Preliminary Clinical Assessment of I.C.R.F. 159 in Acute Leukaemia and Lymphosarcoma

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Summary: I.C.R.F. 159, a new antitumour agent, has been assessed in six patients with acute leukaemia and three with lymphosarcoma. In all but two there was a considerable fall in circulating primitive cells, and in one there was bone-marrow evidence of a partial remission. Severe toxic effects were seen in only one case; they consisted of alopecia and gastroenteritis. It is suggested that I.C.R.F. 159 is worth further examination in all forms of acute leukaemia and lymphosarcoma.

INTRODUCTION

It is generally agreed that certain criteria should be met before a potential anticancer agent receives clinical trial. The most important of these are that the agent should be capable of completely inhibiting the growth of at least one and preferably more than one experimental tumour. Its effect should be consistently reproducible and the response produced should be related to the dose of the agent, which should be active by a clinically acceptable route. The agent should clearly have an adequate margin of safety—that is, a “reasonable” therapeutic index. If in addition to meeting these criteria it is also a representative of a new class of chemical compounds it would be difficult to deny it an adequate clinical trial. The two compounds I.C.R.F. 154 (1,2-bis(3,5-dioxopiperazin-1-yl)ethane) and I.C.R.F. 159 ((±)-1,2-bis(3,5-dioxopiperazin-1-yl)propane) meet most of these requirements. Although their mechanism of action is not known, it seems very likely that they are anti-metabolites, acting as inhibitors of ribonucleic acid or more likely of deoxyribonucleic acid synthesis.

The parent compound I.C.R.F. 154 arose during an investigation of non-polar derivatives of ethylene diamine tetra-acetic acid (E.D.T.A.). In an attempt to synthesize the tetra-amide of E.D.T.A. the vigorous reaction conditions resulted in the formation instead of the cyclic imide, the bisdiketopiperazine, I.C.R.F. 154.

Both I.C.R.F. 154 (R=H) and 159 (R=CH₃) were remarkably effective in totally inhibiting the growth of sarcoma 180 and adenocarcinoma 755, doubling the survival times of mice inoculated with the leukaemia L1210, and significantly inhibiting the growth of the Walker 256, Yoshida and Lewis lung tumours, and the Cloudman melanoma. They also produced

inhibition of growth and distinctive chromosomal abnormalities of mouse L cells in tissue culture at very low concentrations (Carter *et al.*, 1969). Full details of much of the experimental work will be published shortly and a brief summary of some of it will be found elsewhere (Creighton, Hellmann, and Whitecross, 1969).

This paper deals with pilot clinical assessments of I.C.R.F. 154 and 159 in acute leukaemia and lymphosarcoma.

PATIENTS AND METHODS

Patients who received the drugs had come to the end of conventional therapy and were essentially in the terminal stage of their disease. Daily haemoglobin estimations and platelet and white cell counts were done in each case. Bone-marrow examination and chemical, bacteriological, and radiological investigations were also carried out as and where indicated.

Both compounds were administered by mouth in the form of 250-mg. tablets.

I.C.R.F. 154 was given to five patients aged 5 to 81 years. Dosage ranged from 7 to 200 mg./kg./day and was given over a period of 4 to 14 days.

I.C.R.F. 159 was given to nine patients aged 2½ to 75 years. Dosage ranged from 7 to 109 mg./kg./day, and the period of treatment varied from one to 12 days. The types of malignancy treated are shown in the Table.

When it became clear that in patients with a high white cell count extremely rapid falls in total count might occur, patients were always given allopurinol from before treatment until a few days after treatment with I.C.R.F. 159 ceased. Patients taking prednisone continued with it, but attempts were made where appropriate to reduce the dose of the drug.

RESULTS

I.C.R.F. 154

I.C.R.F. 154 was without effect on either acute lymphoblastic, myeloid, or monocytic leukaemia, and there appeared therefore to be no grounds for giving it to patients with lympho-

I.C.R.F. 159 in the Treatment of Acute Leukaemia and Lymphosarcoma

Case	Age	Diagnosis	Total Dose (g.)	No. Days Treated	Average Dose mg./kg./day	Effect on Disease	Side-effects	Comment
1	66	Myelo-monocytic	14	4	40	Significant and continued fall of total W.B.C.	Uric acid deposition in kidneys	Died 5 days after start of treatment of cardiac failure
2	11	Lympho-sarcoma	(i) 2.5 (ii) 1.0 (iii) 1.5 (iv) 1.25	1 2 2 5	71 14 21 7	Sharp drop in primitive cells	Gout	Marrow unchanged. Alive and well 3½ months after start of treatment
3	5	Lympho-sarcoma	7	3	109	Reduction of gland size. Drop in primitive cells	Nil seen	Died 4 days after start of treatment
4	10	Myelo-monocytic	(i) 5.5 (ii) 1.5 (iii) 0.75 (iv) 1.25 (v) 2.25	6 2 1 5 3	30.5 25 25 8 25	Sharp drop in monocytoid cells	Diarrhoea for 7 days. Alopecia	Marrow unchanged. Alive and reasonably well 3 months after start of treatment
5	75	Myelo-monocytic	6	2	43	No effect No effect Nil	Nil seen	Died 3 days after start of treatment of cardiac failure
6	56	Lympho-sarcoma	5	2	27	Reduction of spleen size	Nil seen	Died 10 days after start of treatment
7	4	Acute blastic	4	3	72	Sharp drop in abnormal W.B.C.	Nil seen	Died 5 days after start of treatment
8	5	Acute lymphatic	(i) 1 (ii) 2.625	2 12	31 13	Drop in blast cells and polymorphs	Nil seen	I.C.R.F. 159 replaced by other therapy without avail
9	2	Acute lymphatic	1.25	4	26	Drop of blast cells at first, then rise Partial remission (bone marrow)	Nil seen	Alive and well 3 weeks after start of treatment

sarcoma. Since the large doses of I.C.R.F. 154 administered to these patients had also no overt toxic effect, it is unlikely that even greater doses would have produced a therapeutic effect. This inactivity is attributed to very poor absorption of the compound from the intestinal tract, for which there is evidence (unpublished) in rats and monkeys dosed by mouth.

I.C.R.F. 159

In contrast to I.C.R.F. 154, the analogue I.C.R.F. 159 showed considerable leukopenic activity in nearly all patients. Salient features of three typical cases are summarized below.

Case 2.—Boy aged 11 years. Diagnosis: lymphosarcoma (histologically proved) of four years' duration treated in the past with, and now resistant to, radiotherapy, mercaptopurine, methotrexate, prednisone, cyclophosphamide, vincristine, and rubidomycin. He was treated with 1 g. of I.C.R.F. 159 three times a day for one day, but vomited up half the last dose and therefore received a total of about 2.5 g. spread over 24 hours. This had the effect of reducing his total white cell count from 42,000 (36,540 primitive, 4,200 lymphocytes, and 1,260 neutrophils) to 8,500/cu. mm. within 48 hours; the following day it was less than 1,000 (603 primitive, 90 lymphocytes, and 207 neutrophils), and remained so for one week, when it again rose to over 1,000, though now with no immature cells. Immediately after his treatment he developed an acute attack of gout, which improved rapidly on phenylbutazone and allopurinol. Since then he has required three further courses of treatment, each of which resulted in a reduction of blast cells and neutrophils. Though he is alive and well at the present time, three and a half months after the start of treatment, there are still from 1 to 5% blast cells in his peripheral blood, and the marrow shows leukaemic infiltration. He has required blood transfusions from time to time.

Case 8.—Girl aged 5 years. Diagnosis: acute lymphoblastic leukaemia. Presented two years previously with extreme weakness. Since then she had been treated successively with prednisone, mercaptopurine, vincristine, methotrexate, rubidomycin, cyclophosphamide, cytosine arabinoside, and asparaginase. She became unresponsive at therapeutic doses to each of them in turn and was then given I.C.R.F. 159 0.25 g. twice a day for two days. Within 72 hours her total white cell count was reduced to 0.33 (19,900 to 6,700/cu. mm.) of its value before treatment, and the blast cells to 0.17 (12,600 to 2,200/cu. mm.). At the same time the lymphocyte count fell to only 0.67 (5,400 to 3,620/cu. mm.) and the neutrophil count to 0.61 (1,200 to 735/cu. mm.). Whereas the neutrophil count continued to decline after treatment with I.C.R.F. 159 was stopped, the other cells, particularly the blast cells, began to rise again almost immediately. She was therefore given another course

of treatment with I.C.R.F. 159, this time receiving 0.25 g. twice a day for three days followed after an interval of two days by a maintenance dose of 0.125 g. once daily for nine days. Though initially there was again a drop in blast cells, they began to increase again on the maintenance dose, whereas neutrophils declined further to only 90/cu. mm. Treatment with I.C.R.F. 159 was then stopped, supportive therapy was given, and asparaginase was tried once more, though this time without avail, and she died four days later.

Case 9 (see Fig.).—Boy aged 2 years. Diagnosis acute lymphoblastic leukaemia. He presented six months earlier with an enlarged liver and spleen and palpable lymph glands. He was treated with prednisone, vincristine, methotrexate, mercaptopurine, and cyclophosphamide, either alone or in combination, followed when resistant to all these substances by pertussis vaccine and a further attempt to induce remissions with vincristine and then with mercaptopurine, but without success.

Before treatment with I.C.R.F. 159 began his platelet count was low, and he was pale, listless, and bleeding from the mouth. The total white cell count was 3,200/cu. mm., of which 768 were neutrophils and 192 were blasts. He then received 250 mg. of I.C.R.F. 159 twice a day for four days, but vomited up three out of the eight doses. His total intake over four days was therefore probably 1.25 g. This had the effect of gradually reducing his total white cell count to 320/cu. mm. one week after the start of treatment. One week later his total white cell count had risen to 1,300/cu. mm., with 858 neutrophils and no blast cells. There were 36,000 platelets/cu. mm. The report on his marrow just before treatment with I.C.R.F. 159 was: "Sheets of lymphoblasts; occasional polymorphs. Erythroid cells and megakaryocytes not seen." A marrow specimen examined one week after the start of treatment showed that the sheets of lymphoblasts had disappeared, but some scattered blasts were still present. Regenerating normoblasts and polychromatic red blood cells were seen but little myeloid tissue and no megakaryocytes. A further marrow examination another week later showed the presence of megaloblasts in addition to the remaining lymphoblasts. This partial remission was accompanied by a definite improvement in the patient's general condition.

DISCUSSION

The rapid falls in total white cell counts were accompanied by very little evidence of toxicity. That these rapid falls reflect cell destruction is evident from the deposition of large amounts of uric acid in the renal calices and pelves of Case 1. There was no evidence of the presence of I.C.R.F. 159 in the fine yellow deposit of uric acid removed from the kidneys. It was felt, however, that the degree of renal damage seen at necropsy was greater than could be accounted for by the deposition of uric acid alone.

Apart from the effects of rapid cell destruction, toxic signs attributable to the drug were seen only in Case 4. This patient showed serious side-effects, which must be attributed to the compound, and the diffuse alopecia resembled that produced by some other anticancer agents, notably cyclophosphamide. Though the gastrointestinal effects in Case 4 were severe, they have not been seen in any other patient of this series, though milder gastrointestinal effects have been encountered in two patients in another series of cases (Hellmann *et al.*, 1969). It remains to be seen if administration of the drug by other routes will overcome this problem.

In the six patients who are now dead no toxicity definitely attributable to I.C.R.F. 159 was seen apart from a fall in neutrophils in Case 8. The same effect might possibly have been seen in the others had it not been obscured by their high primitive white cell counts or low initial neutrophil counts. Among the three survivors, one (Case 2) has shown no evidence of drug toxicity, even though he has now had four courses, with a total intake of 6.25 g.

In contrast to the indifferent response obtained with I.C.R.F. 159 in Case 8, Case 9, who had also received all conventional drugs (though not cytosine arabinoside or asparaginase), responded well, went into partial remission, and four weeks after being admitted as moribund was discharged from hospital.

The longest survivors to date are Case 2 and Case 4, who were first given I.C.R.F. 159 on 5 November and 21 November 1968 respectively. Both still have leukaemic infiltration of the bone marrow, but Case 2 has been weaned off corticosteroids and is clinically well. His peripheral blood picture is virtually normal, and, from being in the terminal stages of his disease when treatment with I.C.R.F. 159 began, he has now been sent back to school.

Case 2 has responded to each of four courses of treatment, and, though he was known as a "good responder," this encourages the hope that there may be other patients in whom the compound—either alone or in combination with other drugs—will control the disease even in the presence of some leukaemic bone-marrow infiltration.

It would appear from this preliminary clinical trial that I.C.R.F. 159 is an effective cytotoxic agent, and as such is of potential interest in the treatment of the leukaemias and lymphosarcomas. Because I.C.R.F. 159 belongs to a new class of antitumour compounds, no cross-resistance was expected and none was seen, even in patients who had received a wide range of antileukaemic chemotherapy.

A dose of 25 to 35 mg./kg./day for not more than four days seems to be a reasonably safe and acceptable course. Greater amounts might be given in cases in which a large amount of malignant tissue is present. Further courses may be given depending on the blood picture. It is desirable to give allopurinol at the same time as I.C.R.F. 159 on every occasion. Corticosteroids do not seem to inhibit or promote the activity either experimentally or clinically, and reduction in the dosage of prednisone did not affect the response to I.C.R.F. 159.

Though the number of patients treated so far is too small to be certain of the optimum conditions for administration, it is worth noting that 30 mg./kg./day of I.C.R.F. 159 by mouth is also a very effective antitumour dose in mice. On the other hand, lower doses by mouth of I.C.R.F. 154 are required to achieve the same antitumour effect as I.C.R.F. 159 in mice. It is therefore surprising that in man very large doses of I.C.R.F. 154 were so well tolerated and without any antileukaemic activity. This is probably owing to failure of absorption—a property which, if confirmed, may point to the use of I.C.R.F. 154 in gastrointestinal malignancies.

No direct cytotoxic effects of I.C.R.F. 154 or 159 on resting cells have been noted in animals or in vitro, but it is difficult to believe that the drastic and rapid falls in circulating primitive white cells were due only to an inhibition of their further production, without a direct cytotoxic action on them. This augurs well for the use of I.C.R.F. 159 in other malignancies.

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Medical Memoranda

Renal Medullary Cystic Disease

[WITH SPECIAL PLATE FACING PAGE 809]

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Medullary cystic disease of the kidney is a very rare disorder. It is characterized by the insidious onset of uraemia, usually in childhood or adolescence, and is often accompanied by renal osteodystrophy but rarely by hypertension. The diagnosis is usually made at necropsy, but occasionally it is made during life on the basis of the clinical history and the findings on renal biopsy. These patients have always been uraemic when first seen, and it has not previously been thought possible to obtain an intravenous urogram in any case. With the use of high-contrast dosage and tomography we have obtained x-ray films of useful quality in a patient with this abnormality, giving appearances which are considered diagnostic.

CASE REPORT

A 10-year-old girl was admitted to Margate General Hospital and transferred the following day to the renal unit at Guy's Hospital for the investigation and treatment of her renal failure. For the previous two years she had been generally unwell with increasing pallor, polydipsia, and polyuria; during this time there had been several episodes of fever and dysuria which had subsided spontaneously. For one week she had had vomiting, abdominal pain, and increasing lassitude. There had been no episodes of loin pain or haematuria. In the past she had had two operations for congenital dislocation of the hip. She had four siblings, and neither they nor her parents were known to have renal disease.

On examination she was pale, small for her age (height below the 10th percentile, weight below the 3rd percentile), drowsy, and dehydrated. There was no skeletal deformity. Pulse was 110/min. and B.P. 90/60. The lower pole of the right kidney was palpable but not tender.

Investigations revealed anaemia (haemoglobin 5.1 g./100 ml.; M.C.H.C. 34%) and renal failure (blood urea 520 mg./100 ml.;