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The Clinical Pharmacology of the Adenosine Deaminase Inhibitor 2′-Deoxycoformycin

John F. Smyth[1], Rosanne M. Paine[1], Ann L. Jackman[1], Kenneth R. Harrap[1], Marvin M. Chassin[2], Richard H. Adamson[2], and David G. Johns[2]

[1] The Institute of Cancer Research, Sutton, Surrey, England
[2] Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes Of Health, Bethesda, Maryland 20205, USA

Summary. *2′-deoxycoformycin (2′-dCF; Pentostatin), a stoichiometric inhibitor of mammalian adenosine deaminase (ado deaminase), exhibits immunosuppressive and antilymphocytic activity in animal test systems. A clinical pharmacology/phase I study of 2′-dCF administered as a single agent has been completed (18 patients). Dose levels ranged from 0.1 mg/kg × 1 to 0.25 mg/kg/day × 5; ado deaminase and 2′-dCF were measured spectrophotometrically. Plasma decay curves were bi-exponential (α and $\beta t_{1/2}$ values about 1 and 10 h respectively). Recovery of unchanged 2′-dCF from urine (48 h) was 32%–48% of the administered drug. Major toxic manifestations were lymphocytopenia (all patients) and urate nephropathy (1 patient, with subsequent patients in the series receiving allopurinol, 300 mg/day). Three partial responses were seen in seven patients with acute lymphocytic leukaemia receiving 0.25 mg 2′-dCF/kg/day × 5.*

Introduction

2′-Deoxycoformycin (2′-dCF) is a potent inhibitor of the enzyme adenosine deaminase (ado deaminase) E. C. 3.5.4.4.). Characterised by Woo et al. [27], 2′-dCF is a unique nucleoside obtained from fermentation broths of *Streptomyces antibioticus*. As a transition-state analogue of the ado deaminase-catalysed reaction deaminating adenosine to inosine, 2′-dCF has been shown to be a tight-binding but reversible inhibitor of human ado deaminase with a Ki of 2.5×10^{-12} *M* [2]. Interest in the clinical use of such an effective inhibitor of ado deaminase stems from two considerations – firstly its potential for specific lymphocytotoxicity, immunosuppression, and the treatment of lymphoid malignancies, and secondly for the potentiation of adenosine analogues whose antiviral and antineoplastic activity is restricted by deamination to inactive metabolites.

An association between ado deaminase activity and lymphocyte metabolism was first indicated by distribution studies showing that although the enzyme is detectable in virtually all mammalian tissues, activity is greatest in those of the lymphoid system, such as lymph nodes, spleen, and thymus [5, 6, 10]. Ado deaminase activity has been shown to increase threefold in antigenically stimulated lymphocytes [14, 16] and as much as 23-fold in the blast cells of patients with acute lymphocytic leukaemia [23, 24].

Conclusive evidence indicating the specific requirement of lymphocytes for the presence of this enzyme was provided by the demonstration that the congenital absence of ado deaminase, arising as an inborn error of metabolism, gives rise to a specific defect in lymphocyte maturation and function. Giblett et al. [13] and Dissing and Knudsen [11] described the first cases in which children born with ado deaminase deficiency presented with severe combined immunodeficiency disease (SCID), having a selective defect in the production of both B and T lymphocytes, without deleterious effects on the other haematopoietic elements. A number of other clinical manifestations of ado deaminase-deficient children have been described, including abnormalities of bone and hair growth, but these findings were not confined to ado deaminase-deficient SCID patients and may represent secondary effects resulting from immunodeficiency and failure to thrive [15].

Attempts to mimic the SCID situation arising from ado deaminase deficiency have shown that inhibition of this enzyme with 2′-dCF results in immunosuppression, as measured by inhibition of the

Reprint requests should be addressed to: J. F. Smyth at Medical Oncology Unit, Western General Hospital, Edinburgh EH4 2XU

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delayed hypersensitivity skin response, allograft rejection, and impaired lymphocyte response to mitogen stimulation in vitro [1, 7, 8]. In vivo 2′-dCF is remarkably non-toxic to animals, with the exception of effects seen against the lymphoid system. Parenteral administration to mice [25], dogs, and monkeys[1] results in selective and reversible lymphocytotoxicity. The LD_{50} of 2′-dCF for BDF_1 mice is 100 mg/kg when injected IP, or 55 mg/kg when given by the IV route[1]. At these doses, toxicity was confined to the lymphoid system, with death attributable to overwhelming opportunistic infection. Measuring the duration of inhibition of ado deaminase in BDF_1 mice carrying the L1210 leukaemia, LePage et al. [18] have shown that single doses of 0.25 mg 2′-dCF/kg (1/400 LD_{50}) result in more than 80% inhibition of intracellular ado deaminase for periods in excess of 24 h. Based on these data, 0.1 mg/kg was chosen as the initial dose of 2′-dCF for phase I/clinical pharmacological studies in man.

In this study, 2′-dCF has been administered to patients with advanced refractory cancer, including those with and without malignant disease of the lymphoid system. The objective was to study the toxicity of 2′-dCF in man and to determine the pharmacokinetics of the plasma decay and urinary excretion of the drug. The degree of inhibition of lymphocyte ado deaminase activity was also measured.

Materials and Methods

2′-Deoxycoformycin was supplied as a white crystalline powder by the Natural Products Branch, National Cancer Institute, and prepared as a colourless liquid for IV administration. Because of its instability at acid pH [27], the compound was dissolved in 4.2% sodium bicarbonate and sterilised by filtration. Stock solutions containing 0.5 mg/ml were kept at 4°, and stability testing by measurement of ado deaminase inhibition showed that there was no loss of biological activity over periods in excess of 12 weeks.

Purified calf intestinal adenosine deaminase (specific activity 200 units/mg protein) was obtained from the Boehringer Corporation, London, England, and Indianapolis, Indiana, USA. Adenosine was obtained from the Sigma Chemical Co., St. Louis, Mo., USA.

Adenosine Deaminase Assay

Lymphocytes were separated from freshly obtained heparinised blood on ficoll-hypaque gradients by the method of Böyum [4].

Ado deaminase activity was determined spectrophotometrically in sonicated cell supernatants by the method previously published [24], following the linear decrease in optical density at 265 n*M* as adenosine is deaminated to inosine.

2′-Deoxycoformycin Assay

2′-Deoxycoformycin was measured in plasma and urine by the ado deaminase enzyme inhibition titration assay previously described [9].

Since 2′-dCF is a tight-binding, stoichiometric inhibitor of ado deaminase, equilibrium between the inhibitor and the enzyme is not reached for many hours [2]. The relatively short preincubation time of 10 min used routinely in this assay is accurate to nanomolar concentrations of 2′-dCF, but for enhanced sensitivity at prolonged time periods following drug administration (when plasma levels were predicted to be at subnanomolar levels) the preincubation period was extended to 100 min. Between collection and assay, plasma and urine samples were stored in liquid nitrogen. To prevent any loss of 2′-dCF activity due to instability at low pH, $NaHCO_3$ was added to urine samples prior to storage to raise the pH to neutrality. Standard curves with known concentrations of 2′-dCF were routinely run for both the 10-min and the 100-min preincubation assays. Pharmacokinetic analysis was accomplished with the use of the NIH-22 Pharmacokinetics Program [19].

2′-Deoxycoformycin was administered by direct slow IV injection. The doses used were 0.1 mg/kg, 0.25 mg/kg, and 1.0 mg/kg given once only, and 0.1 mg/kg and 0.25 mg/kg daily × 5. A minimum of three patients were treated at each dose level, with the exception of 1.0 mg/kg × 1 which was tested in only one patient, owing to limited availability of 2′-dCF and to the large quantities required at this dosage. With the exception of two patients who received second courses of 2′-dCF, all patients were treated with only one course of the drug and no patient received a dose escalation of 2′-dCF.

Results

Patients

The patients treated with 2′-dCF in this study were 16 men and two women, all of whom had advanced malignant disease that had failed to respond to previous treatment. All patients gave their informed consent to receiving 2′-dCF. Of the 18 patients, nine had 'solid tumours' and nine had haematological malignancies, namely acute lymphocytic leukaemia or lymphoma (Table 1). The mean age of the solid tumour group was 61 (range 39–75) and that of the leukaemia-lymphoma group 25 (range 18–31).

The major toxicity following the administration of 2′-dCF was profound lymphocytotoxicity (Table 2).

Even following a single dose of 0.1 mg/kg, two out three patients showed over 50% reduction in circulating lymphocytes, as measured 48 h after 2′-dCF was given. The same dose given on 5 consecutive days reduced the lymphocyte count by 88% and 97% of pretreatment levels in two out of three patients, and

1 *Preclinical toxicologic evaluation of 2′-deoxycoformycin (NSC-21831) in dogs, mice, and monkeys.* Progress Report # SORI-KM-79-262, Southern Research Institute, Birmingham, Alabama 35205, May 31, 1979

Table 1. Patient population receiving 2′-dCF

Patient	Sex	Age	Diagnosis[a]	Dose of 2′-dCF
V. M.	F	19	ALL	0.1 mg/kg × 1
A. H.	M	29	ALL	0.1 mg/kg × 1
A. C.	M	64	Ca bronchus	0.1 mg/kg × 1
L. J.	M	62	Ca bronchus	0.25 mg/kg × 1
C. D.	M	20	ALL	0.25 mg/kg × 1
G. F.	M	70	Ca bronchus	0.25 mg/kg × 1
L. B.	F	39	Ca stomach	1.0 mg/kg × 1
J. H.	M	61	Ca stomach	0.1 mg/kg × 5
R. W.	M	75	Ca bronchus	0.1 mg/kg × 5
D. P.	M	75	Ca bronchus	0.1 mg/kg × 5
J. M.	M	55	Ca bronchus	0.25 mg/kg × 5
G. Mg.	M	31	ALL	0.25 mg/kg × 5
A. M.	M	18	ALL	0.25 mg/kg × 5
G. Mt.	M	26	ALL	0.25 mg/kg × 5
L. T.	M	56	Ca bronchus	0.25 mg/kg × 5
R. W.	M	31	ALL	0.25 mg/kg × 5
P. F.	M	27	DPDL[a]	0.25 mg/kg × 5
C. B.	M	26	Hodgkin's disease	0.25 mg/kg × 5

a *Abbreviations used:* ALL, acute lymphocytic leukaemia; Ca bronchus, squamous bronchogenic carcinoma; DPDL, diffuse poorly differentiated lymphocytic lymphoma

the daily administration of 0.25 mg/kg for 5 days resulted in marked lymphopenia in all eight patients treated at this dose level. Lymphocyte counts decreased within 48 h of the start of treatment, the nadir being reached on day 5 in all but two of these nine patients, where in the latter case the lymphocytopenia was maximal by 72 h. Lymphopenia was maintained in this group of patients for 10–12 days from the start of treatment, with recovery to pretreatment proportions by a mean of day 14 (range 11–18). An example of the reversible lymphopenia seen following 0.25 mg/kg daily for 5 days is shown in Fig. 1.

As shown in Table 2, at these dose levels 2′-dCF had no significant effect on neutrophil or platelet count or on the level of haemoglobin, apart from minor decreases in platelets in some of the leukaemic patients treated with 0.25 mg/kg daily for 5 days.

Determinations of the circulating levels of immunoglobulins (IgG, IgA, and IgM) showed no changes consequent on 2′-dCF administration when performed 5 days after the start of treatment. There were no changes in the blood levels of urea, alkaline phosphatase, alanine transaminase, or bilirubin.

Table 2. Haematological toxicity following 2′-dCF

Patient	Dose of 2′-dCF	% of pretreatment values[a]				
		Lymphocytes	Lymphoblasts	Neutrophils	Platelets	Hgb
V. M.	0.1 mg/kg × 1	NC[b]	NC	NC	NC	NC
A. H.	0.1 mg/kg × 1	43	NC	NC	NC	NC
A. C.	0.1 mg/kg × 1	22	–	NC	NC	NC
L. J.	0.25 mg/kg × 1	47	–	NC	NC	NC
L. J.[c]	0.25 mg/kg × 1	NC	–	NC	NC	NC
C. D.	0.25 mg/kg × 1	NC	–	NC	NC	NC
G. F.	0.25 mg/kg × 1	55	–	NC	NC	NC
L. B.	1.0 mg/kg × 1	10	–	NC	NC	NC
J. H.	0.1 mg/kg × 5	12	–	NC	NC	NC
R. W.	0.1 mg/kg × 5	3	–	NC	NC	NC
D. P.	0.1 mg/kg × 5	NC	–	NC	NC	NC
J. M.	0.25 mg/kg × 5	9	–	NC	NC	87
G. Mg.	0.25 mg/kg × 5	12	NC	NC	NC	NC
G. Mg.[c]	0.25 mg/kg × 5	47	30	NC	64	NC
A. M.	0.25 mg/kg × 5	6	0	NC	73	70
G. Mt.	0.25 mg/kg × 5	3	0	2	37	62
L. T.	0.25 mg/kg × 5	19	–	NC	81	NC
R. W.	0.25 mg/kg × 5	14	200	21	64	NC
P. F.	0.25 mg/kg × 5	17	73	66	63	NC
C. B.	0.25 mg/kg × 5	28	–	NC	73	90

[a] Values shown indicate the maximum change observed following 2′-dCF administration
[b] No change following 2′-dCF administration
[c] Second course of treatment

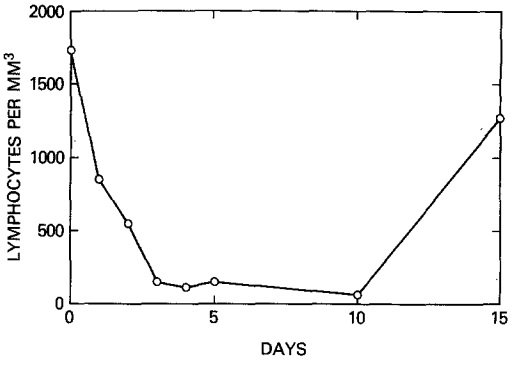

Fig. 1. Peripheral lymphocyte count during and subsequent to the administration of 2′-dCF (patient J. M.; 0.25 mg 2′-dCF/kg/day × 5)

Elevations of uric acid were seen in four patients treated with daily administration of 2′-dCF for 5 days, however. Over the 5-day treatment period, the three patients who received 0.1 mg/kg increased their plasma urate levels by 0.05, 0.14, and 0.19 m*M* respectively. One patient developed life-threatening renal failure presumed to be due to urate nephropathy. Patient J. M., a 55-year-old man with squamous cell carcinoma of the bronchus, developed acute renal failure on day 5 of treatment with 2′-dCF. There was no past history of renal disease, gout, or previous episodes of hyperuricaemia, and his blood urea before treatment was 4.5 m*M* (normal range 3.3–7.5). He was however noted to have blood urate of 0.4 m*M*, which is at the upper limit of normal. By day 5, his urate was 0.6, and concomitant with the development of oliguria his urate rose above 0.7 m*M*, the upper limit of detection in the routine laboratory. Treatment resulted in a diuresis with return of urate, urea, and electrolytes to normal levels by day 14 from the first administration of 2′-dCF. The patient (whose lymphocytopenia is plotted in Fig. 1) had a normal circulating level of 1,740 lymphocytes per mm^3 blood before treatment, whereas 2′-dCF reduced this to 108 per mm^3 by day 5. Following the episode of severe hyperuricaemia seen with patient J. M., and in view of the lymphocytopenia being produced by 2′-dCF, all subsequent patients treated with 0.25 mg/kg × 5 who had lymphoid malignancies were concomitantly treated with allopurinol to block urate formation. Patient L. T., however (squamous cell carcinoma of bronchus), was treated with 2′-dCF alone, and despite a decrease in circulating lymphocytes from 1,380 to 130 per mm^3 blood did not show any significant elevation in urate formation (0.22–0.28 m*M*).

Mild nausea with occasional episodes of vomiting was seen in 11 of the 18 patients and appeared to be dose-related. Seven out of the eight patients treated with 0.25 mg/kg × 5 complained of this symptom, which usually occurred at 36–48 h after the start of treatment. In all instances this was mild and rarely required treatment with anti-emetics. There were no episodes of infection or unexpected exacerbations of the underlying disease seen in this study.

There were three partial responses to 2′-dCF seen in this study in patients with acute lymphocytic leukaemia. Patients G. Mg., A. M., and G. Mt. had progressive disease despite previous treatment with vincristine, prednisolone, 6-mercaptopurine, methotrexate, cyclophosphamide, adriamycin, cytosine arabinoside, and asparaginase. As seen in Table 2, patient G. Mg. had a 70% reduction in circulating lymphoblasts by the end of 5 days of 2′-dCF, but no effects on the marrow were seen. The peripheral lymphoblast count of 644 per mm^3 seen in A. M. before treatment completely cleared by day 4 of 2′-dCF, with an associated 50% reduction in blasts in the marrow. The peripheral blood returned to 10% malignant blasts by day 16. The most marked response was seen in patient G. Mt. At the time of treatment with 2′-dCF, this 26-year-old man had very extensive disease with massive lymphadenopathy, hepatosplenomegaly, a marrow packed with malignant lymphoblasts and 82,000 blasts per mm^3 peripheral blood, representing 84% of the total 98,000 circulating leucocytes. By day 5 of treatment at 0.25 mg 2′-dCF/kg there had been a considerable diminution in organomegaly, and both the peripheral blood and the marrow were clear of malignant lymphoblasts. The patient, who had been thrombocytopenic throughout, died of a cerebrovascular bleed on day 7 from the start of 2′-dCF treatment, at which time the blood urea level was 29.9 m*M* (pretreatment level 6.6 m*M*) and results of the liver function tests considerably elevated: alanine transaminase 232 (pretreatment 38) IU/l; alkaline phosphatase 320 (pretreatment 160) IU/l; bilirubin 71 (pretreatment less than 12) m*M*. In one other patient (P. F.) there was a minor decrease by 27% of circulating lymphoblasts.

Pharmacokinetics and Excretion of 2′-dCF

As was the case in our earlier studies with the dog and rat [4] and the studies of McConnell et al. with the mouse [20], the plasma disappearance of 2′-dCF was characterised by a rapid distribution $\alpha t_{1/2}$, followed by a slower $\beta t_{1/2}$ (5–15 h in man vs 1–2 h in the mouse and dog (Table 3, Figs. 2 and 3).

Since it is likely that in clinical use 2′-dCF will be administered on a multiple-dose schedule, it was of interest to determine the plasma levels to be

Table 3. Plasma disappearance of 2′-dCF in experimental animals and in human subjects

Species	Plasma $t_{1/2}$		References
	$\alpha t_{1/2}$ (min)	$\beta t_{1/2}$ (min)	
Mouse	17–19	64–104	[20]
Dog	12–15	90–120	[18]
Man	30–85	300–900	–

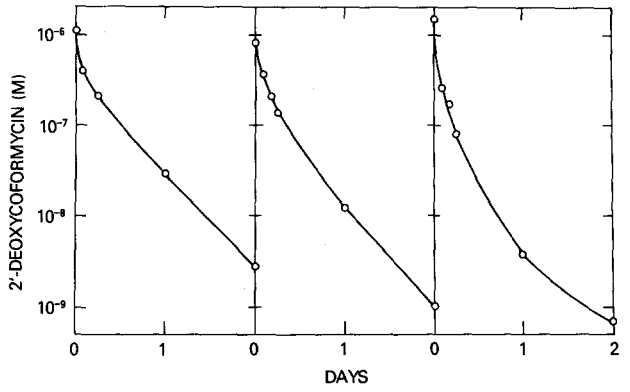

Fig. 2. Plasma concentration of 2′-dCF following IV administration of single doses of 0.10 mg/kg to three individual patients

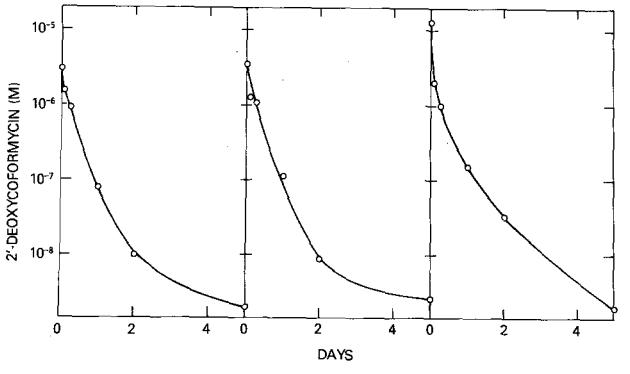

Fig. 3. Plasma concentration of 2′-dCF following IV administration of single doses of the drug to three patients. The *left-hand* and *middle panels* represent doses of 0.25 mg/kg and the *panel* on the *right* represents 1.0 mg/kg 2′-dCF

Table 4. Plasma levels of 2′-dCF in human subjects receiving multiple-dose schedules

Patient	Dose schedule	Treatment day[a]	Plasma 2′-dCF (n*M*)
R. W.	0.1 mg/kg/day × 5	2	21
		3	24
		4	28
		6	30
J. H.	0.1 mg/kg/day × 5	2	6
		3	6
		4	8
		5	14
		6	16
G. Mt.	0.25 mg/kg/day × 5	2	81
		3	78
		4	12
L. T.	0.25 mg/kg/day × 5	2	6
		6	16
		7	<1
A. M.	0.25 mg/kg/day × 5	2	15
		3	18
		4	19
		10	<1
C. B.	0.25 mg/kg/day × 5	2	6
		3	10
		4	12
		11	3
P. F.	0.25 mg/kg/day × 5	2	16
		3	17
		4	16
		5	17
		6	17
		7	4
		10	<1
R. W.	0.25 mg/kg/day × 5	2	102
		5	120
		6	126
J. M.	0.25 mg/kg/day × 5	2	26
		3	31
		5	36
		9	25

[a] Blood samples for 2′-dCF assay were taken immediately before administration of the drug for the treatment day indicated

anticipated. Daily doses of 0.1 and 0.25 mg/kg were therefore administered on a 5-day schedule, with blood being drawn for assay each day immediately before the next administration of the drug. In addition, in several cases daily blood sampling continued for some days after the completion of the 5-day multiple-dose schedule. Plasma levels of 2′-dCF during and after multiple-dose therapy are shown in Table 4. From these data it can be seen that while plasma accumulation of the drug does not occur at these dose levels, persistence of the drug in plasma at readily measurable levels ($> 10^{-9}$ *M*) continues for several days after discontinuation of therapy, and in one patient (C. B.) the drug could still be detected at 11 days, i.e., 6 days after completion of the 5-day course of 2′-dCF.

In our studies, total recovery of administered 2′-dCF was not seen either in the dog or in human subjects (Fig. 4), although fractional recovery at 24 h was considerably greater in the former species

(75%–80% in the dog vs 32%–48% in man). Since the assay method used depends on the retention by the drug of its enzyme inhibitory activity, and since metabolic alteration of 2′-dCF would be likely to result in a partial or complete loss of this property, it is not possible at this time to establish whether the incomplete recovery over this relatively brief time period resulted from metabolism or from long-term tissue retention of the compound. Resolution of this point would require the use of radiolabelled drug or, alternatively, bioassay of retained drug in post-mortem tissue samples.

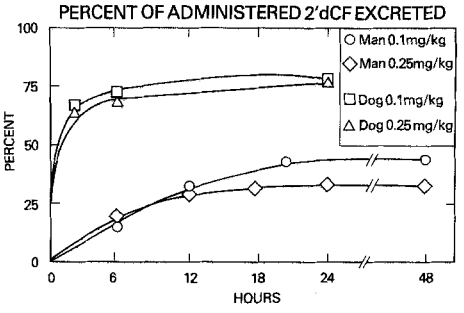

Fig. 4. Cumulative urinary excretion of 2′-dCF in human subjects and in dogs (foxhounds) after IV administration of single doses of the drug

Inhibition of Lymphocyte Ado Deaminase Activity

Ado deaminase activity was measured in the peripheral blood lymphocytes (and blasts where present) before treatment with 2′-dCF and at serial times afterwards (see Table 5). At all doses tested 2′-dCF was effective in inhibiting the lymphocyte ado deaminase of each patient in the study, with the exception of patient L. J. A repeat administration of 0.25 mg 2′-dCF/kg to this patient produced identical results. By 10 min after 2′-dCF injection, the lymphocyte ado deaminase activity was reduced to less than 50% of pretreatment levels in three out of five

Table 5. Effect of 2′-dCF on lymphocyte ado deaminase

Patient	Units of ado deaminase activity[a]						
	0	10 min	2 h	6 h	24 h	48 h	96 h
a) 0.1 mg 2′-dCF/kg, single dose							
V. M.	9.3	3.3	1.5	2.1	2.6	4.0	7.5
A. C.	1.5	< 0.1	< 0.1	< 0.1	< 0.1	1.6	2.5
b) 0.25 mg 2′-dCF/kg, single dose							
L. J.	2.5	1.6	2.5	3.0	1.7	3.0	3.0
G. F.	3.5	0.9	< 0.1	4.6	3.2	2.2	4.2
c) 1.0 mg 2′-dCF/kg, single dose							
L. B.	3.0	2.1	2.0	3.5	1.2	0.5	0.4

	0	Day 1	Day 2	Day 3	Day 4	Day 5
d) 0.1 mg 2′-dCF/kg/day × 5						
J. H.	2.3	1.5	2.1	0.7	0.6	0.3
R. W.	4.8	4.8	2.2	0.5	1.0	1.1
D. P.	2.0	< 0.1	< 0.1	< 0.1	0.7	IS[b]
e) 0.25 mg 2′-dCF/kg/day × 5						
J. M.	3.9	0.4	1.2	IS	0.8	1.3
G. Mg.	4.8	2.7	0.8	< 0.1	< 0.1	< 0.1
A. M.	7.1	IS	IS	< 0.1	< 0.1	< 0.1
G. Mt.	136	IS	0.7	< 0.1	IS	IS
L. T.	6.6	IS	IS	IS	IS	IS
P. F.	22.6	IS	IS	3.4	IS	IS
C. B.	16	1.7	IS	0.8	IS	IS

[a] For assay method, see ref. [24]
[b] Insufficient lymphocytes for ado deaminase assay

of the patients sampled at this early time point. As shown in Table 5, the degree of inhibition of ado deaminase activity was both dose- and schedule-dependent. Only modest inhibition was detected following the single administration of 0.1 mg 2′-dCF/kg, but more pronounced inhibition resulted from 1.0 mg/kg. The repeated daily administration of 2′-dCF resulted in progressive inhibition of ado deaminase activity although the associated lymphopenia frequently prevented investigation of lymphocyte enzyme activity beyond 72 h from the start of treatment. Comparison of the data in Tables 2 and 5 demonstrates that there was a positive correlation between the degree of ado deaminase inhibition and the degree of lymphocytolysis.

Discussion

The major disadvantage of currently available antineoplastic drugs is their poor selectivity, resulting in toxicity to normal host tissues. The identification of adenosine deaminase as an enzyme of selective importance to the lymphoid system, and the demonstration of high levels of ado deaminase activity in human malignant lymphocytes suggested that the pharmacological inhibition of ado deaminase might have selective therapeutic advantages over conventional agents used for the treatment of lymphocytic malignancies. In this study we have shown that 2′-dCF effectively inhibits human lymphocyte ado deaminase, that this effect results in selective lymphocytotoxicity, and that malignant lymphoblasts resistant to conventional antineoplastic agents are sensitive to 2′-dCF.

The dose levels of 2′-dCF examined in this study were relatively low. These low levels were selected purposely, both to permit comparison with earlier animal pharmacological studies and also because of the limited availability of the drug at the time the studies were carried out. Nevertheless, since 2′-dCF is remarkably nontoxic to dogs and monkeys, it was neither necessary nor appropriate to choose a starting dose for this phase I trial based on a conventional proportion of a toxic animal dose. The initial dose of 0.1 mg/kg was selected on metabolic criteria since it was known that in animals this dose would effectively inhibit the target enzyme ado deaminase for periods in excess of 24 h. Of the 18 patients examined, 17 received either single or multiple doses of either 0.1 or 0.25 mg 2′-dCF/kg, while one patient received a single dose of 1.0 mg/kg.

Since conventional dose escalation was not carried out, it is not possible to establish definitively a maximum tolerated dose of the drug or to determine the limiting toxicity of 2′-dCF when used as a single agent. Nonetheless, it would appear that a pharmacologically effective dose for single-agent 2′-dCF was achieved since a partial therapeutic response was noted in three of the patients studied. Therapeutic activity has been confirmed in papers recently presented at the 1980 Annual Meeting of the American Society of Clinical Oncology. Benjamin et al. [3] reported antileukaemic activity in three of six patients treated with continuous infusions of 2′-dCF at doses of 20–160 mg/m^2 over 1–4 days. Significant pulmonary, neurological, and renal toxicity was encountered, as was reported by Kufe et al. [17], who treated 12 patients with daily doses up to 30 mg/m^2. In the latter study there were two partial responses – one a patient with T-cell acute lymphocytic leukaemia, and the other a patient with mycosis fungoides. The difference in toxicity between these two studies and the one presented in this paper may be a reflection of the much higher doses of 2′-dCF used in Benjamin's and Kufe's studies, and the fact that allopurinol was not administered to the patients reported by Kufe et al.

The 2′-dCF used in this study at doses in the effective range for single-agent therapy was remarkably nontoxic. The major haematological effect observed was a marked decrease in lymphocyte count, with little change being noted in other bone marrow elements. In all cases, the lymphocytopenia was reversible although recovery of the circulating lymphocyte count was slow, requiring as long as 10 days after cessation of therapy at the highest dose examined (0.25 mg/kg/day × 5).

Uric acid nephropathy is a potentially troublesome toxic effect of this agent: after this complication was observed in a single patient, subsequent patients in this series received allopurinol in addition to 2′-dCF. It was appreciated that 2′-dCF was resulting in significant lymphocytopenia (Fig. 1), and the increased uric acid production can thus most readily be attributed to specific lymphocyte lysis.

The other toxic effect noted at these dose levels of 2′-dCF was mild nausea and vomiting. These observations do not establish whether the nausea and vomiting seen were due to inhibition of the relatively high level of gastrointestinal ado deaminase or to the central effects of elevated adenosine, 2′-deoxyadenosine and their respective nucleotides consequent upon inhibition of the latter enzyme.

It is not certain at the present time why the congenital absence of ado deaminase results in immunodeficiency or why its inhibition with 2′-dCF causes selective lymphocytolysis. It has been shown, however, that administration of 2′-dCF to mice results in an accumulation of dATP in blood and the

thymus gland, and that this response did not occur in other tissues; such an accumulation of dATP could result either in inhibition of the reduction of ribonucleoside diphosphates or in an accumulation of S-adenosyl-L-homocysteine and consequent inhibition of methylation reactions [21]. As part of the present study we determined the dATP content of erythrocytes in patients receiving 2′-dCF. These results, which have been previously reported [22], demonstrated an inverse relationship between lymphocyte ado deaminase activity and erythrocyte dATP levels, where there was a mean 500-fold increase in dATP in patients treated with 0.25 mg 2′-dCF/kg over 5 days.

The human pharmacological studies were characterised by wider individual variation than noted in earlier studies in the dog, mouse, and rat, an effect to be anticipated since the human subjects studied varied widely in age and physical status, whereas the animal studies were carried out in inbred strains matched in terms of age, sex, and weight. Nonetheless, in the human subjects examined, the two initial plasma clearance half-times noted in other mammalian species were clearly seen. An important difference between the dog and human subjects in the handling of 2′-dCF is the significantly lower cumulative urinary excretion of 2′-dCF in man (32%–48% in man at 24 h vs > 80% in the dog). Since the assay method used depended on the adenosine deaminase inhibitory activity of the drug, it is not possible to establish at this time whether the incomplete recovery in man reflects metabolism of 2′-dCF to compounds inactive in the enzyme inhibitory assay or long-term tissue sequestration of the drug. In the mouse, a small fraction of administered 2′-dCF appears to be converted to the corresponding mononucleotide form [26], but such conversion has not been described in man. An equally likely explanation is greater retention of the drug in the form of the EI complex. Since one of the more likely metabolites is the 5′-monophosphate of 2′-dCF, a nucleotide analogue known to possess biochemical activity as an inhibitor of adenylate deaminase [12], further investigation of the disposition of 2′-dCF in man would appear to be indicated.

The responses noted here in refractory adult acute lymphocytic leukaemia indicate that further exploration of the therapeutic effects of single-agent 2′-dCF in lymphoid malignancies (particularly T-cell malignancies) are warranted, and that its effects as an immunosuppressive agent should be investigated. Such studies are now in progress.

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