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A new combination of two intercalating agents (mitoxantrone + daunomycin) in adult refractory acute leukemia: the DON protocol*

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Summary. A combination of two intercalating agents, mitoxantrone and daunorubicin with vincristine (the DON regimen) was studied in 16 patients with refractory acute leukemia, including three patients with myeloblastic transformation of refractory anemia with excess of myeloblasts after the failure of first-line chemotherapy and one additional patient with AML relapsing while off therapy. All patients had been heavily pretreated prior to receiving the DON regimen, and all but two had previously received high-dose anthracyclines. Of the 17 patients, nine (53%) who achieved complete remissions (CR) had myeloblastic leukemia. The three patients with acute lymphocytic leukemia did not achieve CR. Cardiac toxicity occurred in two patients and contributed to death in one. These results in very poor risk leukemia suggest a possible synergism in the action of the two intercalating agents and absence of increased cardiotoxicity.

Introduction

Refractory acute leukemia in adults (RAL) has been classified into the following categories [11]:

1. A total absence of response to standard chemotherapy (CT)
2. A failure to reach complete remission (CR) after two courses of combination chemotherapy
3. A relapse within 6 months after achieving CR
4. Refractoriness to salvage therapy during relapse

Despite numerous trials using single- and multiple-agent regimens, such as high-dose cytarabine alone (HDARAC) [10, 22] or combined with daunorubicin [10], amsacrine [2, 13], or asparaginase [4], results have been poor in patients classified as above. Even in a group with a better prognosis, such as adult patients with acute leukemia who relapse after intensive chemotherapy, the effectiveness of a salvage therapy is questionable [9]. Similarly poor results have been achieved by various chemotherapy (CT) regimens in patients with transformation of refractory anemia with excess of myeloblasts (RAEB) [15].

Mitoxantrone, an anthraquinone with intercalating properties, has recently been tested in phase II studies on relapsed and refractory acute myeloblastic (AML) and lymphoblastic (ALL) leukemias. The reported CR rates range from 0 to 20% [1, 20]. We designed a new protocol combining daunomycin, vincristine (Oncovin), and mitoxantrone (Novantrone), the DON regimen. Mitoxantrone is only partially cross-resistent with anthracycline [21] and has been shown to be potentiated by vincristine in mice [5]. This protocol was tested in the poorest risk category of both ALL and AML, namely, the adult RAL group of patients defined above, and in patients with leukemic evolution of RAEB. In this paper we report preliminary results for 17 patients; these results indicate a 70% CR rate in patients with refractory AML. No CR was achieved by the three patients with ALL.

Material and methods

Patient characteristics. From January 1985 to July 1987, 17 patients received one course of the DON regimen. Their ages ranged from 17 to 69 years, with a median of 40. There were nine male and 8 female patients. The distribution of the patients according to their diagnosis and the status of the disease has been listed in Tables 1 and 2.

Three patients had ALL and 14 had AML; of the latter, three had transformation RAEB [unique patient numbers (UPN) 6, 14, and 16]. In four patients (UPN 5, 8, 9, and 11), leukemia did not respond to standard CT; another four (UPN 1, 7, 10, and 17) failed to achieve CR after two courses of CT. Relapse occurred within 6 months after the onset of CR in four other patients (UPN 3, 4, 13, and 15), whereas in one patient (UPN 12) the relapse was refractory to salvage therapy. Leukemic transformation of RAEB occurred in three patients.

Prior to receiving the DON regimen, all patients had been heavily pretreated. All but two of the three patients with transformation of RAEB (UPN 14 and 16) had previously received anthracyclines. Twelve patients had been treated with daunorubicin, two with doxorubicin, five with rubidazone, two with bisantrene, and five with successive inoculations of different anthracyclines. Details of the drugs given before the DON regimen are listed in Tables 1 and 2.

Chemotherapy schedule. The DON regimen was delivered over a period of 6 days according to the following sched-

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Table 1. Results of DON in patients with refractory AML

Unique patient no. (UPN)	FAB classification	Age/Sex	Previous chemotherapy		Results (months)
			Anthracyclines before DON mg/m²	Other drugs	
1	M2	17 M	DNR 250	ARAC 6TG VCR Pred Cy × 3	CR 24+
2	M1	53 F	DNR 350 BIS 2250	ARAC 6TG VCR Pred × 2	CR 4
4	M5	40 F	RBZ 800 DNR 100	ARAC VM26 CCNU × 2	Failure
5	M2	69 F	DNR 150	ARAC 6TG VCR Pred	CR 18+
9	M2	23 M	DNR 250	ARAC 6TG VCR Pred	CR 6
11	M5	29 F	RBZ 850	ARAC VM26	CR 5
12	M2	43 F	DNR 250 ADR 100	ARAC 6TG CCNU	CR 2
13	M4	39 F	DNR 250	ARAC 6TG VCR Pred Cy+TBI	CR 3
15	M2	45 M	DNR 250	ARAC 6TG VCR Pred Cy+TBI	Failure
17	M5	23 F	RBZ 1200 DNR 135	ARAC AMSA	Failure

CML, Chronic myelocytic leukemia; RBZ, rubidazone; DNR, daunorubicin; BIS, bisantrene; ADR, adriamycine; 6TG, 6-thioguanine; ARAC, cytosine-arabinoside; ASPA, asparaginase; VCR, vincristine; Pred, prednisone; Cy, cyclophosphamide; TBI, total body irradiation (110 Gy)

Table 2. Results of DON in patients with other refractory leukemias and leukemic transformation of RAEB

Unique patient no. (UPN)	FAB classification	Age/Sex	Previous chemotherapy		Results (months)
			Anthracyclines before DON mg/m²	Other drugs	
3	L2	20 M	DNR 400	Cy ASPA VCR Pred Cy × 2	Failure
7	L1	47 M	RBZ 700	ARAC Cy VCR Pred	Failure
8	M7	59 M	DNR 140	ARAC Pred	Failure
10	Blast crisis of CML (lymphoblastic)	30 M	RBZ 700 ADR 120 BIS 1750	ARAC ASPA VCR Pred	Failure
6	AML RAEB	58 M	DNR 180	Low dose ARAC ARAC 6TG VCR Pred	CR 12
14	AML RAEB	65 M		Low-dose ARAC	CR 5+
16	AML RAEB	57 F		Low-dose ARAC	Failure

CML, Chronic myelocytic leukemia; RBZ, rubidazone; DNR, daunorubicin; BIS, bisantrene; ADR, adriamycine; 6TG, 6-thioguanine; ARAC, cytosine-arabinoside; ASPA, asparaginase; VCR, vincristine; Pred, prednisone; Cy, cyclophosphamide; TBI, total body irradiation (110 Gy)

ule: vincristine (Oncovin), 2 mg total dose i.v. on day 1 in the evening; daunorubicin, 45 mg/m² i.v. over 2 h on days 2–4; mitoxantrone (Novantrone), 12 mg/m² i.v. over 30 min on days 2–6. Mitoxantrone was infused 1 h after daunorubicin when given on the same day. Hyperhydration was started 24 h before chemotherapy and continued until day 7.

Evaluation of response and toxicity. Antileukemic effects were measured according to GALGB criteria [23]; however, patients not in CR were considered failures and none was classified as a partial remission (PR). Bone marrow examination was done on day 15 and on a weekly basis thereafter when necessary. Surveillance for toxicity included daily physical examination and laboratory testing

for liver and renal functions. Prior to and after CT, all patients underwent ECGs and five had echocardiographic examinations.

Results

All patients treated with the DON regimen were evaluable since none died while in the aplastic phase after CT. In nine patients (53%) CR was achieved after a single course of the DON regimen. Of ten patients with refractory AML (Table 1), seven went had CRs and three failed (CR rate, 70%). In contrast, in the group of seven patients with other types of leukemias (Table 2) (three refractory ALL, one megakaryblastic AML, three leukemic transformations of RAEB), only two had CRs (29%). Durations of CRs under maintenance therapy were 2, 3, 4, 4+, 5, 6, 12, 18+, and 24+ months. At the time of writing three patients are alive and well (UPN 1, 5, and 14). Patient 1 was successfully transplanted with bone marrow from his HLA-identical brother while in CR after the DON therapy. The patients who failed to achieve CR died of leukemia.

The immediate toxicity of the DON regime was moderate: mild nausea and vomiting occurred in all patients. A few patients (4/17) experienced transient mucositis of moderate intensity (WHO grade 1), and one patient with AML in relapse following autografting with total body irradiation (TBI) developed a severe mucositis (WHO 3) that persisted for 10 days.

The median duration of aplasia (leukocytes $< 1.10^9/l$) was 29 days (range, 15–43). The infectious complications during aplasia were: two cases of staphylococcemia, two nondocumented cases of reversible pneumonitis, one case of aspergillosis, five herpesvirus (HSV 1) infections, and one case of encephalitis responding to acyclovir. Following DON CT, none of our patients died as a direct consequence of any of the infections listed above.

One patient (UPN 10) developed congestive heart failure responding to digitalis and diuretics. This complication was most likely anthracycline-related; prior to DON, this patient received the highest doses of intercalating agents in our series (700 mg/m^2 rubidazone (RBZ) + 120 mg/m^2 adriamycin (ADR) + 1750 mg/m^2 bisantrene (BIS)). Another patient (UPN 12) died during aplasia of cytomegalovirus (CMV) infection with irreversible cardiac failure after receiving a course of mitoxantrone + VM 26 to consolidate the CR obtained after inducting by DON CT. None of our patients had an increase of liver transaminases greater than 5 times the normal value.

Discussion

Refractory acute leukemia (RAL) in adults has a poor prognosis; most salvage CT regimens tested so far have essentially been applied to heterogeneous groups of patients, a vast majority of whom underwent relapses while either on or off previous therapy rather than when the leukemia was truly end-stage or refractory to chemotherapy. For instance, using mitoxantrone alone at a dose of 12 mg/m^2 daily $\times 5$ in the treatment of so-called refractory acute leukemia, Moore and Olsen have reported an overall response rate of 31% for AML, which can be subdivided into 42% responses in relapsed AML and only 10% responses in truly refractory AML [18]. More recently, in a pilot study of mitoxantrone in combination with ARA-C for AML, Paciucci [19] has reported a response rate of 65% in relapsed patients but only 23% in those classified as refractory. Similar observations of the efficacy of various CT regimens have been made by numerous other teams [3, 4, 22], clearly indicating that RAL is a distinct entity, with the poorest prognosis, to be studied separately. We therefore selected RAL as the most appropriate disease for testing new combinations therapies such as the DON regime. In addition, we tested DON CT in three patients with leukemic evolution of RAEB.

Our rationale for using the DON combination was follows:

1. Mitoxantrone is a potent inhibitor of RNA and DNA synthesis in both proliferating and nonproliferating cells. Although mitoxantrone may have a similar site of action within the cell, it is only partially cross-resistant with anthracycline antibiotics [5, 6, 21]. Furthermore, because there have been indications that anthracyclines and mitoxantrone interact with different base-pairs [8], we postulated that the combination might have a synergistic effect.
2. In the P388 leukemia model [5], vincristine has been reported to potentiate the efficacy of mitoxantrone.
3. Cardiotoxicity may not necessarily be increased, since mitoxantrone does not induce the formation of free radicals in vitro and does not generate lipid peroxidation; it has even been reported to inhibits anthracycline-stimulated lipid peroxidation and microsomal superoxide production [17].

Our results in a limited number of patients indicate an overall 53% CR rate. These results are favorable compared with those of other trials such as, for instance, HDARAC + amsacrine [2] (25% CR), ARAC (conventional doses) + mitoxantrone (2/13 CR) [7], or mitoxantrone + etoposide (35% CR) [14]. On the other hand, they appear similar to those obtained recently in AML with HDARAC + mitoxantrone (53% CR) [12]. In our group of patients with refractory AML, the CR rate was 70%. In addition, two of three patients with leukemic evolution of RAEB went into CR, resulting in an overall CR rate of 69% in patients with leukemic myeloblastic clones. In contrast, of the eight nonresponding patients, two had ALL, one had a lymphoblastic transformation of chronic myelocytic leukemia, one had megakaryoblastic leukemia, and two had an M5 AML with multiple extramedullary sites. It therefore appears that the efficacy of the DON regimen is limited to myeloblastic proliferations.

Clinical indication of possible synergism between the two intercalating agents in our protocol may be suggested by the following:

1. Mitoxantrone alone, as previously tested by numerous investigators [16, 20, 21], is not very effective in RAL (CR rate, 10%).
2. All but two of our patients were considered resistant to anthracyclines by classic clinical criteria, and of the nine patients who underwent CR, seven were specifically resistant to daunomycin.

The high rate of CR observed in our series may be explained by an absence of cross-resistance between mitoxantrone and anthracycline, suggesting a synergistic effect. To the best of our knowledge, the DON protocol is the first regimen reported that combines two intercalating agents: an anthracycline and a relatd synthetic derivative. Although our series is very limited, the high antileukemic

effect we observed in AML as well as the absence of an increased risk of cardiotoxicity, as predicted from in vitro data, suggest that the efficacy of this protocol might further be evaluated in a phase II study on patients with a better prognosis.

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Note added in proof. The authors wish to draw attention to the fact that since this paper was written, one additional patient, a 65 year old woman, with refractory AML put in complete remission by the DON protocol given as salvage therapy, subsequently developed heart failure and died suddenly at home, while in persisting CR.