	290	2	4.5
12	I	4,120	290	8.5	5.8
	II	3,180	350	4.1	5.2
13	I	3,910	260	4.2	7.9
	II	2,640	250	0.5	6
Median	I	2,650	240	3.6	3.9
	II	2,510	350	1.5	3.8
95% confidence limits	I	(2,110; 3,300)	(200; 290)	(2.9; 6.6)	(3.4; 6)
	II	(2,070; 2,910)	(290; 410)	(3.3; 4.9)	(3.3; 4.9)

I, 200 mg prednimustine; II, 20 mg chlorambucil +20 mg prednisolone

plasma of humans who have been given oral prednimustine [5, 13]. Thus, the prednimustine molecule must be metabolized either prior to or during its absorption through the intestinal wall or on its first passage through the liver.

In the present study, large fractions of the alkylating components were present in plasma for a long period following the treatment with prednimustine as compared with the administration of plain chlorambucil. This demonstrates the necessity of extending the time schedule for plasma sampling, especially in the interval between 10 and 20 h post-administration, when the pharmacokinetics of prednimustine are studied. Following the administration of this drug, it is difficult to obtain a reliable estimate of terminal half-lives for the alkylating agents. Therefore, we also chose to characterize the persistency of such compounds in plasma by calculating the fractional area remaining at 10 h post-administration.

In previous studies, the bioavailability of chlorambucil has been estimated to be about 5 times lower when this drug is given as prednimustine as compared with its administration as the free drug [2]. For the prednisolone moiety, somewhat higher figures have been found in pilot studies [15]. Based on these data, prednimustine was given during the present study at a dose that was 5-fold that of plain chlorambucil plus prednisolone. As judged by the AUC values, we found an equal exposure to prednisolone and chlorambucil on both treatment schedules, indicating a relative bioavailability of about 20% for these compounds after the administration of prednimustine. The possible accumulation of metabolites will be examined further in repeated-dose studies using prednimustine.

According to a recent study by Ferry et al. [3], prednisolone is rapidly absorbed after oral administration and its absolute bioavailability is almost 100%. Also, the kinetics of chlorambucil [1, 8, 11] have shown this substance to be well and regularly absorbed, the absolute bioavailability being close to 100%. In the present study, the relative bioavailability of the molecular components of prednimustine was only about 20%. This could be explained by incomplete absorption since prednimustine is only slightly soluble in water. Other factors, such as presystemic metabolism resulting in different metabolic products, should also be considered. Since drugs with poor absorption characteristics are usually associated with a large variation in bioavailability, it is noteworthy that variations of similar magnitude were observed in comparisons of prednimustine administration with the regimen containing prednisolone plus chlorambucil.

When the individual components of the ester molecule as well as one well-known metabolite of chlorambucil were analysed in the present study, several pharmacokinetic differences between prednimustine and its components were found. First, a low rate of metabolite bioavailability was found after prednimustine treatment. Second, although

the doses chosen for this study gave similar exposure (AUCs) to alkylating agents in both of the treatment modalities tested, the peak values observed after the prednimustine treatment were much lower and occurred several hours later than those noted following the administration of chlorambucil plus prednisolone. Third, the terminal elimination phase of chlorambucil and PAM – but not that of prednisolone – was longer after prednimustine treatment. The reason for the comparatively long persistence of alkylating agents in our patients is not yet known, but it is unlikely to be attributable to slow absorption of prednimustine since the elimination of prednisolone was not greatly changed. Instead, we speculate that the disposition of chlorambucil and PAM may be changed. Studies in dogs (Katsura, personal communication) have shown the same profile found in patients. In dogs, another metabolic pathway, transesterification, has been found [6], which might result in prolonged chlorambucil elimination.

In animal experiments, the toxicity of prednimustine has been shown to be reduced as compared with chlorambucil [4, 12]. Such a result could be explained by the low peak values found for cytotoxic metabolites following the administration of prednimustine [12]. Interestingly, our study demonstrates that prednimustine also results in relatively low peak values for chlorambucil and PAM in cancer patients treated with the drug.

The effect of prednimustine is exerted via the release of chlorambucil and prednisolone; thus, the former drug acts as a classic alkylating agent and as a corticosteroid. Prednimustine has demonstrated a wide spectrum of anti-tumour activity both in vitro and in vivo [4, 7, 12] and has exhibited superior clinical efficacy as compared with its components in the treatment of lymphoma patients [9]. The anti-tumour data demonstrate that the activity of prednimustine is higher than that of an equimolar mixture of its components, unless sophisticated split-dose scheduling using chlorambucil plus prednisolone is carried out [12]. Prednimustine is also active against chlorambucil-resistant tumours via its components both in vitro and in vivo. One explanation for these findings is the comparatively long persistence of prednimustine-derived chlorambucil both in the cell-line system [7] and in the animal experimental model [12]. In vitro metabolism studies have indicated that intact prednimustine is inactive but that the prolonged persistence of chlorambucil correlates with the observed potency [7]. This indicates that prednimustine releases chlorambucil and prednisolone in such a way that a cytotoxic profile different from that of its components is found. Our results in cancer patients again show this pharmacokinetic profile of prednimustine, with a prolonged terminal elimination phase being noted for the cytotoxic metabolites.

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