might be due to different rates of irreversible binding of cisplatin or Pt containing species to proteins and/or other reactive nucleophyles (e.g. sulphydryl-containing molecules). This could account for the lower systemic toxicity that was observed in mesothelioma patients. No statistically significant difference was noted by comparing the AUCs for total platinum up to 72 h.

This study further strengthens the rationale for the intrapleural cisplatin treatment schedule given the very high levels of filterable reactive platinum detected in the pleura, the prolonged plasma levels of filterable platinum, and the absence of haematological toxicity and the contained emetic toxicity despite the fact that cisplatin was absorbed systematically up to therapeutic levels.

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Eur J Cancer, Vol. 27, No. 7, pp. 842–845, 1991.
Printed in Great Britain
0277–5379/91 $3.00 + 0.00

Low-dose Cytarabine for Acute Myeloid Leukaemia and Myelodysplastic Syndromes: *in vivo* and *in vitro* Cytotoxicity

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14 patients with acute myeloid leukaemia (AML) and 7 with myelodysplastic syndrome (MDS) were treated with cytarabine in low dosage. In AML a complete remission rate of 43% was found and in all patients profound cytopenia was noticed without any sign of maturation induction. In MDS no effect of low-dose cytarabine could be detected. We also studied the effect of low-dose cytarabine *in vitro* in freshly isolated leukaemic cells of 10 patients with AML. Maturation induction was measured by a comprehensive panel of quantitative and qualitative markers of maturation. No differentiation inducing effect of low-dose cytarabine could be found. We conclude on the basis of our own results and after reviewing the literature that low-dose cytarabine exerts its effect by cytotoxicity instead of maturation induction.
Eur J Cancer, Vol. 27, No. 7, pp. 842–845, 1991

INTRODUCTION

CYTARABINE IS one of the most effective agents in the treatment of acute myeloid leukaemia (AML). Regimens of 100-200 mg/m^2 per day for 7 days induce complete remissions (CR) in 60-80% of patients [1]. Recently, low-dose cytarabine has been introduced as an effective but less toxic treatment. In 846 patients low-dose cytarabine induced a CR in 32% [2]. The CR rate for patients older then 50 years was 56%. The mechanism through which cytarabine in small doses exerts its effect is not clear. Several authors have suggested that low-dose cytarabine promotes differentiation of leukaemic cells [3]. However, our group as well as others showed a cytotoxic effect of low-dose cytarabine without an obvious maturation-inducing effect [4, 5]. We report on *in vivo* data of 21 patients with AML and myelodysplastic syndrome (MDS) treated with low-dose cytarabine together with the *in vitro* effects of low-dose cytarabine in freshly isolated leukaemic cells.

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Revised 15 Apr. 1991; accepted 18 Apr. 1991.

PATIENTS AND METHODS

Patients

14 patients with AML and 7 patients with MDS were treated with cytarabine in a dose of 10 mg/m^2 twice a day subcutaneously over 21 days. After reaching a CR or considerable blast reduction

Table 1. Clinical characteristics of 14 patients with AML and 7 patients with MDS treated with low-dose cytarabine

Case	Sex	Age	FAB classification	Cyto-genetics	WBC (× 10⁹/l) (% granulocytes) Initial	WBC (× 10⁹/l) (% granulocytes) Nadir	Platelets (× 10⁹/l) Initial	Platelets (× 10⁹/l) Nadir	Bone marrow blasts (%) Initial	Bone marrow blasts (%) After 1 course	Bone marrow blasts (%) After 2 courses	Platelet transfusion	CR	Survival *	Major infection
1	F	79	M1	N	1.3(12)	1.0(1)	93	4	52	8	2	4	+	23	+
2	F	76	M5B	AA	40(5)	3.7(1)	90	6	47	–		7	–	1	+
3	M	69	M4	AA	77(23)	0.7(10)	65	2	37	4		16	–	4	+
4	M	72	M1	NM	1.9(22)	1.2(8)	64	5	51	2		11	+	4	+
5	F	75	M2	AA	6.0(11)	1.1(2)	77	9	36	42		12	–	5	+
6	M	76	M4	N	46(15)	19(17)	140	49	42	47		0	–	4	–
7	F	83	M2	N	2.2(47)	0.6(45)	58	3	30	58		30	–	4	+
8	M	84	M5B	N	5.5(50)	1.2(20)	324	18	60	5	2	0	+	8	–
9	F	77	M4	NM	5.3(9)	3.8(5)	114	13	61	65		7	–	26	+
10	F	68	M2	N	17.5(6)	0.3(10)	57	2	70	2		7	+	+10	–
11	M	74	M2	N	24(37)	0.6(6)	171	12	32	3		7	+	+11	–
12	M	54	M2	NM	1.3(14)	0.7(5)	21	2	43	10	2	8	+	+ 8	+
13	M	74	M5B	-	40(23)	2.7(9)	33	12	90	38	7	9	–	8	–
14	M	71	M2	NM	6.2(23)	2.4(2)	397	18	41	58		5	–	2	+
15	M	66	RAEB –t	AA	38.8(52)	5.0(68)	11	1	5	73		33	–	3	+
16	M	78	RAEB –t	AA	4.5(28)	1.0(17)	2	1	16	26		21	–	3	+
17	F	77	RAEB –t	-	16.2(50)	39(21)	35	3	15	3		23	–	2	+
18	F	77	RAEB –t	AA	7.9(48)	5.9(36)	60	10	20	8		6	–	4	+
19	F	55	RAEB –t	-	3.4(8)	1.5(5)	20	2	17	17		20	–	18	+
20	M	65	RAEB	-	1.8(6)	1.2(7)	45	6	8	7		19	–	+20	+
21	M	83	RAEB	N	12.3(75)	4.7(59)	30	8	9	10		15	–	7	–

N = normal, AA = abnormal, NM = no metaphases, – = not performed. * Months after starting therapy.

(> 20%) a second identical course was planned. Maintenance therapy consisting of cytarabine in a dose of 10 mg/m² subcutaneously twice a day for 10 days every 6 weeks for six courses was instituted after a CR was reached. AML and MDS were classified according to FAB criteria [6, 7]. The clinical data are summarised in Table 1. Haemoglobin, platelet count and white blood cell (WBC) count were measured every 2 days. Bone marrow examination was peformed at diagnosis and at least at 18–35 days after the start of therapy. In patients 1, 6, 7, 8, 15, 16 and 17 repeated bone marrow aspirates during therapy were taken. Transfusion of leucocyte-poor red blood cells and platelets were given as required. The platelet count was maintained above 10 × 10⁹/l. Selective decontamination of the gastrointestinal tract was performed, using colistin, amphotericin and cotrimoxazole orally. Fever was treated with broad spectrum antibiotics combining an aminoglycoside and a cephalosporin unless microbiological data allowed more specific treatment. CR was defined as bone marrow containing less then 5% myeloblasts in the presence of a peripheral granulocyte count > 1.5 × 10⁹/l, a platelet count > 100 × 10⁹/l and a haematocrit > 30%. Duration of remission was measured from the date when complete remission was documented until relapse. Survival was measured from start of therapy until death.

The bone marrow specimens of patients 1, 6, 7, 8, 13 and 15 were examined to assess cell phase before therapy and between 4 and 7 days after start of therapy.

In vitro culture studies

Culture. From 10 consecutive patients with AML heparinised peripheral blood or bone marrow specimens were obtained. Mononuclear cells were isolated after centrifuging on a Ficoll–Hypaque density gradient (d 1.077) at 400 *g* for 30 min and washed twice with Hank's medium supplemented with 0.1% bovine serum albumin. The final cell suspensions contained > 80% leukaemic cells as judged by morphological examination of May–Grünwald–Giemsa stained cytocentrifuge preparations. Cell viability as determined by trypan blue dye exclusion was above 80%. These cells were incubated in a concentration of 0.5 × 10⁶/ml in alpha-MEM supplemented with 20% heat inactivated fetal calf serum (FCS), penicillin and streptomycin. Cell cultures were maintained in humidified 5% CO_2 at 37°C for 4 days with and without 10^{-6}, 10^{-7} and 10^{-8} mol/l cytarabine.

Differentiation parameters. Cytochemistry [Sudan Black, naphtol AS-D chloroacetate esterase and alpha naphthyl acetate esterase (ANAE)]; intracellular enzyme activity [myeloperoxidase (MPO), acid esterase and lactate dehydrogenase (LDH)]; nitro blue tetrazolium (NBT) reduction; protein content; DNA analysis and clonogenic survival were performed as described [8].

Statistical methods

Variables expressed as percentages in control cells and treated with cytarabine were compared using the χ^2 statistic for the rate ratio.

Quantitative measurements were compared using the following statistic:

$$2(X_c - X_t)^2/(CV^2 (X_c + X_t)^2)$$

where X_c and X_t denote the control and treated cells, respectively, and CV the coefficient of variation.

RESULTS

Clinical investigation

AML. 3 patients reached CR after one course and another 3 after two courses, for a total of 43%.

The duration of CR was 7, 7, 8+, 10+ and 11+ months,

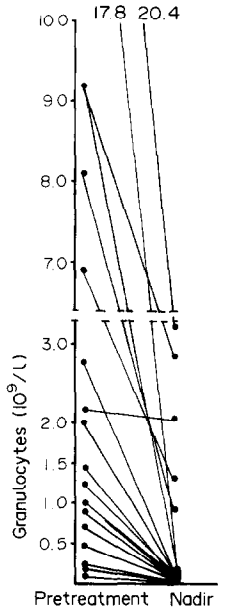

Fig. 1. The effect on granulocyte count of low-dose cytarabine. The absolute number of peripheral blood granulocytes immediately pretreatment and the nadir of granulocytes are indicated.

respectively. 1 patient died in CR at 4 months. The mean overall survival in the non-responding patients was 6.8 months (range 1–27 months, median 4 months). Remissions were reached from 2 to 3 weeks after cessation of therapy. Profound granulocytopenia and thrombocytopenia were seen in both responders and non-responders (Table 1 and Fig. 1). Repeated bone marrow examinations during therapy revealed hypocellularity in all patients tested, without the presence of persistent myeloblasts and maturing myeloid islets, as has been described before. Toxicity was considerable. Infectious complications necessitating admission occurred in 9 patients. Nausea, vomiting and hair loss were noticed in a few patients. There were no bleeding complications.

MDS. None of the 7 patients reached CR, despite induction of severe pancytopenia in all of them.

Table 2. The effect of cytarabine on cell diameter and percent of cells in S-phase

Cell diameter (μm)		S-phase (%)	
Control	Cytarabine	Control	Cytarabine
10.55	11.55	21	26
8.96	8.04	10	10
8.96	10.36	17	26
9.81	11.98	20	43
10.17	9.63	4	4
9.46	10.36	15	25
10.36	14.36	12	20
8.46	8.36	8	6
8.96	9.81	22	27
9.99	10.17	4	5

Freshly isolated leukaemic cells were cultured for 96 h without (control) and with 10^{-7} mol/l cytarabine

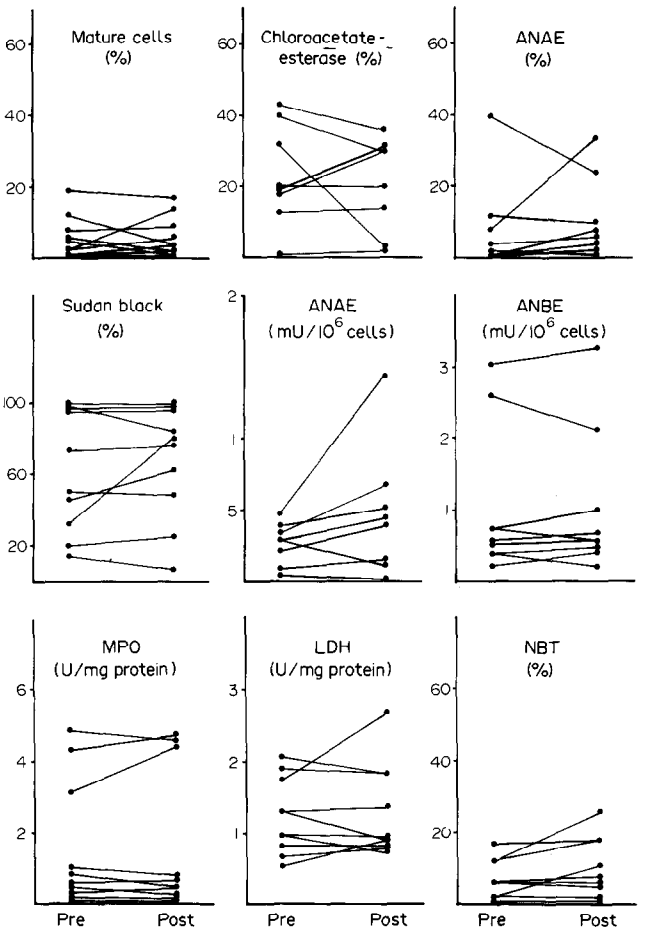

Fig. 2. The effect of 96 h exposure with cytarabine (10^{-7}mol/l) on freshly isolated leukaemic cells of 10 patients. Control values and values after 4 days exposure to cytarabine in the different maturation parameters are shown.

In vitro culture studies

Cytarabine incubation. Incubation of the leukaemic cells with 10^{-6} mol/l cytarabine led to considerable cell death (viability far below 50%) while in the presence of 10^{-8} mol/l cytarabine no differences with the control experiments were measured. The results of culturing in the presence of 10^{-7} cytarabine are thus given.

Morphology (Fig. 2). Cells grown under the influence of cytarabine for 4 days exhibited increased cytoplasmic volumes. No signs of maturation were observed.

Cytochemistry (Fig. 2). In 1 patient the percentage of cells positive for Sudan Black was significantly higher after culturing with cytarabine ($P < 0.01$). In another the same was true for non-specific esterase ($P < 0.01$). In a third patient the percentage of chloroacetate positive cells decreased ($P < 0.05$).

Intracellular enzyme activity (Fig. 2). The activity of lactate dehydrogenase, MPO and alpha naphthyl butyrate esterase (ANBE) in the leukaemic cells after 96 hours culture in the presence of cytarabine did not change significantly. Acid esterase activity with alpha naphthyl acetate used as a substrate revealed a small but significant increase in the leukaemic cells of 4 patients ($P < 0.01$).

NBT (Fig. 2). NBT reduction was not increased to a significant level in the leukaemic cells cultured in the presence of cytarabine.

Table 3. Number of clusters after exposure to cytarabine compared to control

Control	Cytarabine
1005	462
528	140
190	60
16	70
1050	961
308	76
414	104
1159	46
0	0

Freshly isolated leukaemic cells from 9 patients, 96 h culture with cytarabine.

Cell diameter (*Table* 2). 6 out of 10 patients showed a considerable increase in cell diameter.

Pulse cytophotometry (*Table* 2). In 6 out of 10 leukaemic cells the amount of cells in S-phase rose significantly after exposure to cytarabine. In 6 patients the percentage of cells in S-phase was measured before and after 7 days *in vivo* treatment with low-dose cytarabine. In 5 out of 6 patients a rise in the percentage of cells in S-phase was observed.

Clonogenic capacity (*Table* 3). Clusters could be counted in nine experiments. Considerable loss of clonogenic capacity was found in 7 patients.

DISCUSSION

This report confirms that low-dose cytarabine is capable of inducing CR in AML. Our CR rate of 43% is in concordance with reported data [9]. The toxicity in the low-dose cytarabine schedule seems to be milder mainly because of the absence of severe mucositis and the other sequelae of higher doses of cytarabine. However, we observed major infectious complications in 66% of the patients. Profound pancytopenia requiring transfusion of platelets and red cells were found in all patients. No signs of maturation in the peripheral blood smears and bone marrow were found during therapy.

Bone marrow examination during therapy showed invariable hypocellularity and failed to reveal the islets of maturing myeloid cells coincident with persistent leukaemic blasts as has been reported previously [10].

Our *in vitro* studies disclosed that cytarabine did not induce significant maturation. There were no morphological and cytochemical signs of maturation. Development of mature cell function as measured by NBT was not detected. Intracellular enzyme activity of MPO, LDH and non-specific esterase with (ANBE) substrate did not change after exposure of the leukaemic cells to cytarabine, pointing in the direction of granulocytic or monocytic maturation. As can be seen in Fig. 2, the increase in alpha naphthyl esterase activity was very small; the total amount for all but one measurement was below the value found in normal granulocytes and did not reach the amount in normal monocytes or that after obvious monocytic differentiation of HL60-cells and freshly isolated leukaemic cells.

We consider the increase as non-specific because of the extreme low level of activity, not accompanied by a rise in staining for non-specific esterase or an increase in the more specific ANBE activity. Direct evidence against maturation is the increase in cell diameter and the arresting of cells in S-phase. If maturation had occurred, a decrease in cell diameter could have been expected, while more cells should have been found in G_1 phase.

There is much controversy in the literature over whether cytarabine in low doses induces differentiation *in vitro* [10–15].

The reason for those conflicting *in vitro* results could well lie in the techniques used. In most publications very little maturation parameters are taken into account. Often only morphological criteria are used. It is our experience as well as that of others that interpretation of cell morphology is difficult after exposure to differentiation inducers [16, 17].

The weight of our own and published data suggests that low-dose cytarabine exerts its effect by cytotoxicity. Nevertheless, results of this therapy in AML, especially in the elderly, warrant further study and future trials should focus on a better maintenance programme (i.e. low-dose cytarabine together with true differentiation inducing agents such as 1,25 (OH) D_3 to postpone relapses).

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Acknowledgements—We wish to thank W. Jalink, T.v.d. Berg, A. Loonen and G. Broekema for their valuable technical assistance.