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Disposition, Pharmacokinetics, and Metabolism of ^{14}C-Fotemustine in Cancer Patients

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The pharmacokinetics and metabolism of intravenously infused ^{14}C-fotemustine (about 100 mg/m^2) were examined in 2 cancer patients. Plasma levels of radioactivity increased to a maximum of 4.1 and 5.5 μg equivalents per g when the infusion stopped then declined triexponentially with mean half-lives of about ½, 10 and 80 h for the initial, mid and terminal phases, respectively. Plasma levels of intact drug were lower, with maximum levels of 1.1 and 2.8 μg/ml, and declined monophasically with a half-life of about 24 min. Plasma clearance was high (1426 and 764 ml/min) with the volume of distribution based on areas of 47.7 and 26.4 l. Most of the radioactivity was eliminated in urine (50.1 and 61.3%) over 7 days with smaller amounts in the faeces (6.8 and 0.3%) and only minimal quantities (under 0.1%) as expired carbon dioxide. Metabolites of fotemustine were identified as chloroethanol and N-nitroso-1-imidazolone-ethyl-diethylphosphonate in plasma and as 1-hydantoin-ethyl-diethylphosphonate and acetic acid in urine.
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INTRODUCTION

THE NOVEL nitrosourea, fotemustine (diethyl-1-[3-(2-chloroethyl)-3-nitrosoureido] ethyl phosphonate), has significantly increased efficacy and reduced side-effects compared with similar drugs for the treatment of carcinomas such as malignant melanoma [1]. Induction treatment consists of a 1 h constant-rate intravenous infusion at a dose of up to 100 mg/m^2 over 3 consecutive weeks, followed by a 5 week rest, after which maintenance therapy is given every 3 weeks in responding patients. We have investigated the pharmacokinetics and metabolism of fotemustine in cancer patients.

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PATIENTS AND METHODS

Drugs and reference compounds

^{14}C-fotemustine was synthesised at the Commissariat à l'Energie Atomique to a specific activity of 1961 MBq/mmol. The radiochemical purity was established by high-performance liquid chromatography (HPLC) with radiochemical detection as 96.4 and 88.9% for patients 1 and 2, respectively. Non-radiolabelled fotemustine, N-nitroso-1-imidazolidone-ethyl-diethylphosphonate (NIEDP) and 1-imidazolidone-ethyl-diethylphosphonate (IEDP) were supplied by Laboratoires Servier, France, and chloroethanol was from Aldrich.

Patients

Patient 1. (F/65, Caucasian, 52.4 kg, 1.53 m^2). Ovarian carcinoma previously treated with five monthly courses of cyclophosphamide and doxorubicin as well as three subsequent treatments with melphalan. The most recent treatment of cinnarizine was discontinued 1 week before study.

Patient 2. (M/59, Caucasian, 73.9 kg, 1.85 m^2). Prostate adenocarcinoma previously treated with cyproterone acetate which had been changed to goserelin. Current treatment consisted of hydralazine, allopurinol, indomethacin, lactulose, furosemide, amiloride and morphine. This treatment was continued during study.

The patients were fasted from 2100 the previous day until 6 h after the fotemustine infusion started. Food and beverages, apart from water, were not allowed during this period. No alcohol or smoking was permitted for 24 h before and during study. Each subject was given two intravenous doses of metoclopramide (10 mg) at 10 min and about 4½ h after starting the infusion.

Ethical and ARSAC (Administration of Radioactive Substances, Advisory Committee of the Department of Health, U.K.) approval, as well as the signed informed consent of each patient, were obtained before starting the study.

Dosage

^{14}C-fotemustine (about 148 × 10^4 Bq) was dissolved with a known amount of unlabelled drug (about 250 mg) in ethanol. The radiochemical concentration and final specific activity were measured in this solution. Immediately before the infusion, the solution was injected into an infusion bag containing 5% sterile glucose solution (250 ml) with a syringe fitted with a sterile filter, so that the final ethanol concentration was 1.5%. The infusion bag, tubing, and arm of the patient were all protected from light and the solution was infused at a constant rate over approximately 1 h. Patient 1 received 133.9 mg (75 × 10^4 Bq) and patient 2 received 176.1 mg (106 × 10^4 Bq). Thus the final dose was about 100 mg/m^2 (equivalent to 111 × 10^4 Bq).

Collection of samples

Blood and plasma. 20 ml venous blood was collected immediately pre-infusion, heparinised, and 1 ml was retained. Plasma was prepared from the remainder and 1.5 ml was frozen within 3 min of blood collection. The remainder was used to examine the stability of ^{14}C-fotemustine. Further blood samples were removed from the opposite arm to the infusion at nominal times of 5, 15, 30, and 45 min, 1, 1¼, 1½, 2, 2½, 3, 4, 6, 8, 12, and 24, and then daily until 168 h. Those blood samples collected up to 12 h after the infusion started were divided as before. After this time, apart from retention of a sample before plasma preparation, further division was not done. Additional, larger blood samples (50 ml) were collected at 1, 4, and 8 h for measurement of radioactivity in blood and plasma and of intact drug in plasma, with the remainder of the plasma being used for metabolism studies.

Urine, faeces, and vomit. Where possible, urine was collected at about 1, 2, 4, 8, and 24 h and daily thereafter for 7 days. Those samples collected up to 8 h after dosing were immediately frozen. Faeces were collected over 7 days. As is common when treated with nitrosoureas, the patients vomited during the first few hours of infusion despite treatment with metoclopramide. The vomit from patient 1 was collected and analysed for radioactivity; this was not done for patient 2 since only minimal amounts of radioactivity (under 0.1% of the dose) were collected from patient 1.

Carbon dioxide. Expired carbon dioxide was collected at selected times after infusion up to 24 h by the patients exhaling for a known period (about 30 s) via a one-way valve such that expired air was bubbled through 1 mol/l hyamine hydrochloride in methanol diluted with ethanol (3 ml) in a scintillation vial. Exhalation continued until thymolphtalein indicator in the solution went colourless, indicating saturation of the trapping agent [2]. The total amount of radiolabelled carbon dioxide was calculated from the area of the rate of radioactivity expired at each collection time (disintegrations per min/min) versus time curve by the trapezoidal rule [3].

Analysis of samples

Total radioactivity. The samples were analysed for total radioactivity, either after direct addition to scintillation fluid (urine, plasma, vomit) or after combustion (blood, faeces). The samples were counted in a liquid scintillation counter.

Intact fotemustine. All plasma samples collected up to 24 h were analysed for intact drug by HPLC [4]. Fotemustine could be measured with an overall precision ± 5.6% and accuracy of 3.6% down to 20 ng/ml.

Chloroethanol. In common with the metabolism of the chloronitrosoureas in general, it was envisaged that chloroethanol would be a metabolic product of fotemustine. The predose, 1, 4, and 8 h plasma samples were analysed for chloroethanol with the method based on that described by Madelmont *et al.* [5]. The samples (1 ml) were extracted with diethyl ether (2 ml) and 3 µl of the ether extract was injected into a Hewlett Packard '5880 Level 4' gas chromatograph fitted with a 'Carbowax' 10 m × 0.53 mm column (Chrompack, U.K.). Helium carrier flow was 6.7 ml/min with oxygen-free nitrogen gas passed through the detector at 23.3 ml/min. The injector was operated in splitless mode at 125°C with the flame ionisation detector at 300°C, air-flow 450 ml/min and hydrogen flow 30 ml/min. Chloroethanol was separated with an oven temperature gradient of 45°C held for 0.5 min rising to 105°C at 20°C per min, where it was held for 0.5 min then increased to 200°C at 10°C per min. This temperature was held to complete the 16 min run-time. Chloroethanol had a retention time of 3.62 min and could be detected down to 10 ng/ml.

Metabolite identification

Plasma. Other plasma metabolites were identified by HPLC with six different chromatographic conditions, including detection at different ultraviolet wavelengths and comparison with authentic NIEDP, since their low concentrations precluded more conventional techniques.

Urine. The two major metabolic components of fotemusine in urine were isolated by successive extraction with 'SAX' and 'C_{18} BondEluts' until no more radioactivity was retained on the C_{18} cartridge. The unretained fraction from the cartridge contained predominantly a single metabolite designated $H1_U$ and this was purified by passing through a 'SCX BondElut'. The final purification consisted of gradient HPLC with methanol and deionised water (95/5), deionised water, and 0.05 mol/l phosphoric acid at 1 ml/min and a 'Hypersil' 3 µm ODS column (10 cm × 5 mm, Thames Chromatography, U.K.). The fraction retained by the C_{18} BondElut was eluted with methanol (2 × 0.5 ml) and designated $H7_U$. Control urine from each of the patients was subjected to the same procedure. $H7_U$ was examined directly by mass spectrometry, whereas $H1_U$, with acetic acid standard, also first derivatised to corresponding the *p*-bromophenacyl esters with 18-crown-6 ether (0.1 mol/l in acetonitrile)

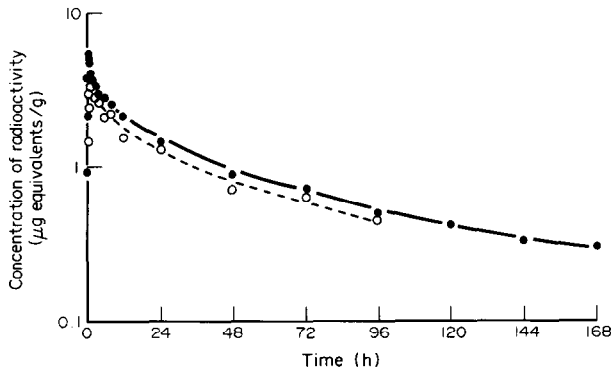

Fig. 1. Plasma levels of total radioactivity from ^{14}C-fotemustine administered as intravenous infusion to patients 1 (○) and 2 (●).

and *p*-bromophenacyl bromide (0.005 mmol/l) (1:1) at 30°C for 30 min.

In vitro stability. Fotemustine (10.25 mg) was added to control plasma (5 ml) and incubated at 37°C for 30 min before extraction through a 'CBA BondElut' cartridge. The extraction products were purified by HPLC with a Hypersil 3 μm column and a gradient profile as for urine. Each of the radioactive products was collected for mass spectral analysis and compared with a control sample where fotemustine had been added to plasma and extracted immediately.

Mass spectrometry. Mass spectral analyses were done on a Nermag 'R10-10' mass spectrometer controlled by a 'Sidar 2026' data system (Delsi Instruments, U.K.). For desorption chemical ionisation (DCI) analysis a probe with either ammonia or isobutane as the ionisation gas was used. Full-scan spectra were obtained over a mass range of 60–750 atomic mass units.

Pharmacokinetic analyses. All plasma level data of total radioactivity or intact fotemustine were fitted to a sum of exponentials with a zero-order input using an iterative least-squares curve-fitting program [6]. Half-lives, area under the curve, clearance, and volume of distribution based on area were calculated using standard equations [7].

RESULTS

Plasma pharmacokinetics

Maximum plasma levels of total radioactivity of 4.12 and 5.49 μg equivalents per g for patients 1 and 2, respectively, were obtained when infusion stopped (Fig. 1). Plasma levels of radioactivity declined triphasically with half-lives of the initial phase (0.49 h), mid phase (7.04 and 12.67 h) and terminal phase (69.7 and 95.5 h) for patients 1 and 2, respectively. Blood levels of radioactivity tended to be more variable than those of plasma, with slightly lower levels at the early times (blood/plasma ratio about 0.7), but slightly higher later on (ratio about 1.3).

The maximum levels of intact fotemustine were lower than those of total radioactivity—1.06 and 2.84 μg/ml for patients 1 and 2. Thereafter plasma levels declined rapidly and monophasically with half-lives of 23.2 and 24.4 min for patients 1 and 2 such that the plasma levels of intact drug were below the level of detection by 4 h after starting the infusion (Fig. 2). The area under the curve for intact fotemustine was less than that of total radioactivity, only increasing by about 1% when extrapolated from the last measured time (3 h) to infinity.

The plasma clearance of intact drug was high for both patients (1426 and 764 ml/min), with volumes of distribution based on areas of 43.7 and 26.4 l.

Excretion of radiolabel

Radioactivity from ^{14}C-fotemustine was mostly eliminated in the urine (50.1 and 61.3% of the dose over 7 days). Most of this was within the first 24 h (Fig. 3). Radioactivity in faeces was only 6.8% for patient 1 and 0.3% of the dose for patient 2. Despite treatment with metoclopramide, both patients vomited about 3 h after the infusion started, but less than 0.1% of the dose was recovered from the vomit from patient 1. The recovery of expired radiolabelled carbon dioxide, although significant, was low (0.09 and 0.15% for patients 1 and 2). Thus the total amount of the radioactive dose recovered in excreta for patients 1 and 2 was 57.0 and 61.7%, respectively.

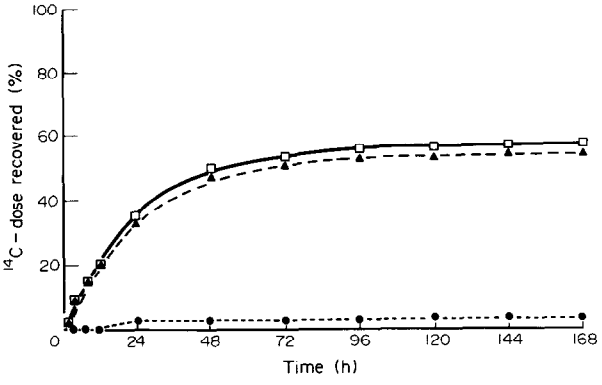

Fig. 3. Mean total (□), urinary (▲), and faecal (●) excretion of radioactivity.

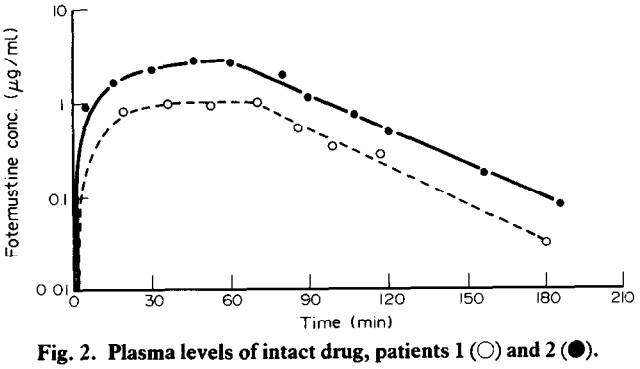

Fig. 2. Plasma levels of intact drug, patients 1 (○) and 2 (●).

Metabolite identification

A peak in the gas chromatograph corresponding to chloroethanol was detected in the 1 h plasma sample of both patients, with concentrations (about 120 ng/ml) above the level of detection (10 ng/ml). However, in the later plasma samples (4 and 8 h) no chloroethanol could be detected.

When the 1, 4, and 8 h plasma samples were also examined by HPLC, there was a product that consistently corresponded in retention time to NIEDP. Moreover, bearing in mind the low concentrations of this product in these plasma samples, when measured at different wavelengths the peak height ratios

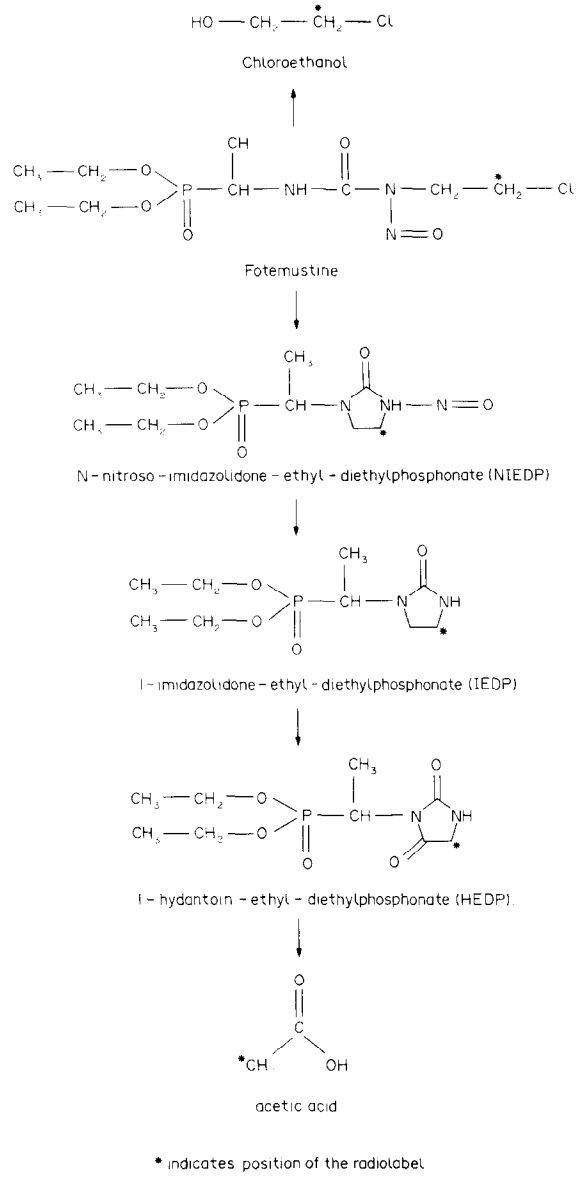

Fig. 4. Metabolism of fotemustine in man.

between 254 and 280 nm (1.3) and between 254 and 215 nm (2.9) were constant and similar to that of authentic NIEDP.

Solid-phase extraction and HPLC purification provided sufficient of the two major urinary metabolites for mass spectral analysis. Control urine treated in the same way showed no interference by endogenous compounds. Examination of the more polar metabolite showed a pseudomolecular ion $(M + H)^+$ at m/z 61 and an ammonia adduct ion $(M + NH_4)^+$ at m/z 78. Furthermore, derivatization to the *p*-bromophenacyl ester yielded mass spectral fragment ions consistent with those from standard *p*-bromophenacyl acetate, which identified $H1_U$ as acetic acid. The less polar metabolite, $H7_U$ showed a pseudomolecular ion at m/z 265 and an ammonia adduct ion at m/z 282. There was also a small fragment ion at m/z 182 indicative of the intact diethylphosphonate moiety. These results identified $H7_U$ as 1-hydantoin-ethyl-diethylphosphonate (HEDP).

In vitro *plasma stability*

Mass spectral analysis of the extract made immediately after the addition of fotemustine to plasma demonstrated a pseudomolecular ion with a chlorine isotope ratio (m/z 316/318). In addition, the corresponding ammonia adduction at m/z 333/335 and fragment ions at m/z 182, 208, 225, and 251 were all characteristic of intact fotemustine. However, similar analysis of an extract taken after a 30 min incubation of fotemustine in plasma at 37°C revealed that the chlorine atom had disappeared. The spectrum now contained a pseudomolecular ion of m/z 280 and an ammonia adduct ion of m/z 297. There were fragment ions at m/z 113, 251, and 268, giving a similar spectrum to that of NIEDP. The intensities of the fragment ions at m/z 251 and 268, however, suggested that 1-imidazolidone-ethyl-diethylphosphonate (IEDP) was also present.

DISCUSSION

As with other nitrosoureas, most of the radioactive dose from our two patients given ^{14}C-fotemustine was eliminated in the urine (50–60%), although a small proportion also appeared in the faeces (up to about 7%). Vomit contained little of the dose which indicates minimal elimination of radioactive products by ion trapping and suggests that the radioactivity in the faeces arose from biliary secreted products. The recovery of radioactivity over the 7 days of study was incomplete with a low but continued excretion of radioactivity in the late urine samples which indicates retention and slow elimination of the remaining radioactivity. This is, perhaps, not surprising since fotemustine is a reactive molecule designed to bind to macromolecules in the tumour.

Plasma levels of radioactivity from ^{14}C-fotemustine declined triphasically after the end of infusion with a half-life of about 70–100 h for the terminal phase. In contrast, the maximum plasma levels of intact drug were lower (1–3 μg/ml) than those of radioactivity (about 5 μg equivalents per ml) and declined monophasically with a half-life of about 24 min. These factors suggest that the drug rapidly degrades to products where the rate limiting stage is their own elimination. The rapid elimination of intact fotemustine, with none of the parent drug appearing in urine, is consistent with high clearance due to extensive metabolism or degradation, as with other nitrosoureas [8, 9]. The volume of distribution is considerably larger than plasma volume, indicating good transfer of fotemustine out of plasma and probably reflecting its high lipophilicity (log P = 1.25) compared with other nitrosoureas. This finding is also compatible with a blood/plasma ratio at early times after dosing approaching unity, when the proportion of intact fotemustine is high.

The *in vivo* elimination of the nitrosourea group of compounds is complex, probably involving both enzymic and non-enzymic reactions [10]. The widely held hypothesis is that the action of the nitrosoureas is by selective alkylation of DNA with interstrand cross-linking, via a chloroethyl carbonium ion that arises during the formation of chloroethanol, which is found in plasma following fotemustine infusion. Another major component of fotemustine in plasma was NIEDP, but this compound is thought to be formed from a deactivation metabolic pathway of dechlorination and cyclisation. NIEDP was further metabolised by denitrosation to HEDP which, with acetic acid produced by hydrolysis of HEDP, is excreted in urine. Interestingly, only minimal quantities of ^{14}C-carbon dioxide were eliminated, suggesting that any ^{14}C-acetic acid not eliminated in urine probably

enters the normal endogenous carbon pool, which would also be consistent with the slow elimination of total radioactivity.

Thus, radioactivity from ^{14}C-fotemustine, given as an intravenous infusion, rapidly appears in the urine over the first 24 h with only small amounts eliminated by other routes. The clearance of intact fotemustine is high with a short half-life and a volume of distribution indicative of good transfer from plasma. The metabolism of fotemustine in man probably occurs both non-enzymically and enzymically, initially resulting in the formation of some chloroethanol and a cyclised product, NIEDP, both of which were identified in plasma. These are then further metabolised to HEDP and acetic acid, which were found in urine (Fig. 4).

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