Clinical Pharmacology of Deoxycoformycin

By P. P. Major, R. P. Agarwal, and D. W. Kufe

Deoxycoformycin (DCF) is an inhibitor of adenosine deaminase (ADA). Twenty-one courses of DCF were administered to 13 patients ranging in age from 15 to 78 yr. Eight patients had T-cell disorders, and five patients had non-T-cell malignancies. The i.v. bolus dose was escalated from 5 to 30 mg/sq m/day, and the duration of the courses ranged from 1 to 5 days. The DCF plasma half-life ranged from 4.9 to 6.2 hr and was independent of dose. The dose-limiting toxicities involved the central nervous system (CNS) and the kidneys. Other toxicities included bronchitis, decreases in hematocrit, arthralgias, and myalgias. Mortality was encountered in three patients. These toxic effects may have been secondary to the accumulation of the metabolites adenosine and deoxyadenosine. Deoxyadenosine and adenosine were both detectable in plasma (10^{-6} M) and in urine (10^{-7} M). Two partial remissions were observed: one in a patient with T-cell ALL and another in a patient with mycosis fungoides. Minimal responses characterized by either declines in peripheral blast counts or partial resolution of adenopathy were observed in five other patients. No responses were observed in six patients. These observations suggest that DCF is effective in the treatment of T-cell lymphoid malignancies.

ADA inhibitors for the treatment of certain lymphoid malignancies was based on this biochemical rationale. Deoxycoformycin (DCF) is a tight-binding inhibitor of adenosine deaminase (ADA; adenosine amino hydrolase, EC 3.5.4.4). It is inactive in the L1210 leukemia model. However, British investigators noted antileukemic activity in their initial human toxicologic evaluation. The mechanism for this antileukemic effect is unclear, although the inhibition of ADA by DCF results in the accumulation of adenosine and deoxyadenosine, and several hypotheses have been proposed to explain the toxic effect of these metabolites.

Recent evidence supports the accumulation of deoxyadenosine triphosphate (dATP) as the main mechanism of toxicity to lymphocytes. The increased levels of dATP can lead to inhibition of DNA synthesis. Furthermore, deoxyadenosine can inactivate S-adenosyl homocysteine hydrolase, which results in the accumulation of S-adenosyl homocysteine, a metabolite toxic to lymphocytes.

Since the first British report, a number of clinical trials have been initiated in this country. This report summarizes the clinical pharmacology data accumulated in treating 13 patients with this agent.

MATERIALS AND METHODS

The diagnosis, age, sex, creatinine, and creatinine clearance of the patients entered in this study are presented in Table 1. The patients had a life expectancy of at least 2 mo, and their performance status on the ECOG scale was 2 or less; the creatinine clearance was at least 50 ml/min, and liver function tests were normal. Patients with a history of coronary disease or congestive heart failure were excluded from the study to avoid problems resulting from fluid and sodium overload. Written informed consent was obtained from all patients. All patients were hospitalized at the Sidney Farber Cancer Institute during treatment.

Drug Administration

Deoxycoformycin (DCF) was supplied by the National Cancer Institute as a lyophilized powder containing sodium phosphate as a buffer and was reconstituted with sterile water immediately prior to i.v. bolus administration. Patients received i.v. hydration and alkalinization to maintain a daily urine output of 3 liters with a pH of between 7.0 and 7.4. The different dose schedules are summarized in Table 2. Patients were treated at 3-wk intervals, provided they had completely recovered from the toxicity of previous courses. None of the patients received allopurinol during treatment.

Clinical Laboratory Studies

Complete blood counts were performed prior to treatment on each day during the infusion and once a week after treatment. Renal function was monitored by daily serum creatinine and urea nitrogen levels. Liver function tests were performed prior to treatment and once a week thereafter. Serum uric acid levels were measured daily during treatment and once a week thereafter.

Urine Sample Collection

All patients had 24-hr urine collections for creatinine clearance before drug administration, on the first day of treatment, and every
day 1 of treatment and cerebrospinal fluid (CSF) and plasma was a modification of Samples were obtained 1, 2, 4, and 8 hr post-drug administration on day 1 of treatment and every other day thereafter. Patients P.G., R.A., and M.C. also had 24-hr excretions measured; these urine collections were kept on ice and the pH adjusted to 7.4 to ensure the stability of DCF.

Blood Sample Collection

Blood samples were collected in preservative-free heparin (Abbott Laboratories, Chicago, Ill.), 200 U/10 ml of blood. Samples were obtained 1, 2, 4, and 8 hr post-drug administration on day 1 of treatment and every other day thereafter when possible.

Determination of DCF Concentrations in Urine and Plasma

The enzymatic assay used to determine levels of DCF in the cerebrospinal fluid (CSF) and plasma was a modification of previously described techniques. All samples were boiled for 5 min to inactivate endogenous ADA activity and to free protein-bound DCF. Samples were then spun at 150,000 g for 90 min in a Beckman L-5-65 Ultracentrifuge (Beckman Instruments, Fullerton, Calif.) using a Ti 40 rotor. Supernatant fluids were then filtered through Amicon CF-25 centriflo membranes (Amicon Corporation, Lexington, Mass.). All samples were stored at -70°C prior to assaying for DCF levels.

A standard curve was constructed for the inhibition of ADA (calf intestinal ADA, 200 U/mg; Boehringer-Mannheim, Indianapolis, Ind.) by DCF. A reaction mixture containing 500 μl of 50 mM potassium phosphate buffer (pH 7.4), 100 μl of DCF (0.5–4.0 x 10⁻⁹M), and 200 μl of ADA (0.5 U of enzyme/ml) was incubated at 25°C for 5 min in a 1-ml cuvette. The clinical samples were assayed for DCF levels by adding 100 μl of the appropriate dilutions of the prepared plasma or CSF samples instead of the DCF standard. After incubation, 200 μl of 0.8 mM adenosine (Boehringer-Mannheim) was added, and the reaction was followed at 265 nm in a recording Beckman model 25 spectrophotometer (Beckman Instruments) at 25°C. The ADA activity remaining after incubation with samples containing DCF is expressed as a percentage of the reaction rate of the control enzyme preparation containing no DCF. Appropriate controls were assayed concurrently with the clinical samples.

Determination of Adenosine and Deoxyadenosine Levels

The levels of adenosine and deoxyadenosine in the plasma and urine were determined by high-pressure liquid chromatography (HPLC). Plasma and urine samples were filtered through Amicon CF-25 centriflo membranes, and 10–20 μl were injected directly on an Altex HPLC machine equipped with an Ultrasphere ODS Altex column and a Hewlett-Packard integrator for analysis. Adenosine and deoxyadenosine were monitored by their retention time and confirmed by the peak-shift method after treatment with ADA.

Table 1. Clinical Characteristics of Patients Treated With DCF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Renal Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.P.</td>
<td>30</td>
<td>M</td>
<td>ALL (T-cell)</td>
<td>0.8*; 78†</td>
</tr>
<tr>
<td>M.M.</td>
<td>15</td>
<td>M</td>
<td>ALL (T-cell)</td>
<td>1.1; —</td>
</tr>
<tr>
<td>P.G.</td>
<td>24</td>
<td>M</td>
<td>ALL (T-cell)</td>
<td>0.8; 153</td>
</tr>
<tr>
<td>M.C.</td>
<td>23</td>
<td>M</td>
<td>Lymphoma (T-cell)</td>
<td>1.2; 78</td>
</tr>
<tr>
<td>T.C.</td>
<td>15</td>
<td>M</td>
<td>Lymphoma (T-cell)</td>
<td>0.6; —</td>
</tr>
<tr>
<td>K.P.</td>
<td>78</td>
<td>F</td>
<td>Mycosis fungoides</td>
<td>1.1; 58</td>
</tr>
<tr>
<td>S.L.</td>
<td>50</td>
<td>F</td>
<td>Mycosis fungoides</td>
<td>0.7; 92</td>
</tr>
<tr>
<td>A.B.</td>
<td>55</td>
<td>M</td>
<td>Thymoma</td>
<td>1.0; 74</td>
</tr>
<tr>
<td>M.W.</td>
<td>37</td>
<td>M</td>
<td>ALL (null-cell)</td>
<td>0.8; 100</td>
</tr>
<tr>
<td>R.A.</td>
<td>20</td>
<td>M</td>
<td>ALL (null-cell)</td>
<td>1.1; 76</td>
</tr>
<tr>
<td>A.M.</td>
<td>35</td>
<td>F</td>
<td>ALL (null-cell)</td>
<td>1.3; 80</td>
</tr>
<tr>
<td>J.K.</td>
<td>25</td>
<td>M</td>
<td>Lymphoma (null-cell)</td>
<td>1.0; 87</td>
</tr>
<tr>
<td>D.C.</td>
<td>23</td>
<td>M</td>
<td>Diffuse histiocytic lymphoma</td>
<td>0.8; 97</td>
</tr>
</tbody>
</table>

*Serum creatinine, mg/dl. †Creatinine clearance, ml/min.

other day thereafter. Patients P.G., R.A., and M.C. also had 24-hr DCF excretions measured; these urine collections were kept on ice and the pH adjusted to 7.4 to ensure the stability of DCF.

Table 2. Dosage and Scheduling of DCF Treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/kg)</th>
<th>Dose (mg/sq m)</th>
<th>Duration (Days)</th>
<th>CNS and Renal Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K.P.</td>
<td>0.10</td>
<td>5</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.50</td>
<td>20</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.75</td>
<td>30</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>A.B.</td>
<td>0.10</td>
<td>5</td>
<td>3</td>
<td>Somnolence</td>
</tr>
<tr>
<td>M.C.</td>
<td>0.25</td>
<td>10</td>
<td>3</td>
<td>Somnolence and rise in creatinine</td>
</tr>
<tr>
<td>M.M.</td>
<td>0.50</td>
<td>15</td>
<td>3</td>
<td>Somnolence</td>
</tr>
<tr>
<td>T.P.</td>
<td>0.75</td>
<td>30</td>
<td>3</td>
<td>Somnolence</td>
</tr>
<tr>
<td>D.C.</td>
<td>0.75</td>
<td>30</td>
<td>3</td>
<td>Rise in creatinine</td>
</tr>
<tr>
<td>M.M.</td>
<td>1.00</td>
<td>30</td>
<td>3</td>
<td>Somnolence</td>
</tr>
<tr>
<td>T.C.</td>
<td>1.00</td>
<td>30</td>
<td>3</td>
<td>Somnolence</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.125</td>
<td>5</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.25</td>
<td>10</td>
<td>5</td>
<td>Lethargy</td>
</tr>
<tr>
<td>M.W.</td>
<td>0.32</td>
<td>10</td>
<td>5</td>
<td>Lethargy</td>
</tr>
<tr>
<td>R.A.</td>
<td>0.25</td>
<td>10</td>
<td>5</td>
<td>Rise in creatinine</td>
</tr>
<tr>
<td>J.K.</td>
<td>0.25</td>
<td>10</td>
<td>5</td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td>A.M.</td>
<td>0.30</td>
<td>12.5</td>
<td>5</td>
<td>Rise in creatinine</td>
</tr>
<tr>
<td>R.A.</td>
<td>0.30</td>
<td>12.5</td>
<td>5</td>
<td>Pulmonary edema</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.375</td>
<td>15</td>
<td>5</td>
<td>Somnolence</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.437</td>
<td>17.5</td>
<td>5</td>
<td>Somnolence and rise in creatinine</td>
</tr>
<tr>
<td>P.G.</td>
<td>0.50</td>
<td>20</td>
<td>5</td>
<td>Somnolence and rise in creatinine</td>
</tr>
<tr>
<td>S.L.</td>
<td>0.50</td>
<td>25</td>
<td>5</td>
<td>Coma and renal insufficiency</td>
</tr>
</tbody>
</table>
Determination of Adenosine Deaminase, Adenosine Kinase, and Deoxyadenosine Kinase Activity

Erythrocytes were washed 3 times in 0.9% NaCl. The washed red cell pellets were resuspended in an equal volume of 0.9% NaCl and the hematocrit was determined. The cells were hemolyzed by adding 3.5 ml of 0.005 M Tris HCl (pH 7.0). The hemolysate was centrifuged for 30 min at 40,000 g and enzyme assays were performed on the supernatant. Washed leukocytes were resuspended in 0.005 M Tris-HCL (pH 7.0) at 1 x 10^8 cells/ml. The cells were freeze-thawed 3 times in dry-ice-acetone and homogenized. The buffer molarity was adjusted to 50 mM. The homogenates were centrifuged at 100,000 g for 30 min and the enzyme activities were determined on the supernatant.

Adenosine deaminase. The adenosine deaminase activity was determined by measuring the rate of decrease in absorbancy at 265 nm resulting from the conversion of adenosine to inosine. The 0.9-mI reaction mixture (in 1-cm cuvette; diameter, 10 mm) containing 50 mM potassium phosphate buffer (pH 7.4) and 0.1 mM adenosine was incubated for 5 min at 37°C. The reaction was started by adding appropriate aliquots of cell supernatants and adjusting the final volume to 1 ml. The decrease in absorbancy was recorded at 37°C.

One unit of adenosine deaminase is the amount of enzyme that catalyzes the deamination of 1 zmole of adenosine/min (A Δ A = 8.6 mm min⁻¹ ml⁻¹) under the conditions of the assay.

Adenosine and deoxyadenosine kinase. The kinase activities were determined by a radioisotope assay. In a standard assay method, a reaction mixture (0.25 ml total) containing 100 mM Tris-HCl (pH 7.0), 0.5 mM MgCl₂, 1.0 mM ATP, 2 mM deoxycoformycin, 9 mM NaF, 5 mM dithiothreitol, and 20-50 μl of enzyme extract was incubated at 37°C in a shaking water bath. The reaction was started by the addition of 50 μM 8-14C-adenosine (10 mCi/mmole) or 8-14C-deoxyadenosine (10 mCi/mmole). Aliquots of 20 μl were withdrawn immediately and after 30 min of incubation at 37°C and applied immediately to 2.5-cm Whatman DE-81 filter paper discs. The discs were placed on a sintered glass funnel attached to a vacuum flask and washed immediately with 10 ml of 1 mM ammonium formate (2 x wash x 5). The determination of total radioactivity was made by withdrawing 20-μl aliquots of the reaction mixture and processing on DE-81 filter paper discs without washing. Discs were transferred to scintillation vials and nucleotides solubilized for 15 min by the addition of 1 ml of 1N HCl. Nine milliliters of Hydrofluor (National Diagnostics, Somerville, N.J.) scintillation fluid were added, and the samples were counted for radioactivity. The formation of nucleotides was determined by the percent radioactivity appearing in that fraction. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmole of nucleotide/hr under the standard conditions.

RESULTS

Deoxycoformycin Pharmacokinetics

Table 3 summarizes the pharmacokinetic data obtained in five patients. Plasma levels 2 hr after drug administration were proportional to dose. The disappearance of DCF from plasma was fitted by the method of least squares to an exponentially decreasing log function of the concentrations obtained at 2, 4, and 8 hr post-DCF administration. The t¹/₂ was similar at all doses and ranged from 4.9 to 6.2 hr. The intercept of the elimination curve with the ordinate on the concentration versus time plot yielded values much lower than those obtained at 1 hr, suggesting an initial equilibrium phase.

The majority of administered DCF is excreted unchanged in the urine. Patient P.G. excreted 50% of the administered dose (10 mg/sq m) in his urine on day 1, 76% on day 3, and 87% on day 5. Patient R.A. excreted 82% of the administered dose (10 mg/sq m) on day 1 and 69% on day 3. M.C. excreted 73% of the administered dose (10 mg/sq m) on day 1.

Accumulation of Adenosine and Deoxyadenosine in Body Fluids

Sequential measurements of plasma adenosine and deoxyadenosine were obtained 2, 4, 8, and 24 hr after drug administration. Rapid rises in plasma adenosine and deoxyadenosine were observed in two patients (P.G. and R.A.) 2 hr after drug administration; these values then declined over the next 6 hr. In other patients (Table 4), the plasma levels increased during the first 8 hr and returned to undetectable levels after 24 hr. The peak plasma adenosine and deoxyadenosine levels progressively increased for each day of DCF treatment (six courses) with the exception of the second course administered to patient R.A. (Fig. 1).

Table 4 lists the peak levels of adenosine and deoxyadenosine achieved in the plasma and urine for the last day of treatment during six courses. These levels vary considerably from patient to patient. Further, certain patients had higher levels of adenosine, while others accumulated more deoxyadenosine. This pattern was maintained during the course of treatment.

Adenosine Deaminase, Adenosine Kinase, and Deoxyadenosine Kinase Activity in Leukemic Cells During Treatment With DCF

Three patients with leukemia were monitored for lymphoblast adenosine deaminase activity prior to and during treatment with DCF. Samples were obtained on days 1, 3, or 5. There was reduction of lymphoblast adenosine deaminase activity in two patients to <10% of control values after 3 days of DCF treatment.
Kinase activities were similar in three patients (M.M., P.G., and T.P.), although the ratio of deoxyadenosine to adenosine kinase was higher in the two responding patients (M.M. and P.G.) than in the nonresponding patient (T.P.).

Toxicity

Table 2 lists the CNS and renal toxicities encountered at different dose levels of DCF. Central nervous system toxicity occurred in 60% of courses and varied from lethargy and somnolence to coma. The CNS depression began several days after DCF administration and persisted for up to 3 wk.

The other dose-limiting toxicity was manifested by renal insufficiency in two patients (J.K. and S.L.). This abnormality was reversible but required dialysis in one patient (J.K.). One patient, A.M., had a 50% decrease in creatinine clearance without renal insufficiency that reversed within 2 wk. Five other patients had minimal elevations of their serum creatinine not exceeding 2.5 mg/dl. There was no increase in uric acid or phosphate levels, except in those patients who developed renal insufficiency.

Decreases in hemoglobin (>15%) were also observed (7 courses) but hemolysis could not be documented. It is possible that volume expansion due to hydration and alkalization is partly responsible for this finding. Platelet declines (16 courses) also occurred but were transient, lasting 3 or 4 days.

Bronchitis (7 courses) and conjunctivitis (7 courses) were encountered but were not dose-limiting. These symptoms resolved within 48 hr after drug administration. Myalgias and arthralgias (5 courses) were also transient, appearing usually during the third day of treatment and clearing within 48 hr of cessation of therapy. The mechanism for these toxic effects is unclear.

Mortality was encountered in three patients entered in this study. One patient (D.C.) developed irreversible shock of unknown cause on day 3 of treatment (30 mg/sq m/day), and a second patient (R.A.) died from pulmonary edema due to fluid overload on the fifth day of a second DCF course. This patient had received adriamycin (600 mg/sq m) and cardiomyopathy was a major contributing factor. A third patient (S.L.) had protracted CNS and renal toxicity that reversed but contributed to death 8 wk after therapy.

Antitumor Effect

Responses were primarily observed in patients with T-cell malignancies. A complete response was observed in a patient with mycosis fungoides (S.L.) who had resolution of extensive skin plaques that persisted for 1 mo. Another patient with T-cell leukemia (P.G.) had a reduction of bone marrow...
involvement to <5% blasts for 3 wk. Minimal responses were observed in three other patients with T-cell malignancies. Patient M.M. had a decrease of >90% in peripheral blast count that lasted for over 3 wk. Skin nodules and peripheral lymph nodes decreased (>50%) in size during treatment of patient M.C., but recurred within 3 wk of therapy. Patient T.C. also had partial (>50%) resolution of mediastinal adenopathy for a period of 3 wk.

Minimal activity was observed in two patients with null-cell disorders. The peripheral blast count declined by >75% for 4 wk in a patient (R.A.) with null-cell ALL and by >90% for 3 wk in a patient (J.K.) with null-cell lymphoma in leukemic phase. Two other patients with null-cell ALL, however, failed to respond.

DISCUSSION

The elimination of DCF from plasma can be fitted by an exponentially decreasing function when using the 2-, 4-, and 8-hr post-DCF administration levels. The existence of an equilibrium phase would explain why the A value (intercept of the excretion half-life curve with the ordinate) was always lower than the plasma level measured 1 hr post-i.v. bolus administration of DCF. There did not appear to be a dose-related variation in the excretion half-lives. The DCF was primarily excreted unchanged in the urine.

Significant amounts of adenosine and deoxyadenosine accumulated in the urine and plasma of patients during DCF treatment. The urine levels of these metabolites were proportional to those in plasma. There was, however, significant variation in the level and pattern of these metabolites in different patients. It should also be noted that the urine of some patients contained a significant amount of an unidentified metabolite that eluted after adenosine and deoxyadenosine on HPLC. The nature and significance of this metabolite requires further study.

Central nervous system effects were the major toxicities of DCF administration. Although the precise mechanism for this toxicity has not been defined, marked elevations of adenosine and deoxyadenosine documented in these patients could have contributed to these effects. Experimental evidence in animals demonstrates that adenosine and deoxyadenosine can produce profound CNS depression.13-18 The brain is known to contain high levels of adenosine deaminase,19 and in a previous report20 we have shown that DCF penetrates the blood barrier. Inhibition of ADA in brain tissue would also render the central nervous system more susceptible to the toxic effects of adenosine and deoxyadenosine.

The renal toxicity was reversible in all cases, but dialysis was required in one patient (J.K.). The mechanism for this toxic effect is also unclear, but the high levels of adenosine and deoxyadenosine in urine (and plasma) might have been toxic to the renal epithelium.

Several dose schedules were employed in this study. A single dose of DCF at 20 mg/sq m was sufficient to inhibit lymphoblast ADA activity in a patient (P.G.) with T-cell ALL. However, this dose failed to yield a decline in peripheral blast count and suggested that duration of therapy might also be important. A 3-day schedule resulted in more significant declines in tumor burden, but these responses were considered minimal. The 5-day schedule, in contrast, resulted in partial responses in two patients. However, the duration of these responses was brief and the associated toxicity was more pronounced. These findings suggested that the longer 5-day course may be more effective and that a dose of 10 mg/sq m/day is sufficient to inhibit lymphoblast ADA activity but does not result in unacceptable toxicity.

DCF as a single agent is thus active against certain T and null-cell malignancies. The finding that T-cell disorders are more sensitive to this agent could also relate to differential patterns of intracellular enzymes. The suppression of lymphoblast ADA activity alone may correlate with therapeutic effect, and this relationship should be explored in phase II studies. However, the relative levels of intracellular adenosine kinase and deoxyadenosine kinase may also be relevant to therapeutic efficacy as they are necessary for the formation of potentially toxic nucleotides. The accumulation of plasma adenosine and deoxyadenosine, for example, could result in enhanced toxicity to cells with higher ratios of kinase to ADA activity.

DCF could be employed to enhance the effectiveness of adenosine arabinoside (Ara-A). Ara-A is rapidly deaminated in the plasma to ara-hypoxanthine by ADA. DCF could, therefore, prolong the half-life of Ara-A and also modulate intracellular deamination. In designing clinical trials for this combination of agents, consideration will have to be given to the possible antagonistic effect of accumulation of deoxyadenosine on the transport and phosphorylation of Ara-A. Ara-A also has neurotoxicity, which may further complicate the CNS effects of DCF. An appropriate dose of DCF might, therefore, significantly prolong the half-life of Ara-A without accumulating adenosine or deoxyadenosine.

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REFERENCES


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