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Original Paper

Amifostine (WR2721) for Dose Escalation in Marrow-ablative Treatment of Leukaemia

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The *in vivo* effect of the radiochemoprotectant Amifostine on the therapeutic efficacy of marrow ablative treatment with cyclophosphamide (CP) and total body irradiation (TBI) followed by bone marrow transplantation (BMT) was studied in normal rats as well as in the Brown Norway rat acute myelocytic leukaemia (BNML) model. In normal rats, when the dose of TBI was escalated and the CP dose was kept constant, pretreatment with Amifostine yielded a positive dose modification factor of 1.26. No significant improvement was found after Amifostine pretreatment when the TBI dose was kept constant and CP dose escalated. When leukaemic rats received CP as the only antileukaemia treatment, Amifostine pretreatment did not lead to a reduction in the antileukaemic efficacy of CP, although protection against treatment-related mortality was observed. In the CP only groups, 9 out of 40 animals died of treatment-related toxicity, compared with none of the 40 animals in the Amifostine pretreatment groups. When applying the maximum tolerated treatment of CP and TBI in various combinations to leukaemic rats, 25 out of 36 rats died from treatment-related toxicity, whilst pretreatment with Amifostine reduced this to 11 out of 36, ($P = 0.002$). Of those animals which survived the CP + TBI conditioning treatment, 10 out of 25 in the Amifostine pretreatment group were cured, versus 8/11 in the CP + TBI only control group ($P = 0.146$). In conclusion, incorporation of Amifostine as a radiochemoprotectant in a marrow-ablative conditioning regimen allows the use of escalated doses of chemoradiotherapy without reducing the antileukaemic efficacy.

Key words: Amifostine, dose escalation, leukaemia, bone marrow transplantation
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INTRODUCTION

Treatment of leukaemia with high-dose chemoradiotherapy followed by a marrow transplant results in cure of 50% of patients, but relapse remains a major cause of death. A prospective study [1] showed that in patients receiving an autologous marrow graft in first remission of acute myelocytic leukaemia (AML), the 3-year actuarial relapse rate was 60% versus 35% in patients who received an allogeneic bone marrow transplantation (allo-BMT). This reduced incidence of leukaemia relapse after allo-BMT is explained by a graft-versus-leukaemia (GvL) reaction associated with graft-versus-host disease (GvHD) that originates from T-cells that are present in the allogeneic graft [2]. Because the allogeneic graft is, by definition, free of leukaemic cells, leukaemia relapses originate from residual leukaemic cells in the host that survived the marrow-ablative conditioning treatment. Reinfusion of genetically marked autologous marrow has provided direct evidence that residual cells in remission marrow contribute to disease recurrence after autologous bone marrow transplantation (ABMT) [3]. Cells escaping treatment were not sensitive to the chemoradiotherapy used, or the drug concentration reached at the maximum tolerated dose level was not sufficient to kill these residual leukaemic cells. When a dose-escalation approach is considered in an attempt to eliminate residual leukaemia, the dose-limiting toxicity is mainly due to the fact that cytostatic drugs or ionising radiation do not differentiate between normal and

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leukaemic cells and, therefore, it is essential to protect normal tissues against life-threatening treatment-related side-effects in which chemoprotectants can play a role [4].

Amifostine or WR2721, a phosphorylated sulphydryl compound, was identified as an agent that can protect a variety of tissues against early damage following total body irradiation (TBI), thereby allowing the tolerated total dose to be increased considerably. Yuhas [5] reported a dose modification factor of 2.6. Amifostine also protects against the late effects of ionising radiation by reducing the risk of secondary tumours after irradiation of experimental animals [6, 7]. Subsequently, the compound was reported also to offer protection against the toxic side-effects of a variety of cytostatic drugs, including cisplatin [8], carboplatin [9, 10], melphalan [11], nitrogen mustard [12] and cyclophosphamide (CP) [13]. A variety of tissues was reported to be protected: the intestinal tract [14–16], neural crest cells [17], lung tissue [18], bone marrow [19] and others [20]. In the bone marrow (BM) the protection was found at the level of progenitor cells in *in vitro* studies as well as *in vivo* in animal models [21]. The recovery of the BM function after supralethal TBI was enhanced by combining Amifostine treatment with G-CSF [22]. Also, in patients receiving hemibody irradiation as palliative treatment for widespread metastases, Amifostine protected the BM function [23]. In BM purging experiments with light-activated Merocyanine 540 phototreatment Amifostine protected multipotential marrow progenitors (CFU-GEMM) but hardly protected small cell lung cancer cells [24]. When using 4-hydroperoxycyclophosphamide (4-HC) for BM purging of breast cancer cells, the combination with Amifostine shortened the engraftment period [25]. Furthermore, the therapeutic index improved when using Maphosphamide in combination with Amifostine for purging leukaemic cells in ABMT [26, 27].

In various studies, it has been reported that the antitumour activity of cytostatic drugs is not affected by pretreatment with Amifostine. It is assumed that normal tissues are more efficient in metabolising the prodrug Amifostine to its active metabolite WR1065 by dephosphorylation and that WR1065 can capture free radicals by donating hydrogen atoms and inhibit DNA damage [28], thereby reducing the damaging effects. Normal tissues have a higher activity of alkaline phosphatase, which is involved in the dephosphorylation of Amifostine to WR1065, whilst a facilitated transport system for WR1065 has been demonstrated in normal, but not in neoplastic tissue [29]. In addition, tumour tissue tends to have a lower pH than normal tissue, leading to a reduced rate of absorption of WR1065 in tumour cells. Hence, selective protection in normal tissues is achieved by a reduced metabolism of WR2721 to the active metabolite WR1065 and a low uptake by tumours of WR1065. This would make it possible to intensify chemoradiotherapy treatment [30]. Yuhas and colleagues [31] first recognised that the maximum tolerated dose of alkylating agents could be increased after pretreatment with WR2721, as recently reported for a variety of experimental animal models for solid tumours. However, information on the *in vivo* response of leukaemic cells to chemoradiotherapy when treatment is combined with Amifostine is scarce. The effect of Amifostine on the protection of leukaemic cells to nitrogen mustard in an experimental mouse model has been reported [32] but, thus far, it is not known to what extent the sensitivity of leukaemic cells *in vivo* for cyclophosphamide (CP) and TBI is influenced by Amifostine pretreatment. CP and TBI are widely used as conditioning agents in the treatment of leukaemia with BM transplantation.

The Brown Norway acute myelocytic leukaemia (BNML) model (see ref. [33] for a review) has been used by us in previous studies to evaluate the influence of Amifostine treatment on the efficacy of CP ± TBI for ablative treatment conditioning prior to BMT [34–36]. The present study reports on dose-escalation experiments with CP and TBI in combination with Amifostine in normal Brown Norway (BN) rats as well as in rats carrying transplantable BNML. The influence of Amifostine pretreatment on treatment-related early mortality and on the antileukaemic efficacy of CP and TBI was evaluated.

MATERIALS AND METHODS

Animals

The SPF inbred (BN) strain BN/Rij, produced in the breeding colony of the TNO Institutes in Rijswijk, The Netherlands, was used. Male rats, 13–16 weeks of age, were used (average body weight 260 g). All experimental protocols used in this study were approved by the Institutional Ethical Committee for Animal Studies.

Leukaemia

The experiments were performed using the BNML model, details of which have been described elsewhere [33]. Rats were injected intravenously (i.v.) with 10^7 BNML cells derived from a leukaemic spleen taken from a terminal stage leukaemic animal. A monocellular spleen cell suspension was prepared in Hanks' balanced Hepes-buffered salt solution.

Drug treatment of animals

CP was dissolved in phosphate-buffered saline (PBS; 20 mg/ml) and administered intraperitoneally (i.p.). The non-leukaemic rats received CP on day 0, i.e. 1 day before TBI. The leukaemic animals received either single-modality treatment with CP only on day 11 after leukaemic cell transfer or CP treatment 1 day before TBI, i.e. on day 11 after leukaemic cell transfer in the case of combination chemoradiotherapy. Amifostine (a generous gift from US Biosciences, West Conshohocken, Pennsylvania, U.S.A.), dissolved in PBS, was given i.p. at a dose of 200 mg/kg 30 min before CP and TBI. This dose level and time interval were previously reported to be optimal [37]. All animals treated with CP only, or those receiving CP followed 1 day later by a TBI, received a routine BM rescue by injection of 10^8 syngeneic BM cells on the same day.

Total body irradiation

Animals either received 300-kV X-rays (Phillips-Muller 300, dose rate 0.34 Gy/min) or were irradiated with γ-rays (1.15 Gy/min) using a caesium source (Gammacel 220, Atomic Energy of Canada). The relative biological effectiveness (RBE) of γ-rays compared with X-rays is 0.85.

Parameters for effect evaluation

The fraction of surviving animals (leukaemic and non-leukaemic) was used to evaluate the influence of Amifostine on the treatment with TBI and/or CP compared with controls not pretreated with Amifostine. Gross pathology was performed on all dead animals.

The antileukaemic effects of dose-escalation chemoradiotherapy with or without Amifostine pretreatment were

evaluated using Kaplan–Meier survival analysis for the animals dying from a leukaemia relapse. In addition, the fraction of animals developing a leukaemic relapse versus the fraction of animals cured were compared among the various experimental groups.

Statistics

Fisher's exact test was used to determine the statistical significance of differences in surviving fraction between the various experimental groups. Kaplan–Meier survival plots of leukaemic animals receiving treatment with CP only versus treatment with CP in combination with Amifostine were analysed for significance according to Peto–Wilcoxon using the StatView® Statistical analysis software program [38] (Abacus Concepts, Berkeley, California, U.S.A.). The SPSS statistical software package (SPSS, Chicago, Illinois, U.S.A.) was used to perform Probit analysis to derive the 50% lethal dose (LD_{50}) values in the dose-escalation studies with CP and TBI.

RESULTS

Dose-escalation studies

The combination of high-dose CP and TBI is widely applied as conditioning treatment prior to BMT; therefore, this combined modality treatment was focused on in the present preclinical rat leukaemia model. Initially, dose-intensification experiments were carried out in non-leukaemic BN rats. Firstly, animals were subjected to CP and TBI with the TBI dose kept constant at 7.0 Gy X-rays, whereas the CP dose levels were increased from 80 to 160 mg/kg i.p. Animals dying of treatment-related toxicity did so before day 11. Gross pathology showed intestinal bleeding and diarrhoea as the causes of death. In Table 1 the fraction of surviving animals at each dose level of CP is shown. At the lower CP dose levels (80–100 mg/kg) 2 animals died in the Amifostine pretreated groups versus 1 in the two control groups, but a larger proportion of animals survived in the three dose groups ≥ 120 mg CP/kg given Amifostine, i.e. 21/24 (88%) compared with 12/24 (50%) in the controls. Statistical analysis using Fisher's exact test revealed no significant differences between the corresponding groups at the various dose levels. Using probit analysis an attempt was made to deduce the LD_{50} values for both treatment groups, but, the data set did not allow a reliable analysis (data not shown).

In a subsequent experiment in normal rats the CP dose was kept constant at 100 mg/kg and the TBI dose was escalated from 8.0 to 13.0 Gy (X-rays). A protective effect of Amifostine was evident in this setting (Table 2). At the lowest dose level (8.0 Gy X-rays), 2 of the 8 control animals died compared with none of 8 in the Amifostine group. At higher TBI dose levels, most rats in the control groups died, i.e. 21 out of 24, whereas only 12/24 (50%) died in the Amifostine pretreated groups, including 7/8 in the 13.0 Gy group. Statistical analysis using Fisher's exact test revealed no significant differences between the corresponding groups at the two lower TBI dose levels (8.0 and 10 Gy, respectively). A significant difference ($P = 0.0035$) was observed at the TBI dose level of 11.5 Gy. Overall, the protective effect of Amifostine was more prominent with TBI dose escalation compared with CP dose escalation. Using probit analysis, the LD_{50} values for both treatment groups were determined (Figure 1). The LD_{50} value for the control group receiving CP-TBI without Amifostine pretreatment was found to be 9.05 Gy, whereas the LD_{50} value for the Amifostine pretreated group was 11.45 Gy, yielding a dose-modification factor of 1.26.

Table 1. *Influence of Amifostine pretreatment on the survival of non-leukaemic rats receiving a fixed dose of total body irradiation (TBI) and escalating doses of cyclophosphamide (CP)*

CP dose (i.p.; mg/kg)	TBI dose (X-rays)	CP + TBI controls (Survivors/total)	Amifostine* + CP + TBI (Survivors/total)	*P*-value†
80	7.0	7/8	7/8	NS
100	7.0	8/8	7/8	NS
120	7.0	4/8	8/8	NS
140	7.0	4/8	7/8	NS
160	7.0	4/8	6/8	NS

*200 mg/kg i.p., 30 min before CP. †Fisher's exact test; NS; no significant difference between corresponding groups.

Table 2. *Influence of Amifostine pretreatment on the survival of non-leukaemic rats receiving a fixed dose of cyclophosphamide (CP) and escalating doses of total body irradiation*

CP (i.p.; mg/kg)	TBI dose (X-rays)	CP + TBI controls (Survivors/total)	Amifostine* + CP + TBI (Survivors/total)	*P*-value†
100	8.0	6/8	8/8	NS
100	10.0	3/8	5/8	NS
100	11.5	0/8	6/8	0.0035
100	13.0	0/8	1/8	NS

*200 mg/kg, i.p. 30 min before CP. †Fisher's exact test; NS; no significant difference between corresponding groups.

Mortality and cures

Single modality treatment with escalating CP doses was invested in leukaemic animals with or without Amifostine pretreatment to study directly the effect of Amifostine on the therapeutic efficacy of CP. On day 11 after injection of 10^7

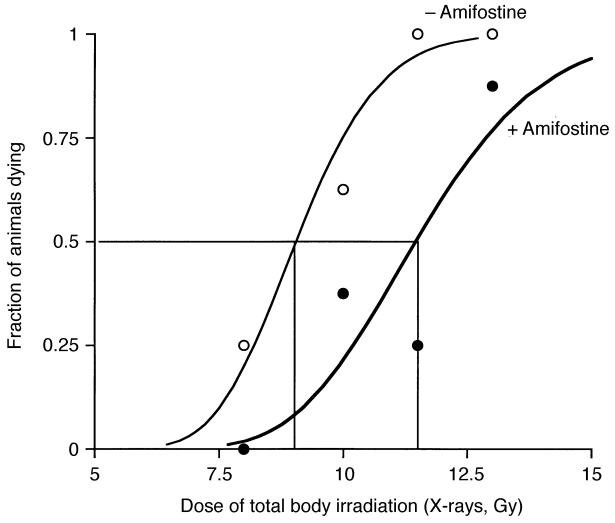

Figure 1. Probit analysis curves of animals dying after conditioning treatment with a fixed dose of cyclophosphamide (CP) with escalating doses of total body irradiation (TBI) before bone marrow transplantation (BMT) with or without Amifostine pretreatment. The extrapolated 50% lethal doses (LD_{50}) values are indicated on the *x*-axis. ●: plus Amifostine; ○: without Amifostine.

BNML cells, the rats were treated with CP at dose levels increasing from 100 to 200 mg/kg. Half of the animals received Amifostine 30 min before CP. In the first 2 weeks after treatment, the rats were monitored for early treatment-related death. Surviving animals were subsequently at risk of leukaemia relapse. From the increase in survival time, the antileukaemia efficacy of CP alone versus Amifostine plus CP could be deduced. Early mortality (9/40 rats) was only encountered in the CP-only control groups (Table 3). 4 animals were cured and these were in the control groups. In the Amifostine + CP groups no rats died from treatment-related toxicity, but no cures were observed (Table 3). All other animals died of a leukaemia relapse.

Survival

At all CP dose levels, with or without Amifostine pretreatment, there was a significant antileukaemia effect, indicated by the increase in the survival time of the CP-treated groups compared with the untreated leukaemic control group. No differences in survival were found between leukaemic controls and leukaemic animals treated with Amifostine only (data not shown). From the increase in the median survival time, the log leukaemic cell kill (LCK) can be deduced since a 4-day increase in survival time corresponds with a 1 log leukaemic cell reduction [31]. The derived LCK values are listed in Table 3. At each dose level the Kaplan–Meier curves were tested for statistically significant differences using the Peto–Wilcoxon test. Slightly shorter median survival times were found for Amifostine-pretreated groups at all CP dose levels except for the 200 mg/kg dose group. At this dose level, 3/8 rats in the CP-only group died from treatment-related toxicity versus none in the Amifostine pretreated group (Table 3; Figure 2). The increase in survival time of the remaining 3 animals in the 200 mg/kg CP-only group was 30, 32 and 42 days, corresponding to a 7.5, 8.0 and 10 LCK, respectively. For the determination of the median survival time in days (MdST) the third animal yielded the 50% value (no. 3 of 5 animals). The difference between the CP-only and Amifostine + CP groups at the 200 mg/kg dose level reached statistical significance ($P = 0.01$) (Table 3).

Marrow-ablative treatment

Dose-escalation experiments combining CP and TBI for marrow-ablative treatment were also performed in leukaemic rats with or without Amifostine pretreatment. Leukaemic animals were treated on day 11 with CP after leukaemic cell transfer, and TBI was given on day 12 followed by a syngeneic marrow transplant on the same day. Before both CP and TBI treatment Amifostine was given i.p. to half the animals. Animals were first at risk of early, treatment-induced mortality and the survivors of the initial period were thereafter at risk of leukaemia relapse. The rats received CP 100 mg/kg followed by either 9.5 Gy γ-rays (with an RBE of 0.85 corresponding to 7.0 Gy X-rays) or 13.5 Gy γ-rays (corresponding to 11.5 Gy X-rays). Following 100 mg/kg CP + TBI 9.5 Gy, there was 1/9 treatment-related death in the control group and 4/9 deaths in the Amifostine pretreated group (Table 4). At the higher TBI level (13.5 Gy) protection by Amifostine was indicated with 6/9 animals dying compared with 9/9 in the control group.

Table 3. Influence of Amifostine pretreatment on the toxicity and antileukaemia efficacy in leukaemic rats treated with various doses of cyclophosphamide (CP)

Early mortality			Antileukaemia effect					Cures	
CP dose (i.p.; mg/kg)	CP controls (Deaths/total)	Amifostine* + CP (Deaths/total)	CP only ΔMdST	CP only LCK	Amifostine + CP ΔMdST	Amifostine + CP LCK	P†	CP controls (cures/total)	Amifostine + CP (cures/total)
100	0/8	0/8	15	3.8	15	3.8	NS	0/8	0/8
120	2/8	0/8	24	6.0	19	4.8	NS	0/8	0/8
140	4/8	0/8	22	5.5	17	4.3	NS	1/8	0/8
160	0/8	0/8	27	6.8	24	6.0	NS	1/8	0/8
200	3/8	0/8	41	10	28.5	7.1	0.01	2/8	0/8

*200 mg/kg i.p., 30 min before CP. ΔMdST, difference in median survival time in days: CP controls or Amifostine + CP versus Brown Norway acute myelocytic leukaemic (BNML) controls; LCK, log leukaemic cell kill. †Kaplan–Meier–Peto. NS, no significant difference between corresponding groups. All animals in the high-dose CP groups (160–200 mg/kg) without Amifostine showed temporary serious morbidity.

Table 4. Influence of Amifostine on treatment-induced early toxicity and on leukaemia relapse after high-dose chemoradiotherapy in the Brown Norway rat acute myelocytic leukaemia (BNML) model

Treatment						
CP (i.p.; mg/kg)	TBI (γ-rays)	Amifostine*	Toxicity (Deaths/total)	At risk of relapse (*n*)	Leukaemia (Deaths/total)	Cures (Cures/total)
100	9.5	Yes	4/9	5	3/5	2/5
100	9.5	No	1/9	8	1/8	7/8
100	13.5	Yes	6/9	3	1/3	2/3
100	13.5	No	9/9	0	—	—
120	8.5	Yes	1/9	8	5/8	3/8
120	8.5	No	9/9	0	—	—
160	8.5	Yes	0/9	9	6/9	3/9
160	8.5	No	6/9	3	2/3	1/3

*200 mg/kg i.p., 30 min before cyclophosphamide (CP) and total body irradiation (TBI).

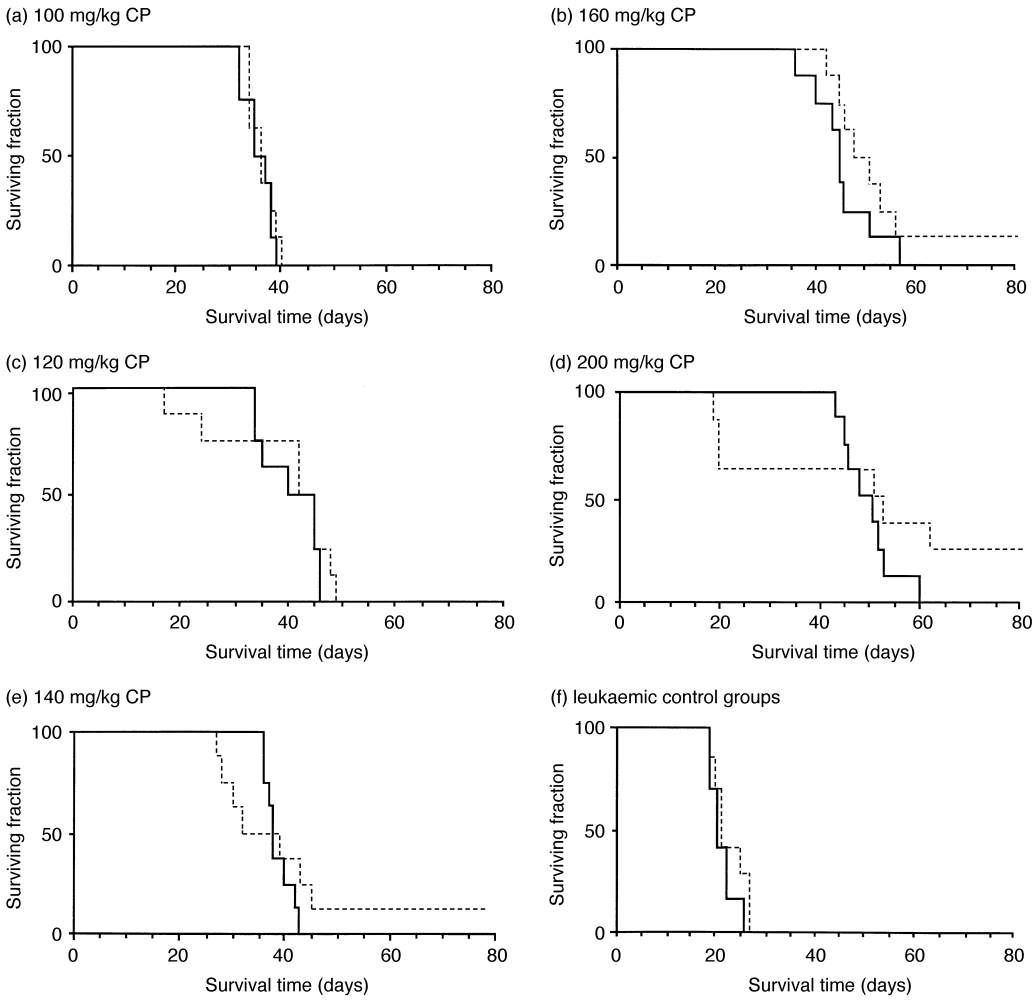

Figure 2. Kaplan–Meier survival curves of leukaemic animals treated on day 11, after 10^7 leukaemic cells i.v., with cyclophosphamide (CP) i.p. at the dose levels indicated, with or without Amifostine pretreatment (200 mg/kg i.p. 30 min before CP). – – –, CP only treated groups; ——, Amifostine+CP-treated groups. The leukaemic control groups received either no further treatment (– – –) or Amifostine treatment only (——).

When the TBI dose was reduced to 8.5 Gy and the CP dose increased to 120 mg/kg, 9/9 animals died from toxicity in the control group whilst in the Amifostine pretreated group only 1/9 animals died (Table 4). When the dose of CP was increased further to 160 mg/kg, 6/9 animals died in the control group while 9/9 animals in the Amifostine group survived. In total, 11/36 (31%) of Amifostine-treated animals and 25/36 (69%) of controls not given Amifostine died from toxicity (Fisher's exact test, $P = 0.002$).

The animals that survived the conditioning treatment were left at risk of leukaemia relapse. The number of animals in the groups varied from 0 to 9, so that statistical analysis at the group level could not be carried out. However, when the animals were divided into two groups, i.e. conditioning treatment with CP and TBI with or without Amifostine, 25 of the original 36 animals (69%) pretreated with Amifostine were survivors at risk of relapse versus 11/36 (31%) not pretreated with Amifostine (31%). 10/25 survivors in the Amifostine pretreated groups were cured (40%) versus 8/11 survivors from the CP + TBI only group (73%) (Fisher's exact test, $P = 0.146$). This implies that when Amifostine is used as a chemoprotectant for escalated ablative conditioning treatment with CP and TBI, the risk of early treatment-related toxicity is decreased, but not at the expense of a higher risk of leukaemia relapse.

DISCUSSION

The protection by Amifostine against ionising radiation-induced damage, which has been reported repeatedly, was also found in these experiments in normal rats when the TBI doses were escalated and the CP dose was kept constant. No statistically significant differences were found when the CP doses were escalated with the TBI dose kept constant, although in the higher dose groups (CP 120–160 mg/kg) given Amifostine 12.5% of the animals died versus 50% in the control groups. The cause of death in animals with escalated CP doses was usually haemorrhage, cystitis and intestinal bleeding in combination with diarrhoea as a result of intestinal tract epithelial damage. Since a marrow transplant was given to restore the bone marrow function, Amifostine may also have acted as a protector of the intestinal tract, thus preventing the loss of animals due to bleeding. The experiments showed that the LD_{50} dose of TBI could be increased from 9.05 Gy to 11.45 Gy, i.e. a factor of 1.26. Since the D_0 of BNML cells is estimated to be in the order of 1.0–1.5 Gy [33], the dose increase from 9 to 11.5 Gy would result in an

additional 1–2 LCK. Because the number of leukaemia cells that survives the marrow transplant-conditioning regimen is estimated to be very low [39], a 1.3-fold increase in the TBI dose might be sufficient to eliminate the residual leukaemic cell population.

For the BM function, it was reported that a dose-modifying factor of 2.4 for colony-forming units–spleen (CFU-S) could be achieved with Amifostine [40]. A protective effect of Amifostine against paclitaxel-induced damage has been reported [41] for BM colony-forming units–granulocyte macrophages (CFU-GM) and burst-forming units–erythroid (BFU-E), which represent haemopoietic progenitor cell subsets of intermediate maturity. In myelodysplastic syndrome, a direct haematological effect was found, illustrated by an enhanced progenitor cell production and a concurring multilineage mature cell production [42]. Subsequently, it became clear that the CFU-S is not a true measure for pluripotent haemopoietic stem cells (PHSC) [43, 44]. Using assays such as the cobblestone-area forming cell (CAFC) assay [45] or the LTC-IC assay [46] will reveal whether Amifostine can indeed protect the PHSC. The shortening of the engraftment period reported previously [25] suggests that the marrow progenitors responsible for short-term engraftment are protected and, although this is in agreement with other studies [41], it does not automatically imply protection of cells with long-term engraftment potential. This could be investigated using the CAFC and LTC-IC assays.

The data collected in the present study concerning the log leukaemic cell reduction are in agreement with the anti-leukaemic effect of CP reported previously for the BNML model [33–36]. Amifostine pretreatment did not significantly influence the response of the leukaemic target population to CP when used as a single treatment modality, although a slightly reduced antileukaemic effect (in the order of 1 LCK) was observed in the highest dose groups (Table 3). Similar observations have been made by other investigators, e.g. in a nude mouse model xenografted with human neuroblastoma, Amifostine pretreatment did not influence the antineoplastic activity of the various drugs tested [47]. When Amifostine preceded CP treatment of high-risk malignant lymphoma, it protected against haematological toxicity but it did not interfere with the tumour response [48]. A potentiation by Amifostine of the antileukaemic efficacy of nitrogen mustard in the AKR mouse leukaemia model has been reported [32] and further confirmed [27]. However, these authors did not apply a marrow-ablative treatment.

The escalated CP dose levels used here for ablative treatment leave few surviving BM stem cells and BM rescue is required. In the high-dose CP groups, the risk of treatment-related early death was reduced since no toxic deaths were observed. Amifostine has been reported to protect the intestinal crypt cells in the small bowel against CP-induced damage and to reduce shrinking of the mucosal wall [16], which could explain the fact that no signs of intestinal tract damage were seen when CP treatment was preceded by Amifostine. The various ablative treatment regimens combining CP and TBI that were applied were at the edge of tolerance for the rats. The overall risk of early mortality was decreased by Amifostine pretreatment. The pattern of leukaemia relapse thereafter did not seem to be affected. In both groups, i.e. Amifostine + CP + TBI versus CP + TBI alone, the risks of leukaemia relapse were similar. This would imply that if Amifostine is used for facilitating dose escalation of the conditioning regimen for BM transplantation for the treatment of leukaemia, it is not to be expected that this will lead to a substantial protection of the leukaemic cell population.

In conclusion, the preclinical studies in the BNML model show that Amifostine reduces the risk of treatment-related mortality, without influencing the antileukaemic efficacy of the CP + TBI regimen. This allows the escalation of the doses of TBI and/or CP, thereby increasing the chance of eliminating residual leukaemic cells and thus of increasing the cure rate.

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