Time dependent protection of amifostine from renal and hematopoietic cisplatin induced toxicity

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Abstract

Efficacy of chemotherapy may be maximized and its toxicity can be minimized if drugs would be administered at specified daily times. The present study was aimed to examine if the protection of amifostine against cisplatin toxicity is time dependent. Amifostine is an organic thiosphosphate that protects selectively normal tissues, but not tumors, against the cytotoxicity of DNA binding chemotherapeutic agents such as cisplatin. ICR male mice which were entrained to Light:Dark (L:D) 14:10 were injected (intrapritoneal bolus) for 5 consecutive days with either: cisplatin, cisplatin plus amifostine (administered 30 minutes prior to cisplatin). Injections were given at either 08:00, 13:00, 20:00 or 01:00. Five days later, on day 10, each set of mice was sacrificed (at the same hour corresponds to the injection hour), blood count, blood creatinine and blood urea nitrogen (BUN) were assayed. Cisplatin treated mice exhibited nephrotoxicity, as indicated by increased blood urea nitrogen values and by high blood urea nitrogen to creatinine ratios, as well as myelotoxicity that was indicated by low levels of hemoglobin and platelets. Co-administration of amifostine-cisplatin reversed both, the nephrotoxicity of cisplatin, and its myelosuppressive effects. For BUN, hemoglobin and platelets, maximal protections were observed at 08:00, (p < 0.05, p < 0.01 and p < 0.01 respectively). For BUN/Cr ratio (p < 0.05), maximal protections was observed at 13:00. These findings show that amifostine exhibits time dependent protection against cisplatin toxicity and thus it...
is recommended to use the protector when treatments are given during morning hours. The results also further validate the notion that chronotherapy is advantageous at least in reducing drug toxicity and thus should be integrated in the design of clinical protocols.

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Introduction

Vast amount of data has been accumulated to substantiate the advantage of chemotherapy, namely, time-dependent drug administration (Wood and Hrushesky, 1996; Levi, 1997). This approach carries high relevance to therapies with narrow efficacy-to-toxicity ratios, as those practiced in most anticancer treatment regimens (Hrushesky et al., 1982a). Time-dependent toxicity has been documented for at least 20 chemotherapeutic agents in many rodent and some human experimental systems (Levi et al., 1982a; Peleg et al., 1989) and the time-dependent efficacy of many of these agents, given alone or in combinations, has been demonstrated in several neoplastic diseases (Wood and Hrushesky, 1996).

Platinum complexes are among the most active drugs which are used against a large spectrum of malignancies. However, they do induce hazardous side effects like gastrointestinal, neural and renal toxicities, as well as myelosuppression. Cisplatin is a heavy metal-based compound that acts as a nonclassical alkylating agent. Its anti-tumoral effect is achieved by binding to DNA, creating intrastrand as well as interstrand cross-links and as a result prevents DNA, RNA and proteins synthesis (Guarino et al., 1979; Treskes et al., 1992; Decatris et al., 2004). The major outcome of its toxicity, even upon low single dose administration, is a dose-related, cumulative renal insufficiency. It’s initial plasma half-life ranges between 25 to 49 minutes, and the post-distribution plasma half-life is 58 to 73 hours, during which, more than 90% of the drug is protein bound. Cisplatin is excreted primarily in the urine and its renal damage is in direct proportion to the drug concentration in the urine (Boisdron-Celle et al., 2001). Pharmacokinetics of platinum-complex in blood and urine, and, presumably, its effect on the proliferative activity of bone marrow exhibit 24 hour rhythmicity (To et al., 2000; Capizzi, 1996). It was observed that maximal tubular damage in rats occurred when cisplatin was given during the peak of urine concentration, and the least toxicity was obtained when cisplatin was administered near the midactivity span, which corresponds to the maximum level of core body temperature (Hrushesky et al., 1982b; Levi et al., 1982b; Peleg et al., 1989).

Amifostine is an organic thiophosphate that selectively protects normal tissues, but not tumors, against cytotoxicity of ionizing radiation and DNA-binding chemotherapeutic agents such as classical and nonclassical alkylating agents. Amifostine (WR-2721) is a pro-drug that undergoes dephosphorylation at the tissue level by membrane-bound alkaline phosphatase to its active metabolite - the free thiol WR-1065 (Bruce, 2001).

Several factors favor the uptake and activity of Amifostine in normal tissues rather than in tumoral tissues. Among these are the localization of alkaline phosphatase and it’s specific activity in normal tissues; the neutral pH of normal cells versus the acidic pH in many tumors, and differences between normal and tumor cells in membrane transport rate (Capizzi, 1996). In normal cells, Amifostine protects
against cytotoxic agents by several mechanisms, including direct binding and detoxifying of platinum agents (Treskes and van der Vijgh, 1993), by reduction of platinum-DNA adduct formation (Treskes et al., 1992), and by scavenging oxygen-free radicals stemming from radiation therapy or specific drugs (Myers et al., 1977).

The protection afforded by amifostine against cisplatin toxicity when administered alone or in combination with other chemotherapies, might enable the therapeutic use of higher doses of cisplatin. The best anti proliferative effect against colon cancer in balb/c mice and the lowest toxicity were found when 5FU and cisplatin were delivered together 30 minutes after amifostine (van der Wilt et al., 1992). Amifostine protected against carboplatin and 5FU-induced thrombocytopenia, but did not prevent leukopenia or anemia in C57B1/6 mice (van Laar et al., 1992). Studies have shown that amifostine addition ameliorates the myelosuppression resulting from treatment with radiation, alkylating agents and platinum analogues (Santini, 2001; Swaab et al., 1996). However, the efficacy of time dependent amifostine treatment on the cytotoxicity of cisplatin has never been examined. The aim of the present study was to determine whether the documented protection of amifostine exhibit time dependency, and if it does, to determine the optimal times at which amifostine administration will protect from cisplatin-induced toxicities in chemotherapy. The ultimate goal of this study is to design improved therapeutic modalities for cancer chemotherapy treatments using chronopharmacology methodology.

Materials and methods

The experimental design was approved by the committee for animal experiments. ICR male mice, 2 months old, with an average weight of 50 mg, were housed 5 per cage (36 × 32 × 11 cm) and exposed to a 14 hours light and 10 hours dark regimen (LD 14:10, lights on at 05:00) for 4 weeks prior to the experiment. Food and water were freely available. These conditions prevailed also during the experiment period.

A total dose of 0.15 mg/mouse/day cisplatin, (Abiplatin®, Pharmachemie BV Haarlem, Holland. Based on calculated dose of 5 mg/kg/day) was injected intraperitoneally (i.p.) for 5 consecutive days (days 1 to 5) to 4 groups (n = 5) of mice. Each group was injected at a different circadian time: 3 HALO (08:00), 8 HALO (13:00), 15 HALO (20:00) or 20 HALO (01:00). The same cisplatin regimen was given to another 4 groups of mice for 5 days, accompanied by amifostine (Ethiol®, U.S. Bioscience, Inc. USA, WR-2721), 1.5 mg/mouse/day (based on calculated dose of 50 mg/kg/day), administered in an i.p. bolus injection 30 minutes prior to the cisplatin injection. All doses of cisplatin and amifostine were adjusted to the doses recommended in the literature (Schiller, 1996), and calculated according to the mean body weight of the mice in the study. Reconstituted solutions of the cisplatin and the amifostine were further diluted with 0.9% sodium chloride injection for dose adjustment to a volume of 1.5 ml/injection/day. A control group of mice was injected i.p. with the same volume of 1.5 ml of normal saline (0.9%) at times parallel to the other two groups of mice.

On day 10, 5 days after the last injection of each group, the mice were sacrificed following induction of coma by 99.8% diethylether at the same hour they were injected during days 1 to 5 (e.g., mice injected with cisplatin at 08:00 were sacrificed at 08:00). Complete blood count, and renal function (measured by blood creatinine (Cr)) and blood urea nitrogen (BUN) levels, were performed immediately afterwards on a blood sample drawn directly from the heart.
Statistical analysis

Differences between the treated groups in levels of complete blood cells count, Cr, BUN levels and BUN/Cr ratio were assessed by the student’s T test. Values of $p < 0.05$ were considered statistically significant.

Results

Renal function tests

Time-dependent effect of cisplatin administration

Cisplatin caused a significant maximal increase of 241% of the BUN level ($17 \pm 2.2$ mg/dl to $41 \pm 12.8$ mg/dl, $p < 0.001$) when given at 13:00 (Fig. 1). No significant changes were detected in Cr levels (Fig. 2). BUN/Cr ratio served as an index for renal function: the higher the ratio, the lower is the perfusion (Stark, 1998; Lafayette et al., 1997), (Fig. 3) and it was found that cisplatin reduced renal perfusion by 60% when given at 08:00 (BUN/Cr ratio: $88 \pm 0.3$ vs $55 \pm 3.7$ in controls, $p < 0.001$) and by 334% at 13:00 (BUN/Cr ratio: $117 \pm 8.7$ vs $35 \pm 4.5$ in controls, $p < 0.001$).

Time-dependent effect of amifostine administration prior to cisplatin injection

The high BUN blood levels caused by cisplatin injection were markedly reduced by prior injection of amifostine in two groups (Fig. 1): in the 08:00-injected group the level fell by 55% from $38.6$ mg/dl to $17.4$ mg/dl ($p < 0.05$); in the 01:00-injected group the level fell by 27% from $22.2$ mg/dl to $16.2$ mg/dl (Fig. 1 $p < 0.01$).

Amifostine administration prior to cisplatin lowered the Cr level (Fig. 2). A 15% decrease was noticed in the 08:00-injected group: $0.374$ mg/dl in the amifostine-cisplatin vs. $0.43$ mg/dl in the control, $p < 0.05$. When the BUN/Cr index was calculated (Fig. 3), it was found that amifostine pretreatment

![Fig. 1. Effect of treatment time on BUN levels. BUN levels were measured in animals that were injected with either saline, cisplatin (5 mg/kg/day for 5 days) or amifostine (50 mg/kg/day for 5 days) 30 min prior to cisplatin injection at 4 circadian times to evaluate the time dependent protection of amifostine pretreatment from cisplatin toxicity. *: $p < 0.05$, **: $p < 0.01$, ***$p < 0.001$, compared to controls. †: $p < 0.05$, ‡: $p < 0.01$, compared to cisplatin treated animals.](image-url)
eliminated the reduction in renal perfusion caused by cisplatin treatment when given at 08:00 (47 ± 7.8 in amifostine treated vs 88 ± 0.3 in cisplatin treated, p < 0.001) and reduced it by 43 % at 13:00 (117 ± 8.7 in cisplatin treated vs 67 ± 25 in amifostine treated, p < 0.05).

**Complete blood cell count results**

**Time-dependent effect of cisplatin administration**

Hemoglobin (Hb) level (Fig. 4) was lowered in all time groups, with significant maximal effect of 78% in the 08:00 group (10.3 ± 1.9 mg/dl in controls vs. 2.3 ± 0.35 mg/dl, p < 0.05) and of 18.4% in the 13:00 group (12.5 ± 0.84 mg/dl in controls vs. 10.2 ± 1.9 mg/dl, p < 0.01).

Fig. 3. Effect of treatment time on cisplatin toxicity on renal function. Early renal damage as expressed by BUN/Cr levels were measured in animals that were injected with either saline, cisplatin (5 mg/kg/day for 5 days) or amifostine (50 mg/kg/day for 5 days) 30 min prior to cisplatin injection at 4 circadian times to evaluate the time dependent protection of amifostine pretreatment from cisplatin toxicity. *: p < 0.05.

Fig. 2. Effect of treatment time on Cr levels. Creatinin levels were measured in animals that were injected with either saline, cisplatin (5 mg/kg/day for 5 days) or amifostine (50 mg/kg/day for 5 days) 30 min prior to cisplatin injection at 4 circadian times to evaluate the time dependent protection of amifostine pretreatment from cisplatin toxicity. *: p < 0.05, **: p < 0.01, ***: p < 0.001, compared to controls. †: p < 0.05, ††: p < 0.01, †††: p < 0.001, compared to cisplatin treated animals.
Total circulating white blood cell count (WBC) (Fig. 5) did not change significantly.

Data on platelets (Plt) level (Fig. 6) showed thrombocytopenia in all time groups. A maximal decrease of 75% compared to control was found in the 08:00 group ($843 \pm 51 \times 10^3/\mu L$ vs. $210 \pm 17.5 \times 10^3/\mu L$, $p < 0.01$). A 70% decrease was noted in the group treated at 13:00 compared to control ($890 \pm 162 \times 10^3/\mu L$ in control vs. $276 \pm 189 \times 10^3/\mu L$, $p < 0.001$). A 62% decrease was noted in the 01:00 group ($829 \pm 262 \times 10^3/\mu L$ in control vs. $321 \pm 151 \times 10^3/\mu L$, $p < 0.01$) and a 42% decrease was seen with the 20:00 group ($905 \pm 164 \times 10^3/\mu L$ vs. $527 \pm 284 \times 10^3/\mu L$, $p < 0.05$).
Lymphocyte (Ly) count (Fig. 7) was lowered by 46% in the 20:00 group (6.9 ± 2.1 \times 10^3/uL in control vs. 3.73 ± 0.46 \times 10^3/uL, p < 0.05).

**Time-dependent effect of amifostine administration prior to cisplatin**

Amifostine injected prior to cisplatin eliminated the severe anemia induced at 08:00 and 13:00 treatment groups (Fig. 4). Mean Hb level at 08:00 was 10.3 gr/dl in the amifostine-cisplatin group vs. 2.3 gr/dl in the cisplatin-treated group (p < 0.01). In the 13:00-injected group, Hb levels were 12.6 gr/dl in the amifostine-cisplatin treated mice vs. 10.2 gr/dl in the cisplatin treated-animals (p < 0.01). No effect was found in the 20:00 and 01:00 treatment groups.
Total WBC count (Fig. 5) remained unchanged in all treated groups. Injection of amifostine prior to cisplatin administration countered (by 96%) the thrombocytopenia (Fig. 6) caused by cisplatin administration alone in the 08:00 group: 412 $10^3$/uL in the amifostine-cisplatin group vs. 210 $10^3$/uL in the cisplatin group, $p < 0.01$. There was no effect in the 13:00, 20:00 and 01:00 groups.

Amifostine administration did not affect the Ly count that had been lowered by cisplatin alone (Fig. 7).

Discussion

Cisplatin is used extensively as a chemotherapeutic agent against a wide range of tumors, including lung cancer, head and neck carcinomas, gynecological malignancies and others. The toxicity of cisplatin, as of many other drugs, varies as a function of time administration. In mice, cisplatin maximal toxicity is known to occur between 0 to 8 hours after light on (Shakil et al., 1993; Boughattas et al., 1990). Numerous clinical studies suggested administering cisplatin in the evening, to reduce renal injury. However in practice, most of the clinical chemotherapy treatments are given during daytime, coinciding with the time when maximal toxicity is induced.

Studies on amifostine, demonstrated statistically significant protection against cisplatin-induced nephrotoxicity and other cisplatin side-effects in mice and in humans (van der Wilt et al., 1992; van Laar et al., 1992; Schiller, 1996; Capizzi and Oster, 2000), and thus the administration of amifostine before cisplatin was accepted for routine use. Thus, the integration of the two drugs in the chemotherapy raises the need to study the time-dependent efficiency of amifostine protection when combined with cisplatin treatment.

Our results demonstrate that maximal toxicity of cisplatin, when administered alone, is observed at 08:00 and 13:00 (3 and 8 hours after light on) in both renal and hematopoietic systems. Minimal toxicity is obtained in groups treated at night (e.g., 20:00 and 01:00). These results are in agreement with other studies (Hrushesky et al., 1982b; Halberg et al., 1979; Boughattas et al., 1990; To et al., 2000).

The optimal times at which amifostine protected the kidneys from cisplatin toxicity were when the combined treatment was given at morning or noon (08:00 or 13:00). These results raise the question regarding the mechanism of amifostine protection from cisplatin toxicity. One explanation is that amifostine addition to cisplatin could improve the tolerability beyond the maximum observed at evenings. Alternatively, amifostine addition could improve cisplatin tolerability in the morning which would result in the reduction of the contribution of cisplatin circadian timing to the variability of its tolerability. Since no effect of amifostine was observed near the circadian peak of cisplatin tolerability our results support the second option in which the amifostine improved tolerability in mornings when cisplatin tolerability is in minimum.

Renal failure may be expressed by elevated plasma creatinine levels, but in the early stages of renal failure, the kidneys can increase creatinine secretion and minimize the rise in creatinine (Rose, 1994). In these early stages, the index of BUN/Cr that we used for renal function reflects reduction in renal perfusion and glomerular filtration rate (GFR). This may indicate the early renal damage, which can lead to renal failure (Lafayette et al., 1997; Stark, 1998). Our results show that pretreatment with amifostine prevents the toxicity of even a single course of cisplatin, as evidenced by reduction of the Bun/Cr ratio elevated by cisplatin (in the 08:00 and 13:00 treated groups).
Hematopoietic suppression by cisplatin was found in hemoglobin, platelets, and lymphocytes. Bone marrow suppression is a common side-effect of cisplatin treatment; however time-dependent toxicity was reported only for leukocytes (Levi et al., 1982b). Our findings show that time-dependent toxicity exists for lymphocytes (20:00) and hemoglobin (08:00 and 13:00).

Despite previous studies demonstrating that amifostine administration has a protective effect on bone marrow suppression, no time dependent study was reported (Capizzi et al., 1993, Kemp et al., 1996). In our study we used amifostine in a time-dependent manner that completely reversed the cisplatin-induced anemia. Since cisplatin was reported to induce significant anemia (Anderson et al., 1988), using the time-dependent protocol may offer an advantage.

Cisplatin was highly toxic for platelets at all examined time-points. However, the amifostine protection was time-dependent and observed only at 08:00.

In conclusion, amifostine administration showed a significant time-dependent protection from cisplatin toxicity both on renal and hematopoietic functions. The optimal time for the combined treatment of cisplatin plus amifostine is the morning to noon. Since clinical administration of cisplatin is usually performed during daytime, in contrast with the recommendation for the use of cisplatin in the evening, the combination of a time-dependent protocol with amifostine may result in fewer side-effects than from daily administration of cisplatin alone.

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References


