CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20221/S012

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 20,221/S#012 **Submission Dates:** December 24,1998
February 10, 1999
March 22, 1999

Drug Name:	Ethyol (Amifostine)
Formulation:	Sterile lyophilized powder (500 mg) for Intravenous administration
Sponsor:	U.S. Bioscience, Inc. West Conshohocken, PA 19428
Reviewer:	N.A.M. Atiqur Rahman, Ph. D.
Type of Submission:	Supplemental New Drug Application
Category:	P

SYNOPSIS

The review of the supplemental NDA, 20-221/S012 recommends deletion of the amifostine and cisplatin drug interaction statement from the Clinical Pharmacology section of the package insert (appendix 1). The sponsor has submitted published literature in support of the proposed statement in the package insert. The short duration of plasma sampling to access the pharmacokinetics of ultrafilterable and total cisplatin in the literature study (appendix 2) fails to provide adequate support for the lack of interaction between amifostine and cisplatin as proposed in the package insert.

In the supplemental NDA the sponsor is seeking approval for the use of Ethyol to reduce the incidence and severity of radiation induced xerostomia. Ethyol is currently approved for the reduction of the cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer or non-small cell lung cancer. The recommended Ethyol dose is 910 mg/m^2 once daily as a 15-minute I.V. infusion starting 30 minutes prior to chemotherapy. The recommended dose of Ethyol for the new indication is 200 mg/m^2 administered once daily as a 3 minute I.V. infusion starting 15-30 minutes prior to radiation therapy.

In the submitted supplemental NDA, the sponsor did not submit any pharmacokinetic study to support the proposed indication since the dose of Ethyol for the new indication is substantially lower than the currently approved dose. Therefore, the effect of radiation on the pharmacokinetics of ethyol is unknown. The sponsor submitted a published article (Influence of Amifostine on the Pharmacokinetics of Cisplatin in Cancer Patients. Korst, et al., Clinical Cancer Research, Vol. 4, 331-336, 1998) in support of the proposed changes in the package insert. The package insert states that "Patients treated with Ethyol and cisplatin showed a minor increase in the ultrafilterable platinum t ½ α (0.8 vs. 0.6h), a decrease on the platinum t ½ β (0.3 vs. 0.4h) but no changes in AUC or Pt-DNA adduct levels, compared to patients treated with cisplatin alone." The plasma sampling was up to 24 hours in the published study. The elimination half-lives of the ultrafilterable platinum and the total platinum are greater than 24 hours. The Agency requires analysis of plasma samples at least up to three half lives for a long elimination half-life drug to adequately characterize the

disposition phase of the drug. Therefore, the study failed to adequately characterize the influence of amifostine on the elimination of ultrafilterable and totalcisplatin.

The sponsor is currently evaluating the pharmacokinetics of cisplatin (120 mg/m^2) following a 15-minute infusion of amifostine (740 mg/m^2). The sponsor provided a partial report of this drug interaction study (Report No. ETH PK2) in the submission. The sponsor is also evaluating the pharmacokinetics of amifostine (910 mg/m^2) administered as a single 15-minute infusion (Report No. ETH PK3). These study reports should be submitted to the Agency for review and update of the Clinical Pharmacology section of the package insert.

Comments:

1. The literature article submitted fails to conclusively support the lack of interaction between amifostine and cisplatin due to shorter duration of plasma sampling compared to the half lives of both ultrafilterable and total cisplatin. Therefore, the proposed information regarding the lack of interaction between amifostine and cisplatin in the Clinical Pharmacology section of the Package Insert should be deleted.

2. The ongoing study evaluating drug-drug interaction between amifostine and cisplatin should be submitted to the Agency for review and to include appropriate cisplatin-amifostine interaction information in the Package Insert.

Recommendations:

The submission fails to support the proposed changes in the Clinical Pharmacology section of the Package Insert. The statement regarding drug-drug interaction between amifostine and cisplatin in the Package Insert should be deleted.

The Recommendation and the Comments should be conveyed to the sponsor.

/S/ 6/11/99

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/S/ 5/24/99

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cc: NDA 20,221 (original file)
HFD-150/Division File
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HFD-150/ GWilliams, IChio
HFD-850/LLesko
HFD-860/ MMehta, Arahman
CDR BMurphy

APPENDIX 2

4175

TITLE: PHARMACOKINETICS OF SINGLE DOSE AMIFOSTINE (WR-2721; Ethyol)

REPORT: ETH PK3

INVESTIGATOR:

STUDY DATES: Ongoing

REPORT DATE: Preliminary, June 1994

0816.

TABLE OF CONTENTS

INTRODUCTION

Amifostine (Ethyol, WR-2721, [S-2-(3-aminopropylamino)-ethyol dihydrogen phosphorothioate] is an organic thiophosphate in clinical development as a protective agent for normal tissues against toxicity of chemotherapy and radiation.

OBJECTIVE

The objective of this study is to evaluate the pharmacokinetics of amifostine (910 mg/m^2) administered as a single 15-minute infusion.

STUDY CONDUCT

The pharmacokinetics of single dose amifostine were evaluated in 13 patients who were participating in clinical trials at either the [ ] (six patients) or the [ ] (seven patients). Each patient received a single 15-minute infusion of amifostine at a dose of 910 mg/m^2. Blood samples were scheduled to be taken at 0, 3, 6, 9, 12, 15, 16, 16.5, 17, 17.5, 18, 20, 30, 45 and 60 minutes after the start of the amifostine infusion. All blood samples were subsequently sent to the [ ] for pharmacokinetic analysis.

[ ] was used to measure parent drug concentration in the blood samples as previously described.(1) Pharmacokinetic parameters were estimated using the [ ] pharmacokinetic model-independent non-linear regression software system. Area under the blood level concentration (AUC) curve was transformed prior to the analysis by taking the $\log_{10}$ of AUC and was transformed back after the analysis by taking the antilog_{10} of the mean $\log_{10}$ of AUC. Type III Sums of Squares were performed testing the significance of each effect after adjusting for all other effects.

RESULTS

TABLE 1 summarizes the pharmacokinetic parameters of the 13 patients who received a single 15-minute infusion of amifostine at a dose of 910 mg/m^2.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 9(4), 845-859 (1986)

MEASUREMENT OF S-2-(3-AMINOPROPYL-AMINO)ETHANETHIOL (WR1065) IN BLOOD AND TISSUE

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ABSTRACT

A procedure for the analysis of blood and tissue specimens for WR1065, the dephosphorylated metabolite of the radioprotective drug WR2721, has been developed. The method includes the use of a perchloric acid/EDTA extraction step at 0°C followed by chromatographic analysis using a mercury/gold thin film electrochemical detection liquid chromatography system. The extraction technique was designed to assure the stability of both WR1065 and any WR2721 present in blood or tissues. Using the described chromatography conditions and an analog of WR1065, 3-(4-aminopropylamino)propanethiol (WR251833), as an internal standard the respective retention times of these two compounds are 6.2 and 8.3 minutes. Experiments showing the applicability of this method to pharmacokinetic studies of WR2721 and WR1065 and to investigation of the kinetics of WR2721 hydrolysis in biological fluids such as stomach juice are described.

INTRODUCTION

WR2721 is an experimental drug that provides significant radioprotection to many normal tissues but provides little or no protec-

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The prepared specimens are analyzed directly with an HPLC method using a mercury/gold amalgam electrochemical detector at the selective potential of + 0.15 volts.

MATERIALS AND METHODS

Apparatus

A Bioanalytical Systems LC-304 liquid chromotagraph including a dual piston pump operated at 3,500 psi and a mercury/gold electrochemical detector was used as previously described (11). Column temperature was maintained at 25°C with a temperature jacket. All teflon tubing was replaced with stainless steel to exclude oxygen. The column used for these studies was the BAS Biophase ODS 5u (4.6 x 250 mm). The mobile phase was continuously purged with nitrogen to remove dissolved oxygen.

Chemicals

S-2-(3-aminopropylamino)ethanethiol (WR1065), S-2-(3-aminopropylamino)ethylphosphorothioate (WR2721), and 3-(4-aminopropylamino) propanethiol (WR251833) were supplied to us by Dr. Lawrence Fleckenstein of the United States Army Medical Research and Development Command at Walter Reed Army Institute of Research. Acetonitrile and methanol were obtained from Fischer Scientific (King of Prussia, PA) and sodium octyl sulfate was from Eastman Kodak Co. (Rochester, NY). All other reagents used were of the highest analytical grade available.

tion to many experimental tumors (1,2). Several reports have shown that WR2721 provides significant protection against the toxic effects of the chemotherapeutic drugs, cisplatin and cyclosphosphamide (3,4). A third pharmacologic action of WR2721 is its hypocalcemic effect (5). The single dose phase I trials of WR2721 as a radioprotector and chemoprotector have been completed (6,7,8) and the drug has been shown to be effective in reducing the serum calcium concentration in a patient with hypercalcemia secondary to parathyroid cancer (9).

WR1065, the dephosphorylated free sulfhydryl metabolite of WR2721, is generally regarded as the active form of the parent drug and/or the precursor of active form(s) of the drug (i.e. the symmetrical disulfide of WR1065 or mixed disulfides of the latter and endogenous sulfhydryl compounds such as cysteine, glutathione and proteins). Thus in order to establish the optimal dosage and time sequence of the protector and treatment in patients it is essential to be able to measure both WR2721 and WR1065 in blood and tissues. Although methods for measuring WR2721 and WR1065 have recently been described (10,11,12, 13) there are no existing methods for satisfactorily measuring both WR2721 and WR1065 in blood and tissues. Efforts to achieve this goal have been hampered by the fact that each compound has stability problems that are different. Thus, the challenge has been to devise a blood and tissue preparation method that assures stability for both WR2721 and WR1065 and which is compatible with the subsequent chromatography methods of analysis. Here we report a sample preparation method that is simple and which assures the stability of WR2721 and WR1065 in biological samples.

Sample Preparation and Chromatography

Unless otherwise noted specimens were processed at 0°C by adding an aliquot of a solution of 1 mol/L perchloric acid in 2.7 mmol/L disodium EDTA to an aliquot of the sample to be analyzed in the ratio 2 to 5, respectively. Samples that contained protein were centrifuged in the cold (4°C) for 15 min in order to prepare the supernatant fraction for injection into the chromatograph. Twenty microliter aliquots of the protein-free specimens were injected onto a Biophase 5 u octadecylsilane column (250 x 4.6 mm) that was maintained at 25°C with a constant temperature jacket. Elution of WR1065 at 6.2 min and the internal standard WR251833 at 8.3 min was achieved isocratically using a 40% (v/v) methanol/water mobile phase containing 0.1 mol/L monochloroacetic acid and 1.0 mmol/L sodium octylsulfate, pH 3.0, at a flow rate of 1.3 mL/min.

RESULTS AND DISCUSSION

Detection, Linearity and Sensitivity

A linear sweep voltammogram of a solution of WR1065 is displayed in Figure 1. The voltammogram shows that the sulfhydryl is oxidized at the surface of the mercury/gold amalgam electrode at a low potential. This is consistent with the observed electrochemical properties of other sulfhydryl compounds such as glutathione and cysteine (14) and shows that the mercury/gold electrode set at a potential of + 0.15 V with respect to a Ag/AgCl reference electrode is a suitable detector for HPLC analysis of WR1065. A typical chromatogram showing detector response versus elution time for WR1065

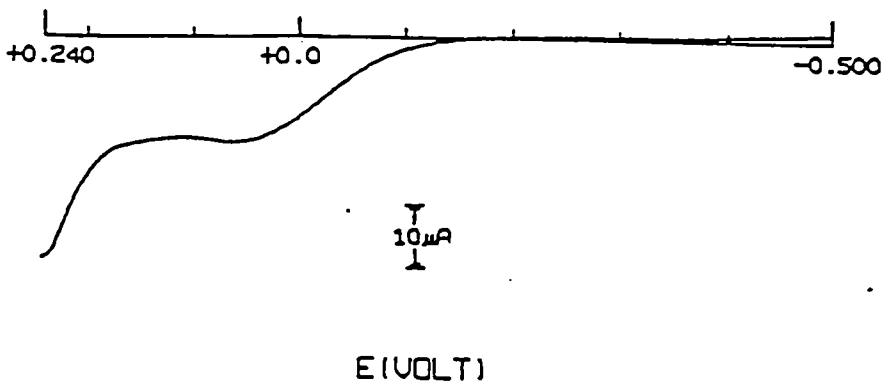

FIGURE 1. A linear sweep voltammogram of 4 mmol/L WR1065 in an aqueous solution of 0.1 mol/L monochloroacetic acid and 1.5 mmol/L sodium octylsulfate, pH 3, using a mercury/gold electrode.

and WR251833 is shown in Figure 2. The ratio of peak heights of varying concentrations of WR1065 to that of the internal standard WR251833, at a concentration of 100 umol/L in an aqueous solution containing 10 mmol/L Tris, pH 7.4, and 1 g/L sodium EDTA, was determined. The increase in the ratio of WR1065 peak heights to that of the internal standard was linear over the WR1065 concentration range of 2.5 to 250 umol/L (R^2 = 0.998). The limit of sensitivity was determined: 2 pmol per injected sample (100 nmoL/L of sample).

Sample Preparation

The use of the perchloric acid/EDTA solution at 0°C for sample preparation is critical to the analysis of WR1065. It has been shown for sulfhydryl compounds such as glutathione that at neutral pH autoxidation occurs in deproteinized samples (15). Further if

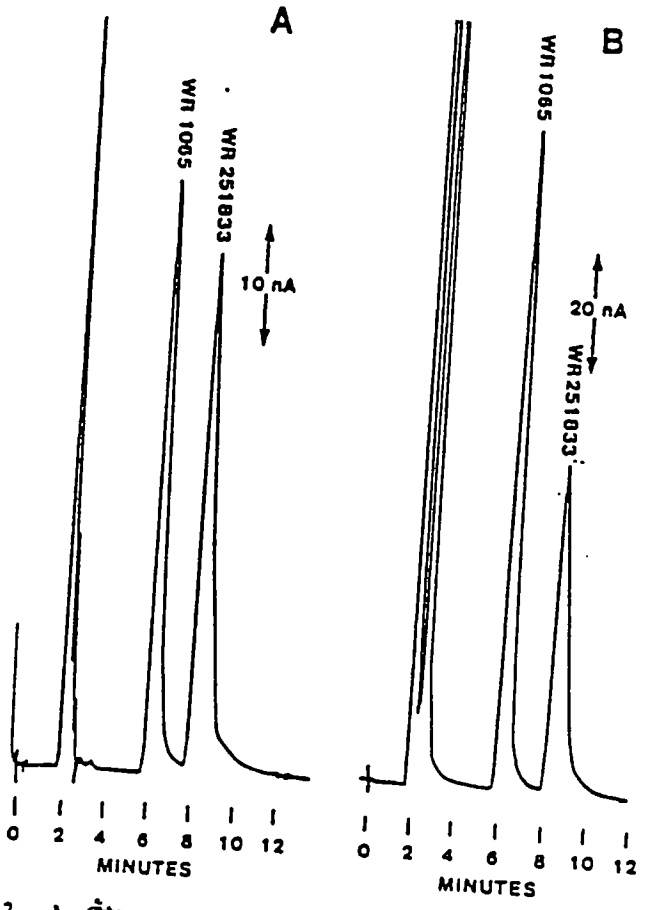

FIGURE 2. A. Chromatogram of a mixture of a solution of 100 umol/L WR1065 and 100 umol/L WR251833 and perchloric acid/EDTA solution in the proportions described in Materials and Methods. Sensitivity is 100 nA full scale. B. Chromatogram of perchloric acid/EDTA extract of normal human blood to which was added 200 umol/L WR1065 and 100 umol/L WR251833. Sensitivity is 200 nA full scale.

TABLE 1

Decrease of WR1065 Concentration in Whole Blood at 0°C

Incubation time (min.)	WR1065 Concentration (umol/L)	Percent Original Concentration, %
0	205 (3.06)[a]	100
30	125 (0.5)	61
60	106 (1.0)	52
120	101 (2.8)	49
180	85 (0.7)	41

Human blood from a healthy subject was collected in EDTA vacutainer tubes. 3.0 mL of a freshly prepared ice-cold solution of 1.0 g/L disodium EDTA, 10 mmol/L Tris, pH 7.4, containing 1.33 mmol/L WR1065 and 0.67 mmol/L WR251833 was added to 17 mL of the ice cold blood sample.

At the indicated times a 1 mL aliquot of the blood sample was taken and immediately added to 0.4 mL of ice-cold 1 mol/L perchloric acid, 2.7 mmol/L disodium EDTA solution. Further sample processing and chromatographic analysis were performed as described in Materials and Methods.

[a] Each result is the average of triplicate determinations. Values in parentheses are standard deviations.

glutathione is added to human plasma and incubated at 37°C, it rapidly disappears to produce glutathione disulfide (16). We have found that WR1065 added to normal human blood and incubated at 0°C rapidly disappears, presumably, to form disulfide and mixed disulfide products (Table 1). This process is prevented by the immediate treatment of blood with the perchloric acid/EDTA solution as described above. It has been shown that autoxidation of sulfhydryl compounds is minimal at low pH and that precipitation of blood proteins removes any possible enzymes that could catalyze sulfhydryl oxida-

TABLE 2

Determination of the Accuracy of the HPLC Method

Spiked-in WR1065 Conc. (umol/L)	Whole Blood: Mean Measured Concentration	Whole Blood: Percent Deviation (D)	Liver: Mean Measured Concentration	Liver: Percent Deviation (D)
5	5.01(0.084)	0.2	4.87(0.17)	-2.6
50	53.6(1.45)	7.2	57.9(1.56)	15.8
100	107(1.92)	7.0	102(0.44)	2.0
200	201(3.91)	0.5	187(3.03)	-6.5
	Average | D = 3.7		Average | D = 6.7	

To ice-cold aliquots of a pool of blood collected from a healthy volunteer were added the indicated WR1065 concentrations. Using the procedure described in Table 1 the blood specimens were processed immediately after addition of the ice-cold WR1065 solution. The same spiking-in procedure was used to determine the accuracy of the WR1065 HPLC analysis method for mouse liver homogenate. The latter was prepared from normal mice by homogenizing a mixture of 1 g of liver in a total volume of 5 mL of 1 g/L disodium EDTA, 10 mmol/L tris, pH 7.4. The concentration of WR251833, the internal standard, was 100 umol/L in all specimens. Each measured concentration is the mean of 4 determinations and the numbers in parentheses are standard deviations.

tion (15). Since the disappearance of WR1065 in blood at 0°C is so rapid it is essential to immediately treat blood samples containing WR1065 with ice-cold perchloric acid/EDTA solution. When processed in this way we obtained very good recovery of WR1065 that had been added to human blood or mouse liver homogenate (Table 2).

Thus it is essential that these low pH conditions be used in order to obtain accurate WR1065 values. Since measurements of WR1065 are often made in the presence of WR2721 it is important to

establish whether or not the perchloric acid/EDTA solution produces significant hydrolysis of the latter at 0°C. The rate of non-enzymatic hydrolysis of WR2721 is strongly dependent on pH (10,17,18) and temperature (18). The rate increases with decreasing pH and decreases with decreasing temperature. We determined the pseudo-first-order rate constant for hydrolysis of WR2721 in the perchloric acid/EDTA solution at 0°C and obtained a value of 0.108×10^{-3} min^{-1} (Table 3). Thus the rate of hydrolysis of WR2721 under these conditions would be 0.011% per minute or about 0.6% per hour. This very slight rate of hydrolysis will not produce significant false increases in WR1065 concentration for the duration of a workday (~4.2% over 7 hours). As shown in Table 3 the WR2721 pseudo-first-order hydrolysis rate constant in the perchloric acid/EDTA solution at 25°C, 8.0×10^{-3} min^{-1}, is 74 times that at 0°C. At 25°C WR2721 hydrolysis would proceed at a rate of 0.8% per minute. Thus, in order to preclude production of significant quantities of WR1065 from WR2721 using the described sample preparation procedure it is essential to maintain samples at 0°C at all times prior to injection onto the HPLC column.

WR1065 blood concentration in a patient given multiple WR2721 doses

The applicability of the described techniques to pharmacokinetic studies is illustrated in Figure 3. WR2721 and WR1065 blood concentrations were plotted versus time in a patient who was given 5 multiple intravenous injections of 150 mg/M^2 WR2721. The first 4 were given every 4 minutes and the fifth and last was administered 3

TABLE 3

Pseudo-First-Order Rate Constants for Hydrolysis of WR2721

	Temp.	pH	First-order rate const. x 10^3 (min^{-1})	T1/2
0.4 mol/L $HClO_4$, 1 mmol/L sodium EDTA	0°C	0.80	0.108	107 hr
0.4 mol/L $HClO_4$, 1 mmol/L sodium EDTA	25°C	0.80	8.0	87 min
Normal human stomach juice	37°C	1.74	22.74	30.5 min

The pseudo-first-order rate constant for hydrolysis of WR2721 was obtained from WR1065 concentrations measured by the described electrochemical liquid chromatography method. For each rate constant determination WR1065 concentrations were measured at 9 consecutive 10 minute intervals for the 25°C study in perchloric acid/EDTA, 4 consecutive 1 hour intervals and at 24 hours for the 0°C study in perchloric acid/EDTA and 5 consecutive 20 minute intervals for the 37°C study in stomach juice. In each study the original concentration of WR2721 was 200 umol/L and one aliquot of the reaction mixture was hydrolyzed completely to WR1065 by incubation of 45°C for 2 hours. The pseudo-first-order rate constant for the hydrolysis reaction was obtained from the slope of the plot of the natural logarithm of the difference between WR1065 produced at each time point and that produced by complete hydrolysis as a function of time.

minutes after the fourth dose. The data shows that WR1065 concentration in blood increased steadily during the time interval of the first four doses, reached a plateau concentration of about 100 umol/L then decreased to a concentration of 35 umol/L 60 minutes after the first dose was given. WR2721 concentration declined rapidly after reaching a concentration of 1200 umol/L 30 seconds after administration of

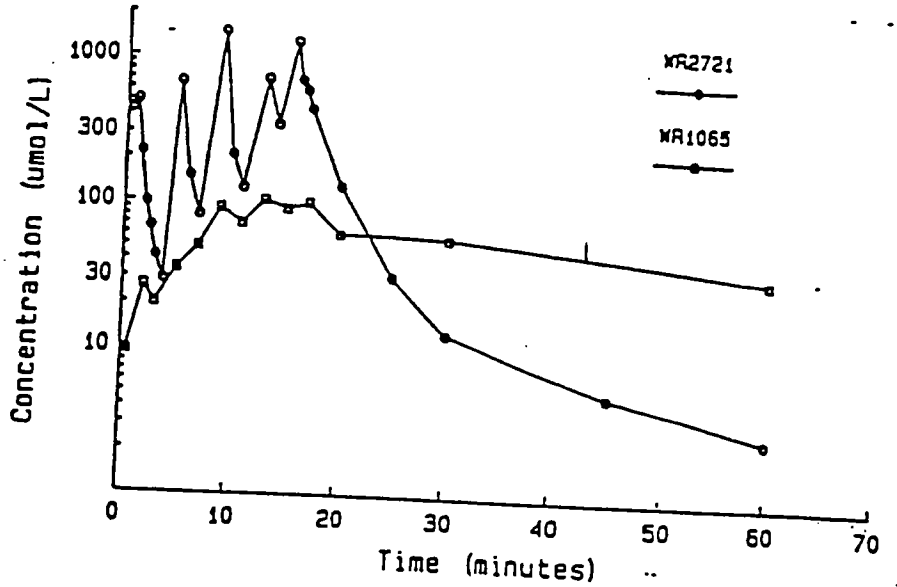

FIGURE 3. A plot of the log WR2721 and WR1065 blood concentrations versus time in a patient who was given four bolus injections of 150 mg/M^2 WR2721 at 4 minute intervals and a fifth bolus injection 3 minutes after the fourth dose. Each point is the average of duplicate determinations.

the fifth bolus dose. 10 minutes after the last dose the WR2721 concentration became lower than that of WR1065. Thus WR1065, the free sulfhydryl metabolite of WR2721, appeared in the bloodstream of the patient shortly after administration of WR2721 and remained in blood at higher concentrations than that of WR2721 for a longer period of time. It has previously been shown that WR1065 appears in various tissues shortly after intravenous administration of WR2721 to mice (12). Furthermore tissue concentrations of WR2721 have been shown by us to be, in general much lower than those of WR1065 in mice

shortly after administration of WR2721 (19). Thus the rapid decrease in WR2721 concentration and rapid appearance of WR1065 in the patient's bloodstream is consistent with the observations of the fast rate of appearance of WR1065 in mouse tissues after WR2721 administration.

It is very likely that WR1065 reacts, in vivo, with other endogenous compounds such as cysteine, glutathione and certain proteins to form mixed disulfides. Future studies will be required to identify and quantitate these.

Production of WR1065 from WR2721 in human stomach juice

It has previously been noted that after oral administration of WR2721 there is a rapid and significant loss of the radioprotective activity of the compound (10). This has been presumed to result from acid-catalyzed hydrolysis of WR2721 to produce WR1065. The latter then presumably was further metabolized to inactive compounds resulting in a loss of radioprotective activity. Our data in Table 3 show that the rate constant for WR2721 hydrolysis in a specimen of normal human stomach juice (pH 1.74) is 22.74×10^{-3} min^{-1}. The half-life for hydrolysis of WR2721 in the stomach juice sample is, therefore, 30.5 minutes. Thus there is a fairly rapid rate of hydrolysis of WR2721 in stomach juice. Although some intact WR2721 might reach the small intestine in subjects given the drug orally it is very likely that the remaining drug would be rapidly dephosphorylated at the surface of intestinal microvilli by alkaline phosphatase. The latter plasma membrane enzyme is present in high concentrations

in small intestinal microvilli (20) and it has been shown to readily catalyze the dephosphorylation of WR2721 (11).

ACKNOWLEDGMENTS

This work was supported by the National Cancer Institute Grant CA-30100 from the Department of Health and Human Services and by an institutional grant from the American Cancer Society. We thank Drs. John Glick, Ronald Shoup and Robert Van Etten for helpul discussions of this work and Drs. Daryll Brown and Ann Ouyang for the mouse liver and normal human stomach juice specimens respectively.

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Intrapleural Administration of Cisplatin and Etoposide to Treat Malignant Pleural Effusions in Patients with Non-Small Cell Lung Cancer.

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Background: To determine the efficacy, toxicity and pharmacokinetics of intrapleural cisplatin (CDDP) and etoposide as a treatment for malignant pleural effusions (MPE) in patients with non-small cell lung cancer (NSCLC). Methods: Seventy patients with MPE associated with NSCLC were enrolled in this study. In 68 patients, a catheter was inserted into the pleural cavity, within 24 h after complete drainage of the pleural effusion, CDDP (80 mg/m2) and etoposide (80 mg/m2) were simultaneously administered successfully via the catheter and the catheter was clamped. Seventy-two hours later, the catheter was unclamped to allow drainage. The catheter was removed when the accumulated intrapleural fluid decreased to 20 ml or less per day. Results: The pharmacokinetic profiles showed high maximum concentrations of CDDP (free form, 88 microg/ml) and etoposide (182.4 microg/ml) in intrapleural fluids. CDDP did not remain for a long period (free form, beta-phase half-life = 10.51 h) in the fluids, while etoposide persisted for a long period (beta-phase half-life = 62.53 h). The overall response rate was 46.2%, the median survival time 32.3 weeks, the 1-year survival rate 28.7% and the 2-year survival rate 12.8%. The most serious adverse reactions were WHO grade 3 anemia (3 patients), grade 3 nausea and vomiting (17 patients), grade 3 constipation (1 patient), grade 3 pulmonary toxicity (1 patient), grade 4 fever (1 patient), grade 3 infection (1 patient) and grade 3 mental disorder (1 patient). Conclusion: Intrapleural administration of CDDP and etoposide was an effective and acceptable regimen for patients with MPE due to NSCLC.

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A LIQUID CHROMATOGRAPHIC ELECTROCHEMICAL ASSAY FOR S-2-(3-AMINOPROPYLAMINO)ETHYLPHOSPHOROTHIOATE (WR2721) IN HUMAN PLASMA

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ABSTRACT

A liquid chromatographic electrochemical method for the determination of the radioprotective drug WR2721 in human plasma has been developed. This method includes the use of a Hg/Au electrochemical detector for the direct measurement of WR2721 concentration. An analog of WR2721, S-3-(4-aminobutylamino) propylphosphorothioate (WR80855) is the internal standard. The retention times for WR2721 and WR80855 are approximately 4.5 and 9 minutes, respectively. WR1065, the free sulfhydryl metabolite of WR2721, is retained on the column under the described chromatographic conditions and therefore does not interefere with the determination of the parent drug. With modification of the mobile phase WR1065 is eluted from the column at a retention time of approximately 20 minutes. This method has good linearity, precision and accuracy, and is free from interference from endogenous plasma substances. Preliminary results showing the applicability of this method to human pharmacokinetic studies and to investigating the enzymatic hydrolysis of WR2721 are presented.

INTRODUCTION

A number of studies in animals have shown that WR2721 provides significant protection of normal tissues from radiation injury (1,2). Recent observations suggest that this experimental

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drug will also protect normal tissues from alkylating agent toxicity (3). On the other hand, the radiosensitivity of a number of solid animal tumors is not altered by the administration of WR2721. The recent observation of facilitated uptake of WR2721 by normal cells compared to passive absorption by solid tumors has been proposed to account for its differential protective effect (4). The protective effect of WR2721 is presumed to result from the appearance, at a very rapid rate, of its dephosphorylated free sulfhydryl metabolite, WR1065, within cells (5). WR2721 is now in clinical trials in the United States and Japan (6,7,8).

In order to pursue pharmacological and pharmacokinetic studies of WR2721 in man an assay for WR2721 is required which is reliable, fast, not subject to interference from endogenous substances and that could readily be adapted to the investigation of metabolites. We describe here our HPLC method. The direct electrochemical detection of WR2721 in the column effluent is based on the finding that it is oxidized at a potential of +0.15 volts at the surface of a mercury/gold amalgam electrode.

MATERIALS AND METHODS

Apparatus

A Bioanalytical Systems LC-154 liquid chromatograph including a dual piston pump operated at 3,000 psi and a single mercury/gold detector was used as recently described by Allison and Shoup (9). Column temperature was maintained at 25°C with a temperature jacket. All teflon tubing was replaced with stainless steel to exclude oxygen. The column used for these studies was the BAS Biophase ODS 5u (4.6 x 250 mm). The mobile phase was continuously purged with nitrogen to remove dissolved oxygen.

Chemicals

S-2-(3-aminopropylamino)ethylphosphorothioate (WR2721), S-2-(3-aminopropylamino)ethanethiol (WR1065) and S-(3-aminobutyl-

amino)propylphosphorothioate (WR80855) were supplied to us by Dr. Lawrence Fleckenstein of the United States Army Medical Research and Development Command at Walter Reed Army Institute of Research. Acetonitrile and methanol were obtained from Fisher Scientific (King of Prussia, PA) and sodium octyl sulfate was from Eastman Kodak Co. (Rochester, NY). Acid phosphatase isoenzyme 2A prepared from human seminal fluid was a gift from Dr. Norman Yang, isoenzyme 5 prepared from human spleen was a gift from Dr. Bill Lam. Human liver alkaline phosphatase was a gift from Dr. Claude Petitclerq and the calf intestine enzyme (Type XXX-TA) was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of the highest analytical grade available.

Sample Preparation and Chromatography

Plasma specimens were prepared, at 4 °C, immediately from blood drawn into EDTA vacutainer tubes which had been stored in an ice bath. To a 1 mL aliquot of each plasma sample was added 0.1 mL of a 1 mmol/L aqueous solution of the internal standard WR80855 and the samples were then stored at -70 °C until they were analyzed. Just prior to analysis the plasma specimens were thawed and maintained at 0 °C in an ice bath. In order to remove plasma proteins prior to liquid chromatographic analysis an equal volume aliquot of ice-cold acetonitrile was added to an aliquot of each thawed plasma specimen. After mixing, the samples were spun in a refrigerated centrifuge at 4 °C. Twenty microliter aliquots of the supernatants were injected onto a Biophase 5μ octadecylsilane column (250 x 4.6 mm) that was maintained at 25 °C with a constant temperature jacket. Elution of WR2721 and the internal standard WR80855 was achieved isocratically using an aqueous mobile phase containing 0.1 mol/L monochloroacetic acid and 1.5 mmol/L sodium octylsulfate, pH 3.0, at a flow rate of 2.0 mL/min.

Pharmacokinetic Study

In order to evaluate the application of the HPLC method for determining WR2721 in human plasma, a patient was given a single 300 mg dose (3.4 mg/kg) as a 10 second intravenous bolus. Blood samples were obtained prior to and after completion of the infusion of WR2721 at 2, 2.5, 3, 4, 8, 12, 20, 30, 45 minutes and 1, 2, 4, 8 and 24 hours. The WR2721 plasma concentration versus time data obtained on the patient were analyzed using the MK MODEL II PLUS version of the Extended Least Squares Nonlinear Regression Program, ELSNLR (10).

Acid and Alkaline Phosphatase Assays

Acid phosphatase catalytic assay conditions are essentially those described by Kachmar and Moss (11). The reaction mixture contained in final concentrations, p-nitrophenyl phosphate, 5 mmol/L; sodium citrate, 100 mmol/L, pH 5.0; and 0.1 mL of enzyme solution in a total volume of 1.0 mL. Incubation was for 30 minutes at 37 °C. The reaction was stopped with 0.1N NaOH and absorbance measured at 405 nm. In testing for the possible hydrolysis of WR2721 by acid phosphatase, the latter was substituted for p-nitrophenyl phosphate at a final concentration of 0.2 mmol/L and the incubation at 37 °C conducted for 30 minutes. A control reaction mixture consisted of all constituents except the acid phosphatase. After 30 minutes the reaction tubes were placed in ice and then 20 µL aliquots were injected onto the HPLC column for WR2721 analysis.

Using p-nitrophenyl phosphate as substrate, alkaline phosphatase activity was measured. The reaction mixture contained in final concentrations: p-nitrophenylphosphate, 16 mmol/L; tris (hydroxymethyl)aminomethane, 50 mmol/L, at pH values of 7.4, 8.0, 8.6, 9.2 and 10.0; magnesium acetate, 2 mmol/L; and 0.02 mL of enzyme in a total reaction mixture volume of 1.0 mL. Incubation was for 15 minutes at 37 °C. Absorbance was measured at 405 nm

after the addition of 0.1N NaOH. In order to determine the rate of hydrolysis of WR2721 by alkaline phosphatase the latter substrate was substituted for p-nitrophenylphosphate at a final concentration of 0.2 mmol/L and the incubation at 37 °C conducted for 15 minutes. Control reaction mixtures consisted of all constituents except for alkaline phosphatase at each of the above pH values. At the completion of the incubation period the reaction tubes were placed in ice and the 20 µL aliquots were injected onto the HPLC column for WR2721 analysis. In a separate chromatographic run these reaction mixtures were analyzed for WR1065.

RESULTS AND DISCUSSION

Detection

A differential pulse voltammogram of a solution of WR2721 in the HPLC mobile phase, (an aqueous solution of 0.1 mol/L monochloroacetic acid and 1.5 mmol/L sodium octylsulfate, pH 3.0), is displayed in Figure 1. From this experimental data it is clear that WR2721 would be oxidized on the surface of a Hg/Au electrode set at an operating potential of +0.15 volts. Thus the HPLC column effluent was monitored with a single Hg/Au working electrode at an operating potential of +0.15 volts. A typical chromatogram showing detector response versus elution time for WR2721 and the internal standard WR80855 is displayed in Figure 2.

It is important to emphasize the fact that in establishing experimental conditions for this HPLC assay for WR2721 considerable precautions were taken to minimize hydrolysis. Since WR2721 is reported to undergo nonenzymatic hydrolysis with increasing rates as pH is lowered, conditions in this method minimize the possibility of any loss of WR2721 due to hydrolysis: plasma specimens (standards and patient specimens) are stored at -70 °C until analyzed; use of a neutral polar organic solvent (acetoni-

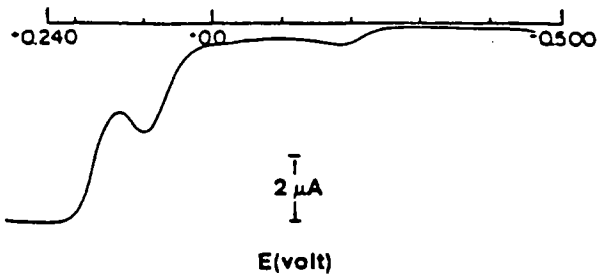

FIGURE 1. A differential pulse voltammogram of 4.5 mmol/L WR2721 in an aqueous solution of 0.1 mol/L monochloroacetic acid and 1.5 mmol/L sodium octylsulfate, pH 3, using a Hg/Au electrode.

trile) instead of an acid, such as perchloric acid, for protein precipitation; a relatively short chromatography time and, therefore, short time in the pH 3.0 mobile phase. Swynnerton, et al. (12) have measured the rate of nonenzymatic hydrolysis of WR2721 as a function of pH at 37 °C. Using a value for k_{obs} of 0.007 min^{-1} and the standard kinetic equation for a first order reaction, it is predicted that in 5 minutes at 37 °C, pH 3.0, 3.44% of WR2721 would be hydrolyzed. Since the retention time for WR2721 is less than 5 minutes and the operating temperature of the HPLC column is 25 °C our experimental conditions should not produce significant losses of WR2721 due to hydrolysis.

Linearity, Recovery, Precision and Accuracy

As shown in Figure 3 the response of the Hg/Au detector was linear over the WR2721 concentration range of 1 to 1000 μmol/L (R^2 = 0.998). The absolute recovery of WR2721 was determined using ^{14}C-WR2721 added to normal plasma (Table 1). 98.4% (68,688/69,774 x 100) of ^{14}C-WR2721 added to normal plasma was recovered in the acetonitrile supernatant. Eighty-nine percent (61,112/68,688 x 100) of the labelled WR2721 in the supernatant aliquot applied to the HPLC column was recovered (a total of sixty 0.4 mL fractions were collected and counted).

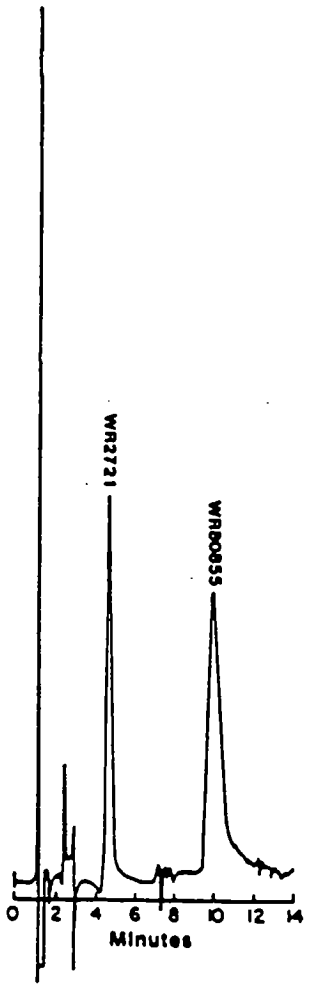

FIGURE 2. Chromatogram of 500 μmol/L WR2721 and 100 μmol/L WR80855 in normal human plasma. Sensitivity is 50 nA full scale through 7.3 min at which point it was changed to 10 nA. The acetonitrile extraction step and other experimental details are described in Materials and Methods.

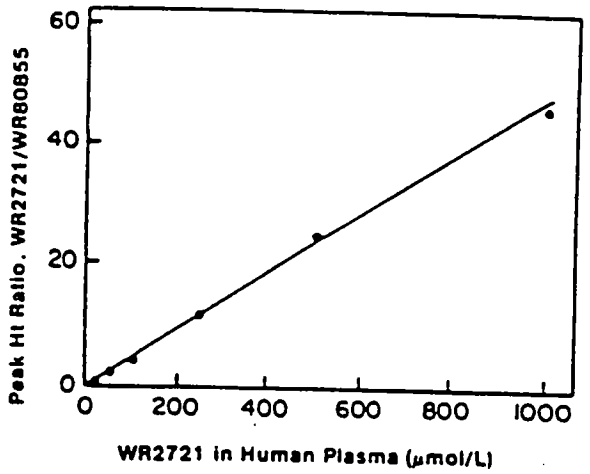

FIGURE 3. The average of duplicate peak height ratios of WR2721 to WR80855 over the range of WR2721 concentrations in normal human plasma of 1 to 1000 μmol/L are plotted against WR2721 concentration.

TABLE 1

Recovery of ^{14}C-WR2721[a] from Spiked Normal Human Plasma[b]

	Counts per min. per 0.02 mL
Plasma containing ^{14}C-WR2721 and unlabelled WR2721 (100 μmol/L)	69,774
Acetonitrile supernatant[c]	68,688
Total counts recovered from chromatograph[c]	61,112
Total counts recovered in WR2721[c] peak	53,388

[a]S-2-(3-aminopropylamino)ethyl-1,2-^{14}C-phosphorothioic acid.

[b]Human plasma from a healthy drug-free subject was collected in EDTA vacutainer tubes. 0.02 mL of a freshly prepared ice-cold solution, in 10 mmol/L phosphate buffer, pH 7.4, of 2.5 mmol/L WR2721 and 1.87 X 10^6 DPM ^{14}C-WR2721 was added to 0.48 mL of the ice-cold plasma pool.

[c]Values corrected for dilution of plasma by an equal volume of acetonitrile.

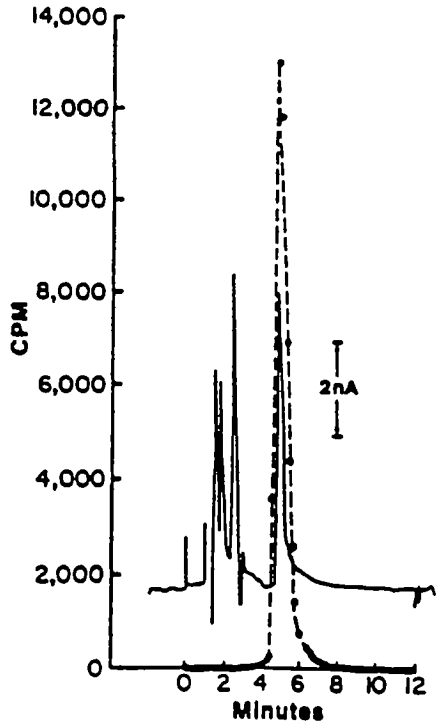

FIGURE 4. Chromatogram of 100 μmol/L WR2721 in human plasma to which ^{14}C-WR2721 had been added. A plot of the ^{14}C cpm for each of sixty collected effluent fractions is displayed as a function of time after the sample was injected. Other experimental details are described in Table 1.

Examination of the chromatogram obtained for this experiment (Figure 4) shows that no radioactivity peak other than that corresponding to the WR2721 chromatographic peak could be detected. Of the total ^{14}C counts recovered from the column, 87% (53,388/61,112 x 100) was recovered in the WR2721 peak. The net recovery of WR2721 carried through all of the steps in the procedure is therefore 76.5% (53,388/69,774). These recovery data compare favorably to those obtained by Swynnerton, et al. (12)

TABLE 2

Determination of the Precision and Accuracy of the HPLC Method

Spiked-in WR2721 Conc. µmol/L	Mean Measured Conc.a	Precision (SD)CVb	Percent Deviation (D)c
2	1.74	(0.11)6.5%	-13.0
5	5.3	(0.36)6.5%	6.0
40	35.8	(1.22)3.4%	-10.5
200	211	(7.38)3.5%	5.5
800	785	(10.1)1.2%	-1.9

Average % deviation = 7.4 (The average of the sum of the absolute values of D)

[a]The mean measured concentrations obtained with our HPLC method for each of four plasma samples at each WR2721 concentration using individually spiked specimens. The internal standard was 100 µmol/L WR80855.

[b](SD)CV, the standard deviation and coefficient of variation of the quadruplicate determinations.

[c](D) is the percent deviation of the mean measured concentration from the spiked-in concentration.

with their recently described fluorescamine derivatization HPLC method.

With WR80855 as an internal standard we obtained the precision and accuracy data summarized in Table 2. Using aliquots of a normal human plasma pool to which WR2721 was added to final concentrations ranging from 2 to 800 µmol/L we obtained an average coefficient of variation of 4.4% and an average deviation from the spiked-in concentration value of 7.4%.

Chromatography of S-2-(3-Aminopropylamino)ethanethiol (WR1065)

WR1065, the free sulfhydryl metabolite of WR2721, does not interfere with the HPLC assay for WR2721. In control experiments we tested for the possible appearance of this compound in our

chromatograms. WR1065 did not elute from the column during 20 minutes after injection of 20 µL of 0.1 mmol/L and 4 mmol/L solutions of this compound in 10 mmol/L tris(hydroxymethyl)aminomethane, pH 7.4, or of 20 µL of 0.1 mmol/L and 4 mmol/L of WR1065 in 10 mmol/L phosphate buffer, pH 7.4.

By modifying the mobile phase used for WR2721 chromatography to include 30% methanol it was possible to elute WR1065 from the Biophase ODS 5µ column in 20 minutes at a flow rate of 1 mL/min (Figure 5).

Human Pharmacokinetic Study

A pharmacokinetic study was performed on a patient treated with 300 mg (3.4 mg/kg) of WR2721 administered as a 10 second intravenous bolus dose. Blood samples were obtained prior to and after completion of WR2721 administration at 2, 2.5, 3, 4, 8, 12, 20, 30, 45 minutes and 1, 2, 4, 8 and 24 hours. A semilog plot of WR2721 concentration versus time is displayed in Figure 6 and a typical chromatogram of this patients' plasma compared to the pre-dose plasma is shown in Figure 7. Examination of the data in Figure 6 shows that the majority of the drug was cleared from plasma within about 5 minutes. Consistent with this conclusion is the very short distribution half-life, T1/2 , of 0.84 minute and the rapid clearance from the central compartment, Cl, of 0.977 L/hr/kg calculated from the data points with the extended least squares nonlinear regression (ELSNLR) program (10).

Enzymatic Hydrolysis of WR2721

It has been shown in experiments with mice that within 15 minutes after administration of an intravenous dose of WR2721 the principal metabolite in most tissues is WR1065 (5). Previous studies with mammalian tissues have raised the possibility that WR2721, or phosphorothioates with similar structure such as cysteamine S-phosphate, are hydrolyzed by either acid phosphatase

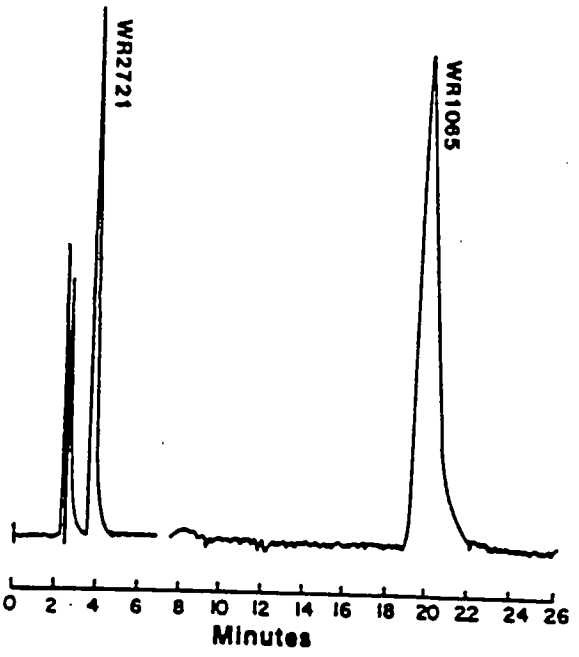

FIGURE 5. Chromatogram of an aqueous solution of 200 µmol/L WR2721 and 200 µmol/L WR1065 in 2.7 mmol/L EDTA and 10 mmol/L tris(hydroxymethyl)aminomethane, pH 7.4. The mobile phase is 30% methanol, by volume, in water. Monochloroacetic acid and sodium octylsulfate, pH 3.0, are in the mobile phase at final concentrations of 0.1 mol/L and 1.5 mmol/L, respectively. The mobile phase flow rate is 1.0 mL/min. Sensitivity was changed from 500 nA full scale to 100 nA full scale at 7 min.

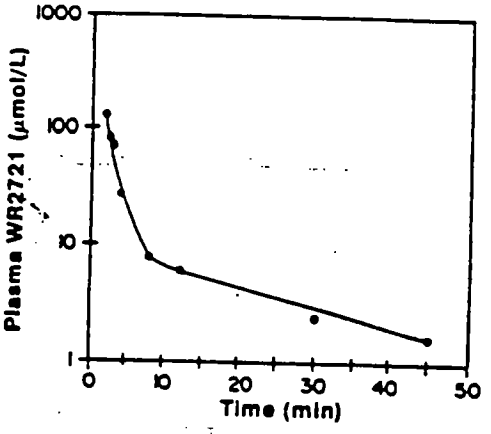

FIGURE 6. A semilog plot of WR2721 plasma concentration versus time. Each point is the average of duplicate determinations. The line fitting the points is the least squares best fit line determined with the ELSNLR program (10).

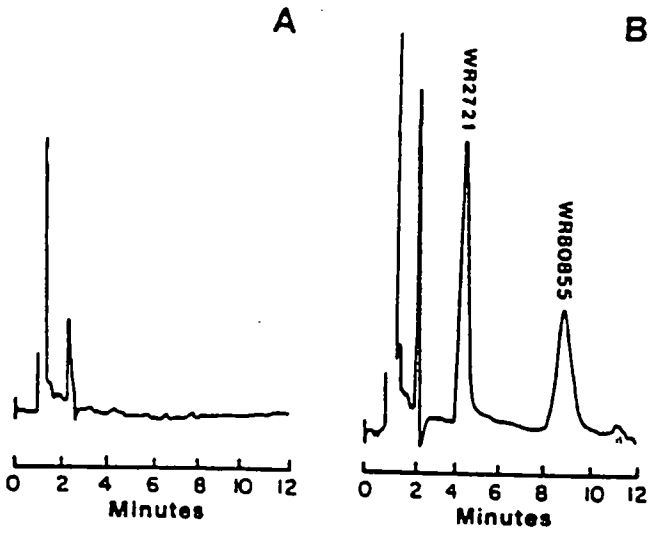

FIGURE 7. Chromatograms of acetonitrile-plasma supernatants prepared from A, plasma obtained from a patient immediately before a 300 mg intravenous bolus dose and B, plasma obtained from the patient two minutes after the dose. The sensitivity is 50 nA full scale.

(13,14) or alkaline phosphatase (15,16,17) to produce the corresponding free sulfhydryl metabolite. As shown in Table 3, WR2721 is not hydrolyzed by either human acid phosphatase isoenzyme 2A prepared from prostatic fluid as previously described (18) or isoenzyme 5 from human spleen (19). On the other hand, human liver alkaline phosphatase, prepared as described by Daigle (20) and calf intestine alkaline phosphatase do catalyze the hydrolysis of WR2721 (Table 3). The fact that the rate of WR2721 hydrolysis was higher than that achieved with PNPP as substrate using the human liver enzyme, but lower using the calf intestine enzyme may result from kinetic differences between the alkaline phosphatase isoenzymes. More detailed kinetic studies will be required to characterize this difference.

TABLE 3

Rates of Hydrolysis of p-Nitrophenylphosphate and WR2721 by Acid and Alkaline Phosphatases

	Hydrolysis Rates[a] (nmol/min/mL reaction mixture)	
	WR2721	p-Nitrophenylphosphate
Acid Phosphatase		
I. Isoenzyme 2A[b]	0.0002	2.75
II. Isoenzyme 5[c]	0	2.07
Alkaline Phosphatase		
I. Human liver[d]	11.49	6.56
II. Calf intestine[e]	11.25	508

[a]Each activity value is the average of duplicate determinations as described in Materials and Methods.

[b]Each reaction mixture contained 2.75 mU of isoenzyme 2A acid phosphatase per mL with p-nitrophenylphosphate as substrate. In testing for possible WR2721 hydrolysis each reaction mixture contained 44,000 mU of isoenzyme 2A acid phosphatase per mL.

[c]Each reaction mixture contained 2.07 mU of isoenzyme 5 acid phosphatase per mL with p-nitrophenylphosphate as substrate. In testing for possible WR2721 hydrolysis each reaction mixture contained 83 mU of isoenzyme 5 acid phosphatase per mL.

[d]Each mL of reaction mixture contained 6.6 mU of human liver alkaline phosphatase.

[e]Each mL of reaction mixture contained 508 mU of calf intestine alkaline phosphatase. All alkaline phosphatase reaction mixtures were incubated at pH 8.6.

The rate of WR2721 hydrolysis as a function of pH using human liver alkaline phosphatase is shown in Figure 8. The maximal rate of hydrolysis catalyzed by human liver alkaline phosphatase was obtained at pH 8.6. In contrast the optimal pH for the synthetic substrate, p-nitrophenylphosphate, is much higher than 8.6 (Figure 8). The highest activity was at pH 10 (the highest pH value tested). Previous studies have obtained pH optima, with p-nitrophenylphosphate as substrate, of 10.2 for alkaline phosphatase extracted from human liver cells grown in tissue culture

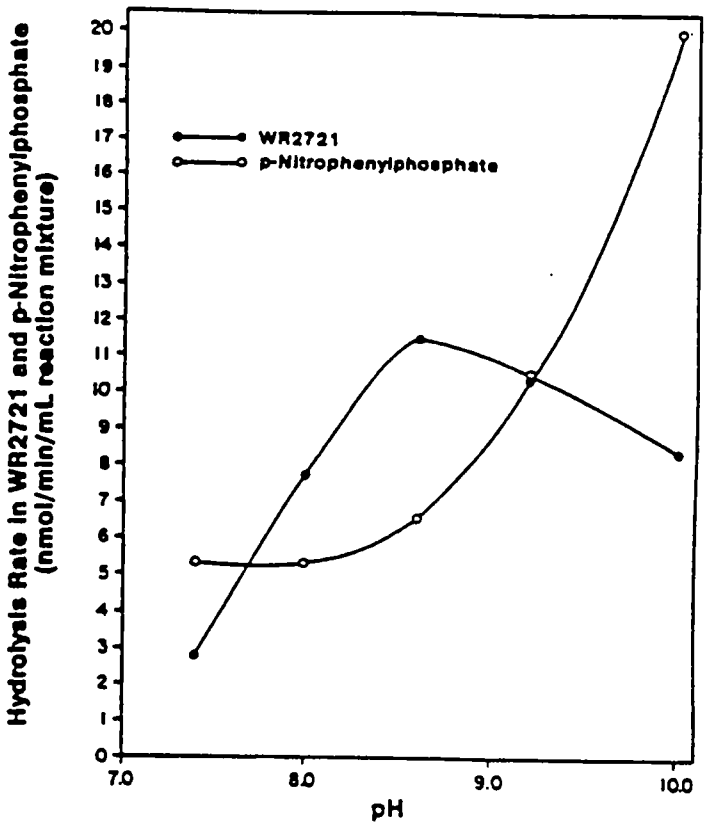

FIGURE 8. A plot of the rates of hydrolysis of WR2721 and p-nitrophenylphosphate by human liver alkaline phosphatase as a function of pH. Each ml of reaction mixture contained 6.6 mU of enzyme.

(21) and 10.4 for alkaline phosphatase purified from human liver obtained at autopsy (22). Our finding of different pH optima for these two alkaline phosphatase substrates is consistent with previous observations of the dependence of the pH optimum for alkaline phosphatase on both the chemical nature of the substrate as well as on the substrate concentration (23).

CONCLUSIONS

The HPLC electrochemical detection method reported here for the measurement of WR2721 has the following advantages: a) it is rapid, since each chromatographic cycle is 15 minutes; b) it is a direct method which does not require additional derivatization steps; c) it is both precise and accurate; d) it eliminates plasma proteins prior to chromatography using a neutral polar organic solvent; e) neither the free sulfhydryl metabolite of WR2721, WR1065, nor endogenous substances from patients' plasma co-elute with either WR2721 or WR80855; f) it is readily applicable to the study of WR2721 pharmacokinetics in humans and to the investigation of its metabolism by mammalian alkaline phosphatases.

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TITLE: PHARMACOKINETICS OF CISPLATIN (CDDP) FOLLOWING A 15-MINUTE INFUSION OF AMIFOSTINE

REPORT: ETH PK2

INVESTIGATOR:

STUDY DATES: Ongoing

REPORT DATE: Preliminary, June 1994

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OBJECTIVE

To determine the effect, if any, of a 15-minute infusion of amifostine (WR-2721) on the pharmacokinetic parameters of cisplatin (CDDP).

METHODS

Pharmacokinetic Methods

RESULTS

A typical concentration-time course for both total plasma platinum and ultrafilterable platinum is shown in FIGURE 1. TABLE 1 lists the pharmacokinetic parameters for the six patients studied.

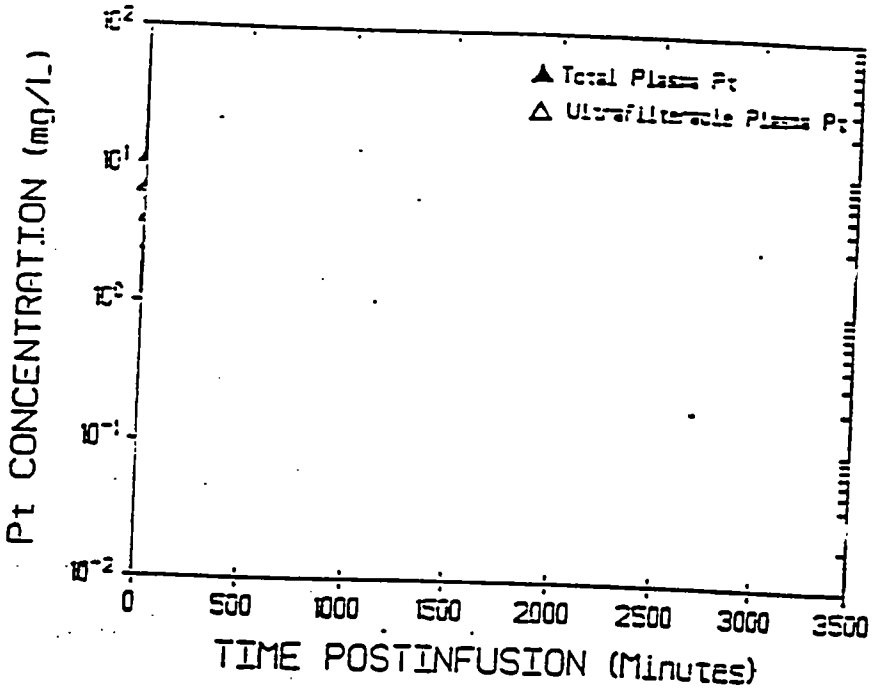

FIGURE 1: Concentration-time course for both total plasma platinum and ultrafilterable platinum following a 30-minute infusion of CDDP at 120 mg/m² 25 minutes after a 15-minute infusion of amifostine at 740 mg/m².

TABLE 1

Pharmacokinetics of Cisplatin (120 mg/m²) Following a 15-Minute Infusion of Amifostine (740 mg/m²) in Six Patients With NSCLC

Parameter	Cisplatin Pharmacokinetics (mean ± SD)	
	Total Plasma Platinum	Ultrafilterable Platinum
Peak Cp (mg/L)	8.3 ± 1.6	5.2 ± 1.0
AUC (mg/L × min)	17410 ± 2796	569 ± 135
Cl_b (ml/min/m²)	4.8 ± 0.9	142 ± 28
t½ α (min)	19.2*	26.8*
t½ β (h)	56.7*	27.0*
V_c (L/m²)	7.0 ± 1.6	11.7 ± 1.5
V_{ss} (L/m²)	23.2 ± 2.1	200 ± 74
% of dose in 24-h urine	30.2 ± 12.5	
% of UF platinum in plasma at end of infusion	58.4 ± 7.5	
% of UF platinum in plasma 2 h after end of infusion	9.6 ± 1.9	

* harmonic mean.

(Abbreviations used are: Cp, plasma concentration; AUC, area under the concentration-time curve; C_b, apparent total body clearance; t½α, distribution phase half-life; t½β, elimination phase half-life; V_c volume of the central compartment; V_{ss}, steady-state volume of distribution)

The AUC, clearance and V_{ss} calculated noncompartmentally were essentially identical to those parameters calculated from the biexponential model for both the total Pt and the ultrafilterable Pt (UF-Pt). For total Pt: 17496 vs. 17410; 4.8 vs. 4.8; and 23.8 vs. 23.2, respectively for UF-Pt: 582 vs. 569; 138 vs. 142; and 196 vs. 200, respectively.

DISCUSSION

The concentration of ultrafilterable Pt (UF-Pt) in plasma declined rapidly so that by 2 hours postinfusion, the concentration of UF-Pt was only 6.4% of that measured at the end of the infusion. The total plasma platinum exhibited a much slower decline in concentration (see FIGURE 1). The low total clearance and long elimination half-life for total plasma platinum indicates a high degree of tissue binding of platinum-containing species as has been extensively described in the literature. Details of total platinum pharmacokinetics in both animals and man have shown a consistent pattern of total platinum plasma clearance, independent of dose and schedule.[11] The long half-life of the UF-Pt reflects metabolism and turnover of tissue proteins containing irreversibly bound platinum.[4]

TABLE 2 includes a comparison of the pharmacokinetic characteristics of cisplatin in the present study with those included in six published reports. This table includes the parameters as described in these reports, some of which have been recalculated from the published data in order to describe the data in similar units. Taken together, these reports indicate that the peak Pt concentrations, degree of protein binding, urinary excretion and elimination of Pt over time reported in the present study are similar to the distribution and clearances of total Pt reported in the literature surveyed in TABLE 2.[5-10] Also, initial distribution half-life and clearance of UF-Pt appears to be similar.

TABLE 2

Clinical Pharmacokinetics of Cisplatin
(Present Study Versus Published Data)

Parameter	Present Study		Bleinck[3]		Preiss[4]		Fracnsso[7]		Vermorken[8]		Gullo[9]		Reece[10]	
	Total	UF-Pt	Total	UF-Pt	Total	UF-Pt	Total	UF-Pt	Total	UF-Pt	Total	UF-Pt	Total	UF-Pt
Peak Cp (mg/L)	8.3±1.6	5.2±1.0	9.3	5.0										
AUC (mg/L ×min)	17410±2796	569±135												
Cl_a (ml/min/m²)	4.8±0.9	142±28			5.8		8.6			121				
t½ α (min)	19.2*	26.8*			15		30				15	22		29
t½ β (h)	56.7*	27.0*			79		66				54			26
V_c (L/m²)	7.0±1.6	11.7±1.5												
V_{ss} (L/m²)	23.2 ±2.1	200±74			36		38		33.5		29			
% of dose in 24-h urine	30.2 ± 12.5		32				24		25					
% of UF platinum in plasma at end of infusion	58.4 ± 7.5								50		50			
% of UF platinum in plasma 2 h after end of infusion	9.6 ± 1.9								10					

* Values based on harmonic mean.

Abbreviations used are: Cp - plasma concentration; AUC, area under the concentration-time curve; Cl_a - apparent total body clearance; t½α - distribution phase half-life; t½β - elimination phase half-life; V_c - volume of the central compartment; V_{ss} - steady-state volume of distribution; total = total plasma platinum; UF-Pt = ultrafilterable plasma platinum

As noted above, amifostine was administered as a 15-minute infusion and the 30-minute cisplatin infusion started approximately 25 minutes after the completion of the amifostine infusion. The pharmacokinetics of amifostine have been previously described; the plasma elimination best fits a biexponential process with the $t_{1/2}\alpha$ <1 min and the $t_{1/2}\beta$ of 8 minutes. Ninety percent of the drug in plasma is cleared within 10 minutes.[n,o] Thus, there is little drug in the plasma that could potentially interact with an anticancer drug that is administered at a later time.

In summary, there does not appear to be any effect of amifostine pretreatment on the pharmacokinetics of cisplatin. This is an ongoing study and further cisplatin pharmacokinetics will be determined in an upcoming randomized Phase III trial of high-dose cisplatin (120 mg/m^2) ± amifostine pretreatment in patients with non-small cell lung cancer.

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Cancer Chemotherapy and Pharmacology

Influence of glutathione administration on the disposition of free and total platinum in patients after administration of cisplatin

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Summary. The kinetics of platinum (Pt) was studied in 12 patients suffering from non-small-cell lung cancer or pleural mesothelioma. Each subject received an infusion of cisplatin (CDDP, 80 mg/m^2), and six patients were pretreated with glutathione (GSH, 2.5 g given i.v.) at 15 min prior to the cisplatin infusion. After a 3- to 4-week interval, all patients were given a second course of treatment on the same schedule. A biexponential model was fitted to plasma concentrations of total and ultrafilterable Pt. The excretion of Pt in urine was evaluated during the first 48 h after the CDDP infusion. Following the administration of CDDP alone or with GSH pretreatment, the pharmacokinetic parameters of Pt did not significantly differ between the treatments. Also, the unbound fraction determined at each sampling time did not vary significantly between the treatments. However, it is noteworthy that the mean values obtained for the terminal half-life, the volume of distribution, the renal clearance, the percentage of the dose excreted in the urine, and the mean residence time of total Pt were higher in patients who had been pretreated with GSH, suggesting that GSH might increase both the rate of Pt elimination and the extent of Pt distribution and, as a consequence of the latter, might prolong the residence time of Pt in the body. In addition, the unbound fraction of Pt from the 4th to the 48th h was higher following the first dose of CDDP+GSH than after treatment with CDDP alone. Because of the rather high variability in the values of the parameters obtained, further work is planned using a larger number of patients.

Introduction

Cisplatin (CDDP) is a widely used antitumor agent that is effective in the treatment of some solid tumors, including lung cancer. The pharmacokinetics of Pt has been studied in cancer patients who have received CDDP treatment [4, 5, 10, 11]. Evidence of a dose-response effect for CDDP has led to the use of high doses of this drug [7]. Since nephro- and neurotoxicity are the major dose-limiting side effects, a variety of strategies have been proposed to protect the kidney and neurological functions following CDDP treatment. It has recently been shown that the administration of glutathione (GSH) provides protection against CDDP-induced nephrotoxicity without reducing the antitumor activity of the cytotoxic agent [3; 12, 13]. The aim of the present study was to investigate the effect of GSH pretreatment on the kinetics of total and ultrafilterable (free) platinum (Pt) in patients receiving CDDP for the treatment of non-small-cell lung cancer or pleural mesothelioma.

Patients and methods

Experimental design. The disposition of free and total Pt after the administration of CDDP was studied in two randomized groups of six patients each. Eligibility criteria included histologically documented, inoperable non small cell lung carcinoma (ten patients) or pleural mesothelioma (two cases), an age of <70 years, no prior chemotherapy, a leukocyte count of >3500 cells/mm^3, a hemoglobin value of >11 g/dl, and a serum creatinine level of <1.5 mg/dl. All subjects were required to show an Eastern Cooperative Oncology Group performance status of <3 and a life expectancy of ⩾4 months. Individuals exhibiting severe metabolic disease or cardiopathy were excluded. Informed consent was obtained from each patient.

The treatment regimen consisted of 120 mg/m^2 etoposide given on days 1, 8, 15, and 22 as a 30-min i.v. infusion in 100 ml normal saline and 80 mg/m^2 CDDP given on day 2 and between days 23 and 30 as a 15-min i.v. infusion in 100 ml normal saline. On a random basis, one of the two groups was also given a 15-min i.v. infusion of 2.5 g GSH in 100 ml normal saline just before the administration of CDDP. Uniform i.v. hydration (1250 ml fluid) without diuretics was used; at 1 h prior to the initiation of the CDDP infusion, patients were hydrated with 250 ml normal saline. Posthydration with 1000 ml normal saline was continued for 4 h. Subjects received prometazine (25 mg given i.m.) and hydrocortisone (200 mg given i.v.) immediately before CDDP administration and dexamethasone (8 mg given i.v.) and alizapride (350 mg given i.v.) after the CDDP infusion as standard antiemetic treatment.

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Table 1. Patients' characteristics

Initials	Sex	Age (years)	Weight (kg)	Height (m)	Total dose of CDDP (mg)
CDDP group:					
M.D.	M	60	72	1.7	144
V.C.	M	68	68	1.67	140
E.O.	M	56	83	1.78	160
L.C.	M	49	82	1.68	152
G.M.	M	47	67	1.69	140
E.P.	M	60	84	1.74	158
CDDP+GSH group:					
G.M.	F	65	69	1.64	140
A.B.	M	53	69	1.7	144
N.Q.	M	52	84	1.8	160
S.C.	M	68	50	1.6	120
A.C.	F	63	53	1.68	124
R.E.	M	51	56	1.7	128

The demographic characteristics of our patients are reported in Table 1. Complete blood cell counts, hepatic and renal functions, fasting blood glucose, plasma total protein, albumin, and electrolytes were evaluated before each CDDP infusion.

Sample collection. Blood samples were drawn into heparinized tubes prior to the administration of CDDP, at the end of the CDDP infusion, and at 5, 15, and 30 min and 1, 2, 4, 7, 24, and 48 h after the infusion. Blood samples were immediately centrifuged at 12,000 *g* for 2 min at room temperature, and the plasma was separated and divided into two aliquots. The first was frozen and stored at −20°C until the analysis of total Pt. The second aliquot was immediately ultrafiltered through Centriflo CF50A cones (Amicon; cut-off, 50,000 Da) by centrifugation at 1,000 *g* for 10 min at 4°C. The ultrafiltrate was frozen and stored at −20°C until the analysis of free Pt.

Total and free Pt assays. The total Pt concentration in plasma samples was determined after a 1:10 dilution of the latter with 0.05% Triton solution. The free Pt concentration was measured in most ultrafiltrates without any dilution; when necessary, the ultrafiltrates were diluted with 0.1% Triton solution. Urine samples were diluted 1:2 with distilled water. Aliquots of 10 µl were analyzed by flameless atomic absorption spectrometry (Varian model 1475-GTA 95). The limit of quantitation was 5 ng/ml for plasma samples and 0.5 ng/ml for urine.

Pharmacokinetic analysis. A biexponential model for i.v. infusion (Eq. 1) was fitted to total and free Pt concentrations using $1/\hat{C}^2$ as a weighting factor (Siphar program; R. Gomeni, Simed, Créteil, France) according to the formula:

$$c = \sum_{i=1}^{2} ci\,(1-e^{-\lambda i T})\,e^{-\lambda i (t-T)}/\lambda i T. \quad (1)$$

where c is the concentration at any time, ci and λi represent the hybrid coefficients and the exponents of each exponential term (had an i.v. bolus dose been given), respectively, and T indicates the infusion time. The platinum concentration at the end of the infusion (c_{max}) was obtained from the experimental data. Other pharmacokinetic parameters were calculated according to standard relationships [6]. The half-lives in the distribution phase ($t_{1/2\alpha}$) and in the elimination phase ($t_{1/2\beta}$) were calculated as:

$$t_{1/2}i = \ln(2)/\lambda i. \quad (2)$$

The area under the concentration-time curves from time zero to 48 h (AUC_{0-48}) and extrapolated to infinity (AUC) were obtained using the following equations in which t' is the post-infusion time:

$$AUC_{0-48} = \sum_{i=1}^{2} ci/\lambda i + ci(e^{-\lambda i T}-1)e^{-\lambda i t'}/\lambda i^2 T \quad (3)$$

$$AUC = \sum_{i=1}^{2} ci/\lambda i. \quad (4)$$

The plasma clearance of total platinum (C and unbound platinum (C_u) were calculated as the ratio of the delivered dose (D) expressed as elemental platinum (D = 52.4 mg/m²) to the respective AUC value for total and unbound Pt. The renal clearance (C_R) was calculated as the ratio of the amount of Pt excreted in the 0- to 48-h urine specimen (A_{0-48}) to the AUC_{0-48} value for total Pt. The mean residence time (MRT) was calculated as the ratio of the area under the first moment curve (AUMC) to the AUC. The initial volume of distribution (V_1) and the steady-state volume of distribution (V_{ss}) were calculated according to the following equations:

$$V_1 = D/(c_{1-2}) \text{ and} \quad (5)$$

$$V_{ss} = C \times MRT. \quad (6)$$

The unbound volume of distribution (V_u) was calculated as the ratio of C_u to λ2 for the unbound Pt. The unbound fraction (fu) was obtained as the ratio of the unbound Pt concentration to the total Pt concentration at each sampling time.

Statistical analysis. Statistical analysis was done by one-way analysis of variance for repeated measurements, with treatments, times (first and second administration), and times × treatments (interaction) as sources of variation.

Results

The temporal profiles of total and ultrafilterable Pt concentrations in plasma following the first i.v. dose of CDDP in the presence and absence of GSH pretreatment are shown in Fig. 1 and 2, respectively. The profiles observed following the second administration were very similar. The mean pharmacokinetic parameters calculated from plasma and urinary data are listed in Tables 2 and 3. Figure 3 illustrates the excretion of Pt during the first 24 and 48 h following the CDDP infusion.

Modest interpatient variability was observed in the plasma concentration values at the different sampling times. The concentration measured in plasma at the end of the infusion (c_{max}) was similar in all subjects and did not depend on the treatment, the mean values ranging from 4.71 (CDDP, first dose) to 5.44 mg/l (CDDP, second dose). Similarly, the mean values for AUC_{0-48} ranged from 72.1 (CDDP+GSH, first dose) to 92.4 mg h l⁻¹ (CDDP+GSH, second dose) and did not differ significantly

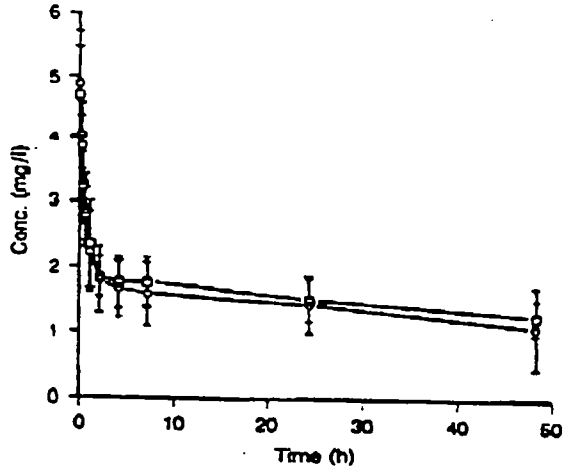

Fig. 1. Temporal profiles of total Pt concentrations (*Conc*) following the administration of the first dose of CDDP alone (□) or with GSH pretreatment (○)

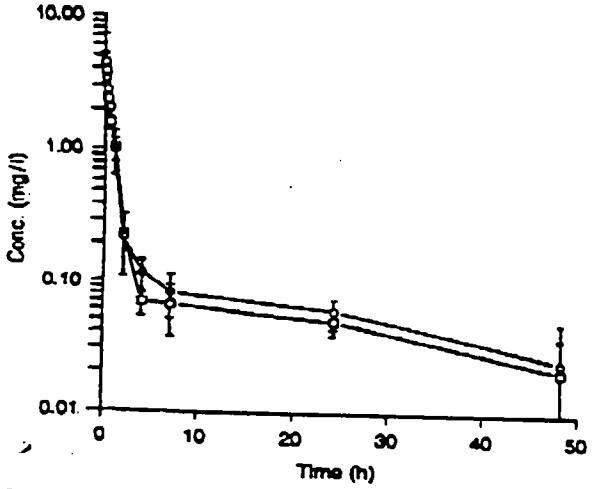

Fig. 2. Temporal profiles of ultrafilterable Pt concentrations (*Conc*) following the administration of the first dose of CDDP alone (□) or with GSH pretreatment (○)

between the treatments. Higher interpatient variability was associated with the other estimated parameters, whose values were dependent on the estimate of the terminal slope of the plasma curves (e.g., AUC, C_l, and $t_{1/2\beta}$).

Following all treatments, the concentration of total Pt subsided after the peak in a biexponential manner; the initial rapid decay ($t_{1/2\alpha}$, from 0.36 to 0.45 h), which was mainly related to the distribution process, was followed by a prolonged and slow apparent terminal phase ($t_{1/2\beta}$, from 85.6 to 143.2 h). Values of ca. 2 mg/l were attained at 4 h after CDDP administration, and at the 48th h the concentration remained as high as >1 mg/l. The c_{max} value for free Pt at the end of the infusion could be superimposed over the concentration of total Pt, indicating that all of the drug in plasma was unbound immediately after its administration. The concentration of unbound Pt decreased in a biexponential fashion. The half-life associated with the distribution phase ($t_{1/2\alpha}$, from 0.43 to 0.46 h) was similar to that of total Pt, whereas the apparent terminal half-life ($t_{1/2\beta}$, from 39.4 to 87.7 h) was much shorter than that of total Pt due to the time dependency of the binding in plasma. The extent of Pt protein binding in plasma increased with time: during the first 4 h, the unbound fraction (fu) decreased from approx. 0.95 to 0.05, and it dropped to 0.03 at the 48th h. Figure 4 shows the time dependency of the fu fraction of Pt. This phenomenon has previously been reported for Pt pharmacokinetics [4].

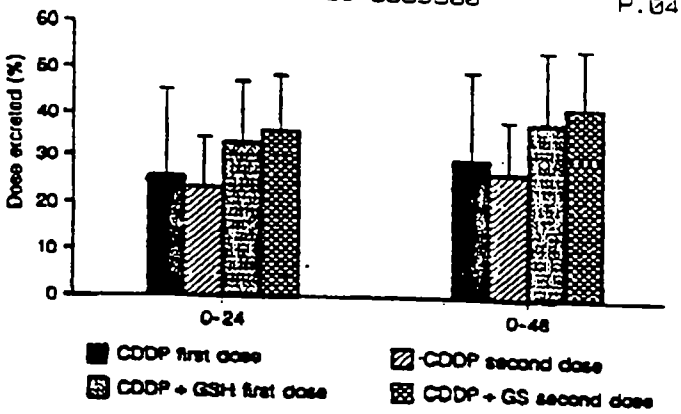

Fig. 3. Excretion of Pt in urine at 24 and 48 h after the CDDP infusion

Table 2. Mean pharmacokinetic parameters of total platinum following the administration of CDDP or CDDP+GSH

	CDDP		CDDP+GSH	
	1st dose	2nd dose	1st dose	2nd dose
c_{max} (mg/l)	4.71 (0.77)	5.44 (1.11)	4.9 (0.82)	5.39 (1.25)
$t_{1/2\alpha}$ (h)	0.36 (0.15)	0.37 (0.15)	0.43 (0.18)	0.45 (0.19)
$t_{1/2\beta}$ (h)	85.6 (16.5)	88.1 (36)	120.7 (57.1)	143.2 (64.4)
AUC (mg h l^{-1})	230.1 (64.7)	262.1 (121.3)	300.8 (164.4)	450.1 (239.3)
AUC_{0-48} (mg h l^{-1})	75.1 (14.5)	82.4 (14.1)	72.1 (19.3)	92.4 (19.9)
MRT (h)	122.6 (24)	126.1 (51.8)	172.9 (82.2)	205.5 (92.8)
C_l (ml h^{-1} kg^{-1})	5.92 (1.4)	5.75 (2.48)	6.17 (3.54)	4.01 (2.05)
C_{lr} (ml h^{-1} kg^{-1})	5.31 (3.67)	4.26 (1.93)	7.51 (2.25)	6.37 (0.68)
A_{e0-48} (%)	29.7 (19.7)	26.8 (11.9)	38.5 (15.6)	42.1 (12.9)
V_1 (l/kg)	0.25 (0.04)	0.22 (0.04)	0.28 (0.06)	0.26 (0.09)
V_{ss} (l/kg)	0.7 (0.1)	0.63 (0.09)	0.89 (0.33)	0.69 (0.23)

SD values are shown in parentheses

Table 3. Mean pharmacokinetic parameters of unbound platinum following the administration of CDDP or CDDP+GSH

	CDDP		CDDP+GSH	
	1st dose	2nd dose	1st dose	2nd dose
c_{max} (mg/l)	4.76 (0.57)	5.02 (1.4)	5.07 (1.58)	4.5 (1.33)
$t_{1/2\alpha}$ (h)	0.43 (0.03)	0.46 (0.06)	0.43 (0.04)	0.43 (0.1)
$t_{1/2\beta}$ (h)	39.4 (11.4)	87.7 (52.8)	42.7 (33.8)	50.1 (42.8)
AUC (mg h l^{-1})	7.02 (0.56)	11.84 (2.07)	9.04 (2.45)	10.87 (7.67)
AUC_{0-48} (mg h l^{-1})	5.4 (0.55)	6.36 (1.12)	6.57 (0.93)	6.58 (1.72)
C_R (ml h^{-1} kg^{-1})	181 (16.3)	108.9 (16.1)	181.6 (33.5)	182.9 (90.2)
V_{ss} (l/kg)	10.37 (3.74)	12.9 (6.31)	7.27 (1.52)	9.17 (3.02)

SD values are shown in parentheses

Platinum proved to be widely distributed in the body: the values for the volume of distribution ranged from 0.63 (CDDP, second dose) to 0.89 l/kg (CDDP+GSH, first dose), indicating that Pt is distributed to both the extracellular and the intracellular fluids. Platinum was slowly cleared from the body, the total clearance ranging from 4.01 (CDDP+GSH, second dose) to 6.17 ml h^{-1} kg^{-1} (CDDP+GSH, first dose).

The occurrence of a high volume of distribution and a low clearance resulted in a long terminal half-life and a high mean residence time (MRT, 122.6–205.5 h) for Pt. In 48 h, the urinary recovery of the administered dose ranged from 26.8% (CDDP, second dose) to 42.1% (CDDP+GSH, second dose), confirming the slow elimination of Pt from the body. The renal clearance values ranged from 4.26 (CDDP, second dose) to 7.51 ml h^{-1} kg^{-1} (CDDP+GSH, first dose) and were similar to those found for total clearance. This indicates that the elimination of Pt from the body occurs mainly by renal excretion. The Pt pharmacokinetic parameters obtained in the present study are consistent with previously published data [5, 10, 11].

Discussion

The effect of GSH pretreatment on the pharmacokinetics of total and free platinum was investigated in 12 cancer patients following i.v. infusions of 80 mg/m^2 CDDP in the presence or absence of GSH pretreatment. For ethical reasons, the last sampling time was at 48 h after the administration, an early cutoff point for a drug that is characterized by a long elimination half-life, and the terminal phase of the plasma curve was defined by a few experimental points. Therefore, some degree of uncertainty might be associated with the estimate of the terminal rate constant and, consequently, with the pharmacokinetic parameters derived. The AUC value calculated using the trapezoidal

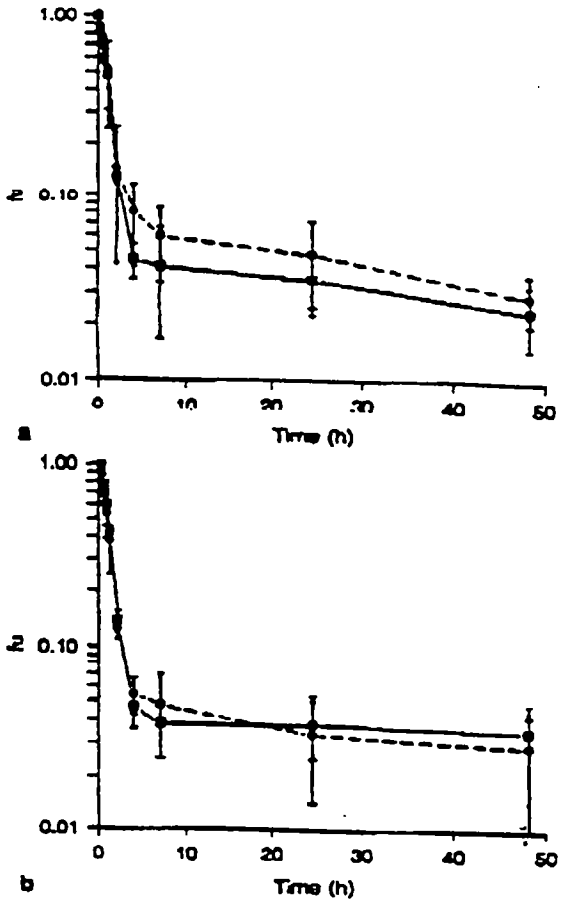

Fig. 4a, b. Time dependency of the unbound fraction of Pt after the a first and b second administration of CDDP (■) and CDDP+GSH (●)

rule from time zero to the last measured concentration accounted for only about 20%–30% of the total AUC, indicating that most of the curve was not experimentally inspected but rather predicted by the fitting model used (Eq. 1).

Influence of GSH pretreatment

Following the administration of CDDP alone or with GSH pretreatment, most of the pharmacokinetic parameters of CDDP were not significantly different between the treatments. Neither the parameters defining the rate ($t_{1/2}$) and the extent (V_1, V_{ss}) of distribution nor those quantifying the rate (C, C_R) and the extent (Ae_{0-48}) of elimination were significantly different following the administration of CDDP in the presence vs the absence of GSH pretreatment. Moreover, the unbound fraction determined at each sampling time did not vary significantly between the treatments.

The relatively high variability in the values of the parameters and the small number of patients treated may explain the lack of statistically significant differences between the treatments. In comparing the mean values ob-

tained for the pharmacokinetic parameters following the administration of CDDP vs CDDP+GSH, some considerations seem noteworthy. The GSH pretreatment appeared to increase both the rate of total Pt elimination and the extent of total Pt distribution and, as a result of the latter, seemed to increase the residence time of Pt in the body. In fact, after the first administration of both medications, the volume of distribution increased from 0.7 to 0.89 l/kg; the renal clearance, from 5.31 to 7.51 ml h^{-1} kg^{-1}; the percentage of the administered dose excreted in the 0- to 48-h urine sample, from 29.7% to 38.5%; the AUC, from 230 to 301 mg h l^{-1}; the MRT, from 122.6 to 172.9 h; and the terminal half-life, from 85.6 to 120.7 h.

The possible influence of GSH on the distribution and elimination of Pt could be the result of the effect of GSH on Pt binding to plasma protein. It is generally recognized that only the free drug is available for tissue distribution and elimination [8]; an increase in the free fraction of Pt results in an increased volume of distribution and clearance, the drug having a low extraction ratio. We actually observed that the unbound fraction of Pt from the 4th to the 48th h was higher following the first dose of CDDP+GSH than after treatment with CDDP alone (Fig. 4). Accordingly, the clearance value for unbound platinum (C_u) did not vary after the first infusion of CDDP and CDDP+GSH (Table 3).

Following i.v. administration, exogenous GSH rapidly disappears from the plasma compartment [1] and is removed by the kidney, in which high concentrations of the thiol compound are achieved [13]. In fact, GSH is not taken up by most of the cells, except for those tissues showing substantial expression of γ-glutamyl-transpeptidase (γ-GT) on the cell membrane surface, mainly in the kidney. Since the nephrotoxicity of cisplatin has been ascribed to its reaction with the thiol groups of membrane proteins of renal tubules [2], GSH may reduce this toxicity by competing with protein sites for reactive platinum intermediates.

Therefore, the observed reduction in the nephrotoxicity of CDDP given with GSH pretreatment might be interpreted as the consequence of both an increase in the renal clearance of the former (due to the increased unbound fraction of CDDP in plasma) and a reduction in CDDP's interaction with the thiol groups of membrane proteins of renal tubules. Extracellular GSH does not interfere with the cytotoxic activity of cisplatin [9] and as the most common tumor histotypes express relatively low levels of γ-GT, GSH uptake by the tumor cells is unlikely. Therefore, the extracellular or intracellular inactivation of toxic platinum species by GSH in the tumor is not expected.

Time dependency of Pt kinetics

In a comparison of the pharmacokinetic parameters obtained following the first and the second dose, no time dependency of Pt pharmacokinetics was found after the administration of CDDP alone or with GSH pretreatment. The parameters obtained after the first administration did not significantly change following the second dose.

Tolerability

The tolerability of the present regimen was evaluated by determinations of blood cell counts, blood urea nitrogen values, and levels of serum creatinine, serum electrolytes, SGOT, SGPT, alkaline phosphatase, and serum total bilirubin. No major side effect was observed following treatment in either of the groups. Nephrotoxicity did not occur in the two groups, as expected due to the low CDDP dose given, to the short duration of treatment, and to the limited number of patients.

Conclusions

In the present study, the pharmacokinetics of cisplatin was studied in cancer patients following the administration of cisplatin in the presence or absence of GSH pretreatment. Due to high variability of the estimates of the parameters and to the limited number of patients, the pharmacokinetic parameters of free and total platinum did not differ significantly between the treatments. However, a comparison of the mean values obtained for most pharmacokinetic parameters calculated for total Pt after both administrations suggested that GSH pretreatment may increase both the extent of CDDP tissue distribution and the rate of the drug's elimination. This may explain some recent observations indicating that GSH pretreatment diminishes cisplatin's nephrotoxicity without reducing its antitumor activity. Further work using a larger number of patients is planned.

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Influence of Amifostine on the Pharmacokinetics of Cisplatin in Cancer Patients[1]

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ABSTRACT

The pharmacokinetics of cisplatin was investigated in 13 patients receiving 18 courses of cisplatin alone or in combination with amifostine to investigate the influence of amifostine (WR 2721; Ethyol) on the pharmacokinetics of cisplatin. Cisplatin was administered as a 1-h i.v. infusion, whereas amifostine was given i.v. over 15 min just before the cisplatin infusion.

An increase in the final half-life of ultrafilterable platinum was observed after treatment with cisplatin and amifostine ($t_{1/2}$, 0.77 ± 0.10 h; $n = 8$), compared to cisplatin alone ($t_{1/2}$, 0.57 ± 0.15 h; $n = 8$). This might be caused by an influence of amifostine on the kidney function, because an increase in the serum creatinine levels was also observed 24 h after treatment with cisplatin and amifostine (13.8 ± 12.6%; $n = 9$), which was not observed after treatment with cisplatin alone (−0.1 ± 6.8%; $n = 9$).

Surprisingly, the final half-life of unchanged cisplatin did not increase, but even showed a slight decrease after treatment with amifostine. *In vitro* data would suggest that this might be due to a chemical interaction between cisplatin and amifostine. Because the AUC values of ultrafilterable platinum and unchanged cisplatin did not change significantly and no change in Pt-DNA adduct (Pt-GG) levels in leukocytes was observed upon addition of amifostine in the treatment schedule, the change in the pharmacokinetics of cisplatin is most probably of minor importance and has no significant impact on the efficacy of cisplatin, as already confirmed by clinical studies.

INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II)] is widely used in the treatment of solid tumors, in particular in testicular and ovarian tumors and in carcinomas of the bladder, lung, cervix and head and neck. Because of its steep dose-response relationship, several studies have focused on efforts to increase the dose intensity of cisplatin and concomitantly to decrease the toxicity of high-dose therapy (1). Most common side effects of cisplatin include nephrotoxicity, neurotoxicity, ototoxicity, and myelosuppression.

Amifostine [*S*-2-(3-aminopropylamino)ethyl-phosphorothioic acid, WR 2721, Ethyol] is one of the most promising chemoprotective agents in the modulation of cisplatin-induced toxicities. In preclinical studies, amifostine reduced cisplatin-induced nephrotoxicity without reducing its antitumor activity (2–5). In clinical trials, amifostine appeared to reduce the incidence of cisplatin-induced nephrotoxicity and hematotoxicity compared to historical data (6–8). Preliminary data from randomized trials showed a reduction in the incidence of neutropenia, nephrotoxicity, and neurotoxicity induced by treatment with cyclophosphamide and cisplatin (9) and a protection against cisplatin-induced bone marrow toxicity, neuropathy, and nephrotoxicity (10, 11). The influence on cisplatin-induced ototoxicity is still unclear. No indications of tumor protection were observed.

Because amifostine changed the pharmacokinetics of carboplatin in patients and mice, manifested by increased platinum levels, which might be related to an increase in the antitumor activity (12, 13), the aim of the present study was to investigate whether amifostine also influences the pharmacokinetics of cisplatin in patients. To this purpose, the pharmacokinetics was studied both in patients who received cisplatin plus amifostine and in patients receiving cisplatin alone. Total platinum, ultrafilterable platinum, and unchanged cisplatin concentrations were determined in plasma and the major cisplatin-DNA adduct, Pt-GG, in WBCs.

PATIENTS AND METHODS

Patients. This pharmacokinetic study was performed within the context of a Phase I/II trial, in which the combination of cisplatin and amifostine was studied. Amifostine was initially administered at a dose of 910 mg/m^2 but was reduced to 740 mg/m^2 during the study because of nausea and hypotension. The pharmacokinetics of cisplatin in patients treated in this Phase I/II trial was compared to the pharmacokinetics of cisplatin in a control group of patients treated with cisplatin alone.

A total of 13 patients, 7 males and 6 females, ages 44–71 years, were entered into this pharmacokinetic study after informed consent had been obtained. These patients received either 70 mg/m^2 cisplatin alone (four patients) or cisplatin plus 910 mg/m^2 amifostine (four patients). The remaining five patients were first treated with cisplatin alone 1 week before they were treated with cisplatin plus 740 mg/m^2 amifostine in the Phase I/II study.

All patients had not received prior treatment with cisplatin,

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except one, who had already received one cisplatin course 1 week before the treatment with cisplatin alone followed by cisplatin plus amifostine.

Except for one patient, who had a low Clcr[3] (32 ml/min), all of the patients had a normal renal function (Clcr > 60 ml/min, calculated with the Cockroft formula; Ref. 14). The mean Clcr in patients sampled during treatment with cisplatin alone was comparable to that in patients sampled during treatment with cisplatin in combination with amifostine (84 *versus* 91 ml/min). Similar serum creatinine levels were found at the start of the first and the second courses in patients who were sampled during two subsequent courses.

In total, 18 cycles were studied, 9 cycles of cisplatin and 9 cycles of cisplatin and amifostine. The pharmacokinetic data of the patient with the impaired renal function, who was treated with cisplatin alone followed by cisplatin plus amifostine, could not be integrated in the results of the two treatment groups. These data were only used for an intrapatient comparison. Thus, in total, eight cycles of cisplatin were compared to eight cycles of cisplatin plus amifostine. Also, a comparison was made between the two treatment groups including only the first treatment cycles to exclude a possible influence of prior treatment, which includes 7 cycles of cisplatin *versus* 4 cycles of cisplatin plus amifostine.

Drug Administration. Cisplatin (10, 25, or 50 mg/vial; Bristol Myers Squibb, Woerden, the Netherlands) was dissolved in 330 ml of 3.0% NaCl. Amifostine (500 mg of lyophilized amifostine/vial with 500 mg of mannitol; USB Pharma, Nijmegen, the Netherlands) was dissolved in 55 ml of 0.9% NaCl. Both drugs were given as i.v. infusions by using syringe infusion pumps. Cisplatin was administered for 1 hour. Amifostine was given as a 15-min infusion immediately before the cisplatin administration. Patients received 1 liter of normal saline before the start of treatment and 4 liters of normal saline at the end of the cisplatin infusion.

Sampling. For the pharmacokinetic studies, blood samples of 6 ml were taken in cooled heparinized tubes just before treatment; at 30 min after the start of the cisplatin infusion; at the end of the cisplatin infusion; and at 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 9, 21, and 24 h after the end of the cisplatin administration. At 5 and 21 h after the end of the infusion, an additional 18 ml of blood were collected for the analysis of cisplatin-DNA adducts in the leukocytes. Urine was collected during the first 25 h after the cisplatin administration had started.

Sample Pretreatment. Blood samples were immediately placed on ice and centrifuged at 2000 × *g* for 5 min at 4°C. Plasma was ultrafiltrated (1500 × *g* for 30 min at 4°C) using MPS-1 systems provided with YMT filters (Amicon, Capelle a/d yssel, the Netherlands). The plasma ultrafiltrate was chromatographed in duplicate (2 × 100 μl) on an anion exchange column [MCI gel CDR10 (Mitsubishi Chemical Industries, Ltd., Düsseldorf, Germany), 100 × 4.6 mm; mobile phase, 15 mM NaCl; flow rate, 1.5 ml/min], and the fractions containing unchanged cisplatin were collected. The RBCs were washed once with PBS. Plasma, plasma ultrafiltrate, cisplatin fractions, and RBCs were stored at −20°C until platinum analysis. For the analysis of Pt-GG adducts, 18 ml of whole blood were stored at −80°C until analysis. The urine fractions were pooled, and after measuring the total volume, an aliquot was stored at −20°C for platinum determination.

Analytical Methods. Plasma samples were diluted 10 times with 0.38 M NaCl in 0.5 M HCl and 0.1% Triton X/Antifoam B. Plasma ultrafiltrate samples were diluted 2.5 times with 0.15 M NaCl in 0.2 M HCl. The unchanged cisplatin-containing column fractions were evaporated and reconstituted in water. The urine samples were diluted 10 times with 0.15 M NaCl in 0.2 M HCl. RBCs were destroyed overnight at 55°C with 0.5 ml of benzethonium-hydroxide (Sigma, Zwyndrecht, the Netherlands) and then diluted with 4.25 ml 0.2 M HCl. Calibration standards and quality control samples, prepared by adding cisplatin to blank plasma, plasma ultrafiltrate, urine, and RBCs, were treated the same way as the patient samples. Platinum concentrations were measured with flameless atomic absorption spectrophotometry (Spectra AA-300 Zeeman AAS, Varian, Houten, the Netherlands).

For the analysis of Pt-GG adducts, the leukocytes were isolated from the thawed blood samples (15), followed by isolation of the DNA after inactivation of free and monofunctionally bound cisplatin with thiourea (16). Then, after digestion of the DNA and chromatography of the digest, the Pt-GG adducts were quantified with specific antibodies in an ELISA (15, 16). The levels of other cisplatin-DNA adducts were not detectable.

Pharmacokinetic Analysis. The pharmacokinetic parameters of total platinum, ultrafilterable platinum, and unchanged cisplatin were calculated with a two-compartmental model by the pharmacokinetic data analysis program Topfit 2.0 (Gustav Fischer, Stuttgart, Germany). The results were compared with the results obtained from the noncompartmental data analysis. For total platinum, the pharmacokinetic analysis included the data collected during the first 25 h after the start of the treatment, whereas for ultrafilterable platinum and unchanged cisplatin, only data were included that had been obtained during 4 and 3 h, respectively, after the start of the cisplatin treatment. For the calculation of the final half-lives in the noncompartmental data analysis, the four final data points of total platinum and the three final data points of ultrafilterable and unchanged cisplatin were used.

For the calculation of the AUC values (from $t = 0$ to infinity) of total platinum in plasma and RBCs of patients who had previously been treated with cisplatin, the calculated values were corrected for the contribution of the platinum still present from the preceding treatment. The fraction of the AUC value originating from the preceding treatment was calculated by the platinum concentration at the start of the treatment under study and the elimination half-life established between 24 h after the preceding treatment and the start of the treatment under study. These half-lives were comparable to the values previously reported in the literature (17).

In a few patients, the two-compartmental curve fitting of ultrafilterable or unchanged cisplatin resulted in an unrealistically long half-life, when compared to the results from the

[3] The abbreviations used are: Clcr, creatine clearance; AUC, area under the plasma concentration-time curve; MRT, mean residence time.

Table 1 Pharmacokinetic parameters (means ± SD) of total platinum (TPt), ultrafilterable platinum (UFPt), and intact cisplatin in plasma after treatment with cisplatin alone (CIS) or in combination with amifostine (CISWR) in all patients with normal renal function (Clcr >60 ml/min)[a]

			Plasma pharmacokinetics						RBCs		Urine		Clcr (Cockcroft; Ref. 14)	
Platinum species	Treatment	*n*	AUC (μM · h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	Cl (ml/min/1.73 m²)	Vss (liters/kg)	*n*	AUC (nmol/g·min)	*n*	Ae (% of dose)	*n*	ml/min
TPt	CIS	8	691 ± 104	0.39 ± 0.04	57.2 ± 14.0	81.1 ± 20.3	9.5 ± 2.5	0.69 ± 0.11	8	44.8 ± 8.4	6	34.3 ± 4.4	8	84 ± 17
	CISWR	8	614 ± 157	0.32 ± 0.07[b]	53.9 ± 15.6	76.5 ± 22.7	8.8 ± 3.6	0.58 ± 0.14	8	52.0 ± 6.9	4	33.3 ± 6.1	8	91 ± 18
UFPt	CIS	8	16.1 ± 1.6	0.20 ± 0.13	0.57 ± 0.15	0.70 ± 0.06	420 ± 37	0.28 ± 0.03						
	CISWR	8	17.6 ± 1.9	0.29 ± 0.13	0.77 ± 0.10	0.78 ± 0.10	383 ± 39	0.28 ± 0.04						
Cisplatin	CIS	8	13.8 ± 2.3	0.16 ± 0.14	0.47 ± 0.12	0.63 ± 0.05	501 ± 87	0.31 ± 0.07						
	CISWR	8	13.2 ± 2.1	0.17 ± 0.18	0.39 ± 0.07	0.61 ± 0.07	520 ± 77	0.30 ± 0.04						

[a] $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, initial elimination half-life; Cl, total body clearance; Vss, apparent volume of distribution at steady state; Ae, cumulative urinary excretion over the first 24 h; *n*, number of patients.
[b] $P < 0.05$.
[c] $P < 0.01$.

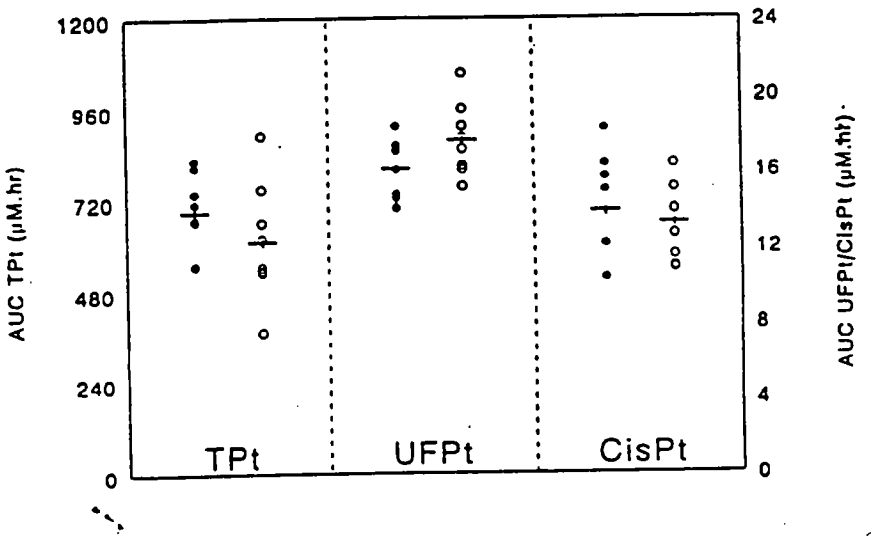

Fig. 1 AUC values of total platinum (*TPt*), ultrafilterable platinum (*UFPt*), and unchanged cisplatin (*CisPt*) in patients with a normal renal function (Clcr >60 ml/min) after treatment with cisplatin alone (●) and cisplatin in combination with amifostine (○). *Horizontal lines*, means.

noncompartmental analysis. In those cases, the curves were fitted again with the two-compartmental model after fixation of the value of the elimination rate constant to the value obtained from the noncompartmental analysis.

Statistics. For the statistical evaluation of the results, Student's *t* test (unpaired) was used.

RESULTS

To investigate a possible influence of amifostine on the pharmacokinetics of cisplatin, the mean values of the pharmacokinetic parameters of the three platinum species [total platinum, ultrafilterable (not protein-bound) platinum, and unchanged cisplatin] after treatment with cisplatin in combination with amifostine were compared with the data after treatment with cisplatin alone (Table 1). In Figs. 1 and 2, the individual data of the most important parameters, the AUC and the final half-life in patients with a normal renal function, are shown. In Table 2, the mean values of the pharmacokinetic parameters are given for patients who were not treated previously with cisplatin, to exclude a possible influence of prior treatment.

No significant changes in the AUC values of total platinum, ultrafilterable platinum, and unchanged cisplatin were observed after treatment with amifostine. For total platinum, a decrease in the initial and final half-life was observed, of which only the first was significant. For unchanged cisplatin, too, a trend for a decrease in the final half-life was seen, whereas for ultrafilterable platinum, the final half-life significantly increased after treatment with amifostine. No significant changes were observed for MRT, total body clearance, distribution volume, AUC of total platinum in RBCs, or cumulative urinary excretion, although trends for an increase in AUC and MRT and a decrease in total body clearance were seen for ultrafilterable platinum, in agreement with the increase in the final half-life.

The same trends were observed when comparing the data

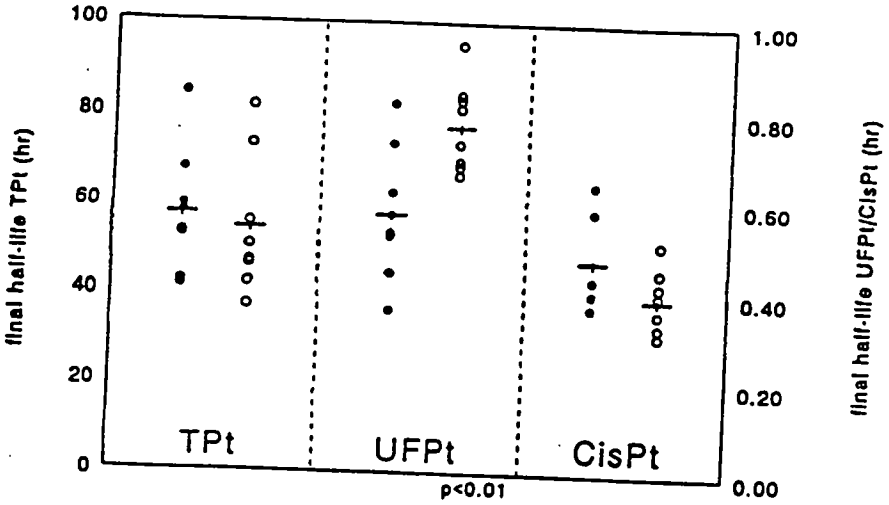

Fig. 2 Final half-lives of total platinum (*TPt*), ultrafilterable platinum (*UFPt*), and unchanged cisplatin (*CisPt*) in patients with a normal renal function (Clcr >60 ml/min) after treatment with cisplatin alone (●) and cisplatin in combination with amifostine (○). *Horizontal lines*, means.

Table 2 Pharmacokinetic parameters (means ± SD) of total platinum (TPt), ultrafilterable platinum (UFPt), and intact cisplatin in plasma after treatment with cisplatin alone (CIS) or in combination with amifostine (CISWR) in previously untreated patients with normal renal function (Clcr >60 ml/min)[a]

		Plasma pharmacokinetics						RBCs	Urine		Clcr (Cockcroft; Ref. 14)	
Platinum species	Treatment	*n*	AUC (μM · h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	Cl (ml/min/1.73 m²)	Vss (liters/kg)	*n*	AUC (nmol/g·min)	*n*	Ae (% of dose)	*n*	ml/min
TPt	CIS	7	671 ± 95	0.38 ± 0.04	53.3 ± 9.4	75.4 ± 13.4	10.2 ± 1.6	0.72 ± 0.10	7	44.8 ± 9.1	5	34.2 ± 4.9	7	87 ± 17
	CISWR	4	559 ± 41	0.29 ± 0.06*	43.7 ± 5.6	61.5 ± 8.3	12.0 ± 0.8	0.69 ± 0.09	4	51.6 ± 6.8	1	38.6	4	102 ± 16
UFPt	CIS	7	16.0 ± 1.6	0.18 ± 0.12	0.55 ± 0.15	0.69 ± 0.05	423 ± 39	0.28 ± 0.03						
	CISWR	4	17.1 ± 1.4	0.20 ± 0.09	0.77 ± 0.08*	0.73 ± 0.08	393 ± 32	0.27 ± 0.03						
Cisplatin	CIS	7	13.3 ± 2.1	0.11 ± 0.09	0.48 ± 0.12	0.64 ± 0.05	518 ± 86	0.33 ± 0.07						
	CISWR	4	12.4 ± 2.6	0.12 ± 0.16	0.33 ± 0.02*	0.56 ± 0.04*	556 ± 96	0.29 ± 0.06						

[a] $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, initial elimination half-life; Cl, total body clearance; Vss, apparent volume of distribution at steady state; Ae, cumulative urinary excretion over the first 24 h; *n*, number of patients.
* $P < 0.05$.

of patients who were not treated previously with cisplatin (Table 2). When comparing the pharmacokinetic data of cisplatin with and without amifostine within a representative patient, an increase in the final half-life of ultrafilterable platinum was seen as well (Fig. 3). In the patient with the impaired kidney function (Clcr, 32 ml/min), the same trends were observed: after treatment with amifostine, the AUC of ultrafilterable platinum increased from 17.5 to 19.9 μM · h, whereas the AUC values of total platinum and unchanged cisplatin decreased from 2002 to 1223 μM · h and from 18.2 to 15.1 μM · h, respectively. These data correspond to an increase in the final half-life of ultrafilterable platinum from 0.58 to 1.01 h; to a decrease in the final half-life of total platinum (from 131 to 80 h); and for unchanged cisplatin, to a small decrease from 0.63 to 0.51 h.

In relation to the observed increase in the final half-life of ultrafilterable platinum, a possible influence of amifostine on the renal clearance was investigated by measuring the serum creatinine levels just before treatment and 25 h after the start of the treatment with cisplatin. In the cisplatin-treated group, the serum creatinine level did not change (change in serum creatinine: −0.1 ± 6.8%; $n = 9$), whereas in patients treated with cisplatin in combination with amifostine, the serum creatinine levels at 25 h after the start of treatment were significantly higher than the levels just before the start of treatment (change in serum creatinine, +13.8 ± 12.6%; $n = 9$; $P = 0.01$).

In Table 3, the Pt-GG adduct levels are shown for blood samples collected at 6 and 22 h after the start of the cisplatin administration from patients who were not treated previously with cisplatin. No influence of amifostine on the formation or repair of the cisplatin-DNA adducts was observed. In the patients who had received prior treatment with cisplatin, elevated Pt-GG levels were observed, due to adducts still remaining from the preceding treatment. In patients receiving two subsequent courses of cisplatin, the Pt-GG levels in the WBCs were higher

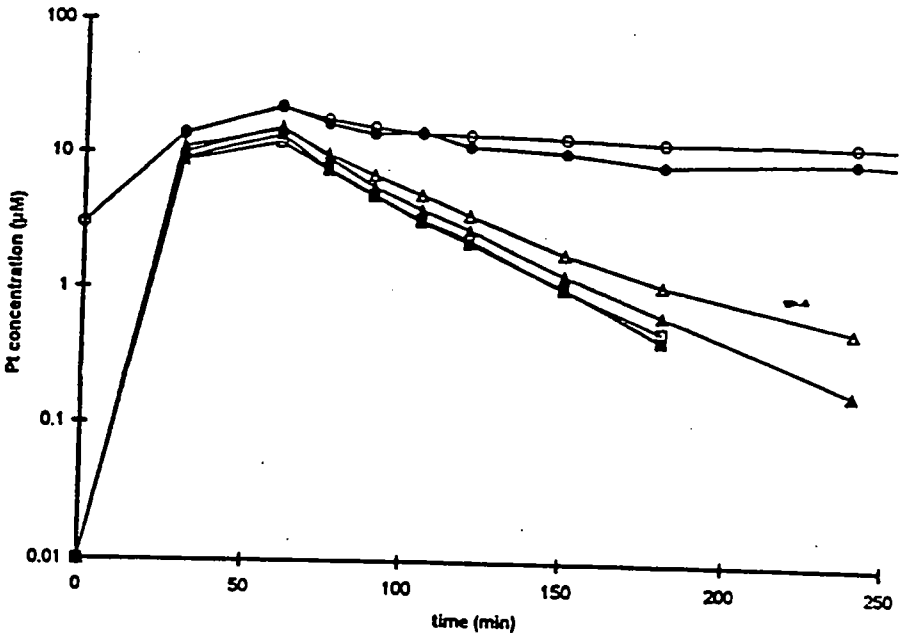

Fig. 3 Plasma concentration-time curves of total platinum (● and ○), ultrafilterable platinum (▲ and △), and unchanged cisplatin (■ and □) in one patient who was first treated with cisplatin alone (●, ▲, and ■) and 1 week later with cisplatin in combination with amifostine (○, △, and □).

Table 3 Pt-GG adduct levels (mean ± SD) in leukocytes of previously untreated patients at 6 and 22 h after receiving cisplatin alone (CIS) or in combination with amifostine (CISWR)

Time (h)	Treatment	*n*	Pt-GG (fmol/μg DNA)
6	CIS	6	1.27 ± 0.42
	CISWR	4	1.31 ± 0.36
22	CIS	6	0.89 ± 0.24
	CISWR	2	0.92

than after the first course, *i.e.*, 1.63 ± 0.25 fmol/μg DNA (n = 5) and 1.17 ± 0.25 fmol/μg DNA (n = 5) at 6 and 22 h after the start of the second treatment, respectively.

DISCUSSION

Previously, we reported a pharmacokinetic interaction between amifostine and carboplatin (13). An increase in the final half-life and the AUC value of ultrafilterable platinum in plasma was observed after treatment with carboplatin in combination with amifostine. Therefore, we investigated whether amifostine had the same influence on the pharmacokinetics of cisplatin.

In this study, a 1.3–1.4-fold increase in the final half-life of ultrafilterable platinum was observed after treatment with cisplatin and amifostine, which was comparable to the observed increase in the final half-life when carboplatin was combined with amifostine. This explains the small increase found for the AUC and MRT values, because only the relatively low concentrations at the end of the curve were enhanced. For the total body clearance of ultrafilterable platinum, a slight decrease was observed, indicating that the increase in the final half-life might be due to a decreased renal elimination of cisplatin, which is the major pathway of excretion of cisplatin (17). As we found in the carboplatin study (13), this was confirmed by an increase in the serum creatinine concentration observed after treatment with amifostine. In the cisplatin study, the increase was smaller than that observed in the carboplatin study (14 *versus* 34%), most probably due to the number of administrations of amifostine, because carboplatin was combined with three doses of amifostine. However, the more intensive hydration in the cisplatin study might also play a role in the reduced influence on the serum creatinine levels when compared to the carboplatin study. In both studies, the increase in serum creatinine was completely reversible, because within 1 week, the serum creatinine levels returned to the level measured before treatment. Therefore, this effect will probably not have a negative influence on the protection by amifostine against the cisplatin-induced nephrotoxicity.

The pharmacokinetics of total platinum was not influenced by amifostine. No increase in the final half-life was observed, but on the contrary, a trend for a decrease was seen when amifostine was administered. This difference in effect of amifostine on the pharmacokinetics of total platinum and ultrafilterable platinum suggests a possible change in protein binding by cisplatin. *In vitro*, however, amifostine and its active metabolite, WR 1065, only have a small impact on the protein binding of cisplatin. Only after 6 h, when most of the cisplatin is already bound to proteins, the ongoing binding is slightly reduced (data not shown). *In vivo*, in most cases no detectable ultrafilterable platinum levels are observed at 6 h after treatment, and therefore, an influence of amifostine on the protein binding of cisplatin is not likely. The fact that the final half-life of total platinum did not increase by amifostine confirms the hypothesis that the influence on the final half-life of ultrafilterable platinum was caused by an influence on the renal elimination, because it was reported earlier that the pharmacokinetics of total platinum

is unaffected by renal impairment (18). The final half-life of total platinum is largely dependent on the turnover rate of the proteins to which the platinum compound binds irreversibly. The small decrease in the final half-life could not yet be explained.

Surprisingly, the final half-life of unchanged cisplatin did not increase by the amifostine treatment, as was observed for ultrafilterable platinum, but showed even a slight decrease. This may partly be caused by the fact that the influence of amifostine on the renal elimination is not an immediate effect, and therefore, the influence will be less pronounced when a shorter observation period is used, which is the case for the final half-life of unchanged cisplatin.

One reason for the difference in the influence of amifostine on the pharmacokinetics of cisplatin in comparison to carboplatin might be the difference in the pharmacokinetic behavior between cisplatin and carboplatin itself (19). Although both drugs are excreted predominantly by the kidneys, they have different elimination pathways: carboplatin is excreted mainly by glomerular filtration, whereas in the case of cisplatin, active tubular secretion and reabsorption play a role as well (19).

In the case of cisplatin, the clinical relevance of the observed pharmacokinetic interactions is probably small, because no significant influence on the AUC values of total platinum, ultrafilterable platinum, and unchanged cisplatin was seen, nor did the observed pharmacokinetic interactions lead to any changes in the level of the main Pt-DNA adduct, Pt-GG, in the leukocytes of the patients. However, the influence of amifostine on the platinum concentrations and the Pt-DNA adduct levels in tissues, especially tumor tissue, is still unknown.

In conclusion, amifostine has only a minor influence on the pharmacokinetics of cisplatin in plasma, resulting in an increase of the final half-life of ultrafilterable platinum, comparable to that found for ultrafilterable carboplatin. This may be due to a direct influence of amifostine on the renal function, as indicated by an increase in serum creatinine levels. The influence on the pharmacokinetics of unchanged cisplatin was not comparable to that of unchanged carboplatin, which might be due to some chemical interaction between cisplatin and amifostine. Most probably, this has no influence on the efficacy of cisplatin in the tumor, because no indication of any reduced efficacy of cisplatin was found in this study or in other clinical studies (9, 10).

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