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Amifostine (WR-2721) Protects Normal Haematopoietic Stem Cells Against Cyclophosphamide Derivatives' Toxicity Without Compromising Their Antileukaemic Effects

L. Douay, C. Hu, M.-C. Giarratana and N.-C. Gorin

We compared the effects of amifostine (WR-2721) on the cytotoxicity of mafosfamide or 4-hydroperoxycyclophosphamide (4-HC) in normal marrow progenitor cells (CFU-GM) and leukaemic progenitor cells (CFU-L) during *ex vivo* purging for autologous bone marrow transplantation (ABMT). Mononuclear cells (MNC) were incubated with amifostine 3 mg/ml for 15 min, washed, and subsequently tested for their sensitivity to mafosfamide or 4-HC (20–200 μg/10^7 MNC/ml). The LD_{95} was significantly higher among amifostine-treated cells for PCM-CFU-GM in 6 of 13 patients and for 5R-CFU-GM in 4 of 10 patients ($P < 0.05$). In contrast, amifostine exhibited no protective effects upon CFU-L. The results of this study show that amifostine protects normal late and early progenitor cells from the toxic effects of cyclophosphamide derivatives while preserving their antileukaemic effects. These results suggest that amifostine has therapeutic value as a protective agent for normal marrow progenitor cells during *ex vivo* purging of bone marrow for ABMT.

Key words: amifostine, bone marrow transplantation, stem cells, leukaemia
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INTRODUCTION

CYCLOPHOSPHAMIDE AND its derivatives are the chemotherapeutic agents most commonly used for elimination of residual leukaemic cells from human autologous bone marrow grafts (Figure 1), following the observation by Sharkis and associates [1] that they are able to eliminate residual marrow leukaemic cells in the Brown Norway myelocytic leukaemia rat model. Indirect evidence has also been provided in favour of purging by a report that *in vitro* treatment of the marrow was associated with a significant decrease in relapse [2]. However, although these agents effectively kill tumour cells, they are also toxic to normal progenitor stem cells (CFU-GM). The degree of depletion of CFU-GM content of purged marrow has been demonstrated to be positively correlated with the length of time to engraftment, especially in acute nonlymphoblastic leukaemia (ANLL) [3].

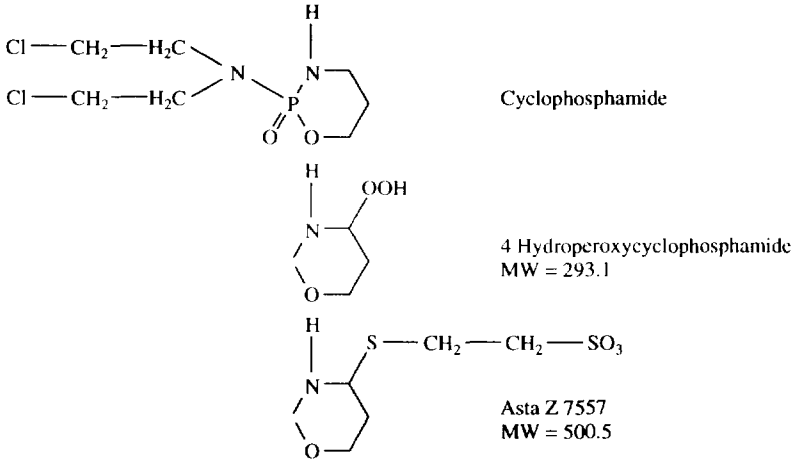

Figure 1. The structure of cyclophosphamide and its metabolites.

Correspondence to L. Douay.
All authors are at the University of Paris-Saint-Antoine Medicine School, Hôpital d'enfants, Armand-Trousseau, 26 Av. du Dr. Arnold-Netter, 75012 Paris, France.

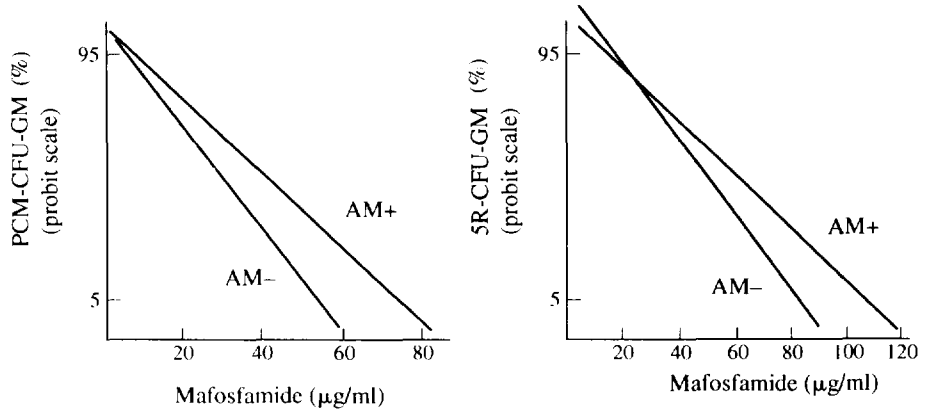

Figure 2. Dose–response curves for late (CFU-GM-PCM) and early (5R-CFU-GM) progenitor cells treated with mafosfamide in the presence (+) or absence (−) of amifostine (AM).

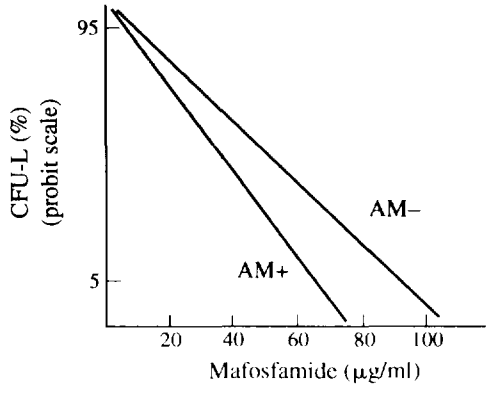

Figure 3. Dose–response curves for leukaemic progenitor cells (CFU-L) treated with mafosfamide in the presence (+) or absence (−) of amifostine (AM).

Table 2. LD_{95}*s of mafosfamide in leukaemic progenitor cells (CFU-L) in the presence or absence of amifostine*

	LD_{95} (μg/ml)	
Diagnosis	Amifostine−	Amifostine+
ANLL	42	38
ANLL	34	18
ANLL	50	45
ANLL	48	41
ANLL	16	13
ANLL	59	50
AP-CML	64	51
AP-CML	53	30
ALL	36	28

ANLL, acute nonlymphoblastic leukaemia; AP-CML, acute phase chronic myelogenous leukaemia; ALL, acute lymphoblastic leukaemia.

Table 1. LD_{95}*s of mafosfamide or 4-hydroperoxycyclophosphamide (4-HC) in late (PCM-CFU-GM) and early (5R-CFU-GM) progenitor cells and in long-term culture-initating cells (LTC-IC) in the presence (+) or absence (−) of amifostine (AM)*

			LD_{95}*					
			PCM-CFU-GM†		5R-CFU-GM‡		LTC-IC	
Patient number	Diagnosis	Drug	AM−	AM+	AM−	AM+	AM−	AM+
1	Normal	Mafosfamide	49	46	145	149	221	255§
2	Normal	Mafosfamide	40	40	150	150	208	184
3	Normal	Mafosfamide	23	24	80	93§	124	114
4	Normal	Mafosfamide	63	61	106	209§	263	232
5	Normal	Mafosfamide	39	53§	ND	ND	ND	ND
6	NHL	Mafosfamide	42	58§	ND	ND	ND	ND
7	NHL	Mafosfamide	37	38	67	74	114	154§
8	NHL	4-HC	25	35§	ND	ND	ND	ND
9	ALL	4-HC	28	32§	28	38§	ND	ND
10	ANLL	4-HC	23	38§	35	55§	ND	ND
11	ANLL	Mafosfamide	34	33	68	67	140	156
12	ANLL	Mafosfamide	36	37	90	78	ND	ND
13	ANLL	Mafosfamide	45	48§	87	79	ND	ND

* Estimated dose (μg/ml) allowing 5% survival of progenitor cells; † CFU-GM grown in agar in presence of placenta conditioned medium; ‡ CFU-GM grown in methylcellulose in presence of SCF + G-CSF + GM-CSF + IL-3 + EPO; § Significantly different from AM−, $P < 0.05$; Fisher's exact test. ND, not done; NHL, non-Hodgkin's lymphomas; ALL, acute lymphoblastic leukaemia; ANLL, acute nonlymphoblastic leukaemia.

Amifostine is a cytoprotective agent that has been found to protect bone marrow from the toxic effects of chemotherapy [4]. To determine whether amifostine would protect normal progenitor/stem cells from the toxicity of cyclophosphamide derivatives without interfering with their antileukaemic activity, we compared the effect of amifostine on the survival of CFU-GM and leukaemic progenitor cells (CFU-L) exposed to 4-hydroperoxycyclophosphamide (4-HC) or mafosfamide during purging for autologous bone marrow transplantation. The degree of protection in late differentiated and earlier immature progenitor cells was also studied.

MATERIALS AND METHODS

Patients

Fresh normal bone marrow progenitor cells were obtained from 5 normal donors and from 8 patients with haematological malignancies in complete remission (4 with ANLL, 3 with non-Hodgkin's lymphoma (NHL), and one with acute lymphoblastic leukaemia (ALL). Fresh leukaemic cells were obtained from 9 patients at the time of diagnosis (6 with ANLL, 2 with AP-CML (acute phase chronic myelogenous leukaemia), and one with ALL). Cells were processed immediately after collection.

Amifostine protection

Mononuclear cells (MNC) were separated on a Ficoll gradient, incubated (2×10^7 MNC/ml medium) in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% autologous plasma with 3 mg/ml amifostine at 37°C for 15 min. Cells were washed and exposed to increasing doses (20–200 μg/ml) of 4-HC or mafosfamide at 37°C for 30 min. A dose–response curve was established for normal and leukaemic progenitor cells, and the LD_{95} doses were determined.

Normal progenitor cells

Three levels of progenitor stem cells were studied. Late CFU-GM (PCM-CFU-GM) (2×10^5 MNC/ml) were cultured in agar in the presence of placenta conditioned medium and scored on day 10. Early CFU-GM (5R-CFU-GM) (5×10^4 MNC/ml) were grown in 0.92% methylcellulose (MC) in the presence of 10 ng/ml GM-CSF, 10 ng/ml interleukin 3 (IL-3), 100 ng/ml stem cell factor (SCF), and 3 units/ml EPO and scored on day 14. Long term culture-initiating cells (LTC-IC) were determined as follows: 2.4×10^5 MNC were maintained over a preformed irradiated stroma layer in 20–30 0.6 cm^2 microwells, in a culture medium made of IMDM plus fetal calf serum (FCS), horse serum (HS), hydrocortisone (OH cortisone), folic acid, L-glutamine, inositol and thioglycerol. At week 6, the adherent layer was treated with trypsin for LTC-IC determination according to the number of adherent and non-adherent detected progenitors.

Leukaemic progenitor cells

Irradiated leucocytes (2×10^6) were suspended in a feeder layer. Leukaemic MNC (2×10^5) were cultured in an overlay of growth medium consisting of 0.72% MC supplemented with 2.5% PHA ± 25 units/ml interleukin 2 (IL-2). Scoring was done on day 7. Identification of the leukaemic origin of the clones was performed as follows: cells were stained with May–Grünwald–Giemsa and examined by light microscopy. Cytospin slides were prepared from pooled harvested colonies and cytology compared with that of specimens prior to culture. All tests to determine the immunophenotypic patterns of cultures and uncultured cells were performed by flow cytometric direct immunofluorescence (Becton Dickinson Facsort). We used the following monoclonal antibodies: for the myelomonocytic panel, CD13 (My-7), CD14 (My-4) and CD15 (80H7); for the T-cell panel, CD2 (0275B), CD3 (T3), CD5 (K39), and CD7(I-21); for the B-cell panel, CD9 (BA2), CD10 (J5), CD19 (B4) and CD20 (B1); for HLA-DR, G-157; and for negative control, L191 (against human epithelium).

RESULTS

The effects of amifostine on cyclophosphamide toxicity in late and early progenitor/stem cells in marrows from normal donors and patients with haematological malignancies in complete remission are detailed in Table 1. The LD_{95} was significantly higher when cells were treated with amifostine for PCM-CFU-GM in 6 of 13 cases, for 5R-CFU-GM in 4 of 10 cases, and for LTC-IC in 2 of 6 cases. In the responder group, the median per cent differences in LD_{95} between amifostine-treated cells that were protected by amifostine and corresponding cells that were not treated with amifostine were 38% (range, 7–65%) for PCM-CFU-GM (n = 6/13), 47% (range, 16–97%) for 5R-CFU-GM (n = 4/10) and 25% (range, 15–35%) for LTC-IC (n = 2/6). The dose–response curves from responders for late (PCM-CFU-GM) and early (5R-CFU-GM) progenitor cells treated with mafosfamide in the presence or absence of amifostine are shown in Figure 2. Cells protected by amifostine had a significantly lower sensitivity to mafosfamide than cells not exposed to amifostine.

The effects of CFU-L treated with mafosfamide are shown in Table 2. In all 9 cases, the LD_{95} was lower for cells treated with amifostine than for cells not treated. This was especially true for cells from patients in the acute phases of CML. That the LD_{95} was lower in amifostine-treated cells than in the untreated cells in all 9 patients suggests that amifostine potentiated the cytotoxic effect of mafosfamide against the tumour cells. Figure 3 presents the dose–response curve for mafosfamide in CFU-L in the presence or absence of amifostine. The CFU-L cells exposed to amifostine had a significantly higher sensitivity to mafosfamide than cells that were not exposed to amifostine ($P < 0.05$).

CONCLUSIONS

The results of this study show that amifostine protects normal late and early progenitor cells from the toxic effects of cyclophosphamide derivatives while preserving their antileukaemic effects. These results suggest that amifostine has therapeutic value as a protective agent for normal marrow progenitor cells during *ex vivo* purging of bone marrow for autologous bone marrow transplantation.

1. Sharkis SJ, Santos GW, Colvn OM. Elimination of acute myelogenous leukemia cells from marrow and tumour suspensions in the rat with 4-hydroperoxycyclophosphamide. *Blood* 1980, 55, 521–523.
2. Laporte JP, Douay L, Lopez M, *et al.* 125 adult patients with primary acute leukemia autografted with marrow purged by mafosfamide. A 10 year single institution experience. *Blood* 1994, **84**, 3810–3818.
3. Rowley SD, Zuehlsdorf M, Braine HG, *et al.* CFU-GM content of bone marrow graft correlates with time to hematologic reconstitution following autologous bone marrow transplantation with 4-hydroperoxycyclophosphamide-purged bone marrow. *Blood* 1987, **70**, 271–275.
4. McColloch W, Scheffler BJ, Schein PS. New protective agents for bone marrow in cancer therapy. *Cancer Invest* 1991, **9**, 279–287.