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SHORT COMMUNICATION

Annelies E. C. Korst · Helen E. Gall · Jan B. Vermorken
Wim J. F. van der Vijgh

Pharmacokinetics of amifostine and its metabolites in the plasma and ascites of a cancer patient

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Abstract The pharmacokinetics of amifostine, a protector against chemotherapy and radiation-induced toxicities, was investigated in the plasma and ascites of a cancer patient. A high-performance liquid chromatography (HPLC) procedure with electrochemical detection was used to measure amifostine, its active metabolite, WR 1065, and the disulfides (symmetrical plus mixed disulfides). Both amifostine and WR 1065 were rapidly cleared from the plasma (95% and 50% of the peak concentration within 1 h, respectively). The disulfides, which were rapidly formed from WR 1065, were cleared much more slowly (final half-life 13.6 h). Multiple dosing resulted in a tendency toward increasing peak levels of WR 1065 and decreasing peak levels of the disulfides. Only 1% of the delivered dose appeared in the ascites. Therefore, it is not plausible that the presence of ascites or other third spaces would have an impact on the pharmacokinetics of amifostine.

Key words Pharmacokinetics · Amifostine · WR 1065 · Disulfides · Ascites

Introduction

Amifostine [S-2-(3-aminopropylamino)ethylphosphorothioic acid, Ethyol, WR 2721] has been approved as a protector against chemotherapy-induced toxicity to the bone marrow and kidneys and is under investigation as a protector against radiation-induced toxicities. The active metabolite, which is responsible for this protection, is the aminothiol WR 1065 [1]. It is formed by dephosphorylation in the presence of alkaline phosphatase. WR 1065 can be further oxidized to disulfides (symmetrical disulfide WR 33278 or mixed disulfides with endogenous thiols and thiol-containing proteins) [7] (Fig. 1). In preclinical studies in mice, amifostine protected against carboplatin-induced myelosuppression and potentiated the antitumor activity of carboplatin [10]. This selective protection is based on a preferential formation and uptake of the active metabolite WR 1065 in normal tissues [8, 11, 12]. On the basis of these results, clinical studies were started to investigate the clinical benefits of combining carboplatin with amifostine. In one of these studies we had the opportunity to investigate the pharmacokinetics of amifostine and its main metabolites in the plasma and ascites of a cancer patient to determine whether an accumulation of amifostine or its metabolites would occur in third-space fluids such as ascites.

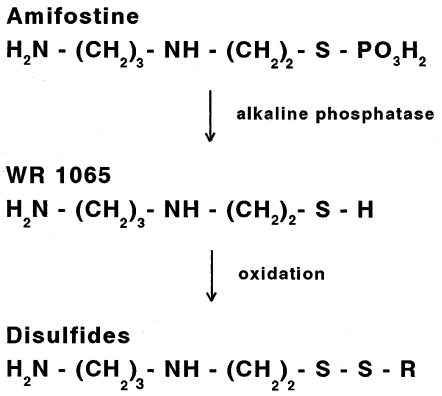

Fig. 1 Structural formula of amifostine and its conversion into the active metabolite WR 1065 and the disulfides [with WR 1065 itself or with endogenous thiols (*RSH*)]

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A. E. C. Korst (✉) · H. E. Gall · J. B. Vermorken · W. J. F. van der Vijgh
University Hospital Vrije Universiteit, Clinical Research Laboratory of Oncology, BR232, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands
Fax! + 31-20-4443844

Materials and methods

Patient's characteristics

A 64-year-old man (body surface 1.8 m^2, weight 72 kg) was referred to our institution for the treatment of an adenocarcinoma of the stomach. Ascites fluid was present in the peritoneal cavity: during the first course, about 5.2 l; during the second course, approximately 2.2 l. After giving informed consent the patient was entered in a phase I study of carboplatin in combination with the modulating agent amifostine.

Drugs

Carboplatin was provided in vials containing 150 mg in the presence of 150 mg mannitol (Bristol Myers-Squibb, Woerden, The Netherlands). Amifostine (Ethyol) was provided in vials containing 500 mg in the presence of 500 mg mannitol (USB Pharma, Nijmegen, The Netherlands).

Treatment

In the phase I study, carboplatin and amifostine were given at 4-week intervals. Before each treatment cycle the patient was adequately hydrated with normal saline. The patient received 900 mg carboplatin (500 mg/m^2) reconstituted with 150 ml 5% dextrose and three doses of 1350 mg amifostine (740 mg/m^2) reconstituted with 50 ml normal saline. Both drugs were given as 15-min i.v. infusions using a syringe infusion pump. Amifostine was given three times; just before and at 2 and 4 h after the carboplatin infusion. Dexamethasone, ondansetron, and torecan were given before and after the amifostine infusion to reduce nausea and vomiting. The patient received three courses, but samples were obtained only during the first and the second course.

Sample collection

During the first course, ascites samples were obtained at the following time points: before treatment and at 0, 0.25, 0.5, 2, 2.25, 2.5, 4. 4.25, 4.5, 4.75, 5, 5.5, 6, 7, 10, 22, and 24 h after the end of the first amifostine infusion. During the second course (4 weeks later), blood samples were obtained at the same time points. Both ascites and blood samples were collected in cold tubes containing ethylenediaminetetraacetic acid (EDTA) and were immediately pretreated.

Analytical method

A high-performance liquid chromatography (HPLC) method with electrochemical detection was developed for the combined analysis of amifostine, WR 1065, and the disulfides (including the symmetrical disulfide WR 33278 plus mixed disulfides with endogenous thiols such as glutathione and cysteine plus mixed disulfides with plasma proteins) [3]. For the analysis of WR 1065, cold perchloric acid was immediately added to the sample to precipitate the proteins. The supernatant was injected onto the analytical column and the WR 1065 concentration was quantified by an electrochemical detector provided with a Au electrode maintained at +1.00 V versus Ag/AgCl. The conversion of amifostine to WR 1065, which would result in a putative increase in the WR 1065 concentration during the collection and processing period, was minimized by maintenance of the sample on ice and its immediate analysis after the pretreatment procedure. Amifostine and the disulfides could be analyzed by the same HPLC procedure by the use of sample-preparation procedures during which amifostine and the disulfides were quantitatively converted into WR 1065. Amifostine was converted into WR 1065 by incubation of the acidic supernatant (after the precipitation of proteins) for 5 h at 37 °C. Reduction of the disulfides to free WR 1065 was accomplished by incubation of plasma with dithiothreitol for 15 min at 20 °C. The within- and between-day accuracy of the assay for WR 1065 varied from 3% to 16% (from 10 to 0.25 μ*M*, respectively), whereas the precision varied from 0.6% to 8%. The lower limit of quantification of WR 1065 was 0.15 μ*M*.

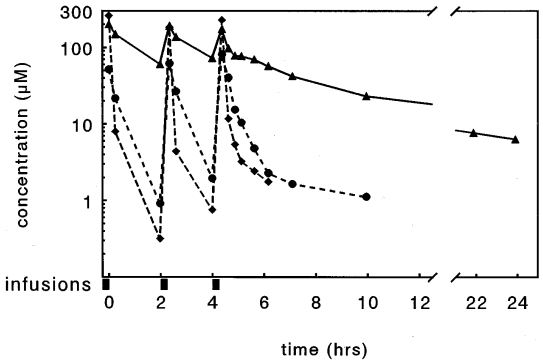

Fig. 2 Plasma concentration-time curves generated for amifostine (*diamonds*), WR 1065 (*circles*), and the total disulfides (*triangles*) in a patient treated with 3×740 mg/m^2 amifostine

Results

Pharmacokinetics

The plasma concentration-time curves generated for amifostine, WR 1065, and the disulfides are shown in Fig. 2. For all three compounds, peak values were observed by the end of each 15-min infusion, indicating that amifostine was rapidly converted into WR 1065 and the disulfides. Plasma peak values recorded for amifostine and the disulfides were

Table 1 Pharmacokinetic data obtained on amifostine, WR 1065, and the disulfides in the plasma of a patient after repeated 15-min infusions of amifostine[a]

Compound	Postinfusion concentrations at zero (peak) and 15 min after each infusion (μ*M*)						Approximate half-lives (h) after 3rd infusion		
	I		II		III				
	Peak	15 min	Peak	15 min	Peak	15 min	$t_{1/2\alpha}$	$t_{1/2\beta}$	$t_{1/2\gamma}$
Amifostine	260.1	8.0	184.6	4.4	229.0	11.8	0.05	0.83	–
WR 1065	51.7	21.7	61.8	26.7	81.6	40.6	0.19	2.73	–
Disulfides	201.3	149.9	192.8	137.6	174.9	98.2	0.05	1.94	13.6

[a] 3×740 mg/m^2; infusions were carried out at –0.25, 2, and 4 h

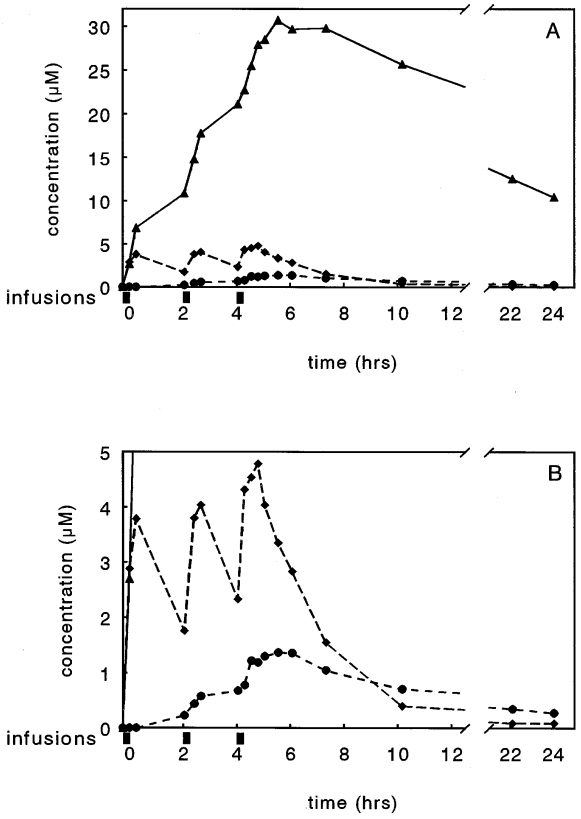

Fig. 3 **A** Ascites concentration-time curves generated for amifostine (*diamonds*), WR 1065 (*circles*), and the total disulfides (*triangles*) in a patient treated with 3×740 mg/m² amifostine. **B** Enlarged section of **A**

of the same magnitude, whereas the peak plasma levels noted for WR 1065 were lower than these values (Table 1). At 15 min after each infusion the plasma concentrations of amifostine and WR 1065 were 2–5% and 42–50% of the peak postinfusion concentrations, respectively, indicating rapid plasma clearance, especially of amifostine itself. After repeated administration, peak plasma values obtained for amifostine and the disulfides showed a trend toward a small decrease, whereas the peak plasma values noted for WR 1065 showed a slight increase after repeated administration. Amifostine and WR 1065 almost disappeared from the plasma within 2 h, but the total disulfides, which probably were to a large extent protein-bound, remained detectable for at least 24 h. The approximate half-lives calculated from the limited amount of concentrations measured after the third administration are given in Table 1.

The ascites concentration-time curves generated for amifostine and its metabolites are shown in Fig. 3. Repeated administration of amifostine resulted in an accumulation of amifostine, WR 1065, and the disulfides. After the third infusion, peak amifostine values were observed at 30 min after drug administration, whereas maximal concentrations of WR 1065 and the disulfides were seen at 75 min after the end of the infusion. The levels recorded for all three compounds in ascites were much lower than those measured in plasma. After the third infusion the peak ascites concentrations of amifostine, WR 1065, and the disulfides were 2.1%, 1.7%, and 17.6% of the peak plasma concentrations, respectively. In contrast to the levels observed in plasma, the levels of amifostine measured in ascites remained higher than those of WR 1065. As in plasma, the total disulfide levels observed in ascites were relatively high in comparison with the levels of amifostine and WR 1065. The final half-life of the disulfides in ascites was 11.1 h. The total amount of the compounds present in ascites at 75 min after the third administration was equivalent to 35.5 μ*M* amifostine, which represents 1.0% of the total delivered dose. The drug concentrations measured at the end of each infusion and the peak values are summarized in Table 2.

Table 2 Pharmacokinetic data obtained on amifostine, WR 1065, and the disulfides in ascites after repeated dosing of amifostine[a]

Compound	Values at the end of the infusion (μ*M*)			Peak values (μ*M*) after 3rd infusion (time after end infusion)
	I	II	III	
Amifostine	2.9	3.8	4.3	4.8 (30 min)
WR 1065	0.0	0.4	0.8	1.4 (75 min)
Disulfides	2.7	14.8	22.8	30.7 (75 min)

[a] 3×740 mg/m²; infusions were carried out at –0.25, 2, and 4 h

Toxicity

The patient received three courses of 500 mg/m² carboplatin given each time in combination with 3×740 mg/m² amifostine at 4-week intervals. Side effects experienced by the patient were more or less similar for each course and consisted of mild flushing, dry mouth, anorexia, some fatigue, and myalgia. During the amifostine infusions, hypotension was observed, which necessitated interruption of the infusion for several minutes once during the first course, once during the second course, and more than once during the third course. This did not lead to reduction of the drug dose. No nausea or vomiting was experienced. The general condition of the patient deteriorated during therapy; this was not drug-related but resulted from disease progression.

Discussion

The pharmacokinetics of *amifostine* in plasma has previously been described in patients receiving a single dose of amifostine [5, 6]. A biphasic decrease with very short half-lives was described for amifostine ($t_{1/2\alpha}$ 0.88 min, $t_{1/2\beta}$ 8.8 min). More than 90% of the dose was cleared from the plasma compartment within 6 min [5]. In our patient, 95–98% of the peak plasma concentration was removed from the plasma within 15 min of the last infusion. In this study the sampling frequency was too low for an accurate determination of the half-lives as described by Shaw et al. [5]. From our data an approximation of the initial half-life

of 3 min could be determined, which is probably a combination of the two phases comprising the biphasic decrease described in the literature. The extent to which amifostine was rapidly cleared from the plasma compartment was comparable with the data reported by Shaw et al. [5]. Because our pharmacokinetics study included sample collection during 24 h, whereas Shaw et al. [5] investigated the pharmacokinetics over the first 45 min only, we determined a terminal elimination phase with a half-life of approximately 0.83 h. However, because the concentrations of amifostine measured at 1 h after the administration were very low, the clinical relevance of this elimination phase is not clear. Because of these very low concentrations, no accumulation of the amifostine concentration was observed in plasma after treatment of the patient with multiple doses at 2-h intervals.

Very little is known about the pharmacokinetics of *WR 1065*, the active metabolite, when amifostine has been given to patients. A rapid formation and uptake of WR 1065 in tissues has been described in mice [7, 11]. In our patient, peak WR 1065 values were observed in plasma as early as at the end of the infusion, indicating that the formation of WR 1065 is also very rapid in patients. Therefore, the fast decrease in the amifostine concentration is probably due to its rapid conversion into WR 1065. WR 1065 was also rapidly cleared from the plasma compartment, probably due to its fast uptake in normal tissues [7, 11] and to its conversion to disulfides. Within 15 min, 50–58% of the peak concentration had disappeared. After a fast initial phase a slower second phase with a final half-life of approximately 2.7 h was observed. As with amifostine, very low concentrations were present just before the next infusion (2–3% of the peak concentration). Therefore, no accumulation) was expected using this multiple dosing schedule. However, a trend toward a slight increase in the peak concentrations of WR 1065 from 51.7 to 61.8 to 81.6 μ*M* was observed. This has also been observed in other patients treated on the same schedule [4]. This might suggest a possible saturation of the formation of the disulfides or the distribution of WR 1065.

The conversion of WR 1065 to *disulfides* was investigated by quantification of all the disulfides in plasma, i.e., the total amount of the symmetrical disulfide WR 33278 and the mixed disulfides (with endogenous thiols such as glutathione, cysteine, and plasma proteins). It is known that the symmetrical disulfide has some protective properties, albeit to a lesser extent than WR 1065 [9]. The protective properties of the mixed disulfides would probably be even weaker than those of the symmetrical disulfide. Therefore, the clinical meaning of the total concentration of disulfides is unknown. However, they may serve as an exchangeable pool of WR 1065 [7]. In plasma the formation of disulfides was very fast, as peak values were observed in plasma as early as at the end of the infusion. This would at least in part explain the fast decrease in the WR 1065 concentration observed in plasma, which is probably also due to its fast uptake in tissues [7, 11]. As compared with amifostine and WR 1065, the total disulfides were cleared rather slowly from the plasma compartment, displaying a final half-life of 13.6 h. The observation that amifostine was cleared more rapidly than WR 1065, which in turn decreased more rapidly than the disulfides, is comparable with the pharmacokinetics observed in the rhesus monkey [2]. High concentrations of disulfides remained present before the start of the next infusion in the multiple dosing schedule (60.8 μ*M* before the start of the second infusion and 73.3 μ*M* before the start of the third infusion). However, no accumulation of the peak values was observed. On the contrary, a tendency toward a decrease was noted (from 201.3 μ*M* to 192.8 μ*M* to 174.9 μ*M*). This has also been observed in other patients treated on the same multiple dosing schedule [4]. A saturation of the formation of disulfides might be an explanation for the lack of accumulation. However, the observed increase in WR 1065 peak concentrations was not high enough to explain it. This might be due to a higher uptake of WR 1065 in tissues.

Peak concentrations were observed later in *ascites* than in plasma. Peak levels of amifostine appeared at 30 min after the end of the i.v. infusion, whereas those of WR 1065 and the disulfides occurred at 75 min after the infusion. This was later than expected from earlier pharmacokinetics studies in tissues of mice, which reported peak WR 1065 values at 10–30 min after the administration of amifostine [7, 11]. These peak values corresponded to 2.1%, 1.7%, and 17.6% of the peak plasma concentrations of amifostine, WR 1065, and the disulfides, respectively. Amifostine, WR 1065, and the disulfides accumulated slightly in ascites after multiple dosing. At the time of the peak concentration after the third administration, only approximately 1% of the delivered dose was present in the ascites. It is therefore not plausible that the presence of ascites or other third-space fluid accumulations would have an impact on the plasma or tissue pharmacokinetics of amifostine. It is clear from our data that a small amount of amifostine itself is transported from the plasma to the ascites compartment. Whether the metabolites in ascites are originating from this amifostine or from direct diffusion/transport from the plasma cannot be eludicated from our data.

In conclusion, both amifostine and WR 1065 were briefly present in the circulation, whereas the total disulfides were observed at higher concentrations for a longer time. Amifostine and its metabolites were present in the ascites, but the presence of ascites does not have an impact on the plasma pharmacokinetics of amifostine, as only 1% of the dose appeared in the ascites.

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