Influence of Amifostine on the Pharmacokinetics of Cisplatin in Cancer Patients

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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-diaminedichloroplatinum(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, neutropathy, 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/m² but was reduced to 740 mg/m² 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/m² cisplatin alone (four patients) or cisplatin plus 910 mg/m² amifostine (four patients). The remaining five patients were first treated with cisplatin alone 1 week before they were treated with cisplatin plus 740 mg/m² 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 (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 za 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/anti-foam 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 benzenthionium-hydroxide (Sigma, Zwijndrecht, 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 thiouracil (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
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 alone (●) and 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
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...increased from 17.5 to 19.9...cumulative urinary excretion over the first 24 h: total platinum and unchanged cisplatin decreased from 2002 to...treatment with cisplatin alone (Clcr, 32 ml/min), the same trends were observed: after treatment with cisplatin alone (○) and cisplatin in combination with amifostine (●). Horizontal lines, means.

**Fig. 2** Final half-lives of total platinum (TPt), ultrafilterable platinum (UFPt), and intact cisplatin (CisPt) in patients with a normal renal function (Clcr >60 ml/min) after treatment with cisplatin alone and without amifostine, the AUC of ultrafilterable platinum in-

...increase in the final half-life of ultrafilterable platinum was seen...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).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatment</th>
<th>n</th>
<th>Pt-GG (fmol/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>CIS</td>
<td>6</td>
<td>1.27 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>CISWR</td>
<td>4</td>
<td>1.31 ± 0.36</td>
</tr>
<tr>
<td>22</td>
<td>CIS</td>
<td>6</td>
<td>0.89 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>CISWR</td>
<td>2</td>
<td>0.92</td>
</tr>
</tbody>
</table>

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 alone (CIS) or in combination with amifostine (CISWR).

In this study, a 1.3–1.4-fold increase in the final half-life of ultrafilterable platinum was observed after treatment with cisplatin alone (CIS) or in combination with amifostine (CISWR), which was comparable to the observed increase in the final half-life when carboplatin was combined 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 alone (CIS) or in combination with amifostine (CISWR). 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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Influence of amifostine on the pharmacokinetics of cisplatin in cancer patients.

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