Pergamon

European Journal of Cancer Vol. 33, No. 9, pp. 1425–1429, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00138-X

Original Paper

Pharmacokinetics of Amifostine and its Metabolites in Patients

A.E.C. Korst, C.M. Eeltink, J.B. Vermorken and W.J.F. van der Vijgh

Department of Medical Oncology, University Hospital of Vrije Universiteit, PO Box 7057, 1007 MB Amsterdam, The Netherlands

The pharmacokinetics of the cytoprotective agent amifostine (Ethyol^R; WR 2721) and its main metabolites (WR 1065 and the disulphides) were studied in patients participating in two phase I trials concerning carboplatin or cisplatin in combination with amifostine. Patients were treated with a single dose or three doses of amifostine (740 or 910 mg/m^2). The single or first dose was given as a 15 min i.v. infusion just before administration of the chemotherapeutic agent. The additional two infusions were administered 2 and 4 h thereafter. Amifostine was rapidly cleared from the plasma, due to, at least in part, the fast conversion into WR 1065. A biphasic decrease with a final half-life of 0.8 h was observed. The active metabolite WR 1065 was cleared from the plasma with a final half-life of 7.3 ± 3.6 h. The short initial half-life of WR 1065 can be explained by its fast uptake in tissues and the formation of disulphides. The disulphides were cleared with a final half-life of 8.4–13.4 h and were detectable for at least 24 h after treatment. They may serve as an exchangeable pool of WR 1065. The amifostine peak values at the end of each 15 min infusion did not accumulate in the multiple dosing schedule. For WR 1065 a trend towards an increase in the peak levels was observed [$C_{1,max}$: 47.5 ± 11.9 μM, $C_{2,max}$: 79.0 ± 13.2 μM, $C_{3,max}$: 84.8 ± 15.1 μM, (n = 6)], whereas a trend towards a small decrease was observed for the peak levels of the disulphides [$C_{1,max}$: 184.2 ± 12.6 μM, $C_{2,max}$: 175.0 ± 23.7 μM, $C_{3,max}$: 166.0 ± 17.2 μM, (n = 6)]. This latter finding might suggest a saturation of the disulphide formation or a change in the uptake or elimination of WR 1065, which would result in higher WR 1065 levels in plasma and tissues, after multiple doses of amifostine. © **1997 Elsevier Science Ltd.**

Key words: pharmacokinetics, amifostine, ethyol, WR 2721, WR 1065, disulphides

Eur J Cancer, Vol. 33, No. 9, pp. 1425–1429, 1997

INTRODUCTION

AMIFOSTINE [S-2-(3-aminopropylamino)ethylphosphorothioic acid, Ethyol, WR 2721], originally developed as a radioprotector, is successfully being used as a modulating agent. In preclinical studies, amifostine protected against chemotherapy-induced toxicities without reducing its antitumour activity [1–3]. This selectively is thought to be based on the preferential formation and uptake of the active metabolite WR 1065 in normal tissues, as the result of higher alkaline phosphatase activity and a higher pH compared to hypoxic tumour tissues (Figure 1) [4–7]. WR 1065 can be further oxidised to disulphides, i.e. the symmetrical disulphide WR 33278 or mixed disulphides with endogenous thiols or thiol-containing proteins. These disulphides may serve as an exchangeable pool of WR 1065 [8].

For a better understanding of the mechanism of action and the metabolism of amifostine, pharmacokinetic studies of amifostine itself as well as its main metabolites WR 1065 and the disulphides may be of great importance. Until now pharmacokinetic studies in humans have been mainly limited to analysis of the parent drug [6, 9–11]. Preclinical data on the pharmacokinetics of the active metabolite WR 1065 have been obtained in mice [7, 8] and rhesus monkeys [12, 13]. Only a few data on the human pharmacokinetics of WR 1065 and the symmetrical disulphide WR 33278 are available [9, 11, 14].

Correspondence to W.J.F. van der Vijgh.
Received 15 Oct. 1996; revised 6 Feb. 1997; accepted 5 Mar. 1997.

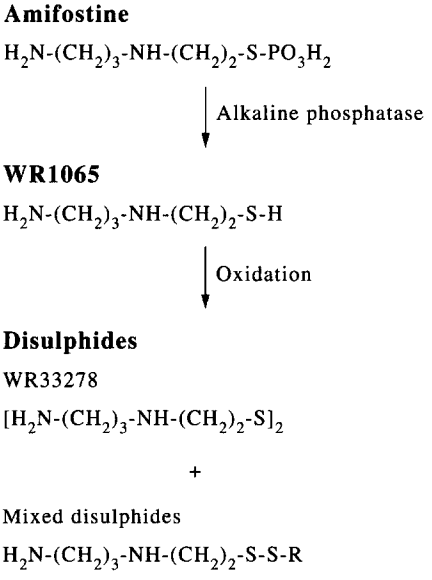

Figure 1. Structural formula of amifostine and its conversion into the main metabolites WR 1065 and the disulphides (with WR 1065 itself or with endogenous thiols (RSH)).

Therefore, the aim of our study was to determine the pharmacokinetics of amifostine, WR 1065 and the disulphides in plasma of patients participating in two phase I trials concerning carboplatin or cisplatin in combination with amifostine.

PATIENTS AND METHODS

Patients

Twelve patients, 4 males and 8 females, aged 39–64 years, participated in two phase I studies after informed consent had been obtained. Patients were treated with carboplatin ($n = 7$) or cisplatin ($n = 5$) in combination with amifostine. Blood samples were collected to study the pharmacokinetics of amifostine and its metabolites. Six patients were sampled after receiving a single dose of 740 or 910 mg/m^2 amifostine as a 15 min i.v. infusion followed by a 1 h i.v. infusion of cisplatin ($n = 5$) or a 15 min i.v. infusion of carboplatin ($n = 1$). Six patients were sampled after receiving three doses of amifostine (3×740 or 910 mg/m^2 amifostine as a 15 min i.v. infusion at $t = 0$, $t = 2$ h 15 min and $t = 4$ h 15 min, in combination with one i.v. infusion of carboplatin over 15 min at $t=$ 15 min). All patients had a normal function (creatine clearance >70 ml/min).

Drug administration

Amifostine was given at a dose of 910 ($n = 5$) or 740 ($n = 7$) mg/m^2. The infusion solution was prepared by reconstituting amifostine (500 mg lyophilised amifostine with 500 mg mannitol per vial; USB Pharma, Nijmegen, The Netherlands) with normal saline up to a total volume of 55 ml. The drug was given as a 15 min i.v. infusion with a syringe infusion pump. Carboplatin was administered as a 15 min infusion (in 150 ml 5% dextrose), whereas cisplatin was given over one hour (in 300 ml 3% NaCl). Dexamethasone, ondansetron and torecan or lorazepam were administered to reduce nausea and vomiting. Desamethasone and ondansetron were administered 30 min before the start of treatment and 4 and 8 h later. Torecan or lorazepam were given 4.5 or 6 h before treatment, respectively. All patients received at least 1 litre of normal saline before treatment.

Sampling

Blood samples were always taken from the non-infused arm by venipuncture using prechilled glass tubes containing ethylenediaminetetraacetic acid (EDTA). Patients treated with three doses of amifostine were sampled just before and immediately after each infusion i.e. just before and at 0, 2, 2.25, 4 and 4.25 h after the end of the first amifostine administration. Patients treated with single dose of amifostine were more extensively sampled i.e. just before and at 0, 0.5, 1, 2, 3, 4, 5, 6, 10, 22 and 24 h after the end of the amifostine infusion. The samples were pretreated and analysed immediately.

Analytical methods

Three different pretreatment procedures were performed to measure amifostine, WR 1065 and the disulphides [15, 16]. For the analysis of WR 1065, plasma was immediately added to cold perchloric acid to precipitate the proteins. After neutralising the acidic supernatant to pH 2, the WR 1065 concentration was determined by HPLC with electrochemical detection. Separation of the WR 1065 peak from endogenous components could be obtained on a C18 column (Customsil 5 ODS-4, 100×4.6 mm) with a mobile phase consisting of a mixture of monochloroacetic acid and methanol with hexanesulphonic acid as the ion-pairing agent. WR 1065 was quantified in the effluent with a static mercury drop electrode at +0.15 V versus Ag/AgCl (PAR 303, EG&G, Princeton, U.S.A.) or a digital amperometric detector (Decade, Antec, Leiden, The Netherlands) equipped with a gold working electrode at +1.0 V versus Ag/AgCl.

Amifostine could be analysed by incubating the acidic deproteinised supernatant for 5 h at 37°C in order to convert amifostine quantitatively into WR 1065. After neutralising the incubation mixture to pH 2, the total amount of WR 1065 was quantified by the above described HPLC procedure. The total amount of WR 1065 corresponds to native WR 1065 plus the amount of amifostine. After subtracting the concentration of native WR 1065 measured according to the first procedure, the concentration of amifostine was obtained.

For the analysis of the (mixed) disulphides, dithiothreitol was added to the plasma sample. The disulphides were quantitatively reduced to free WR 1065 after 15 min of incubation at ambient temperature. Subsequently, the proteins were precipitated by the addition of perchloric acid and the total concentration of WR 1065 was determined as described above. The total concentration of WR 1065 represents native WR 1065 plus the disulphides. The concentration of the disulphides could be calculated by subtracting the concentration of native WR 1065, as determined with the first procedure, from the total concentration of WR 1065.

The lower limit of quantitation for all three compounds was 0.15 μM. The within-day and between-day precisions were ≤4.4% and ≤8.2% for WR 1065, ≤4.9% and ≤13.1% for amifostine, ≤8.5% and ≤5.5% for the disulphides, respectively. The within-day and between-day accuracies were 97.2–109.8% and 97.6–101.5% for WR 1065, 88.3–110.7% and 99.4–101.5% for amifostine and 99.2–110.2% and 103.3–104.9% for the disulphides, respectively.

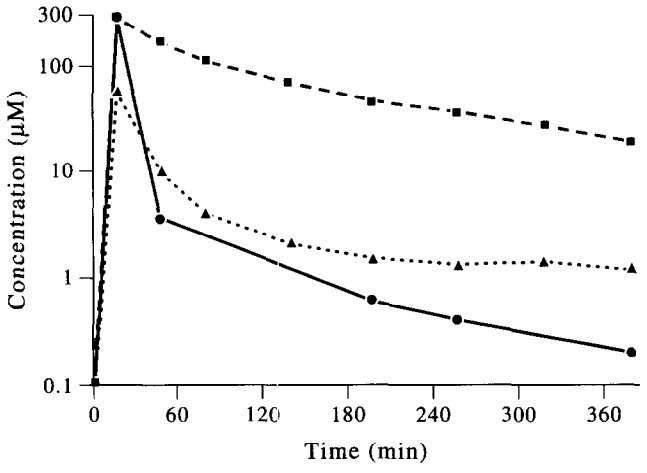

Figure 2. Plasma concentration–time curves of amifostine (•), WR 1065 (▲) and the disulphides (■) in a representative patient treated with 910 mg/m² amifostine.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with the pharmacokinetic data analysis program Topfit 2.0 (Gustav Fischer, Stuttgart, Germany). Pharmacokinetic analysis was performed for amifostine over a time period of 3 h and for WR 1065 and the disulphides over 6 h. The pharmacokinetic parameters of the disulphides were also calculated over 24 h when data were available.

Statistics

The Mann–Whitney test was used for statistical evaluation of the results. P values were given when significant differences ($P < 0.05$) were observed.

RESULTS

Figure 2 shows the plasma concentration–time curves of amifostine, WR 1065 and the disulphides of a representative patient treated with a single dose of amifostine. In general, amifostine and WR 1065 could not be detected for longer than 3 and 6 h after the start of treatment, respectively. The disulphides were still detectable 24 h after the start of infusion (mean concentration: 3.1 μM ($n = 2$)). Unfortunately, in most patients, data were only available until 6 h after treatment. For all three components, peak values were observed at the end of the infusion, indicating that amifostine was rapidly converted into WR 1065 and the disulphides. The mean peak values (±S.D.) were 234.7 ± 45.2 μM ($n = 10$) for amifostine, 45.2 ± 13.5 μM ($n = 12$) for WR 1065 and 178.0 ± 34.4 μM ($n = 11$) for the disulphides when all values were normalised to a dose of 740 mg/m² amifostine. No notable differences were observed when comparing the normalised peak values after treatment with 740 mg/m² amifostine with those after treatment with 910 mg/m² amifostine [amifostine: 236.1 ± 42.3 ($n = 8$) versus 229.3 ± 77.4 ($n = 2$); WB 1065: 41.6 ± 15.0 ($n = 8$) versus 52.6 ± 6.2 ($n = 4$); disulphides: 170.8 ± 27.4 ($n = 7$) versus 190.7 ± 45.9 ($n = 4$)].

For all three components, a two-compartmental analysis resulted in the best curve fit. The main pharmacokinetic parameters calculated for amifostine and its metabolites after a single dose of amifostine are shown in Table 1. These results were comparable with those obtained from non-compartmental data analysis. The AUC values, which were normalised to 740 mg/m² amifostine, were comparable between the two dose levels [WR 1065: 50.4 (740 mg/m², $n = 1$) versus 44.6 ± 7.3 μM/h (910 mg/m², $n = 5$); disulphides: 363 ± 48 (740 mg/m², $n = 3$) versus 403 ± 107 μM/h (910 mg/m², $n = 5$)]. The AUC value obtained for the disulphides was much higher than those for amifostine and WR 1065 ($P < 0.001$), due to a higher plasma concentration for a longer time.

The initial half-lives of the three compounds were very short with the lowest value for amifostine. $T_{1/2\beta}$ was highest for WR 1065 (range 2.7–11.7 h) ($P < 0.02$). However, when considering the concentration–time curves over 24 h, which was only possible for the disulphides, then the final half-lives obtained with a three compartmental model ranged from 8.4 to 13.4 h, which were comparable with the final half-lives of WR 1065.

Table 2 summarises the peak plasma concentrations of amifostine and its metabolites in patients treated with three doses of 740 mg/m² amifostine. Comparable peak plasma levels of amifostine were observed after multiple dosing. The peak values of WR 1065 showed a trend towards an increase [47.5–84.8 μM ($P < 0.001$ for $C_{2,max}$ when compared to $C_{1,max}$)], whereas the peak values of the disulphides showed a trend towards a small decrease (184.2–160.0 μM) after each dose. Table 2 also shows the plasma levels just before the second and the third infusion ($C_{2,min}$ and $C_{3,min}$), which were very low for amifostine and WR 1065 (0.2 μM and 0.7–2.4 μM, respectively). The trough plasma levels of the disulphides were much higher at these time points (55.5–59.9 μM, $P < 0.001$).

DISCUSSION

Pharmacokinetic data of amifostine in patients have been previously reported by Shaw and associates [6, 9–11]. However, in these studies, mainly the pharmacokinetics of the parent drug were investigated, whereas data on the metabolites were very limited. We investigated in patients

Table 1. Pharacokinetics parameters calculated for amifostine, WR 1065 and the disulphides after a single dose of 740 or 910 mg/m² amifostine (mean ± S.D.)

Compound	Time interval pharmacokinetic model	n	$AUC^{(0-\infty)}$ (μMh)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	MRT (h)	Total body clearance (l/min/1.73 m²)	Distribution volume (l/kg)
Amifostine	0–3 h, 2-comp	3	61.0 ± 15.8	0.04 ± 0.01	0.8 ± 0.0		0.14 ± 0.08	1.72 ± 0.51	0.21 ± 0.11
WR 1065	0–6 h, 2-comp	6	45.5 ± 6.9	0.18 ± 0.01	7.3 ± 3.6		6.8 ± 4.6		
Disulphides	0–6 h, 2-comp	8	388 ± 87	0.17 ± 0.12	2.5 ± 0.6		3.2 ± 0.7		
	0–24 h, 3-comp	2	594 ± 10	0.03 ± 0.03	2.2 ± 0.3	10.9 ± 3.6	8.7 ± 5.1		

AUC values were normalised to 740 mg/m² amifostine; comp, compartment.

Table 2. Plasma concentrations (mean ± S.D., n = 6, μM) of amifostine, WR 1065 and the disulphides just before ($C_{i,min}$) and immediately after ($C_{i,max}$) each infusion in patients treated with 3 × 740 mg/m² amifostine

Compound	$C_{1,min}$	$C_{1,max}$	$C_{2,min}$	$C_{2,max}$	$C_{3,min}$	$C_{3,max}$
Amifostine	0	240.2	0.2	221.9	0.2	247.1
		±30.3	±0.3	±36.8	±0.4	±53.3
WR 1065	0	47.5	0.7	79.0	2.4	84.8
		±11.9	±0.8	±13.2	±2.1	±15.1
Disulphides	0	184.2	59.9	175.0	55.5	166.0
		±12.6	±30.1	±23.7	±19.3	±17.2

the pharmacokinetics of amifostine, the metabolite WR 1065 and the (mixed) disulphides. These investigations coincided with clinical phase I studies in which both a single dose and multiple doses of amifostine (administrations with 2 h intervals) were given.

A rapid biphasic plasma clearance of amifostine had been reported in patients ($t_{1/2\alpha}$0.88 min, $t_{1/2\beta}$8.8 min) [6, 9]. These two phases were observed within 45 min. Our sampling frequency was clearly too low to distinguish these two phases. Therefore, the initial half-life we observed (0.04 h, 2.4 min) is most probably a combination of the two phases described by Shaw and associates [9]. In addition, we determined a terminal elimination phase with a half-life of 0.8 h, (48 min), which was not observed by Shaw because he investigated the pharmacokinetics over the first 45 min only. However, the plasma levels during this final elimination phase were very low and may not be of clinical relevance. Because of these low concentrations, repeated treatment of amifostine with 2 h intervals did not lead to increasing peak values at the end of each infusion ($C_{1,max}$: 240.2 μM, $C_{2,max}$: 221.9 μM, $C_{3,max}$: 247.1 μM).

Other data from Shaw and associates suggest the presence of a saturable metabolism of amifostine [10], because disproportional higher C_{max} and area under the curve (AUC) values were observed after treatment with 910 mg/m² in comparison with those after 740 mg/m². Also the clearance of amifostine was twice as low after 910 mg/m² than after treatment with 740 mg/m². In our study, we did not observe such a difference in the C_{max} values when comparing the two dose levels, although our data only concerned 2 patients treated with 910 mg/m² versus 8 patients treated with 740 mg/m². However, our C_{max} ($n = 10$) and AUC values ($n = 3$), which were normalised to the 740 mg/m² dose level, were comparable to the values of Shaw at a dose level of 910 mg/m². An explanation for this difference is unknown.

Most pharmacokinetics data about the active metabolite WR 1065 have been obtained in mice. In these animals, WR 1065 was rapidly formed and distributed to the tissues [7, 8]. In patients, only a few data on the pharmacokinetics of WR 1065 have been reported. Maximal concentrations of the metabolite were observed shortly after a bolus injection of amifostine [9] or at the end of a 15 min infusion [11] which also indicates that amifostine is rapidly converted into WR 1065. This corresponds to the fast decrease of the amifostine concentration in plasma. The mean maximal concentration of WR 1065 of 45.2 μM in our study is in agreement with that reported by Shaw and associates (50 μM) after 740 mg/m² amifostine [11]. WR 1065 was rapidly cleared from the plasma compartment with an initial half-life of 0.18 h, followed by a slower second phase with a half-life of 7.3 h. The short initial half-life can be explained by the fast uptake in tissues [7, 8] and the fast conversion of WR 1065 into disulphides. During the second phase, only low plasma concentrations were present. Therefore, hardly any accumulation was expected after multiple dosing with 2 h intervals. However, a trend towards a slight increase in the peak concentrations of WR 1065 was observed ($C_{1,max}$: 47.5 μM, $C_{2,max}$: 79.0 μM, $C_{3,max}$: 84.8 μM). This observation might suggest a saturation of the uptake of WR 1065 in tissues or its conversion into disulphides. Such a saturation of the pharmacokinetics of WR 1065 had also been suggested by Mangold and associates [13]. They observed a decrease in the volume of distribution of WR 1065 in rhesus monkeys when the infusion time of amifostine was decreased, possibly due to a saturation of binding in plasma and tissue. The observation in our study of accumulating WR 1065 concentrations with successive administrations might have some clinical relevance because Ryan and associates showed that amifostine-induced hypotension in rats was mediated by WR 1065 [17]. Indeed, we observed more frequent and more severe hypotension during the third amifostine administration in comparison with the first (J.B. Vermorken, University Hospital, Vrije Universiteit, Amsterdam, The Netherlands). It is attractive, though speculative, to relate the pharmacokinetic data to our clinical observation.

Up to now, no data are available about the pharmacokinetics of the (mixed) disulphides in patients after treatment with amifostine. Although the protective properties of the disulphides (symmetrical and mixed disulphides) are doubtful, they could be of clinical relevance when they would serve as an exchangeable pool of the active metabolite WR 1065 [8]. A parallel decrease in the plasma concentration–time curves of WR 1065 and the disulphides would confirm the existence of an exchange between WR 1065 and the disulphides. We did not observe such a parallelism during the first 6 h after the administration of amifostine. However, the final half-life of the disulphides, observed in the time period of 6–24 h after treatment, was comparable to the $t_{1/2\beta}$ of WR 1065. This observation supports the suggestion that the (mixed) disulphides serve as a pool of exchangeable WR 1065.

Peak values of the disulphides at the end of the infusion indicate that the conversion of WR 1065 into the disulphides must have been very fast. Also, Shaw and associates mentioned a fast formation of the symmetrical disulphide WR 33278 with peak plasma levels of 7–11 μm at 1–3 min after a bolus injection of 150 mg/m² amifostine [9]. Our peak values (178.0 ± 34.4 μM; $n = 11$) were much higher than the peak values of WR 33278 reported by Shaw and associates, indicating that a principle part of the total disulphides, as we determined, consisted of mixed disulphides with endogenous thiols. In contrast to amifostine and WR

1065, the disulphides were detectable in the plasma compartment for at least 24 h after a single dose of amifostine. In the multiple dosing schedule, rather high plasma concentrations (55.5–59.9 μM) were still present at the beginning of the next infusion. Therefore, an increase in the peak values of the disulphides was expected. However, a trend towards a decrease in the subsequent peak concentrations was observed ($C_{1,max}$: 184.2 μM, $C_{2,max}$: 175.0 μM, $C_{3,max}$: 166.0 μM). This might be due to a saturation of the disulphide formation, which was also suggested by the unexpected increase in the subsequent peak concentrations of WR 1065. However, if this suggestion is correct it would be expected that the increase in the peak concentrations of WR 1065 would have been higher. Therefore, the uptake of WR 1065 in tissues and/or its elimination might also be enhanced in parallel with the relatively lower disulphide formation.

The observed rapid decrease of amifostine and WR 1065 concentrations in plasma and the low reaction rate between amifostine or its metabolites with platinum compounds [19] suggest that no chemical inactivation of the platinum drug will take place in the circulation when amifostine is administered close to the platinum drug. However, this does not exclude the possibility for any other than chemical interaction between amifostine and the platinum drugs, as observed in clinical and preclinical studies [20, 21].

It can be concluded that amifostine is rapidly converted into the active metabolite WR 1065, which in turn is rapidly oxidised to the disulphides. A rapid clearance from the plasma was observed for amifostine and WR 1065, whereas the disulphides were cleared much slower. Multiple dosing (2 h intervals) resulted in an increase of the peak levels of WR 1065 but not of the disulphides. This might suggest a saturation of the disulphide formation or changes in the uptake of tissues or the elimination of WR 1065.

1. Treskes M, Boven E, Holwerda U, Pinedo HM, Van der Vijgh WJF. Time dependence of the selective modulation of cisplatin-induced nephrotoxicity by WR-2721 in the mouse. *Cancer Res* 1992, **52**, 2257–2260.
2. Treskes M, Boven E, Van de Loosdrecht AA, *et al.* Effects of the modulating agent WR2721 on myelotoxicity and antitumor activity in carboplatin-treated mice. *Eur J Cancer* 1994, **30A**, 183–187.
3. Van der Vijgh WJF, Peters GJ. Protection of normal tissues form the cytotoxic effects of chemotherapy and radiation by amifostine (ethyol): preclinical aspects. *Semin Oncol* 1994, **21**, 2–7.
4. Brown DQ, Graham WJ, Mackenzie LJ, Pittock JW, Shaw LM. Can WR-2721 be improved upon? *Pharmacol Ther* 1988, **39**, 157–168.
5. Calabro-Jones PM, Aguilera JA, Ward JF, Smoluk GD, Fahey RC. Uptake of WR-2721 derivatives by cells in culture: identification of the transported form of the drug. *Cancer Res* 1988, **48**, 3634–3640.
6. Shaw LM, Glover D, Turrisi A, *et al.* Pharmacokinetics of WR-2721. *Pharmacol Ther* 1988, **39**, 195–201.
7. Utley JF, Seaver N, Newton GL, Fahey RC. Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. *Int J Radiat Oncol Biol Phys* 1994, **10**, 1525–1528.
8. Shaw LM, Bonner HS, Brown DQ. Metabolic pathways of WR-2721 (Ethyol, amifostine) in the BALB/c mouse. *Drug Metab Dispos* 1994, **22**, 895–902.
9. Shaw LM, Turrisi AT, Glover DJ, *et al.* Human pharmacokinetics of WR-2721. *Int J Radiat Oncol Biol Phys* 1986, **12**, 1501–1504.
10. Shaw L, Bonner H, Nakashima H, Lieberman R. Pharmacokinetics of amifostine in cancer patients: evidence for saturable metabolism. *Proc Am Soc Clin Oncol* 1994, **13**, 144.
11. Shaw L, Bonner H, Schuchter L, Schiller JH, Nakashima H, Lieberman R. Population pharmacokinetics of amifostine in cancer patients. *Proc Am Soc Clin Oncol* 1996, **15**, 478.
12. Mangold DJ, Miller MA, Huelle BK, *et al.* Disposition of the radioprotector ethiofos in the rhesus monkey: influence of route administration. *Drug Metab Dispos* 1989, **17**, 304–310.
13. Mangold DJ, Huelle BK, Miller MA, *et al.* Pharmacokinetics and disposition of WR-1065 in the rhesus monkey. *Drug Metab Dispos* 1990, **18**, 281–287.
14. Korst AEC, Gall HE, Vermorken JB, Van der Vijgh WJF. Pharmacokinetics of amifostine and its metabolites in the plasma and ascites of a cancer patient. *Cancer Chemother Pharmacol* 1996, **39**, 162–166.
15. Korst AEC, Snel J, Kedde MA, Van der Vijgh WJF. A sensitive HPLC-ECD assay for amifostine and its metabolites in plasma. *Proc Int Symp Column Chromatogr Rel Tech* 1995, **19**, 225.
16. Korst AEC, Vermorken JB, Van der Vijgh WJF. An HPLC-ECD assay for the combined measurement of amifostine, WR 1065 and the disulfides in plasma. *J Chromatogr B*, **in press**.
17. Ryan SV, Carrithers SL, Parkinson SJ, *et al.* Hypotensive mechanisms of amifostine. *J Clin Pharmacol* 1996, **36**, 365–373.
18. Turrisi AT, Glover DJ, Hurwitz S, *et al.* Final report of the phase I trial of single-dose WR-2721 [*S*-2-(3-aminopropylamino)ethylphosphorothioic acid]. *Cancer Treat Rep* 1986, **70**, 1389–1393.
19. Treskes M, Holwerda U, Klien I, Pinedo HM, Van der Vijgh WJF. The chemical reactivity of the modulating agent WR 2721 (ethiofos) and its metabolites with the antitumor agents cisplatin and carboplatin. *Biochem Pharmacol* 1991, **42**, 2125–2130.
20. Korst AEC, Boven E, Van der Sterre MLT, Fichtinger-Schepman AMJ, Van der Vijgh WJF. Influence of single and multiple doses of amifostine on the efficacy and the pharmacokinetics of carboplatin in mice. *Br J Cancer* **in press**.
21. Korst AEC, Van der Sterre MLT, Eeltink CM, Fichtinger-Schepman AMJ, Vermorken JB, Van der Vijgh WJF. Pharmacokinetics of carboplatin with and without amifostine in patients with solid tumors, *Clin Cancer Res.* **in press**.