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# Deoxycoformycin Toxicity in Mice After Long-term Treatment

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**Summary.** *Deoxycoformycin, a tight-binding inhibitor of the enzyme adenosine deaminase, is a potent lymphocytotoxic agent. To examine the effect of deoxycoformycin on mouse tissues the drug was administered IP either by single or by repeated injections at one of two dose levels (0.2 or 10.0 mg/kg). Treatment with repeated injections at the higher dose caused retardation of growth and increases in lung and splenic mass. Body temperature, hematocrit, and total leukocyte count remained constant. Single injections at the lower dose caused complete inhibition of adenosine deaminase in liver and blood, and partial inhibition in jejunum and spleen, but at the higher dose complete inhibition of the enzyme in all tissues was obtained. Dosage appeared to have no effect on the rate of recovery of the deoxycoformycin-inhibited enzyme but marked tissue differences were observed. The enzymic activity recovered rapidly in jejunum (100% in 1 day) but slowly in other tissues (after 28 days, about 60% in spleen and liver; about 85% in kidney and blood, and 100% in lungs). These observations suggest that the recovery of inhibited enzyme depends largely upon the rate of proliferation of cells and protein synthesis. These tissue differences in recovery rates may play a role in the pharmacological and chemotherapeutic behavior of this drug.*

## Introduction

Deoxycoformycin, a tight-binding inhibitor of adenosine deaminase [3], is potentially of clinical use in the treatment of lymphoid malignancies [12]. Recent phase I and II clinical trials of DCF in various malignancies indicate that it is cytotoxic to T-lymphoblasts and suggest that further evaluation in diseases such as T-cell acute lymphoblastic leukemia is indicated [4–7, 10, 12]. In contrast to the earlier report of Smyth et al. [12], where no drug toxicity was apparent, drug-related toxicities such as CNS depression, renal failure, diffuse pulmonary infiltrates, decline in hematocrit, hyperuricemia, and conjunctivitis have been observed in recent clinical trials [4, 5, 7, 10, 12]. In vivo toxicity to lymphoid tissues in BDF mice has been reported by Smyth et al. with 100 mg DCF/kg given by a single IP injection [11]. This dose was much higher than is used clinically (0.1–1.0 mg/kg/day). Since there is great interest in DCF as a chemotherapeutic agent, we have examined the effects on a number of mouse tissues and the recovery of ADA from inhibition following administration of the drug at clinically used dosages. This was done over a 4-week period following the administration of DCF.

## Materials and Methods

DCF was obtained from the Drug Synthesis and Development Branch, National Cancer Institute.

Male CDF mice weighing 20–25 g were divided into six groups. Treatment with IP injection of either normal saline or DCF at 0.2 or 10.0 mg/kg was by one of two treatment schedules: a single injection or repeated injections given twice weekly to a total of eight injections.

Each animal was marked and weighed at the start of the experiment. Rectal temperature, total leukocyte count, and hematocrit were determined weekly and body weight fortnightly. Food and water were allowed ad libitum.

Animals were sacrificed at intervals for determination of ADA activity in tissues and organ weights. The spleen, liver, kidneys, and lungs were excised, cleaned of connective tissue, and weighed. After weighing, a small portion of each tissue was saved

*Abbreviations used in this paper are:* ADA, adenosine deaminase (adenosine aminohydrolase, E.C. 3.5.4.4); DCF, 2′-deoxycoformycin [3-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetra-hydroimidazo (4,5-*d*) (1,3)-diazepin-8-(R)-ol] or Pentostatin

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**Table 1.** Normal values of weights, protein contents, and adenosine deaminase activity of CDF mouse tissues

| Organ | Organ weight mg/g body weight | Protein mg/g tissue | Adenosine deaminase Units[a]/g tissue |
|---|---|---|---|
| Liver | 51.8 ± 1.8[b] ($n = 4$) | 120.6 ± 5.8[b] ($n = 10$) | 0.75 ± 0.06[b] ($n = 6$) |
| Spleen | 5.0 ± 0.5 ($n = 3$) | 87.7 ± 10.9 ($n = 10$) | 3.50 ± 0.72 ($n = 6$) |
| Kidney | 18.3 ± 1.6 ($n = 4$) | 89.5 ± 4.7 ($n = 10$) | 0.65 ± 0.11 ($n = 6$) |
| Lungs | 6.5 ± 1.1 ($n = 4$) | 94.5 ± 7.6 ($n = 9$) | 0.52 ± 0.13 ($n = 6$) |
| Intestine | – | 93.5 ± 7.5 ($n = 5$) | 25.0 ± 5.9 ($n = 4$) |

[a] One unit is defined as the amount of enzyme catalyzing the deamination of 1μmole adenosine per min
[b] Mean ± SD (number of animals)

for determination of ADA activity. An 8-cm segment of jejunum (measured from the duodeno-jejunal junction) was also excised, flushed with normal saline, and assayed for ADA activity.

In a separate experiment, similar animals were given a single injection of either saline or DCF (0.2 or 10 mg/kg), and the ADA activity in blood, liver, jejunum, and spleen was determined 4–72 h after treatment.

*Determination of ADA Activity*

Tissue homogenates (10%, w/v) were prepared by homogenization of tissues for 2 min in 0.1 *M* Tris-HCl, pH 8.0, in a glass homogenizer. The samples were kept cold during homogenization by immersion in crushed ice. The homogenates were centrifuged at 100,000 *g* for 30 min. The ADA activity in the supernatant fluids was determined by a spectrophotometric method following decrease in absorbance at 265 nm at 37° C. An ammonia liberation method [9] was used to assay ADA activity in whole blood. Under these conditions, the contribution from plasma to the total activity of whole blood was < 2% [2].

**Table 2.** Effect of deoxycoformycin on the growth of CDF mice

| | Percent change in weight after | |
|---|---|---|
| | Day 14 | Day 28 |
| Single injection | | |
| Saline | 20.1 ± 4.7 (5)[a] | 29.7 ± 4.4 (4)[a] |
| DCF (0.2 mg/kg) | 15.7 ± 4.8 (5) | 18.6 ± 5.4 (5)[b] |
| DCF (10 mg/kg) | 19.0 ± 3.4 (5) | 28.4 ± 3.1 (5) |
| Repeated injections | | |
| Saline | 17.3 ± 3.0 (5) | 22.2 ± 5.3 (4) |
| DCF (0.2 mg/kg) | 15.6 ± 2.4 (4) | 20.3 ± 1.1 (4) |
| DCF (10 mg/kg) | 5.2 ± 1.2 (6)[c] | 14.5 ± 4.6 (4)[d] |

[a] Mean ± SD (number of animals)
[b] Different from results following single injection of saline or DCF (10 mg/kg) ($P < 0.05$)
[c] Different from results in all other groups on day 14 ($P < 0.01$)
[d] Different from results following single injection of either saline or DCF (10 mg/kg) ($P < 0.01$)

## Results

Normal values for organ weight, protein content, and ADA activities in CDF mouse are presented in Table 1. Among the tissues examined, the ADA activity per gram of tissue was highest in the intestine, followed by spleen, liver, kidney, and lungs. Since the protein contents are higher in liver than in other tissues the specific activity (units/mg protein) of the enzyme was as follows: intestine > spleen > kidney > liver > lungs. This order is in agreement with the published data for CD-1 mice [13].

Effects of DCF treatment on the growth of animals and tissue weights are presented in Tables 2 and 3. The growth of animals receiving repeated injections was generally slower than that of the animals receiving single injections, suggesting that the trauma of multiple injections slowed the growth.

DCF 10 mg/kg by single injection or 0.2 mg/kg by repeated injections did not affect growth compared with similarly treated controls. With the single-injection groups there was significant retardation of growth in the animals treated with 0.2 mg DCF/kg but not in the group treated with 10 mg/kg. This phenomenon cannot be explained at present.

No significant change in the body temperature, total leukocyte count, or hematocrit values was

**Table 3.** Effect of deoxycoformycin on tissue weights of CDF mice[a]

| | Single injection | | | Repeated injections | | |
|---|---|---|---|---|---|---|
| | Saline | DCF | | Saline | DCF | |
| | | 0.2 mg/kg | 10 mg/kg | | 0.2 mg/kg | 10 mg/kg |
| Liver | | | | | | |
| mg/g body weight | 52.2 ± 1.1[b] | 48.8 ± 1.2 | 54.0 ± 0.4 | 53.8 ± 0.4 | 53.2 ± 0.6 | 54.9 ± 3.6 |
| % Change[c] | | −6.5 | +7.3 | | −1.1 | +2.0 |
| Spleen | | | | | | |
| mg/g body weight | 5.6 ± 0.4 | 5.9 ± 1.9 | 7.3 ± 3.5 | 5.6 ± 0.4 | 5.6 ± 0.8 | 7.3 ± 1.2 |
| % Change | | +2.7 | +30.4 | | 0 | +30.4 |
| Kidney | | | | | | |
| mg/g body weight | 16.8 ± 1.2 | 19.5 ± 0.6 | 17.4 ± 1.3 | 17.3 ± 0.1 | 18.7 ± 0.5 | 18.7 ± 0.3 |
| % Change | | +16.1 | +3.6 | | +8.0 | +8.0 |
| Lungs | | | | | | |
| mg/g body weight | 6.1 ± 0.2 | 5.7 ± 0.1 | 6.1 ± 1.1 | 4.8 ± 0.4 | 6.3 ± 0.8 | 8.6 ± 2.3 |
| % Change | | −6.6 | 0 | | +31.3 | +79.2 |

[a] Weight at day 28 from the start of experiment
[b] Mean ± SD. Two animals were used in each group
[c] From saline-treated controls

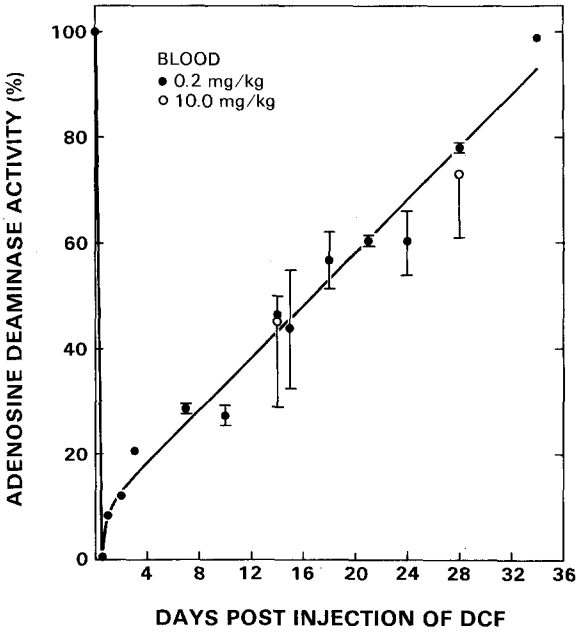


**Fig. 1.** Recovery of DCF-inhibited ADA in mouse blood. DCF (0.2 mg/kg or 10 mg/kg) was injected IP. Blood (50 µl) was collected in heparinized (sodium salt) capillary tubes by retro-orbital puncture. A reaction mixture in 3 ml containing blood (50 µl), potassium phosphate buffer (50 m*M*, pH 7.4), NaCl (75 m*M*), $MgCl_2$ (2 m*M*), glucose (10 m*M*), penicillin (30 units), streptomycin (30 µg), heparin (150 units), and adenosine (1 m*M*) was incubated at 37° C. Aliquots (0.5 ml) were withdrawn at 0, 10, and 20 min for determination of liberated ammonia. The percentage activity was calculated form the values of similarly treated saline controls. Two animals were used per point

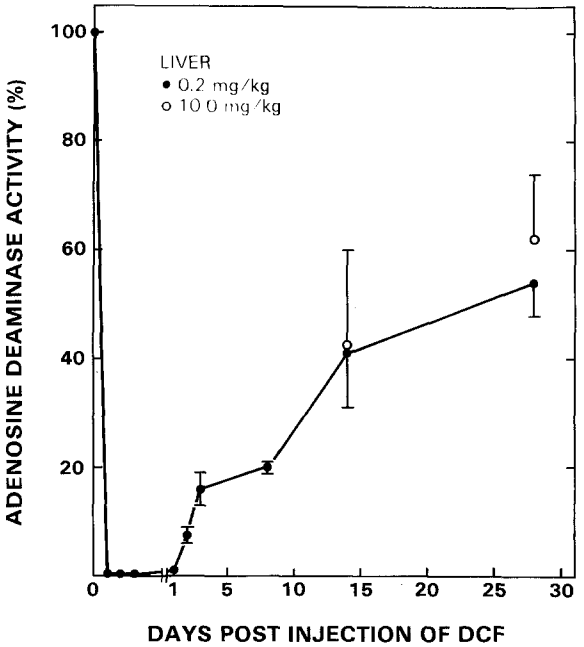


**Fig. 2.** Recovery of DCF-inhibited ADA in mouse liver. After IP injection of DCF (0.2 mg/kg or 10.0 mg/kg), two animals were sacrificed by cervical discolation at intervals as indicated. The organs were excised, cleaned of connective tissue, and homogenized (10%, w/v) in 0.1 *M* Tris-HCl, pH 8.0, in a glass homogenizer. ADA activity was determined in the 100,000 X *g* supernatant fluids by a spectrophotometric method at 265 nm at 37° C. The percentage recovery was calculated from similarly treated saline controls

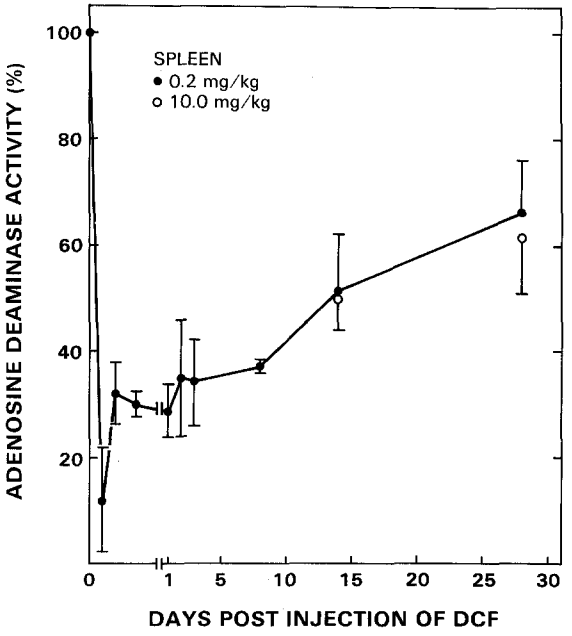


**Fig. 3.** Recovery of DCF-inhibited ADA in mouse spleen. Conditions were similar to those described for Fig. 2

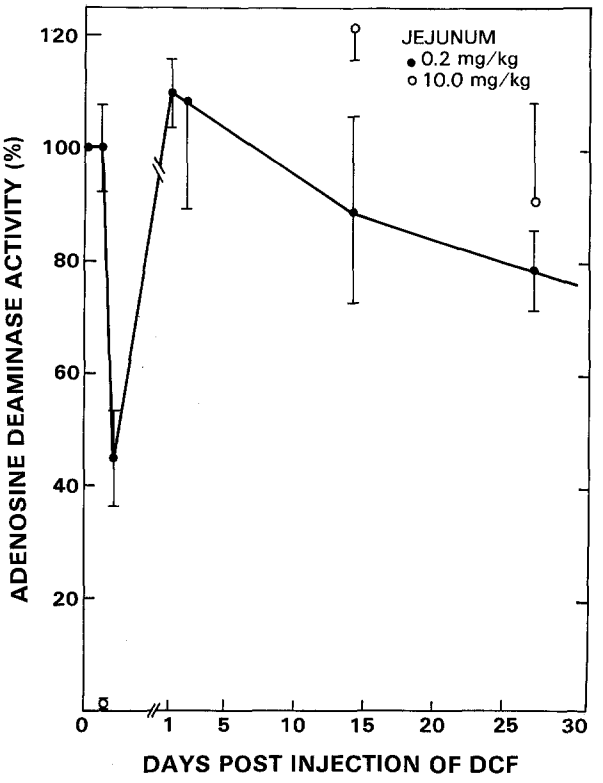


**Fig. 5.** Recovery of DCF-inhibited ADA in the jejunum of mouse. Conditions were similar to those described for Fig. 2

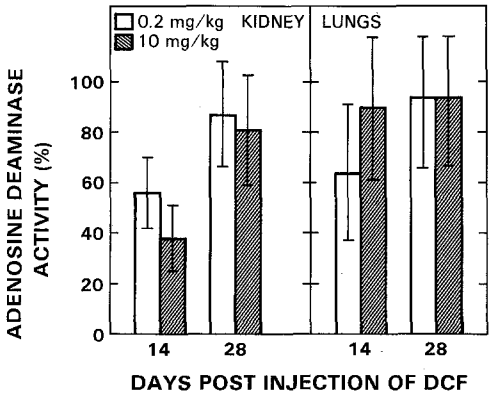


**Fig. 4.** Recoveries of DCF-inhibited ADA activities in mouse lung and kidneys. Conditions were similar to those described for Fig. 2

observed as a result of drug treatment. Also, no toxic deaths occurred in any of the groups during the study period.

The most striking change occurred in lung mass. With a single-injection schedule there was no change in this tissue, but the repeated-injection schedule resulted in increases (31% at 0.2 mg/kg; 79% at 10 mg/kg) in the tissue weights at both dosages. The other tissue that increased in mass on DCF treatment was the spleen. Liver and kidneys did not change significantly. Increased kidney and lung weight and decreased spleen and whole-body weight of animals 10 days after DCF treatment at a dose of 100 mg/kg by single IP injection have been reported [11].

Recoveries of DCF-inhibited ADA (after a single injection of the drug) in whole blood, spleen, liver, kidney, lungs and jejunum are shown in Figs. 1–5. Although marked tissue differences were observed, dosage appeared to have no effect on the rate of recovery of the inhibited enzyme.

DCF at a dose of 0.2 mg/kg completely inhibited the ADA activity in blood and liver, but the inhibition at this dose was only partial in spleen (88%) and small intestine (57%). The inhibition was almost complete (98%) in all the tissues examined at 10 mg DCF/kg by 4 h following treatment.

Recovery of enzymic activity was slow in spleen, liver, kidney, lungs, and blood. About 35 days were required for 100% recovery in blood. The recovery 28 days after treatment was about 60% in spleen and liver and 85% in kidney. In lung, the enzymic activity recovered 65%–90% in 14 days and > 95% in 28 days. In contrast, 100% recovery was observed in 1 day in the jejunum (Fig. 5). A large variation in ADA activity from one animal to another was also observed in this particular tissue.

## Discussion

The results described in this study indicate that in mice the major toxicity of DCF results in retardation of growth and change in lung weight. Furthermore, there are marked differences in the ability of different tissues to recover from DCF-induced ADA inhibition.

Differences in the recovery of DCF-inhibited ADA activity between L1210 cells and mouse blood have been reported earlier [1], when it was hypothesized that rapid reactivation seen in L1210 cells was due to new protein synthesis and rate of cell proliferation. The results of the present study are consistent with this hypothesis. The recovery of the enzyme was rapid in jejunum (100% in 24 h), whereas it was slow in other tissues. Intestinal mucosa has a high rate of cellular turnover and protein synthesis. Tissues such as spleen, liver, lungs, and kidney synthesize proteins but do not have a high degree of cellular turnover, and recoveries were slow in these tissues. This suggests that resting cells may not replace the enzyme. This is in agreement with our earlier observations with sarcoma-180 cells and human erythrocytes [9]. Although sarcoma-180 is a rapidly dividing cell and synthesizes protein, no recovery of the enzyme could be detected in this tumor over a period of 3 days in vitro when maintained under non-proliferating conditions. Similarly, no recovery was observed in human erythrocytes, which cannot synthesize protein or proliferate. It is interesting that it takes about 35 days for recovery of 100% activity in mouse blood (Fig. 1), i.e., about 3.0% per day. The average life-span of murine erythrocytes is 42 days i.e., about 2.2% of cells are renewed per day. Therefore, the 65%–70% recovery in blood observed in these studies is probably due to the production of young erythrocytes. The remainder of the difference in recovery may be accounted for by dissociation of ADA-DCF complexes in the older erythrocytes and other blood components.

Multiple injections, in general, resulted in more growth retardation than the single-injection schedule. Weight loss has been reported with a single dose of 100 mg/kg [11] and at multiple dosages (0.5 mg/kg) of DCF [8]. In agreement with these reports we have observed marked retardation in growth at 10 mg DCF/kg given by multiple injections.

The major toxicity observed in the present study was in the lung. This is consistent with the observations of Smyth et al. [11] in BDF mice. Furthermore, body temperature, total leukocyte counts, and hematocrit values did not change as a result of DCF treatment. Therefore, it appears that under these conditions, DCF is not toxic to tissues other than the lungs. Recent results from clinical trials in cancer patients have revealed that in addition to the marked T-cell lymphocytotoxicity, DCF causes hemolysis and CNS, renal, and pulmonary toxicities [4–7, 10]. Observed toxicities in clinical trials raise an important question: Is there a relation between the disease and DCF toxicity? In most human studies, patients have advanced disease, massive cellular breakdown, and elevated levels of adenosine and deoxyadenosine in their plasma, cerebrospinal fluid, and urine. It is possible that toxicities seen in human studies could be due to adenosine and deoxyadenosine rather than DCF. An examination of the relationship of adenosine and deoxyadenosine levels to tissue toxicity might answer this important question.

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