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Amifostine (Ethyol®): Pharmacokinetic and Pharmacodynamic Effects *In Vivo*

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Amifostine (Ethyol®) administered to cancer patients is rapidly cleared from plasma by a biphasic decay with an alpha half-life ($T_{1/2\alpha}$) of 0.88 min and a $T_{1/2\beta}$ of 8.8 min. The result is that more than 90% of the drug has disappeared from the plasma compartment 6 min after intravenous (i.v.) administration. Only approximately 1% of the dose appears in the ascites. Animal studies indicate that amifostine is primarily excreted in urine—approximately 6% of the dose is excreted in the urine as amifostine and its metabolites WR-1065 and disulphides—which means that a large percentage of the dose is taken up by the tissues. Maximal tissue concentrations of WR-1065 and the disulphides were obtained between 10 and 30 min after an intraperitoneal injection of amifostine in mice, with the lowest concentrations in tumour tissues. Because WR-1065 gives protection to normal tissues rather than rescue, the pharmacokinetic data indicate that amifostine must be given shortly before administration of the cytostatic drug or radiation from which protection is required. For these reasons, amifostine is given to patients as a 15-min i.v. infusion before cisplatin and carboplatin to protect against their dose-limiting toxicities. In some regimens carboplatin is combined with three doses of amifostine because of the high concentration of the active carboplatin species during the first 4 h after administration. When carboplatin was administered as a 15-min i.v. infusion of 400 mg/m^2 and amifostine as a 15-min i.v. infusion of 740 mg/m^2 just before and 2 and 4 h after carboplatin, the area under the plasma concentration–time curve for ultrafilterable platinum increased from 253 ± 45 μM·h (n = 6) for carboplatin alone to 305 ± 63 μM·h (n = 11) for carboplatin + three doses of amifostine. Experiments in nude mice bearing OVCAR-3 xenografts showed that amifostine, given once before cisplatin or three times in combination with carboplatin, did not affect the antitumour effect of these drugs. When amifostine was only given just before carboplatin, it even stimulated the antitumour effect of carboplatin significantly.

Key words: amifostine, Ethyol®, WR-2721, WR-1065, cisplatin, carboplatin, pharmacokinetics, pharmacodynamics, patient, animal

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INTRODUCTION

It is thought that cisplatin and carboplatin have steep dose–response curves for their antitumour activity in humans, which means that an increase of the maximal tolerable dose by using modulating agents may allow the safe administration of a higher dose of both drugs and thus improve therapeutic efficacy [1].

At present, it is generally accepted that the antitumour activity of platinum compounds occurs through their binding to DNA (Pt–DNA adduct formation) [2, 3]. It is thought that nephrotoxicity of cisplatin is caused by interaction of the platinum compound with cellular proteins (enzymes), whereas it is assumed that carboplatin-induced bone marrow suppression is caused by the inhibition of the division of the rapidly proliferating myeloid progenitors through Pt–DNA adduct formation. Modulators such as thiosulphate diethyldithiocarbamate, mercaptoethanesulphonate glutathione (GSH) and others, all containing nucleophilic sulphur, mostly as a thiol group, are supposed to protect against platinum-induced toxicities by inactivating the platinum compound before reaching the target or by removing platinum from the Pt–DNA or platinum protein complexes. These modulators do not seem to have any selectivity regarding their uptake to either healthy or tumour tissue. In addition, they can have chemical interaction with platinum compounds. Therefore, they do not only protect healthy tissues, but may also reduce the antitumour activity of the platinum compounds [4].

It has been shown that amifostine (Ethyol®, ethiofos, WR-2721, or S-2-[3-aminopropylamino]ethylphosphorothioic acid), which was developed as a radioprotector, protects normal tissue selectively against radiation-induced damage [5] through selective formation and uptake of the active metabolite WR-1065 (containing a free thiol group) [6]. This property makes

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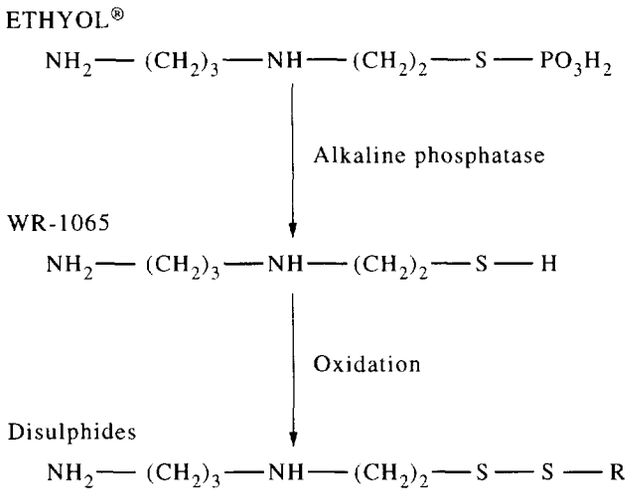

Figure 1. Conversion of amifostine (Ethyol®) into its main metabolites WR-1065 and (mixed) disulphides.

amifostine an interesting compound for investigation of its potential use as a selective protector against toxicities induced by platinum compounds. This paper reviews the pharmacokinetics of amifostine and that of carboplatin alone and in combination with amifostine as well as their pharmacodynamic effects.

PHARMACOKINETICS AND PHARMACODYNAMIC EFFECTS OF AMIFOSTINE (ETHYOL®)

Pharmacokinetics of amifostine in humans and mice

In vivo, amifostine (Figure 1) is converted by alkaline phosphatase into the free thiol WR-1065, which consequently can be oxidised to WR-33278, the symmetric disulphide of WR-1065 and the mixed disulphides of WR-1065 with L-cysteine, GSH and possibly other endogenous thiols [7]. Amifostine and its metabolites are principally quantified by high performance liquid chromatography with electrochemical detection [7, 8].

Pharmacokinetic aspects of amifostine and its metabolites in cancer patients are summarised in a review by Shaw and associates [6]. During an intravenous (i.v.) infusion of 740 mg/m^2 over 15 min, a steady-state plasma level of amifostine of approximately 100 μM was obtained within a few minutes. Half-lives of amifostine measured after 10-s bolus injections of 150 mg/m^2 were 0.9 and 8.8 min for distribution and elimination, respectively. In the 13 patients investigated, more than 90% of amifostine was cleared from plasma within 6 min after amifostine administration. The mean steady-state volume of distribution of amifostine was 6.4 l, which means that amifostine itself is principally distributed throughout the intravascular system only. When 750 mg/m^2 of amifostine was given to a patient in five repeated (150 mg/m^2) i.v. infusions over 15 min, steady-state plasma levels of 1mM of amifostine were achieved. WR-1065 reached a plasma level of 100 μM, which declined to 35 μM after 1 h [9]. Korst and coworkers [10] treated 6 patients with three doses of amifostine: i.v. infusions of 740 mg/m^2 over 15 min at 15 min before and 2 and 4 h after carboplatin. Mean peak plasma concentrations of amifostine (240 μM), WR-1065 (48 μM) and the (mixed) disulphides (184 μM) were obtained immediately after the first infusion. Comparable peak concentrations were obtained after the second and third administrations. The disulphides were still detectable (3.4 μM) 24 h after a single dose of amifostine. After a single bolus dose as well as after a 15-min infusion of amifostine, the urinary excretion of amifostine, including its metabolites WR-1065 and the disulphides, summated to 6% of the administered dose [6]. Only approximately 1% of the dose appeared in ascites [10].

Tissue distribution studies of amifostine and its metabolites were performed in several animal species [7, 11]. Maximal tissue concentrations of WR-1065 and the disulphides were obtained between 10 and 30 min after an intraperitoneal (i.p.) injection of amifostine in mice [7]. As an example, the blood and tissue concentrations of WR-1065 and the symmetric disulphide WR-33278 are shown in Table 1. Tissue concentrations were the lowest in tumour tissue, which is indicative of the selective uptake by normal tissues. Studies in the rhesus monkey indicated that amifostine was primarily excreted in the urine [12].

Pharmacokinetic rationale for amifostine administration schemes

Because WR-1065 gives protection to normal tissues rather than rescue them [13, 14], this active metabolite must already be present in tissues when protection against a cytostatic drug is required. Therefore, amifostine must be given shortly before the cytostatic drug or radiation because maximal tissue levels of WR-1065 are rapidly reached after amifostine administration. For these reasons, amifostine is given to patients as a 15-min i.v. infusion 15–30 min before cisplatin or carboplatin to protect against their dose-limiting toxicities. In some regimens, carboplatin is combined with three doses of amifostine (not only just before but also 2 and 4 h after carboplatin) because the reactive ultrafilterable platinum species of carboplatin are present at much higher concentrations and for a longer period than after cisplatin [15].

Pharmacokinetics of carboplatin with and without amifostine in humans

In a phase I study at our institute, carboplatin is currently given in combination with amifostine to patients with solid tumours and normal renal function ($Cl_{Cr} \geq 80$ ml/min/1.73 m^2). The pharmacokinetics of carboplatin were investigated in 6 patients treated with 400 mg/m^2 of carboplatin and 11 patients treated with 400 or 500 mg/m^2 of carboplatin in combination with three doses of 740–910 mg/m^2 of amifostine [16] given just before and 2 and 4 h after the start of the

Table 1. *Blood and tissue concentrations* (μmol/kg *tissue, mean* ± *S.D.*) 10 min *after intraperitoneal injection of amifostine (Ethyol®)* 365 mg/kg *in mice*

Tissue	WR-1065*	WR-33278*
Blood	318 ± 28	42 ± 13
Liver	839 ± 165	151 ± 53
Kidney	2016 ± 666	87 ± 18
Heart	321 ± 109	ND
Small intestine	335 ± 61	18 ± 0.1
Tumour (I-347T)	112 ± 6	ND
Tumour (EMT-6)	62 ± 12	—

* WR-1065 is the free thiol of amifostine; WR-33278 is the symmetric disulphide of WR-1065.
ND, none detected.
Adapted with permission from Shaw and coworkers *Drug Metab Dispos* 1994, 22, 895–902.

Table 2. Pharmacokinetic parameters of ultrafilterable platinum after carboplatin without and with amifostine (mean ± S.D.) in humans

Treatment (*n*)	AUC* (μmol/l·h)	$T_{1/2\gamma}$ (h)	Ae (% D)
Carboplatin (6)	253 ± 45†	3.4 ± 0.5‡	73.1 ± 5.8
Carboplatin + amifostine (11)	305 ± 63†	5.4 ± 1.1‡	74.6 ± 9.3

* Normalised to 400 mg/m^2 of carboplatin.
† $P = 0.05$.
‡ $P = 0.02$.
Ae, amount excreted in the urine; AUC, area under the concentration–time curve of ultrafilterable platinum; D, dose.
Reproduced by permission of W.B. Saunders Company, from Korst AEC, *et al.*, *Proc Am Soc Clin Oncol* 1995, Vol. 14, 172 (Abstract).

carboplatin infusion. Both drugs were given as a 15-min i.v. infusion with a syringe infusion pump. Preliminary data on the principal pharmacokinetic parameters of ultrafilterable platinum are given in Table 2. In contrast with an earlier report [17], the area under the plasma concentration–time curve (AUC) significantly increased when carboplatin was given in combination with amifostine, which corresponded with the significant increase of the final half-life, but not with the constant value of the amounts excreted in the urine. The relative change in serum creatinine (SCr) at 24 h after treatment (SCr, 24 h – SCr, 0 h/SCr, 0 h) did not change after treatment with carboplatin alone (−1 ± 11%), but significantly increased ($P = 0.01$) after treatment with carboplatin combined with amifostine (35 ± 19%). It is supposed that the transient hypotension occurring during amifostine infusions may lead to transient decreases in creatinine and platinum clearances, which may result in an increased SCr at 24 h, a longer final half-life of free platinum species and therefore an increased AUC [16]. Because most carboplatin is excreted in the urine during the first few hours after administration, transient changes in the renal platinum excretion at later times will go unnoticed. The change in AUC might lead to an increased antitumour activity in the clinic as observed earlier in murine experiments [18].

Pharmacodynamic effects of amifostine on cisplatin and carboplatin in mice

Beneficial effects of amifostine on cisplatin-induced nephrotoxicity and carboplatin-induced myelotoxicity have been shown in mice [14, 18], rats [6] and humans [19].

During amifostine administration, the antitumour activity of cisplatin and carboplatin was shown not to be compromised. Treskes and coworkers [14] showed that the antitumour effect of cisplatin was not reduced when nude mice inoculated with OVCAR-3 xenografts were treated with 5 mg/kg of cisplatin i.v. + 200 mg/kg of amifostine i.p. in comparison with 5 mg/kg of cisplatin i.v. alone (Figure 2a). In these experiments, amifostine was given 5 min before cisplatin (twice, with an interval of one week). In the presence of amifostine, it was possible to raise the dose of cisplatin to 8 mg/kg, which, in comparison with a dose of 5 mg/kg, resulted in a significant decrease in T/C% [= (mean volume of treated tumours/mean volume of control tumours) × 100%] at day 29 (2.7% versus 1.4%).

When nude mice inoculated with OVCAR-3 xenografts were treated with carboplatin 60 mg/kg i.v. in combination with 200 mg/kg of amifostine i.p. 5 min prior to the platinum drug, T/C% decreased from 9.4% (for mice treated with carboplatin 60 mg/kg only) to 2.2%, which indicated that a significant potentiation of the antitumour activity of carboplatin occurred under the influence of amifostine [18]. Under these circumstances of protection, it was possible to raise the dose of carboplatin to 90 mg/kg, which resulted in a further significant decrease of T/C% to 1.4% (Figure 2b).

In another experiment, mice were treated with 60 mg/kg of carboplatin in combination with 200 mg/kg of amifostine i.p. given three times at 5 min before and 2 and 4 h after carboplatin, and then compared with mice given carboplatin alone or in combination with one dose of 200 mg/kg of amifostine at 5 min before carboplatin [20]. T/C% values were 11.5% for carboplatin alone, 7.3% for carboplatin with one dose of amifostine and 10.1% for carboplatin with three doses of amifostine. These values indicate that carboplatin combined with amifostine once was significantly more active than carboplatin alone ($P < 0.0001$) or carboplatin with three times amifostine ($P < 0.01$). The antitumour activity of carboplatin with three times amifostine was similar to that of carboplatin alone. A probable explanation for the reduced potentiation by three doses of amifostine of the antitumour activity of carboplatin compared with a single dose of amifostine might be the severe hypothermia induced by the highest dose of amifostine in the strain of nude mice used for this experiment [21]. As a consequence, tumour blood flow and consequently drug uptake by the tumour might have been affected.

Pharmacokinetics and Pt–DNA adduct formation of carboplatin with and without amifostine in nude mice

The pharmacokinetics of carboplatin and Pt–DNA adduct formation have been investigated in mice bearing subcutaneous OVCAR-3 xenografts [20]. Mice were treated on days 0 and 7 with 60 mg/kg of carboplatin i.v. alone or in combination with 200 mg/kg of amifostine i.p. given at 5 min before and 2 and 4 h after carboplatin. Of each group, three mice were sacrificed at 1, 3, 5, 8, 12 and 24 h after carboplatin. Platinum concentrations and Pt–DNA adducts were determined in various organs. Table 3 shows the AUC of platinum in plasma ultrafiltrate and tissues during the first 24 h after carboplatin administration. Amifostine did increase the AUC of ultrafilterable platinum in plasma (3.1 fold) and the AUC of total platinum in liver (1.72 fold), kidney (1.47 fold) and tumour (1.36 fold). The AUC values of the Pt–DNA adducts measured after treatment with carboplatin alone and those after treatment with carboplatin combined with three doses of amifostine were comparable. This means that the higher exposure of the tissues due to the increase of the AUCs in the tissues (see above) was compensated for by selective reduction of Pt–DNA adduct formation. These results correspond with the selective uptake of WR-1065 by normal tissue compared with tumour tissue.

Table 3. Areas under the concentration–time curves of platinum in plasma ultrafiltrate (PUF) (μM·h) *and tissues* (nmol/g·h) *in OVCAR-3-bearing nude mice during the first* 24 h *after carboplatin administration with and without three times amifostine (at* −5 min, 2 *and* 4 h)

Treatment	PUF	Kidney	Liver	Tumour
Carboplatin	5.2	287	177	129
Carboplatin + 3× amifostine	16.1	421	304	175

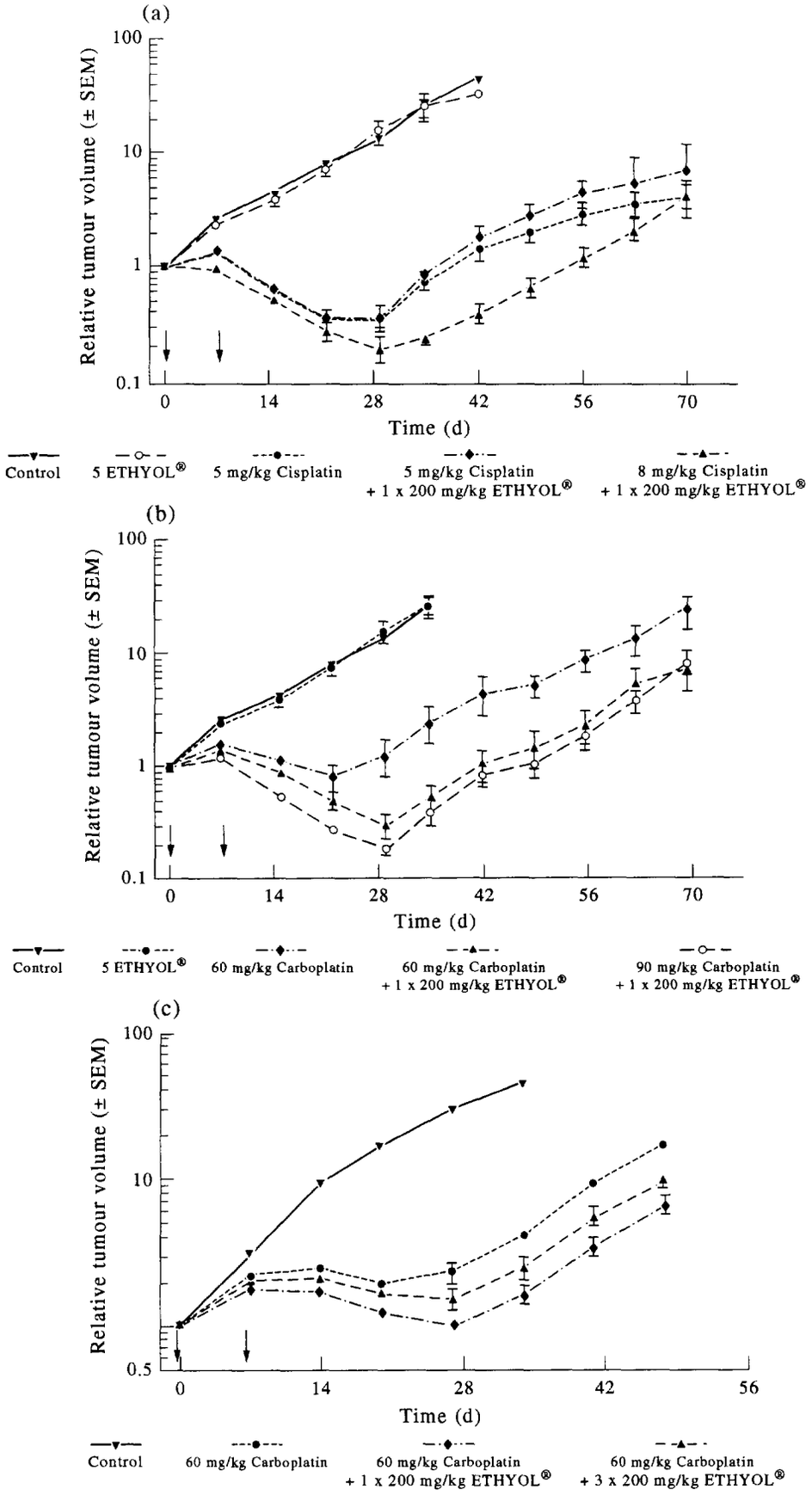

Figure 2. Relative tumour volumes (± S.E.M.) of subcutaneous OVCAR-3 xenografts grown in female nude mice. Treatments are indicated in the figures. Each treatment was given twice with an interval of one week. Each group consisted of five–six mice with two tumours per mouse. Reproduced by permission of the American Association of Cancer Research, Inc., from Treskes M, *et al.*, *Cancer Res* 1992, Vol. 52, pp. 2257–2260.

CONCLUSIONS

The results of these studies indicate that amifostine must be given shortly before cytostatic drug or radiation; that when combined with carboplatin, amifostine increases the AUCs of the platinum concentration in plasma ultrafiltrate (human, mice) and in tissues (mice); and that amifostine protection is selective for normal compared with tumour tissue and does not interfere with the antitumour effects of cisplatin or carboplatin. Finally, amifostine may even potentiate the antitumour effects of carboplatin.

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