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Stem Cell Disorders

Amifostine in the treatment of low-risk myelodysplastic syndromes

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ABSTRACT

Background and Objectives. **The phosphorylated aminothiol agent amifostine (Ethyol) protects bone marrow and other tissues from toxicity due to ionizing radiation and antineoplastic drugs, and stimulates progenitors from normal and myelodysplastic bone marrow. Contrasting results have been published so far on the effectiveness of amifostine in correcting cytopenia in patients with myelodysplastic syndromes (MDS).**

Design and Methods. **In a pilot phase II study we treated 26 patients with low risk MDS (13 RA, 2 RARS, 2 CMML, 9 RAEB with blasts < 10%) with amifostine (200 mg/m² × 3/week for 4 weeks).**

Results. **Hemoglobin concentration, reticulocyte, neutrophil and platelet counts increased respectively in 6 (23%), 11 (42%), 13 (50%) and 9 (34%) of patients. Red cell transfusions were reduced (> 50%) in 4/26 patients and abolished in 1/26. Unexpectedly a significant decrease in soluble transferrin receptor level at week 4 of therapy, compared to the basal level ($p<0.04$), was observed in the whole population of patients.**

Interpretation and Conclusions. **Amifostine can ameliorate cytopenia in some patients with MDS, with few and mild side effects. Neutropenia is more likely to be corrected than anemia or thrombocytopenia. Mechanisms underlying this biological effect remain to be clarified.**

Key words: myelodysplastic syndromes, amifostine, soluble transferrin receptor, erythropoietin

Myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by highly ineffective hematopoiesis, whose treatment relies heavily on supporting measures. Infections, bleeding complications, iron overload and evolution to acute leukemia are common causes of death in MDS patients. Ineffective hematopoiesis possibly due to apoptosis is a primary factor in determining cytopenia in MDS, and inadequate production of endogenous erythropoietin (Epo) contributes to anemia in about 30% of patients. Several studies have shown that chemical inducers of cell differentiation[1] and hematopoietic growth factors[2-4] may overcome these mechanisms and possibly stimulate the residual normal hematopoiesis in specific subsets of patients. However, in no instance was the overall response rate higher than 30%. Aggressive cytoreductive therapy and bone marrow transplantation have a role, especially in high risk MDS.[2] Unfortunately only a few patients are candidates because age in MDS is frequently advanced. The aminothiol prodrug amifostine, a compound developed to protect tissues from toxicities of ionizing radiations and cytotoxic drugs, has been found to stimulate normal hematopoiesis in canine models.[5] Moreover, *in vitro* it was found to be able to stimulate proliferation of normal hematopoiesis,[6] suppress apoptosis and enhance growth of progenitor cells from patients with MDS. Reports of limited clinical phase I/II trials[7,8] have been published so far. We report herein the results obtained with amifostine in the treatment of 26 patients with MDS.

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Design and Methods

Patients and treatment

Twenty-six patients with low-risk MDS (LR-MDS), defined on the basis of bone marrow blasts (<10%), were treated with amifostine (Ethyol) (200 mg/m² ×3/week) for four weeks, either after having unsuccessfully received prior therapies (not less than a month before) or not. A further course with amifostine at 300 mg/m² for 2 weeks was planned for non-responders. Characteristics of the patients are reported in Table 1. According to the FAB classification 13 patients with refractory anemia (RA), 2 with refractory anemia with ringed sideroblasts (RARS), 2 with chronic myelomonocytic leukemia (CMML) and 9 with refractory anemia with excess of blasts (RAEB) with bone marrow blasts <10% were treated. According to the International Prognostic Scoring System (IPSS) risk score,[9] the 16 evaluable patients were classified as follows: low 10 (FAB: RA 8; RARS 2); intermediate-1 3 (FAB: RA 2; RAEB 1); intermediate-2 3 (FAB: RAEB 2; CMML 1). Patients were treated with alizapride prior to each infusion to reduce nausea and vomiting, and amifostine was infused intravenously over 15 minutes. Supplementation with oral folate (15 mg/day) was also given.

Laboratory studies

Pre-treatment evaluation included bone marrow study, complete blood count (CBC) with leukocyte differential count, reticulocytes, kidney and liver function tests, and serum folate and B12 levels. Serum Epo concentration was also measured by an immunoradiometric assay (COATRIA, Bio Merieux), and enzyme immune assays were used for thrombopoietin (Tpo) and soluble transferrin receptor (sTfR) (Quantikine Human TPO; Quantikine HUMAN sTfR, R&D, Minneapolis, USA). For chromosome analysis, BM cells were G-banded with Wright's stain after 24-hour culture, and at least 15 metaphases were karyotyped according to the *International System for Human Cytogenetic Nomenclature*. The analysis was available for 16/26 patients and aberrations were found in 4/16. CBC, leukocyte differential and reticulocyte counts were checked twice a week.

Response criteria

Evaluations of data were carried out at weeks 4 and 8. Similarly to works published by List *et al.* and Bowen *et al.*,[7,8] we aimed exclusively at recording changes in CBC induced by amifostine. Appreciable responses were considered increases ≥ 1 g/dL in Hb, ≥ 30% in neutrophil and ≥ 15% in platelet and reticulocyte counts, of at least 4 weeks' duration after therapy had been stopped. For Epo, Tpo and sTfR evaluations, statistical analysis was carried out by Wilcoxon's non-parametric test, and a p value < 0.05 was considered statistically significant.

Response was also evaluated according to the IPSS risk score,[9] when cytogenetic analysis was available.

Table 1. Patient characteristics.

No. of enrolled patients	26
Male/Female	13/13
Mean age, years (range)	70 (50-85)
Median age, years	72
Diagnosed more than 6 months before therapy	19/26
Pre-treated patients	15/26
Pre-transfused patients	15/26
FAB classification	
Refractory anemia	13
Refractory anemia with ringed sideroblasts	2
Chronic myelomonocytic leukemia	2
Refractory anemia with excess of blasts	9
Cytogenetic aberrations	4/16
Refractory anemia	del 5 (q12q33)
Chronic myelomonocytic leukemia	+8
Refractory anemia with excess of blasts	-6;-15; +21
Refractory anemia with excess of blasts	del 5 (q22q33); -X

Results

Response to treatment

Patients were evaluated after four weeks of treament and at the end of the observation period, at the 8th week. Hemoglobin concentration, reticulocyte, neutrophil and platelet counts respectively increased in 6 (23%), 11 (42%), 13 (50%) and 9 (34%) of 26 patients (Table 2). A second course of amifostine at 300

Table 2. Individual response to treatment.

Id.	*Sex*	*Age (years)*	*Diagnosis*	*Hemoglobin (g/dL)*		*Reticulocytes (x10⁹/L)*		*Neutrophils (x10⁹/L)*		*Platelets (x10⁹/L)*	
				day 0	*day 28*	*day 0*	*day 28*	*day 0*	*day 28*	*day 0*	*day 28*
1	F	75	RAEB	7.9	7.9	16.5	15.2	0.37	0.69	33	20
2	M	71	RAEB	8.1	11.6	76.6	60.6	0.59	0.68	108	187
3	F	75	RA	8.0	7.8	36.0	41.6	5.34	5.18	169	135
4	M	58	RA	13.4	12	48.3	95.5	0.88	0.67	88	85
5	F	66	RA	11.4	10.2	83.4	84.0	0.91	0.8	17	13
6	M	70	RAEB	7.6	9.2	89.2	102.6	1.51	4.6	19	15
7	F	85	RAEB	8.0	6.0	104.2	149.5	0.87	0.48	27	13
8	F	74	RA	8.5	8.3	8.70	5.3	1.03	1.55	108	138
9	M	76	RA	8.0	8.3	18.6	28.2	4.32	6.0	320	332
10	M	79	RA	13.4	12.6	77.7	74.0	6.69	3.84	39	59
11	F	66	RARS	6.4	7.8	30.4	35.0	1.26	1.45	193	234
12	F	81	RA	9.6	9.5	45.1	39.7	0.38	0.28	42	37
13	M	78	RA	8.5	8.5	21.1	30.4	2.58	3.3	276	231
14	M	71	CMML	11.9	10.4	201.0	136.0	1.08	1.26	21	18
15	F	67	RARS	7.5	8.6	16.6	75.5	0.76	1.15	235	238
16	F	72	RA	7.0	8.7	118.0	136.0	0.5	1.2	30	58
17	F	66	RA	14.3	13.7	66.0	69.4	0.58	1.3	253	254
18	F	60	RA (5q-)	8.4	8.0	43.4	41.2	3.0	3.9	664	412
19	M	72	RA	8.0	9.1	122.3	65.5	4.2	5.46	370	426
20	M	72	CMML	8.6	8.6	74.2	44.9	1.12	1.80	78	121
21	F	76	RA	6.1	6.6	15.0	10.0	0.92	0.8	11	15
22	M	76	RAEB	8.0	7.5	141.2	124.8	0.34	0.31	152	175
23	M	73	RA	8.0	7.5	84.7	97.7	1.57	2.58	169	350
24	F	50	RAEB	9.0	9.3	50.6	76.4	0.9	1.40	20	21
25	M	69	RA	10.3	9.3	63.8	52.2	4.2	3.2	533	495
26	M	53	RAEB	9.7	9.0	21.0	34.9	0.44	0.2	44	43

Table 3. Results according to the International Prognostic Score System (available for 16/26 patients).

	Amelioration of cytopenia			
Risk	*monolineage*	*bilineage*	*trilineage*	*no response*
Low (10/16)	5	2	1	2
Intermediate-1 (3/16)		3		
Intermediate-2 (3/16)	1	2		

Table 4. Side effects of amifostine therapy.

Asymptomatic hypocalcemia	6/26
Nausea	2/26
Fever	2/26
Pruritus	1/26
Fatigue	1/26

mg/m^2 x 3/week for 2 weeks in 10/26 non-responders did not improve the response to treatment. In responders amelioration of cytopenia lasted at least 4 weeks from the end of treatment and duration was independent of the cell lineage; in a patient with the 5q– syndrome thrombocytosis progressively decreased during treatment and returned to baseline shortly after it was stopped. Transfusion need was reduced (>50%) in 4/26 patients, and eliminated in one who was still unsupported 10 months after treatment. Minimum time to response was 3 weeks from the beginning of therapy. As shown in Table 3, responses to amifostine according to IPSS score were not significantly different in low, intermediate-1 and intermediate-2 risk groups.

Special laboratory studies

Serum thrombopoietin (Tpo) and Epo levels were not found to be significantly different before and after completion of therapy (Tpo: 27.5±22.6 vs 32.2±23.9 fmol/mL; Epo: 183.6±238.2 vs 159.2±208.7 mU/mL) in the whole population of patients. Nevertheless final values of Tpo and Epo were found increased respectively in 9/26 and 15/26 evaluable patients. In contrast, in the overall population of patients sTfR levels were found to be lower on day 28 than at baseline ($p < 0.04$) (Figure 1). Taking into account patients with hemoglobin or reticulocyte response, sTfR final levels were higher in only two hemoglobin responders. No correlation was found between changes in sTfR and in reticulocytes or hemoglobin in the overall population of patients.

Side effects and evaluation

Only a few, mild side effects were recorded, including asymptomatic hypocalcemia, nausea, fever and fatigue (Table 4). We also noted a reduced frequency of bleeding episodes, even in two patients whose platelet count was not affected by amifostine. Performance status, evaluated according to the ECOG scale, improved by one grade or more in 8/26.

Progression to AML was observed in 5/26 patients; time from diagnosis ranged from 9 to 28 months.

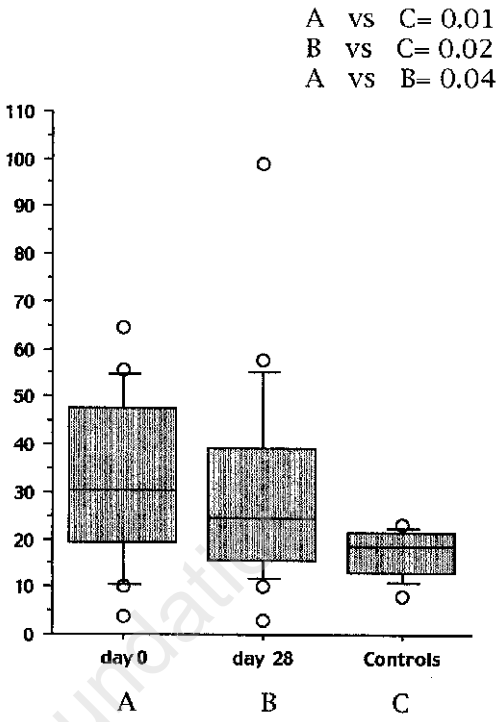

Figure 1. Changes in sTfR levels induced by treatment with amifostine in patients with MDS.

Discussion

Hematopoiesis is largely ineffective in MDS and several approaches to prolong progenitor cell survival, induce differentiation, and possibly stimulate residual normal hematopoiesis have been attempted. Differentiating agents, such as retinoids and vitamin D3, and recombinant human cytokines have been employed for these purposes. This topic has recently been reviewed.[1]

The thiol compound amifostine stimulates *in vitro* proliferation of normal human hematopoietic progenitors, in particular BFU-E and the more immature CFU-GEMM,[7] and improves hematopoiesis in MDS.[6] In the present study amifostine ameliorated cytopenia in some patients with MDS, with neutropenia being more likely to improve (50% of patients) than anemia (23%, only one long-lasting unsupported responder) or thrombocytopenia (34%). It should be noted that only in 2 platelet responders was pre-therapy thrombocytopenia severe ($< 50\times10^9$/L). This observation agrees with the report by List *et al.*,[7] that correction of platelet count is likely to occur in patients with only slightly reduced baseline values. This finding would suggest that stimulation of residual normal thrombocytopoiesis may occur. On the other hand, the observed progressive decrease of thrombocytosis during treatment in a patient with the 5q– syndrome suggests that clonal hematopoiesis might have been temporarily inhibited by amifostine.

Despite the unmodified platelet counts, bleeding episodes were reduced in two thrombocytopenic patients; this effect may have been due to either a trophic action on vessel wall or to an amelioration of platelet function, typically impaired in MDS.[10]

The possible mechanism(s) underlying the therapeutic effect of amifostine in MDS is still unclear. Serum Epo levels are reported to be inadequate for the degree of anemia in about 30% of patients with MDS.[11] In our population, pre- and post-therapy values were not significantly different, therefore a stimulation of endogenous production does not seem to have supported the hemoglobin increase in responders. Nevertheless, at week 4 of treatment serum Epo was found to be increased in 15 of our patients, therefore further evaluations on this topic seem worthwhile. More intriguing were the changes observed in sTfR, whose levels typically rise whenever a stimulation of erythropoiesis occurs, even if highly ineffective.[12] In a recent report on the treatment of MDS with recombinant human Epo (rHuEpo),[4] an increase in sTfR levels at week 4 of treatment of less than 18% compared to basal values, predicted non-response. Therefore stimulation of erythropoiesis was necessary in order to have an increase in hemoglobin levels in rHuEpo treated patients. Surprisingly, in the patients treated with amifostine, sTfR values at week 4 were significantly lower than the pre-therapy values (p=0.04) (Figure 1). Data published by Cazzola *et al.*[13] indicate that the number of transferrin receptors on cell membrane decreases following induction of differentiation and slowing of cell proliferation, while the number typically increases after Epo stimulation. Our observations on sTfR (a truncated form of cell receptor probably produced by a proteolytic mechanism) levels could be explained by an amelioration of ineffective erythropoiesis due to the antioxidant activity of amifostine through its ability to reduce free radicals[14] and/or by polyamine-like effects.[15] On the other hand, the absence of correlation between sTfR and reticulocyte or hemoglobin values might also be explained by an inhibition of the dysplastic clone. Most of our patients refused to undergo a new biopsy, so we could not evaluate post-therapy bone marrow. If our data were further confirmed, they would indicate that recombinant Epo and amifostine could ameliorate erythropoiesis in MDS through different pathways.

Data published by List *et al.*[7,15] and our own results may caution against the use of amifostine because of the risk of MDS evolution into AML. Nevertheless the frequency of evolution and time from diagnosis were comparable with data observed in other series.[16,17] In conclusion a percentage of patients with MDS may have a partial benefit from treatment with amifostine, especially if neutropenic. This drug seems particularly suitable in the elderly because of the absence of toxicity and significant side effects at the dose used in this study. Future issues remaining to be addressed are the identification of MDS patients who are likely to respond to amifostine, and the effect of chronic administration. Finally, in a preliminary study we observed that the combined use of amifostine and LDARA-C in 5 patients with RAEB with blasts > 10% induced complete remission with cytogenetic normalization in one case, and partial remission in two more patients. It, therefore, seems worth studying further the effectiveness of amifostine in combination with differentiating and cytotoxic drugs, and with hematopoietic growth factors.

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Preparation of the protocol and enrollment of patients: AG, AF, VS, FL. Amifostine administration: AG, AF. Cytogenetic and special laboratory studies: CN, SC, GP. Analysis of laboratory data: GL. Preparation of manuscript: AG, AF. Revision of the manuscript: VS, FL, GL. Final approval: PRF.

In assigning the order of authorship, the specific role of each co-author, and his/her participation during the development of the study were taken into account.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

- Amifostine is a non-toxic and well tolerated drug (at the dose employed in this study) that could be usefully employed in the treatment of low risk MDS, especially to improve neutropenia.
- Data obtained from analysis of soluble transferrin receptor levels suggest that evaluation of the combined use of amifostine and recombinant human erythropoietin in the treatment of low-risk MDS would be worthwhile.
- The promising data observed in a small group of patients with RAEB treated with amifostine and low-dose ARA-C (treatment was effective and extremely well tolerated), suggest that it would be worth trying a larger study on amifostine in combination with cytotoxic agents.

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