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Original Paper

Pharmacokinetics of Cisplatin With and Without Amifostine in Tumour-Bearing Nude Mice

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Amifostine (Ethyol, WR-2721) is in use in the clinic as a protector against platinum-induced toxicities. We have previously reported that amifostine induced a potentiation of the antitumour activity of carboplatin in human ovarian cancer xenografts. An influence of amifostine on the pharmacokinetics of carboplatin, resulting in higher platinum concentrations in plasma and tissues of the tumour-bearing nude mice, was thought to be the cause of enhancement of the antitumour activity. Therefore, the pharmacokinetics of cisplatin were investigated in tumour-bearing nude mice treated with cisplatin alone or in combination with amifostine. A significant increase in the area under the curve (AUC) of the total platinum concentration in mice treated with amifostine was only observed in the kidney (from 355 to 398 nmol h/g), whereas in the other tissues and plasma no significant changes were measured. The selective protection of normal tissues by amifostine was confirmed by a decrease in the AUC of the cisplatin-DNA adduct levels in normal tissues. The decrease was only significant in the liver (282–240 fmol h/μg DNA), whereas in tumour tissue a slight increase in the AUC of the cisplatin–DNA adducts could be detected (91.3–110.1 fmol h/μg DNA). The minor influence of amifostine on the pharmacokinetics of cisplatin may be the reason why amifostine did not potentiate the antitumour activity of cisplatin. The influence of amifostine on cisplatin–DNA adduct levels in normal tissues versus tumour tissues is further evidence for the usefulness of this toxicity modulator in cancer patients.

Key words: amifostine, cisplatin, ethyol, pharmacokinetics, WR-2721
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INTRODUCTION

AMIFOSTINE (*S*-2-(3-AMINOPROPYLAMINO)ETHYLPHOSPOROTHIOIC acid, WR 2721, Ethyol[R]), initially developed as a protector against radiation toxicity, has been shown to protect non-tumour tissues from chemotherapy-induced damage by its thiol metabolite WR 1065 [1]. The selectivity of this protection may be explained by a preferential formation and uptake of WR 1065 in normal tissues [2–4]. As a result, the therapeutic index of the anticancer agents will be increased, which might be of advantage for the patient.

In preclinical studies, a protection against the dose-limiting nephrotoxicity of the cytostatic agent cisplatin has been observed after administration of amifostine [5–8]. Protection was obtained when amifostine was administered 5 or 30 min prior to cisplatin, whereas no protection was observed when amifostine was administered 30 min after cisplatin treatment [8]. This observation is in agreement with both *in vitro* data, indicating that the modulating activity of amifostine was due to protection rather than to rescue [9], and *in vivo* data, showing a rapid uptake and clearance of WR 1065 in normal tissues [4, 10]. In the clinic, the administration of amifostine just before cisplatin has led to a reduction of cisplatin-induced nephrotoxicity, myelosuppression and neurotoxicity [11–16].

The use of modulating agents can only be successful when such drugs do not interfere with the antitumour activity of the cytotoxic agent. In tumour-bearing nude mice and in patients, amifostine did not reduce the antitumour activity of cisplatin [8, 14], but enhanced the antitumour activity of

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carboplatin [17]. This seemed to be related to an influence of amifostine on the pharmacokinetics of carboplatin, resulting in higher platinum concentrations in plasma and tissues [18]. Therefore, the aim of the present study was to investigate whether amifostine also influences the pharmacokinetics of cisplatin in tumour-bearing nude mice.

MATERIALS AND METHODS

Chemicals

Platinol^{R} (0.5 mg/ml cisplatin in 0.9% NaCl) was obtained from Bristol-Myers Squibb (Woerden, The Netherlands). Amifostine vials (500 mg lyophilised WR-2721 and 500 mg mannitol) were obtained from U.S. Bioscience (West Conshohocken, Pennsylvania, U.S.A.). Each vial was reconstituted in 9.3 ml sterile water. Prior to use the compound was further diluted in 0.9% NaCl to a final concentration of 20 mg/ml.

Mice

Female athymic nude mice (Harlan/Cpb, Zeist, The Netherlands), housed and fed as previously described [19], were inoculated subcutaneously (s.c.) in both flanks with fragments (2–3 mm in diameter) of the human ovarian cancer xenograft OVCAR-3, a poorly differentiated serous adenocarcinoma. The pharmacokinetic study was started at the time tumours had reached a mean size of approximately 150 mm^3 (range 20–300 mm^3).

Pharmacokinetics

Mice were treated with 5 mg/kg cisplatin alone or in combination with 200 mg/kg amifostine. Amifostine was administered i.p. 5 min before cisplatin, which was injected in the tail vein. Of each group 3 mice per time point were bled from the axillary vein under ether anaesthesia at 0.5, 1, 3, 5, 12 or 24 h after cisplatin administration. Thereafter, liver, kidney and tumours were removed. Bone marrow was collected by flushing both femurs with RPMI medium and pooled from the 3 mice per time point of each group. Plasma was ultrafiltrated by MPS-1 systems provided with YMT filters (Amicon, Capelle a/d IJssel, The Netherlands). Plasma, plasma ultrafiltrate and parts of liver, kidney and tumours were stored at −20°C until analysis of total platinum. Bone marrow and parts of liver, kidney and tumours were stored at −80°C until the Pt–DNA adducts were analysed.

For platinum analysis the samples were pretreated as follows. Plasma samples were diluted 2.5 times by adding 100 µl plasma sample to 25 µl 0.38 M NaCl/0.5 M HCl and 125 µl 0.1% triton X (Merck, Darmstadt, Germany)/0.1% antifoam B (Sigma, St Louis, Missouri, U.S.A.) (1:1) prior to measurement of the platinum concentration. Plasma ultrafiltrate samples were diluted 1:1 with 0.15 M NaCl/0.4 M HCl. Tissue samples of 100 mg were digested in 0.5 ml of hyaminehydroxide (Sigma) at 55°C for 24 h and then diluted with 4.25 ml 0.2 M HCl. Platinum concentrations were analysed by flameless atomic absorption spectrophotometry using a Spectra AA-300 Zeeman AAS (Varian, Houten, The Netherlands). Standards of blank plasma and tissue spiked with cisplatin were treated in the same way as the samples.

In order to determine the Pt–DNA adducts, tissue samples were ground to cell suspensions in 2 ml Tris-EDTA and DNA was isolated after inactivation of free and monofunctionally bound cisplatin with thiourea [20, 21]. From the liver samples the cell nuclei were isolated prior to DNA isolation according to Roggeband and associates [22]. Then, after digestion, the isolated DNA samples were chromatographed on a Mono Q anion-exchange column (Pharmacia, Woerden, The Netherlands) and appropriate column fractions were analysed in a competitive ELISA [20]. With this procedure three platinum-containing (di)nucleotides could be identified: Pt–CG and Pt–AG (bifunctional adducts of cisplatin with two adjacent guanines and with adenine adjacent to guanine) and G–Pt–G (bifunctional adduct of cisplatin with two non-adjacent guanines either in the same strand (intrastrand cross-link) or with two guanines in the opposite DNA strands (interstrand cross-link)).

Area under the curve (AUC) values of the concentration–time curves of total platinum and of Pt–DNA adducts were calculated from the mean concentrations at each time point, over 0.5–24 h after cisplatin administration, using the trapezoidal rule.

Statistics

Statistical analysis of the calculated AUC values was performed as follows: at each time point the variance (VAR(C_i)) of the mean concentration (C_i) of the data obtained from three mice was calculated. The AUC values were calculated by the trapezoidal rule:

$$\mathrm{AUC} = \sum_{i=1}^{n} [0.5 * (t_i - t_{i-1}) * (C_i + C_{i-1})]$$

which can be rewritten as:

$$\mathrm{AUC} = \sum_{i=1}^{n} (W_i * C_i)$$

in which factor W_i is calculated as follows:

$$W_1 : 0.5 * (t_2 - t_1)$$
$$W_2 : 0.5 * (t_3 - t_1)$$
$$W_3 : 0.5 * (t_4 - t_2) \ldots etc$$
$$W_8 : 0.5 * (t_8 - t_7)$$

The standard deviation of the calculated AUC value is calculated by:

$$\mathrm{S.D.} = \sqrt{\left(\sum_{i=1}^{n} (W_i^2 * \mathrm{VAR}(C_i)) \right)}$$

For evaluation of the mean AUC values and the standard deviation Student's *t*-test was used.

RESULTS

The platinum concentration–time curves in tissues and plasma after treatment with cisplatin alone or in combination with amifostine are shown in Figure 1. In Figure 2 the AUC values of these curves are shown. In kidney, the AUC value of total platinum was 12% higher after treatment with cisplatin and amifostine than that after treatment with cisplatin alone ($P = 0.05$). In the other tissues, plasma and erythrocytes, no significant differences between the AUC values were obtained, although in most a slight increase in the AUC was observed after treatment with amifostine (6–8%). A rather

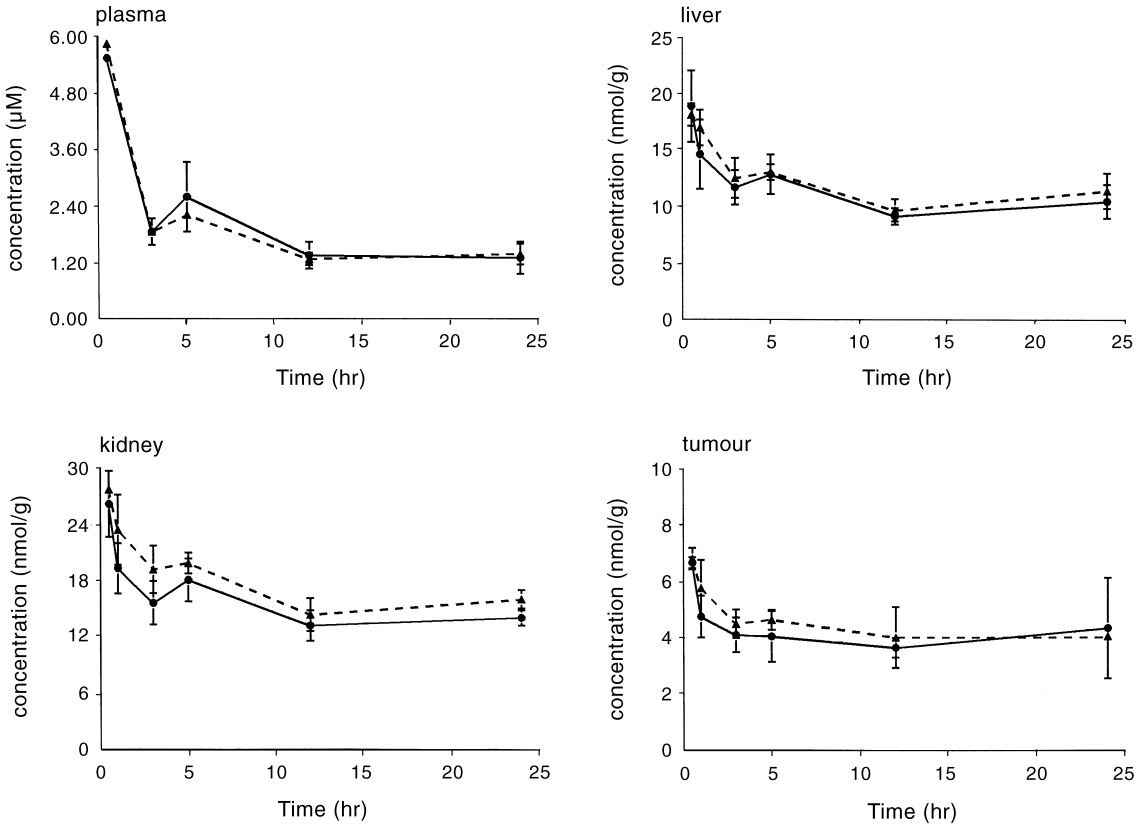

Figure 1. Platinum concentration–time curves (over 0.5–24 h) (± S.D.) in plasma, liver, kidney and OVCAR-3 tumour tissue from mice treated with 5 mg/kg cisplatin i.v. alone (•) or in combination with 200 mg/kg amifostine i.p. (▲). Each point represents the mean and the S.D. of the concentrations obtained from three mice.

large variation in tumour sizes was observed. However, no differences were observed between the total platinum concentrations in small and large tumours. Due to two missing data points at 30 min the S.D. of the AUC value of total platinum in plasma could not be calculated. In plasma ultrafiltrate, the platinum concentration was only detectable up to 1 h after cisplatin administration. No significant differences were observed between the ultrafilterable platinum concentrations at 30 min and 1 h after treatment with cisplatin alone and the levels after treatment with cisplatin and amifostine.

The AUC values of Pt–DNA adduct levels are shown in Figure 3. Each column represents the sum of the three bifunctional adducts determined in the digested DNA samples. In liver, a significant decrease of 15% in the platination of DNA was observed ($P = 0.03$), mainly due to a decrease in the major adduct Pt–GG. A slight, but statistically insignificant decrease in Pt–DNA adduct levels (9–10%) was also observed in kidney and bone marrow. The S.D. of the AUC value of Pt–DNA adduct levels in bone marrow could not be calculated because the bone marrow was pooled at each time point in order to obtain enough material. In tumour tissue,

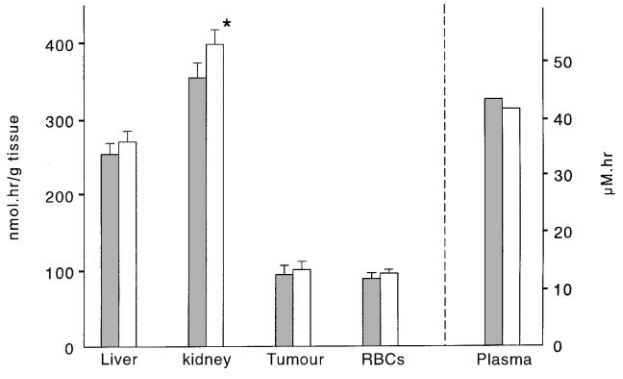

Figure 2. AUC values (over 0.5–24 h) (± S.D.) of total platinum in liver, kidney, OVCAR-3 tumour tissue, red blood cells and plasma from mice treated with 5 mg/kg cisplatin i.v. alone (shaded bars) or in combination with 200 mg/kg amifostine i.p. (open bars). *$P = 0.05$.

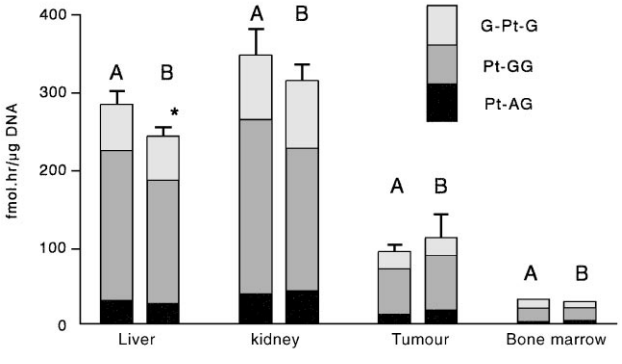

Figure 3. AUC values (over 0.5–24 h) (± S.D.) of Pt–DNA adducts in liver, kidney, OVCAR-3 tumour tissue and bone marrow after treatment with 5 mg/kg cisplatin i.v. alone (treatment A) or in combination with 200 mg/kg amifostine i.p. (treatment B). *$P = 0.03$.

on the contrary, an increase in Pt–DNA adduct levels was seen (27%). Due to the high variation, however, this difference was not statistically significant.

DISCUSSION

Amifostine, one of the most promising modulating agents of side-effects, has been shown to selectively protect against platinum-induced toxicities [8]. No interference with antitumour activity has been observed, which was to be expected from the low reaction rates of platinum compounds with amifostine and its main metabolites [23]. In addition, the uptake of the active metabolite WR 1065 appears to be selective for normal tissues [2–4, 10]. Surprisingly, amifostine potentiated the antitumour activity of carboplatin in tumour-bearing nude mice, whereas no influence on the antitumour activity of cisplatin was observed in the same human tumour model [8, 17]. A pharmacokinetic interaction between amifostine and carboplatin was likely to be the cause of this potentiation, because higher total platinum concentrations were observed in normal and tumour tissues when amifostine preceded the administration of carboplatin. In the present study, amifostine did not have the same influence on the pharmacokinetics of cisplatin as observed earlier for carboplatin.

In our experiments, amifostine had only a minor influence on the pharmacokinetics of cisplatin. As shown in Figure 3, a small, but significant increase (1.1-fold) of the AUC of total platinum was observed in kidney, whereas in plasma, erythrocytes, liver and tumour the AUC value of total platinum was not significantly affected. This slight pharmacokinetic interaction was much less pronounced than the influence of amifostine on the pharmacokinetics of carboplatin reported by our group [18, 25]. In the carboplatin study, a 1.3–1.7-fold increase was observed in the AUC value of total platinum in plasma and tissues after administration of the same dose of amifostine as we used in the present study. Evidently, the insignificant influence of amifostine on the pharmacokinetics of cisplatin was the reason that the antitumour activity did not change, whereas the antitumour activity of carboplatin was enhanced when preceded by amifostine [8, 17]. The different pharmacokinetic interactions between amifostine and cisplatin versus carboplatin might be related to the pharmacokinetic properties of the two platinum compounds [24]. It is our hypothesis that the increase in the AUC values of total platinum after addition of amifostine to carboplatin is at least in part caused by the amifostine-induced hypothermia. A single dose of 200 mg/kg amifostine was shown to induce a decrease in the body temperature of mice from 37°C to a minimum of 34.5°C at 1.5 h after the injection, which only recovered after 7 h [25]. It is known that free carboplatin is present in the circulation for a much longer time (12 h) [25] than ultrafilterable platinum after cisplatin (about 1 h, present study). This means that in contrast to free carboplatin, most of the ultrafilterable platinum after cisplatin will have been eliminated or bound to proteins before the effects of hypothermia will have become manifest.

In order to investigate the possible consequence of the small increase in total platinum, we measured the effect on the DNA being the target molecule of platinum compounds. Pt–DNA adduct levels, Pt–GG, Pt–AG and G–Pt–G, were determined in liver, kidney and tumour tissues and also in bone marrow. In liver a significant decrease in the Pt–DNA adduct levels was observed, whereas in the other tissues, such as the kidney, a slight trend for a decrease in the Pt–DNA adduct level was seen, despite the increase in total platinum. This suggests a protection against DNA damage by amifostine in the normal tissues (kidney and liver), as was also observed in clinical and preclinical studies [5–8, 11–16]. This decrease in DNA platination was mainly apparent from a reduction in the major adduct Pt–GG. This corresponded with the results of *in vitro* studies, in which amifostine mainly reduced the Pt–GG levels as well [9]. However, it is not yet clear which Pt–DNA adduct is responsible for the cytotoxicity. It has been suggested that, if only one of the adducts exclusively causes the cytotoxic effect, Pt–AG would be the most likely candidate [21].

After treatment with cisplatin, the AUC values of total platinum in tissues were comparable to the values after an equitoxic dose of carboplatin [18]. However, the Pt–DNA adducts in liver and kidney were much higher after cisplatin than after carboplatin, whereas in bone marrow the adduct levels were higher after treatment with carboplatin. This is in agreement with the dose-limiting toxicity for cisplatin and carboplatin, being nephrotoxicity and myelosuppression, respectively.

In contrast to the small decrease in the Pt–DNA adduct levels in normal tissues, in tumour tissue a slight increase in Pt–DNA adduct levels was observed after treatment with cisplatin and amifostine, indicating that the protective effect of amifostine was restricted to the normal tissues. The small increase in Pt–DNA adduct levels, corresponding to a small increase in total platinum, was probably not sufficient to give a measurable improvement of the growth inhibition induced by cisplatin alone.

In conclusion, amifostine has only a minor influence on the pharmacokinetics of cisplatin in tumour-bearing nude mice, resulting in a slight increase in total platinum concentrations in plasma and tissues. The Pt–DNA adduct levels confirmed a selective protection of cisplatin-related side-effects in normal tissues without affecting the antitumour activity. The influence of amifostine on the Pt–DNA adduct levels is further evidence for the value of this compound for the modulation of cisplatin-induced toxicities in the cancer patient.

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