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Clinical Trials with Daunorubicin-DNA and Adriamycin-DNA in Acute Lymphoblastic Leukemia of Childhood, Acute Nonlymphoblastic Leukemia, and Bronchogenic Carcinoma

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Summary. *Treatment with daunorubicin-DNA (DNR-DNA) or adriamycin-DNA (ADM-DNA) has been evaluated in acute lymphoblastic leukemia of childhood (ALL), acute nonlymphoblastic leukemia (ANLL) and bronchogenic carcinoma (BC). The Five-year survival rate in 69 children with ALL was 73.7% when ADM-DNA was introduced in the treatment and 38% with DNR-DNA ($P = 0.03$).*

A randomization between free DNR and DNR-DNA for remission induction in 26 patients with ANLL has shown that the drugs were of equivalent effectiveness. The one-year survival rate was 66% for the DNR group and 64% for the DNR-DNA group.

In 59 patients with BC, a randomized trial between ADM-DNA and cyclophosphamide-vinblastine (CTX-VLB) did not show an advantage in favor of one of these treatments. In anaplastic BC (51 patients), there was no difference in survival rate or remission rate between patients treated with ADM or ADM-DNA.

No cardiotoxicity was noted among the patients treated with the complexed drugs. ADM-DNA and DNR-DNA are as effective as the free drugs. Cardiotoxicity appears to be reduced.

Introduction

The basic hypothesis of a lysosomotropic cytostatic drug implies more selective tumor cell killing and less toxicity for normal tissues. In this way, cardiotoxicity of adriamycin and daunorubicin could be reduced, and perhaps mucous, cutaneous, and bone marrow toxicity, also (Trouet et al., 1972).

These tempting prospects, the promising results obtained in animal tumors, and phase I studies in leukemia and solid tumors which showed that the cytostatic properties of the free and the complexed drugs were at least similar (Sokal et al., 1973; Cornu et al., 1974; Lie et al., 1975) prompted us to use DNA-complexed cytostatic drugs in childhood lymphoblastic leukemia, acute nonlymphoblastic leukemia, and bronchogenic carcinoma.

Materials and Methods

The daunorubicin-DNA and adriamycin-DNA complexes were prepared as described by Trouet et al. (1972). The complexed drugs as well as the free drugs were administered at a rate of 20 mg/h for daunorubicin (DNR) and 10 mg/h for adriamycin (ADM). The actuarial survival curves were drawn from standard life-table formulae (Cutler et al., 1958).

A. Acute Lymphoblastic Leukemia of Childhood. Sixty-nine children were treated with cytostatic drug complexes. The children were randomized between treatment with ADM-DNA or DNR-DNA. Of these children, 25 had one or more poor prognostic feature, i.e., age over ten, high peripheral blood blast count, or organ enlargement.

Two slightly different regimens were in use during this randomized study.

The 1972 protocol includes 36 children and consisted of the infusion of 200 U/kg/day of L-asparaginase for 21 days. Prednisone (40 mg/m^2/day) was added from day 4 until day 21. Vincristine, at a dose of 2 mg/m^2, was injected on days 8, 15, 22, and 29 and either ADM-DNA (12 mg/m^2) or DNR-DNA (25 mg/m^2) on days 10, 17, 24, and 31.

After central-nervous-system prophylaxis with cranial irradiation (2400 rad) and intrathecal methotrexate (12 mg/m^2 on days 10, 12, 17, 24, and 31), maintenance treatment was started. It consisted of 6-mercaptopurine (1 mg/kg/day) and methotrexate (15 mg/m^2/week) with reinduction on day 45 and at the 3th, 6th, 9th, and 12th months and every 6 months for 2 years.

Reinduction treatment consisted of prednisone (40 mg/m^2) for 10 days, vincristine (2 mg/m^2) on days 1 and 8, ADM-DNA (12 mg/m^2) or DNR-DNA (25 mg/m^2) on days 3 and 10, and L-asparaginase (200 U/kg/day) from day 4 to day 10.

The 1975 protocol (33 children) differs in that at induction L-asparaginase was administered for 14 days, either when starting treatment or once remission was achieved. Vincristine was injected on days 1, 8, 15, and 22 and either ADM-DNA or DNR-DNA on days 3, 10, 17, and 24.

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B. Acute Nonlymphoblastic Leukemia. This group includes acute myeloblastic leukemia, acute monoblastic leukemia, promyelocytic leukemia, and myelomonocytic leukemia. Patients over 65 years of age were not included.

The results of a phase I study have already been reported (Cornu et al., 1974). Afterward, we intended to compare regimens with ADM-DNA or DNR-DNA, and a protocol with DNR or DNR-DNA.

The 1973 protocol included two courses at 3-week intervals of vincristine (2 mg/m^2) on day 1 and either DNR-DNA (75 mg/m^2) or ADM-DNA (35 mg/m^2) on days 3, 4, 5, and 6. Cytosine arabinoside was substituted for vincristine in the third and fourth courses. Eight patients were treated with DNR-DNA and four patients with ADM-DNA.

Courses of continuous single-drug infusion (ADM-DNA, 10 mg/m^2/24 h, or DNR-DNA, 20 mg/m^2/24 h for 6 days) were given to nine patients.

The 1975 regimen includes three courses of DNR and cytosinearabinoside. DNR, either free or complexed, was infused over 24 h at a dose of 150 mg/m^2 on day 1. Cytosine arabinoside (150 mg/m^2/day as continuous infusion was administered on days 5, 6, and 7. This group includes 26 patients, 13 patients being randomly allocated to the free drug or the complex. Consolidation therapy consisted of three cyclophosphamide injections (300 mg/m^2 each) given every 2 days during a week. Maintenance treatment consisted in weekly methotrexate (15 mg/m^2) and daily 6-mercaptopurine (50 mg/m^2).

C. Bronchogenic Carcinoma. Fifty-nine patients with inoperable bronchogenic carcinoma were randomized within two series. In 29 patients, cyclophosphamide (900 mg/m^2) and vinblastine (9 mg/m^2) were infused every 3 weeks. The 30 other patients were randomized either to ADM-DNA (75 mg/m^2 every 3 weeks; 16 patients) or to free ADM for three infusions which were followed by infusions of the complexed drug (14 patients).

Fifty-two patients with anaplastic bronchogenic carcinoma were randomly allocated to either free ADM followed by complexed ADM or to complexed ADM. The administration modalities were as described earlier.

Results

1. Acute Childhood Lymphoblastic Leukemia

The five-year survival rate of 69 patients treated with regimens including complexed cytostatic drugs (DNR or ADM) was 56%. Treatment with ADM-DNA achieved better results than treatment with DNR-DNA. The difference is statistically significant ($P = 0.02$) (Fig. 1).

The same trend in favor of ADM-DNA persisted, whether good or poor prognostic features are considered; in patients with good prognosis, the five-year survival rate was 85% in the ADM-DNA group (27 patients) and 45% in the DNR-DNA group (17 patients). Patients with poor prognostic features had a 25% five-year survival rate when treated with DNR-DNA and a 30% five-year survival rate when treated with ADM-DNA ($P = 0.3$). It might be useful to note that the survival rate was practically identical to the complete remission rate.

2. Acute Nonlymphoblastic Leukemia

Since only 12 patients were allocated to the 1973 protocol comparing DNR-DNA and ADM-DNA, this study is of little value. Two patients (50%) treated with ADM-DNA had a one-year survival rate, whereas only one patient (12.5%) treated with DNR-DNA lived more than one year. Continuous infusion of DNR-DNA or ADM-DNA (nine patients), did not bring on a single remission.

The 1975 protocol, which included a randomization between free (13 patients) and complexed DNR (13 patients), resulted in an 88% complete remission rate. The remission rates of the two groups were identical. The one-year complete remission rate was of 48% for the free DNR group and 44% for the complexed DNR group. The one-year survival rate did not differ very much either: 66% for the free DNR group and 64% for the complex group (Fig. 2).

3. Bronchogenic Carcinoma

The randomized study between ADM-DNA or ADM and vinblastine with cyclophosphamide did not show an

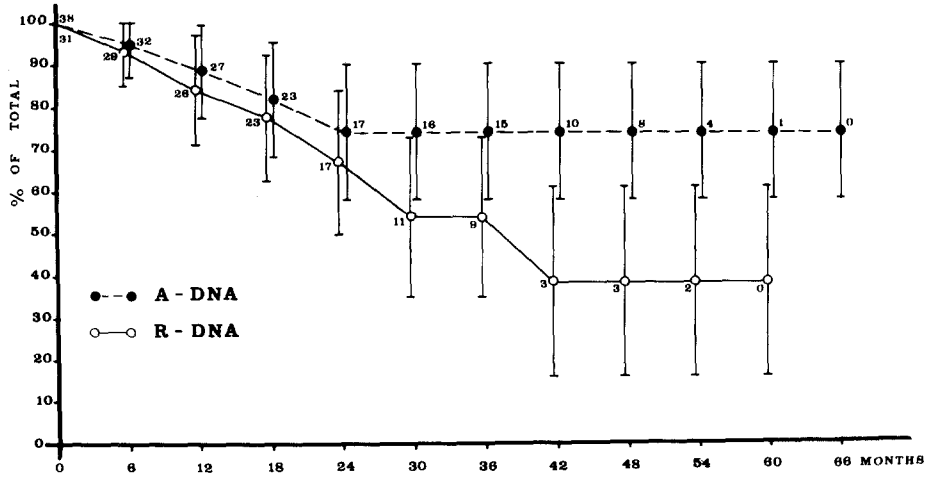

Fig. 1. Actuarial survival (actual numbers also given) in children with ALL. A better survival rate is observed in children who were infused with ADM-DNA

advantage in favor of a single regimen; 5 of the 29 patients treated with cyclophosphamide-vinblastine and 7 of 30 treated with ADM achieved remission (Table 1).

In anaplastic bronchogenic carcinoma, there was no difference in remission rates between the patients treated with the complexed drug alone (11/25: 44%) and the patients treated with both the complexed drug and the free drug (17/26: 65%) (Table 2). The survival rates between the two groups were similar (ADM 17.5%, ADM-DNA 11.5%) (Fig. 3).

Table 1. Chemotherapy in bronchogenic carcinoma

	Total group I	Total group II	Subgroup II_A ADM-DNA	Subgroup II_B ADM + ADM-DNA
	CTX-VLB			
Remissions	5	7	2	5
Failures	27	23	14	9
Remissions	5/29	7/30	2/16	5/14
Remission rate	17.2%	23.3%	12.5%	35%
	$P = 0.2$		$P = 0.71$	

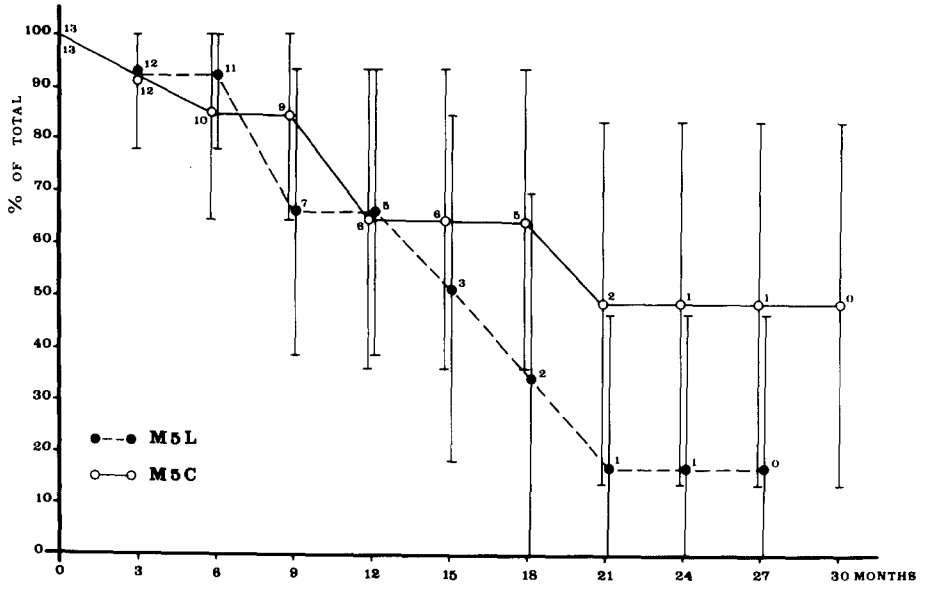

Fig. 2. Actuarial survival (actual numbers also given) of 26 patients with ANLL; randomized trial between DNR-Ara-C* (M5L) and DNR-DNA-Ara-C (M5C) at induction. The difference in survival rate between the two patient groups is not statistically significant

* Ara-C = cytosine arabinoside

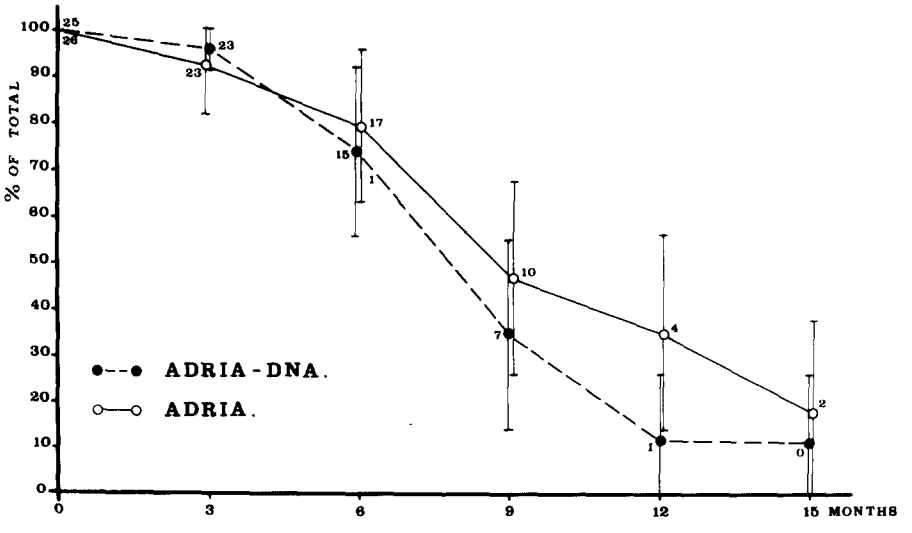

Fig. 3. Survival in anaplastic bronchogenic carcinoma as calculated by the actuarial technique. The randomized trial between patients treated with ADM and patients treated with ADM-DNA does not show a statistically significant advantage in favor of one of these treatments

Table 2. Anaplastic bronchogenic carcinoma: response to chemotherapy

	Group I ADM-DNA	Group II ADM
Cases	25	26
Remissions	11	17
Failures	14	9
Remission rate	44%	65%

Discussion

High endocytic capacity of many tumor cells led to the development of DNA complexes with cytostatic drugs (Trouet et al., 1972). DNA acts as carrier for the drug and should be degraded by lysosomes.

When L 1210 leukemic cells were injected intravenously in DBA_2 mice, the complex was considerably more effective (Trouet et al., 1974; Rozencweig et al., 1975; Staquet et al., 1977). Preliminary trials in human leukemia with DNR-DNA and ADM-DNA have shown that the therapeutic effectiveness was at least similar to that of the free drug. Absence or reduced cardiotoxicity was noted notwithstanding doses considered to be toxic to the heart (Sokal et al., 1973; Cornu et al., 1974; Benjamin et al., 1977; Michaux et al., 1975). These findings suggested that lower toxicity but equivalent therapeutic effectiveness could be achieved with DNA-linked cytostatic drugs.

Meanwhile, striking differences in initial plasma levels between free and complexed DNR and ADM were demonstrated (Hulhoven et al., 1977; Staquet et al., 1977; Rozencweig et al., 1977; Kummen et al., 1978; Hulhoven, 1978). After infusion of ADM-DNA, the total plasma fluorescence remained higher than that for the free drug for a period of 8 h. High-performance liquid chromatographic determination of DNR and daunorubicinol, its active reduced metabolite, in human and rabbit plasma also suggested a slower disposition of the DNR-DNA complex (Hulhoven and Desager, 1976; Hulhoven et al., 1977a + b).

In acute lymphoblastic leukemia of childhood, the five-year survival rate is significantly higher in the group where ADM-DNA was introduced than in the DNR-DNA group. Even when the patients are subdivided into groups with good or poor prognosis, ADM-DNA-treated patients survive better, whatever their prognostic factors. Nevertheless, the statistical significance is adversely affected by dividing the patient group. The fact that a higher remission and survival rate is achieved in ADM-DNA-treated patients with favorable prognostic factors incites us to use such regimens to treat these patients.

The reason for the advantage of ADM-DNA over DNR-DNA is not obvious. It might be possible that ADM becomes more effective when linked to DNA. In this respect, it appears that DNR-DNA dissociates into the circulation (Hulhoven, 1978), and should only be considered as a 'slow-release' DNR. The possibility that ADM-DNA could be more effective than DNR-DNA when treating acute nonlymphoblastic leukemia (ANLL) is not excluded. No firm conclusions can be drawn from our study, since only a few ANLL patients were treated according to the protocol comparing DNR-DNA with ADM-DNA.

The latest induction protocol for ANLL includes the combination of cytosine arabinoside and DNR or DNR-DNA. The remission rate (88%) is excellent and the 64% one-year survival rate compares favorably with other regimens. At this moment, the therapeutic effectiveness of DNR and DNR-DNA appears to be identical.

Since no randomized study on the use of the complexes in solid tumors has been reported, and since promising results were obtained when treating metastatic breast carcinoma with ADM-DNA (Longueville and Maisin, 1975), a randomized trial was started in squamous cell bronchogenic carcinoma and anaplastic bronchogenic carcinoma (Bosly et al., 1978). Similar results were obtained with the combination of vinblastine and cyclophosphamide versus ADM-DNA. Furthermore, a similar therapeutic effectiveness between free and complexed ADM was noted in anaplastic bronchogenic carcinoma. This might suggest that a highly vascularized environment may be required to obtain a higher activity for the drug complexes (Staquet et al., 1977).

It has been advised that the total dose of ADM should not exceed 550 mg/m^2. The limiting dose for DNR is 750 mg/m^2. Our limited experience indicates that reduced cardiotoxicity, while therapeutic effectiveness remains identical, is the main important advantage when using DNR-DNA or ADM-DNA rather than the free drugs. Decreased cardiotoxicity could be due to lower uptake of the complexed drug by heart muscle (Langslet et al., 1974; Hulhoven, unpublished results).

Further pharmacologic investigations are needed to unravel the ways in which the complexes act. Indeed, a preferential incorporation into the lysosomes, as initially postulated, is not likely (Hulhoven, 1978). The slower disposition of the complexed drug could change the therapeutic effectiveness in certain circumstances, and the disappearance rate of the metabolites could be modified and influence the effectiveness when the drug is used as a complex. These points need further evaluation.

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