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Disposition of orally administered ^{14}C-prednimustine in cancer patients

Robert C. Gaver[1], George Deeb[1], Kenneth A. Pittman[1], Brian F. Issell[2], Arnold Mittelman[3], and Robert D. Smyth[1]

[1] Departments of Metabolism and Pharmacokinetics and
[2] Clinical Cancer Research, Bristol-Myers Co., PO Box 4755, Syracuse, NY 13220-4755 USA
Roswell Park Memorial Institute, Buffalo, NY 14263, USA

Summary. *A single oral solution dose (40 mg/m^2) of ^{14}C-prednimustine was administered to each of four cancer patients. Plasma, urine, and feces were collected at appropriate times and analyzed for total radioactivity. Plasma samples were analyzed for prednimustine. Peak plasma levels of radioactivity (1–3 μg ^{14}C-prednimustine equivalents) occurred at 1.5–3 h in three patients and at 5–6 h in one patient. No intact prednimustine was detected in the plasma; this means that if present, it would be at a concentration of 0.02 μg/ml or less and would account for less than 1% of the total drug-related material at the time of peak plasma levels. Solvent-extractable metabolites had a plasma half-life of about 8 h or less. By 24 h essentially all the plasma radioactivity appeared to be covalently bound, and it was eliminated slowly with an estimated terminal elimination half-life of about 10 days. Rapid urinary excretion occurred in the first 24 h, and 40%–60% of the dose was recovered in the urine in 72 h. Although prednimustine was well absorbed, the ester was subject to extensive presystemic metabolism and was not present in the systemic circulation after oral administration.*

Introduction

Prednimustine (LEO-1031, NSC-134087) is an ester of chlorambucil and prednisolone [11β,17α, 21-trihydroxy-pregna-1,4-diene-3,20-dione,21-4-*N*,*N*-bis(1-chloro-1,2-di-^{14}C-ethyl)-*p*-aminophenyl butyrate] (Fig. 1). The synthesis and relationship between activity and chemical structure of this class of cytostatic compounds have been described [13]. Prednimustine has activity against a number of experimental tumor systems [12], including lymphoid leukemia L1210 and osteosarcoma [7] and Yoshida ascites sarcoma [21]. Preliminary investigations have indicated prednimustine to be effective in patients with chronic lymphocytic leukemia [4, 6, 10, 20], non-Hodgkin lymphomata, mainly of the lymphocytic type [6, 8, 10, 16, 17, 20), breast cancer [18], acute myelocytic leukemia [2, 3], ovarian carcinoma [9, 15], and prostatic cancer [5]. Small doses of prednimustine given as an oral solution were well absorbed in cancer patients, relative to IV administration, but prednimustine appeared to be quantitatively hydrolyzed following oral administration, with the metabolites being excreted primarily in the urine [11]. The oral availability of the ester from tablets was quite variable based on the fecal excretion of intact prednimustine [14]. We report here the disposition of carbon-14-labeled prednimustine in cancer patients following the oral administration of 40 mg/m^2 as a solution in polyethylene glycol.

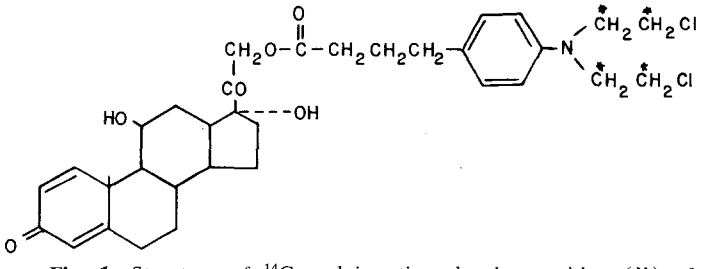

Fig. 1. Structure of ^{14}C-prednimustine showing position (✲) of carbon-14

Reprint requests should be addressed to R. C. Gaver
Abbreviations used in this paper are: HPLC, high-performance liquid chromatography; PEG, polyethylene glycol

Materials and methods

Chemicals. Carbon-14-labeled prednimustine (Fig. 1) was obtained from A.B. Leo Research Laboratories, Helsingborg, Sweden. The radiochemical purity, based on the distribution of radioactivity on HPLC, was 93% and the specific activity was 11.0 μCi/mg. The compound was dissolved in polyethylene glycol 400 (PEG-400) to give 3.7 mg prednimustine (91% radiochemically pure) and 7.6 μCi radioactivity/ml of the first lot (patients 1 and 2) and 3.9 mg of prednimustine (92% radiochemically pure) and 7.3 μCi radioactivity/ml of the second lot (patients 3 and 4). Analysis of the prednimustine content and radiochemical purity of the PEG-400 solutions showed them to be stable for at least 30 days when stored at 18–22° C in a closed container. Unlabeled prednimustine was from A.B. Leo; hexanes, pesticide grade, was from Fisher Scientific; phenylmethylsulfonyl fluoride from Calbiochem-Behring Corp.; triphenylmethane was from Eastman Kodak Co.; glass-distilled acetonitrile and methanol were from Burdick and Jackson Laboratories, Inc.; Insta-Gel, Soluene 350, and Dimilume 30 were from Packard Instrument Company, Inc.; and all water was purified in a Millipore Corp. MilliQ water purification system. Control plasma was obtained from volunteers.

Study design. Patients with the characteristics shown in Table 1 were fasted overnight, and the following morning were given a single dose of 40 mg prednimustine/m^2 body surface area. The prednimustine was dissolved in PEG-400 and administered via

Table 1. Characteristics of patients given ^{14}C-prednimustine

Patient			Body		Stage	Prednimustine dose		
No.	Age	Sex	Wt (kg)	SA (m^2)		(mg)	(mg/kg)	(mg/m^2)
1	58	F	85	1.9	D[a]	78	0.9	41
2	69	F	38	1.5	D[b]	60	1.6	40
3	36	M	85	2.1	D[c]	82	1.0	39
4	55	M	79	2.1	D[d]	82	1.0	39

[a] Adenocarcinoma of colon
[b] Adenocarcinoma of colon/rectum
[c] Adenocarcinoma of unknown origin
[d] Adenocarcinoma of rectum

a nasogastric tube. The tube was rinsed with an additional 10 ml PEG-400. Patients 1 and 4 were confined to a bed or chair during the study, while patients 2 and 3 were ambulatory during the daytime hours. Water (200 ml) was drunk at 1 h prior to drug administration and at 2, 4, 6, 8, and 10 h. A liquid diet was given 3–4 h after drug administration. A signed informed consent was obtained from each patient and laboratory tests were performed prior to and at the end of the study. Blood samples were obtained at appropriate times and total urine and fecal output were collected for at least 72 h.

Sample collection and processing. Blood samples (10 ml) were collected in Vacutainer tubes containing 20 mg potassium oxalate and 25 mg sodium fluoride (B.D. #4726) 15 min before and at the following times after drug administration: 0.1, 0.15, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, 24, 48, and 72 h. The blood was centrifuged immediately at 1,000 *g* for 10 min at 5° C. The plasma was transferred to labeled polypropylene tubes in an ice-water bath and duplicate 0.5-ml samples were placed in scintillation vials. Duplicate 1.0-ml samples were transferred to labeled 100 × 13-mm tubes containing 1.0 ml cold 0.02 *M* acetate buffer (pH 5). The latter, plus the remaining plasma, were immediately frozen in a dry-ice/ethanol bath, placed in a freezer and maintained frozen until analyzed. Urine samples were collected just prior to and over the following intervals after drug administration: 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–24, 24–48, and 48–72 h. The samples were collected in containers immersed in ice and the pH and volume were determined. Duplicate 0.5-ml samples were transferred to labeled scintillation vials. The remaining urine was frozen. Total 0–72 h fecal output was collected in individual plastic containers, placed in a freezer and stored frozen until analyzed. Feces were thawed, mixed, and diluted to a total volume of 250 ml with 0.02*M* acetate buffer (pH 5). The mixture was homogenized in a Sorvall Omni-Mixer (Dupont Instruments) and duplicate 0.5- or 1.0-ml samples were transferred to quartz combustion boats.

Analysis of radioactivity. Solutions of prednimustine, HPLC effluents, urine, and plasma were counted in 10 ml Insta-Gel (Packard Instrument Company, Inc.) in a Beckman LS9000 liquid scintillation spectrometer equipped with an automatic data reduction system. A set of Packard quench standards were used to generate a quench curve of H number vs counting efficiency [1]. Fecal samples were combusted in a Harvey model OX-300 Biological Oxidizer and the $^{14}CO_2$ was collected in 15 ml R.J. Harvey Instrument Company, Inc. ^{14}C-cocktail. Appropriate standards of feces and labeled compound were combusted to determine the efficiency of combustion (96% ± 3%). All samples were counted for 50–100 min or to a 2% 2-sigma error and corrected for background (50 cpm). All results were expressed in dpm. Total dpm/ml of plasma were divided by the specific activity of the prednimustine (4,511 dpm/μg for patients 1 and 2; 4,155 dpm/μg for patients 3 and 4) to give microgram equivalents of prednimustine-^{14}C/ml plasma.

High-performance liquid chromatography. The HPLC system consisted of the following components: Waters Associates, Inc. (Milford, MA), Model U6K injector, Model 6000A pump, Model 440 (254 nm) detector, a 300 × 4-mm (id) μBondapak C-18 column, and an Omniscribe recorder (Houston Instruments, Austin, TX). The mobile phase was methanol/water (4 : 1, v/v) at a flow rate of 2 ml/min. The retention times for chlorambucil, triphenylmethane, and prednimustine were 2.1, 6.6, and 8.0 min, respectively. In stability studies with unlabeled prednimustine the mobile phase was methanol/0.01 *M* phosphate buffer (pH 7) (81.5 : 18.5 v/v) at a flow rate of 2.5 ml/min. Triphenylmethane and prednimustine eluted at 5.6 and 6.8 min, respectively.

Analysis of ^{14}C-prednimustine in plasma. Plasma samples were thawed overnight at 3–7° C. Then 5 ml 7% (v/v) isoamyl alcohol in hexanes was added to 1.0 ml plasma plus 1.0 ml of 0.02 *M* sodium acetate buffer (pH 5) in a 100 × 13-mm screw-capped tube. After thorough mixing the tubes were centrifuged at 1,000 *g* for 10 min, and 4.0 ml of the organic phase was transferred to a clean 100 × 13-mm conical tube. The solvent was evaporated under a gentle stream of nitrogen. Immediately before chromatography, 0.250 ml methanol/water (4 : 1, v/v) was added to the residue, mixed, and placed in an ultrasonic bath for 3 min. After centrifugation at 2,750 *g* for 5 min, two 25-μl samples were placed in scintillation vials. A 150-μl sample was analyzed by HPLC. Two 1-min fractions, corresponding to the elution time of prednimustine, were collected in scintillation vials. Appropriate standards of ^{14}C-prednimustine were added to control plasma on the day of analysis and analyzed with the samples. The best-fit straight line for a plot of ^{14}C-prednimustine (μg) added per milliliter control plasma vs dpm recovered in the column effluent was determined by linear regression. The correlation coefficient of the standard curves over a concentration range of 0.05–1.0 μg prednimustine/ml plasma was greater than 0.999 and the slopes were $1.7–1.8 \times 10^{-3}$. No radioactivity was detected when control plasmas were analyzed. The limit of detection was about 0.01–0.02 μg (20–40 dpm) prednimustine/ml plasma,

and the relative standard deviation for 10 replicates (0.4 μg/ml) was 0.03. Prednimustine was stable under the conditions of storage and analysis.

Prednimustine stability in plasma. Samples of freshly drawn human serum or plasma containing 0.4–0.6 μg unlabeled prednimustine/ml were stored at −10° C and incubated at 37° C. At appropriate times, 1.0 ml acetonitrile, containing 10–20 μg triphenylmethane/ml, was added to 1.0 ml plasma in 100 × 13-mm tubes to precipitate plasma proteins. The tubes were mixed on a vortex mixer for 10 s and centrifuged at 2,500 *g* for 10 min. A 1.0-ml sample of the clear supernatant was transferred to a separate tube and 250 μl was analyzed by HPLC. Experiments with ^{14}C-prednimustine showed 98% of the added prednimustine was recovered in the supernatant as prednimustine. Standards of prenimustine covering a range of 0.05–1.2 μg/ml plasma were prepared fresh daily and analyzed with the samples. The peak height ratios of prednimustine to internal standard were calculated and the best-fit straight line of these ratios for the standards vs micrograms of prednimustine per milliliter of plasma was determined by least-squares regression analysis. The sample concentrations were determined by inverse prediction. The correlation coefficient of the standard curves were greater than 0.999 and the slopes were 0.58–0.63. The mean (± SD) concentration of prednimustine in triplicate samples stored at −10° C for 10 days (0.42 ± 0.02 μg/ml) was the same as the initial concentration (0.42 ± 0.01 μg/ml). The half-life of prednimustine in fresh human plasma was 1.5 h at 37° C and was not significantly prolonged by the addition of phenylmethylsulfonyl fluoride, $CsCl_2$ or NaF at concentrations of 2 mg/ml. Carbon-14-labeled prednimustine was added to ice-cold fresh human plasma at a concentration of 0.2 μg/ml and stored at 3–7° C. At appropriate times samples were analyzed as described under *Analysis of ^{14}C-Prednimustine in Plasma.* Prednimustine was stable for at least 24 h in fresh human plasma stored at 3–7° C, and had a half-life of about 8 days at this temperature.

Binding of radioactivity to plasma constituents. Samples of plasma (1.0 ml) from patient 1 were diluted with water (1.0 ml) and extracted twice with acetonitrile (5.0 ml) and once with methanol (5.0 ml). The samples were centrifuged to separate the insoluble material and portions of the extracts were removed for determination of total radioactivity. The residues were sonicated for 5 min with water (0.2 ml) and 1.0 ml of a tissue solubilizer, Soluene 350, left at room temperature for 16 h, and then counted with 15 ml Dimilume 30 as the scintillation fluid. Mean (± SD) recovery of total plasma radioactivity from 25 samples was 96% ± 2%. Samples of plasma (0.5 ml) from patient 2 were mixed with methanol (5.0 ml) and left at 7° C for 20 min. After centrifuging to separate the insoluble material the solvent phase was removed and counted. The procedure was repeated, and the residue was treated and counted as above. Mean (± SD) recovery of total plasma radioactivity from 19 samples was 101% ± 4%.

Results

The plasma concentrations of total radioactivity, expressed as microgram equivalents of ^{14}C-prednimustine per milliliter of plasma, for each of the patients are shown in Fig. 2. Peak concentrations of 1–3 μg equivalents/ml occurred at 1.5–3 h in three of the patients and at 5–6 h in the other (patient 1).

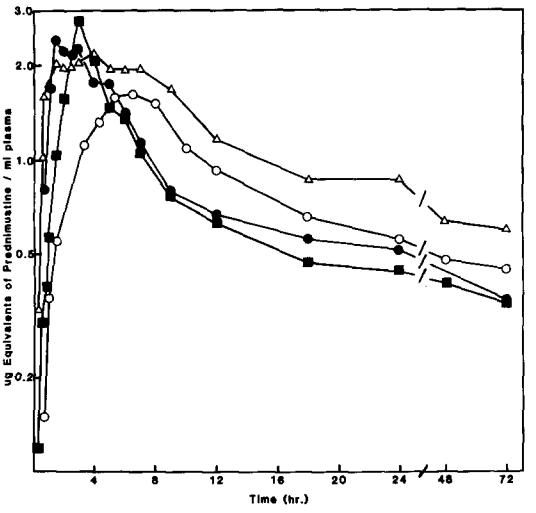

Fig. 2. Plasma concentrations of ^{14}C, expressed as microgram equivalents of prednimustine per milliliter, as a function of time after oral administration of ^{14}C-prednimustine (40 mg/m^2) to four cancer patients: 1 (○), 2 (△), 3 (■), 4 (●)

The levels of radioactivity decreased very slowly to about 0.1 μg equivalent/ml over 15–23 days, with an estimated terminal elimination half-life of 10 days.

HPLC analysis of plasma extracts for prednimustine showed that none of the radioactivity up to 12 h post-administration was associated with prednimustine. A standard of 0.10 μg ^{14}C-prednimustine/ml plasma gave 185–200 dpm in the fractions eluting at the retention time of prednimustine so the lower limit of detection, corresponding to 20–40 dpm above background, was about 0.01–0.02 μg prednimustine per ml. With blank plasma samples and all study samples, no more than 3 dpm above background (50 dpm) was detected in the column effluents at the retention time of prednimustine. If prednimustine was present in the plasma it accounted for less than 1% of the total drug-related material in the plasma at the time of peak concentrations of radioactivity.

In the analyses of prednimustine in plasma it was observed that as the time after drug administration increased, the percentage of the plasma radioactivity recovered in the organic solvent decreased. By 3–6 h only about 50% of the plasma radioactivity was extracted and by 12–18 h less than 10% was recovered, compared with 70%–75% during the first hour after administration. To assure removal of more polar metabolites, separate plasma samples were extracted several times with acetonitrile and methanol or with methanol alone. Both solvents removed 90% or more of the radioactivity present in the plasma within the first 2 h. However, by 12 h only 50%–60% was recovered by either solvent and by 24 h only 10% or less of the plasma radioactivity was solvent-extractable. The amounts of solvent-extractable and -non-extractable radioactivity, expressed as microgram equivalents of prednimustine per milliliter of plasma, as a function of time are shown in Fig. 3. Free, non-covalently bound metabolites of prednimustine (chlorambucil portion) decreased steadily over the first 24 h, with an estimated terminal elimination half-life of about 8 h or less. The concentration of covalently bound metabolites (non-solvent extractable), however, increased steadily over the first 8–12 h and remained relatively constant up to 24 h, when they accounted for essentially all of the radioactivity in the plasma. The approximately 10-day terminal elimination half-life for plasma radioactivity can be attributed

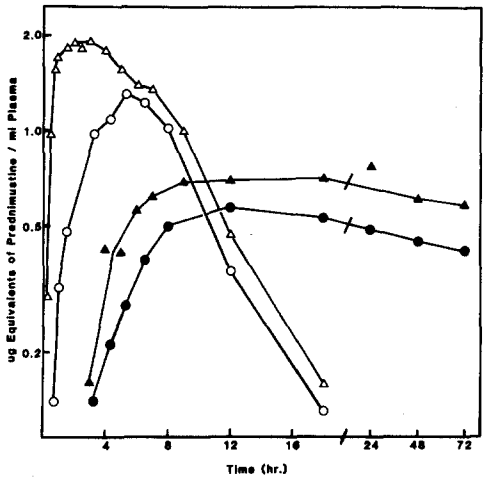

Fig. 3. Plasma concentrations of solvent-extractable (*open symbols*), and -non-extractable (*solid symbols*) radioactivity, expressed as microgram equivalents of prednimustine per milliliter, as a function of time after oral administration of ^{14}C-prednimustine (40 mg/m^2) to cancer patients: extractable, patient 1 (○), patient 2 (△); non-extractable, patient 1 (●), patient 2 (▲)

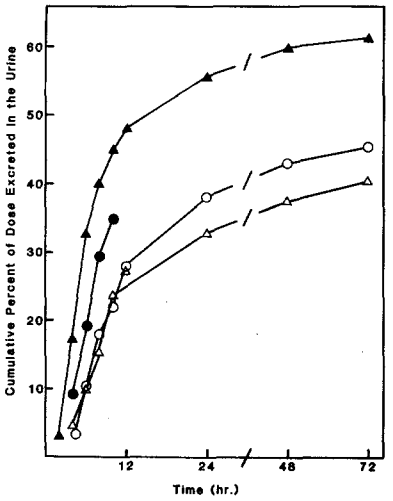

Fig. 4. Cumulative percentage of dosed radioactivity excreted in the urine as a function of time after oral administration of ^{14}C-prednimustine (40 mg/m^2) to four cancer patients: 1 (○), 2 (△), 3 (▲), 4 (●)

to these covalently bound metabolites being eliminated from the body at rates dependent on the half-life of the protein or other molecule to which they are bound.

From 40% to 60% of the dosed radioactivity was excreted in the urine in 72 h, the majority of this being excreted within 24 h (Fig. 4). An incomplete urine collection from patient 4 precluded calculations of the total dose excreted. Presumably this subject would have excreted the same total as the other patients, since the rates of early urinary excretion were similar to the other patients.

Fecal recoveries of radioactivity were variable. Two of the patients excreted less than 10% of the dosed radioactivity in the feces in 72 h, while a third excreted 22% for overall recoveries of 42%–83%.

Discussion

The results of this study show that at least 40%–60% of an oral solution dose of prednimustine in PEG-400 was absorbed. These results are in agreement with those of Kirdani et al. [11], who administered small doses of prednimustine labeled with carbon-14 in the chlorambucil moiety and tritium in the prednisolone moiety. They found 25%–51% and 4%–14% of the carbon-14 was excreted in the urine and feces, respectively, after oral administration of a small dose of prednimustine (1.5 mg) in fruit juice. Although the labeled chlorambucil portion of the molecule entered the general circulation, intact prednimustine did not. The compound for all practical purposes was completely hydrolyzed and/or metabolized either prior to or during absorption through the intestinal wall or on its first pass through the liver. The same result was reported by Newell et al. [19] following the oral administration of 20 mg prednimustine to Hodgkin's patients. They detected no prednimustine or chlorambucil in the plasma, but they did detect chlorambucil in the plasma after the oral administration of 10 mg chlorambucil to the same patients. The rationale for the synthesis of prednimustine was that the steroid portion of the molecule would carry the alkylating agent to specific steroid-binding sites. It was also possible that prednimustine would have a different tissue distribution than either prednisolone or chlorambucil. These proposed advantages of prednimustine over individual administration of chlorambucil and prednisolone, however, cannot be realized in man using the oral route of administration.

The results of this study also show that chlorambucil and/or its metabolites are covalently bound to plasma constituents and are maintained in the body for a considerable period of time. This is not unexpected, since chlorambucil and one of its human metabolites [19], phenylacetic mustard (*p*-bis(2-chloroethyl)amino phenylacetic acid), are both alkylating agents. Since these compounds are irreversibly bound and cannot be extracted by solvents, analyses of plasma metabolites based on solvent extraction will not detect them and therefore will not be a valid measurement of the plasma concentrations of all forms of the administered drug. The 10-day elimination half-life for the bound plasma radioactivity indicates that drug-related material will accumulate in the body on repeated administration of either prednimustine or chlorambucil.

Since orally administered prednimustine is not bioavailable, the efficacy reported following administration of prednimustine tablets is mot likely due to prednisolone and/or chlorambucil or metabolites of these compounds. However, the low toxicity seen in clinical studies using tablets of prednimustine, compared with equivalent doses of chlorambucil, as was confirmed by Newell et al. [19], indicated that the bioavailability of chlorambucil from the tablets was poor.

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