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Clinical and Pharmacologic Studies with Adriamycin-DNA Complex in Children with Malignant Disease

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Summary. *During the last 4 years, we have studied the adriamycin-DNA complex originally developed by Trouet and co-workers (1972). This paper summarizes the results of our pharmacologic and clinical studies.*

The complex is taken up by cells through an adsorptive pinocytosis, with DNA as the binding molecule. Excess DNA prevents uptake of the drug. Administration of the drug as the complex results in much higher serum concentration and a reduced urinary excretion. The complex is well tolerated, but side effects are probably of the same order as those seen with the free drug. An exception may be the heart. The acute toxicity is not seen when infusing the complex. Our experience with 20 children who have received more than 500 mg/m² indicates that the chronic cardiac toxicity may be reduced, too.

Spectacular, but anecdotal, results have been observed in a variety of solid tumors. Of 16 children with acute myelogenous leukemia, 14 went into a complete remission on a protocol of cytosine arabinoside in combination with the complex. Three of these children are now off therapy, with the longest observation period being 4 years and 4 months.

Introduction

Adriamycin is an anthracycline antibiotic closely related to daunorubicin, which has produced encouraging results in the treatment of neoplastic disease (Blum and Carter, 1974; Goldin and Johnson, 1975). The drug is toxic, and the most consistent side effects are alopecia, stomatitis, and myelosuppression. Perhaps the most disturbing side effects involve the heart, where both acute and chronic cardiomyopathy have been observed (von Hoff et al., 1977; Lefrak et al., 1973; Propper et al., 1975). The frequency of chronic heart failure is clearly related to the cumulative dose given, and an incidence of 30% has been reported in patients receiving more than 500 mg/m² body surface.

One of the main actions of these anthracycline drugs is that they form tightly bound intercalating complexes with DNA, thereby interfering both with DNA and RNA synthesis (Calendi et al., 1965; di Marco and Arcamone, 1975). Trouet et al. (1972, 1974, 1975) have developed a new principle in cancer chemotherapy based on the ability of these drugs to form stable complexes with DNA. Such complexes, formed in vitro, are unable to permeate cell membranes and are consequently not toxic to cells as such. However, the complexes can be taken up by cells through pinocytosis and delivered to the lysosomes of the cells. Here, the acid hydrolases will digest the DNA carrier, while the anthracyclines are resistant to the action of the enzymes. The drug molecules so liberated are now free to permeate membranes and diffuse to target areas of the cells. Cells with high pinocytic activities and DNA synthesis will be most affected. Since there is some evidence that the malignant cells are more active in pinocytosis than their normal counterparts (Easty, 1964; Gey, 1956; Ghose et al., 1962), this could lead to a selective effect on the malignant cells and reduce the toxic side effects. In mice, it has been shown that the complexes are superior to the free drug in the treatment of L 1210 leukemia (Atassi et al., 1975; Trouet et al., 1975). Sokal et al. (1973) have shown that the complex is well tolerated when tested in patients with leukemia.

We were stimulated by these observations and decided to investigate the effect of the complex in children with resistant malignant disease. This communication will review our experience during the last 4 years. The results show that the complex is well tolerated, is particularly effective in myelogenous leukemia, and that complexing may reduce the cardiotoxic effects of the free drug. Preliminary results have been presented elsewhere (Lie et al., 1975, 1977).

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Materials and Methods

Initially, only children who by independent physicians were considered to be beyond the help of available therapy were included in the study. As experience accumulated, children with myelogenous leukemia were given the complex as part of the initial protocol together with cytosine arabinoside. Informed consent from the parents was always obtained.

Adriamycin-DNA was prepared according to the method described by Trouet et al. (1972). In brief, DNA (herring sperm, type VII, Sigma, or calf thymus, highly polymerized, type V, Sigma) was dissolved in 0.15 M NaCl to a final concentration of 2.34 mg/ml, autoclaved at 120° C for 15 min, and cooled slowly. DNA solutions were used within 2 days.

Adriamycin (Farmitalia) was dissolved in distilled water and mixed with the DNA solution to a final concentration of 20 mg/100 ml. The complex so formed was administered as a slowly running infusion. The pyrogenicity of each DNA batch used was examined in rabbits. Complex formation was controlled by measuring the quenching of the fluorescence of the free drug when bound to DNA (Trouet et al., 1972).

Blood and urine concentrations of adriamycin were measured as described earlier (Arena et al., 1971; Bachur et al., 1970, 1974; Benjamin et al., 1974; Kummen et al., 1978). Uptake studies in cells in vitro were performed using freshly isolated leukemic cells or fibroblasts grown in culture (Lie and Lie, 1977).

Results

Pharmacologic Studies

Cellular Uptake. The cellular uptake of free adriamycin and of adriamycin-DNA complex has been studied in our laboratory, using both fibroblasts in culture and freshly isolated leukemic cells from patients with dominantly blast cells in peripheral blood (Lie and Lie, 1976, 1977). The results in these two cell types are similar and are summarized below.

Uptake of the free drug is temperature dependent and proportional to the concentration of the drug in the tissue culture fluid. Uptake is strongly pH dependent, increasing progressively as the pH of the medium is increased. Fluorescence microscopy of the cells has shown that the drug is located mainly in cytoplasmic granules and nuclei.

The uptake of the complexed drug is remarkably similar to that of the free drug at low medium concentrations (less than 1 μg/ml). At increasing concentrations, however, a saturation level is obtained, so that further increase in medium concentration of the drug complex does not lead to any further increase in uptake velocities (Fig. 1). Analysis of the data has shown that this uptake is probably receptor mediated, with DNA binding to a limited number of receptors on the cell surface. The presence of excess DNA in the tissue culture thus prevents uptake of adriamycin. Uptake of the complex (and thereby the DNA molecule) is again strongly related to the pH of the medium, increasing progressively as the medium is made more alkaline.

Efflux of the drug from cells in vitro is strongly temperature dependent. When nontoxic concentrations were used during uptake (less than 1 μg/ml), no fluorescence remained after 8 h when the cells were kept in drug-free medium at 37° C. In contrast, no chase could be detected if the cells were kept at 4° C during this interval. The results were the same whether the cells received the drug in complexed or free form.

Studies in several different leukemic cells show in principle the same results, but quantitatively great differences in uptake were detected. Table 1 shows the uptake in various leukemic cells when the same cells have been exposed to free and to complexed drug. There is a great variation in uptake characteristics between cell lines.

Observations in Children. Detailed analysis of these studies has recently been published (Kummen et al., 1978). Six children with malignant disease were given adriamycin in free or complexed form. The two types of the drug were given to the same child in the same manner with 3–4 weeks interval, thereby excluding genetic

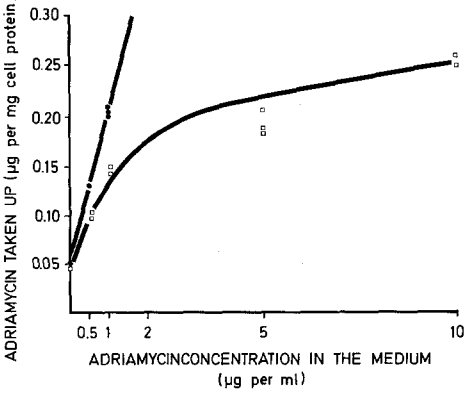

Fig. 1. Adriamycin uptake in leukemic blast cells when exposed to free (●—●) or complexed (□—□) drug

Table 1. Uptake of adriamycin in leukemic blast cells when given in free and complexed form

Patient	Adriamycin (1 μg/ml)	Adriamycin-DNA (5μg/ml)
KS	0.17[a]	0.43
BN	0.25	0.20
ETB	0.13	0.18
KU	1.0	0.77
TF	0.06	0.04
GE	0.18	0.22

[a] Expressed as μg adriamycin taken up per mg cell protein per 8 h

variations when comparing the results. Blood and urine were collected at specific intervals and total fluorescence measured as described.

Children who were administered free drug as a continuous infusion obtained a serum concentration of about 0.2 μg/ml plasma. The concentration obtained is rather similar from one patient to another when comparable dosages are given. However, in children receiving the complexed drug, a much greater variation in serum concentrations is observed. Table 2 shows the peak values obtained in plasma in these six children. A spread from 0.7 to 8.2 μg/ml plasma indicated that the capabilities of clearing the complex from plasma vary markedly from one child to another.

In urine, approx. 8% of the total dose given was recovered in children receiving the free drug, while only 4% was found after complex infusion. A smaller glomerular filtration of the complex probably explains this greater retention.

Table 2. Peak serum concentrations obtained when infusing adriamycin in complexed or free form (μg/ml plasma)

Patient	Adriamycin	Adriamycin-DNA
AT	0.2 (60)[a]	2.2 (60)
KS	0.3 (40)	0.8 (32)
SH	0.3 (42)	0.7 (21)
AF	0.3 (52)	6.0 (52)
EFI	0.2 (39)	8.2 (39)
RB	0.2 (59)	3.0 (35)

[a] Dose given per m^2 body surface

Clinical Effects

Toxicities. In the beginning of our study, our principal aim was to test the tolerance and efficiency of the complex. All patients were monitored for the presence of DNA antibodies, antinuclear antibodies, and LE cell phenomenon. The results for the first patients treated are presented in Table 3. During the first 2 years, only children who by independent physicians were considered to be beyond the help of available therapy were included in the study. After 200 infusions of the drug, the following conclusions can be established.

The complex is well tolerated, and no immediate, serious side effects have been observed.

Only freshly autoclaved DNA should be used. DNA solutions that have been kept for more than 2 days before use tend to give febrile reactions. With newly autoclaved DNA, an occasional febrile episode is easily controlled by antipyretics and does not indicate cessation of further therapy. No evidence of allergic or anaphylactic reactions have been observed, and we have not found any indication of anti-DNA activity in any of the first 30 patients monitored closely for such reactions.

The complex is time consuming both to prepare and to infuse.

The degree of alopecia, stomatities, and bone marrow depression is probably not different in patients receiving the free drug compared with those receiving the complex. In children receiving both forms at equal dosages, the nadir of white cell count was not significantly different.

The most serious side effects of adriamycin involve the heart. We have previously published data showing that the acute cardiotoxic effect of adriamycin is com-

Table 3. Effect of adriamycin-DNA (A-DNA) in children with solid tumors in relapse

Diagnosis	Age (years)	Previous therapy (months)	Total dose of A-DNA (mg/m^2)	Response	
				Type	Duration (days)
Neuroblastoma					
JEM	$1^1/_2$	2	329	P.R.	60
RK	6	3	760	P.R.	180
SMI	3	2	150	N.R.	–
KL	2	0	380	P.R.	160
Wilms' tumor					
EG	7	6	390	C.R.	180
AF	6	6	80	C.R.	240
Embryonic testis tumor	3	6	325	C.R.	230
Hepatoblastoma	13	1	360	P.R.	120
Retinoblastoma	3	3	416	P.R.	90
Ewing's tumor	4	24	331	C.R.	250
Rhabdomyosarcoma	4	0	240	P.R.	14
Malignant lymphoma	11	1	180	P.R.	21

pletely eliminated when the drug is complexed with the DNA molecule (Langslet et al., 1974). In this system, the isolated rat heart was used, and various parameters such as pulse frequency, ECG changes, and contractile force were continuously recorded. The presence of DNA in the perfusate completely inhibited the gross changes induced by the free drug, provided that enough DNA was present to bind the drug molecules.

The chronic progressive cardiomyopathy is the important side effect today and is clearly related to the accumulated dose administered (von Hoff et al., 1977; Lefrak et al., 1973; Propper et al., 1975). An upper limit of 500 mg/m^2 is therefore practiced in most centers, and it may well be that children are more sensitive to this complication than adults (von Hoff et al., 1977; Propper et al., 1975). Because the complex has no cardiotoxic effects in the rat model, we have not had any strict upper limit in children whose disease responds to adriamycin. Table 4 shows the experience with the children who received more than 500 mg/m^2. One child developed a slight cardiopathy that was rapidly reversed on cessation of therapy and digitalis. She finally died from her disease – and no changes attributable to adriamycin were found in the heart. In the other patients, no clinical signs of cardiac disturbance were detected. Sixteen children relapsed and died from their disease, and a detailed pathologic examination of the heart did not reveal any signs of cardiac damage. Although no firm conclusions can be drawn from this limited experience, the data seem to indicate that a certain degree of protection is offered when the drug is administered as a complex bound to DNA.

Table 4. Cardiac toxicities in children receiving more than 500 mg/m^2 of adriamycin in complexed form

Patient	Sex	Age (years, months)	Total dose in mg/m^2	Clinical signs of cardiac toxicity	Autopsy changes
1. Acute leukemias					
TF	♀	2, 6	1200	0	0
KS	♀	11, 0	934	0	0
OMAS	♂	4, 11	827	0	0
BN	♂	6, 10	760	0	0
SAL	♂	7, 9	700	0	0
BH	♀	0, 11	700	0	Alive
LEB	♂	8, 6	550	0	Alive
RBH	♀	1, 1	500	0	Alive
2. Neuroblastomas					
RK	♂	6, 8	675	0	0
JAS	♀	2, 5	600	Mild degree of cardiac decompensation	0
AS	♀	1, 2	584	0	0
HÅ	♂	5, 9	560	0	0
DG	♂	0, 8	540	0	Alive
3. Malignant lymphomas					
KL	♂	14, 0	1175	0	0
SH	♂	3, 1	710	0	Alive
TS	♂	10, 4	557	0	0
4. Other solid tumors					
AF	♀	5, 10	870	0	0
KAJ	♂	13, 1	795	0	0
KØ	♀	1, 4	557	0	0
IMH	♀	5, 1	532	0	0

Therapeutic Effects. Spectacular, but anecdotal, results have been observed in a variety of embryonic tumors in children. Complete regression of metastasis in three cases of Wilms' tumor, one case of testicular embryonic carcinoma, and one case of neuroblastoma with cerebral metastasis has been observed. However, all cases relapsed after 6–12 months, and no conclusion can be drawn from these observations. More detailed investigations have been done in children with acute myelogenous leukemia.

Since January 1974, 16 children with acute myelogenous leukemia in our department have been treated according to a protocol consisting of cytosine arabinoside (100 mg/m^2 × 2 on days 1, 2, and 3) and adriamycin-DNA complex (60 mg/m^2 on day 4). The courses were repeated with 14–16 days interval three or four times. Maintenance therapy did not follow any strict protocol. Most patients received the same course as during induction therapy, once monthly for the first year and every sixth week during the second year. However, one child who is still in remission ceased therapy after having gone into remission and is still free of disease 4 years later. Six children used 6-mercaptopurine in addition to the maintenance courses, but all have relapsed later.

With this therapy, 14 of the 16 children went into complete remission (remission rate of 87%). Most of the

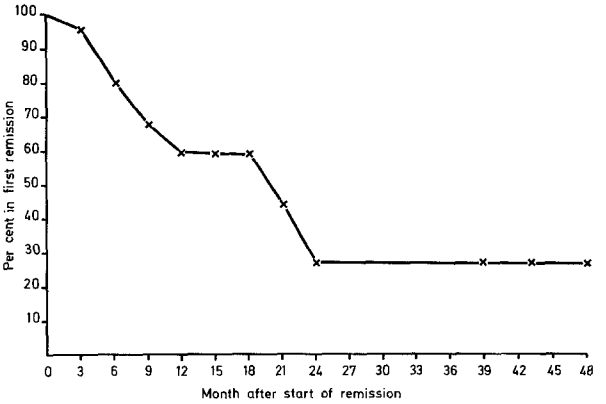

Fig. 2. Actuarial duration of remission in children with acute myelogenous leukemia

children tolerated the protocol well, and three children went into remission with only one week's stay in the hospital. Figure 2 shows the actuarial remission duration curve. Three children (19%) are now off therapy with the longest observation time being 4 years and 4 months. It is worth mentioning that two of these children developed a pneumocystis pneumonia during induction therapy, while the third developed an erysipelas and later an osteomyelitis. Our results compare favorably with the best that have been published, and detailed analysis of our experience will be published later.

There is some evidence to suggest that the complex might be superior to the free drug in the treatment of leukemic patients. Table 1 presents the results of uptake experiments using freshly isolated blast cells from patients and measuring uptake of the drug given in either the free or the complexed form. It can be seen that although the serum concentration when receiving free drug very seldom exceeds 0.2 μg/ml, concentrations of even 1 μg/ml seldom lead to more uptake than complexed drug when present in 5 μg/ml which is a concentration frequently observed in children receiving the complex. The presence of high concentrations of the drug in the circulation for a prolonged period of time might give a greater uptake in the leukemic cells. However, only controlled clinical trials will prove whether the complex indeed is superior to the free drug in the treatment of leukemia.

Discussion

Despite all the progress we have witnessed in the treatment of childhood malignancies in recent years, it remains a fact that half of the children die of their disease and that the intensive therapy offered today involves much morbidity. The need for better drugs and better use of existing drugs is therefore a continuous challenge. This paper has described our experience with the adriamycin-DNA complex developed by Trouet et al. (1972).

We have found that the complex is well tolerated by all our patients and many of them have been treated on a wholly outpatient program. No anti-DNA activity has been observed. The side effects, apart from the cardiotoxicity, are probably the same as with the free drug.

In a previous study, we showed that the presence of DNA dramaticly protects isolated rat hearts from the cardiotoxic effect of adriamycin (Langslet et al., 1974). Twenty children have received more than 550 mg/m^2 with one child developing an easily controlled cardiomyopathy. In 16 children receiving more than 550 mg/m^2 who finally died of their disease, no change in the heart could be detected. This could indicate a certain degree of protection, especially since leukemic children have been reported to develop cardiotoxicity very early (Propper et al., 1975). However, the number of patients treated is small, and no definite conclusions may be drawn from our results so far.

One patient with acute myelogenous leukemia developed a rapidly progressive retinopathy 1 year after he went into a complete remission. It is impossible to say whether this was related to the therapy or to the disease. We have not found any evidence of retinal damage in the other patients treated, and it has not been observed in the patients treated by Sokal et al. (Trouet, personal communication) in Belgium.

The tumors that should respond most favorably to the lysosomotropic principle should of course be those with high pinocytic abilities and located so that the complexes can reach the malignant cell easily. A priori, one might expect different malignant cells to differ in their pinocytic activities, and some conflicting evidence in the literature could support this notion (Easty, 1964; Gey, 1956; Ghose et al., 1962). The most dramatic responses that we observed in solid tumors were with Wilm's tumor, embryonic testis carcinoma, and Ewing's tumor. However, all responders relapsed within a year – and no effect of the complex could be detected in these children at these stages. In summary, our experience in solid tumors does not allow conclusion as to whether the complex is superior to the free drug, but resistance certainly may develop.

In acute myelogenous leukemia, 14 of 16 new cases went into complete remission, while one went into a partial remission lasting 1 year. The only nonresponder was a retarded 13-year-old boy with skeletal anomalies, where constitutional factors might have been of importance. We have been impressed with the ease with which these children have gone into remission. Three children are now off therapy.

In published studies, the use of free adriamycin and cytosine arabinoside have induced remission in 60–80% (Evans et al., 1975; Gale and Cline, 1977; Preisler et al., 1977). Although children are better responders than adults (Ansari et al., 1975), our results certainly justify further work on this combination.

In conclusion, we have found that adriamycin-DNA complex is a well tolerated and most effective cytostatic principle in resistant childhood malignancies. Especially encouraging results have been obtained in acute myelogenous leukemia, Wilms' tumor, embryonic testis tumor, and Ewing's sarcoma. However, although our results suggest that the complex is more active (and perhaps less toxic) than free adriamycin in some cases, we believe that only controlled clinical trials in selected malignancies can settle this question.

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