Cancer Chemother. Pharmacol. 1, 49–51 (1978)

Cancer Chemotherapy and Pharmacology

In vivo Toxicity to Lymphoid Tissue by 2′-Deoxycoformycin

John F. Smyth, Robert C. Young, and David M. Young

Medicine Branch and Laboratory of Toxicology,
National Cancer Institute, National Institutes of Health,
Bethesda, Maryland 20014, USA

Summary. *The enzyme adenosine deaminase has an essential role in lymphocyte metabolism. To examine the in vivo effects of inhibition of this enzyme healthy BDF_1 mice were injected intraperitoneally with 2′-deoxycoformycin, a stoichiometric tight-binding inhibitor of adenosine deaminase. Of the treated animals, 20% died of overwhelming infection, and histopathological examination of these, and surviving animals sacrificed 10 days following treatment indicated a selective toxicity to lymphoid cells. No toxicity to tissues other than the lymphoid system was observed, which is consistent with the hypothesis that 2′-deoxycoformycin offers a new and selective approach to the treatment of lymphoid malignancies.*

Introduction

Agents that exert anti-lymphocytic effects are widely used for the treatment of lymphoid malignancies and for immunosuppression. Unfortunately, the alkylating agents, antimetabolites, and other compounds in current clinical use for this purpose, impair cell proliferation nonspecifically, resulting in toxicity to tissues other than the lymphoid system, particularly to the bone marrow and gastrointestinal tract. These side effects compromise the utility and effectiveness of such drugs when the immunosuppressive effect is the only one desired. We present here evidence to suggest that a new class of compounds, which is about to be introduced into clinical trials, may exert selective toxicity on lymphocytes, and spare other host tissues; this new class of compounds functions by inhibiting the enzyme adenosine deaminase (ADA) [EC 3.5.4.4.].

Several observations indicate that lymphocytes require ADA for maintenance of proper function, proliferation, and viability. Although present in almost all mammalian tissues, ADA levels are highest in lymphoid organs such as the spleen, thymus, and lymph nodes (Conway and Cook, 1939; Brady, 1942; Brady and O'Donovan, 1965). Furthermore, the activity of this enzyme has been shown to increase three-fold in antigenically stimulated lymphocytes (Hall, 1963; Hovi et al., 1976) and 23-fold in blast cells from patients with acute lymphocytic leukaemia (Smyth and Harrap, 1975; Smyth, 1976). The most conclusive evidence establishing the specific requirement of lymphocytes for ADA comes from the observation that the genetic deletion of this enzyme results in a selective defect in lymphocyte development and function. Children with congenital ADA deficiency are impaired in the lymphoid system only, but present clinically with severe combined immunodeficiency disease (Giblett et al., 1972; Dissing and Knudsen, 1972) and die as a result of infection from opportunistic pathogens (Cohen, 1975).

If the activity of ADA is specifically required for lymphocyte function, then inhibition of this enzyme might, by mimicking the inborn error of metabolism referred to above, cause specific lymphocytotoxicity. A number of compounds are known to inhibit ADA, the most potent being 2′-deoxycoformycin (DCF), a tight-binding stoichiometric inhibitor of the enzyme (Johns and Adamson, 1976). We have treated normal healthy mice with DCF to observe the effects of in vivo inhibition of ADA on lymphoid tissue, the peripheral blood, and other host tissues.

Material and Methods

Twenty BDF_1 male mice were injected on day 1 with DCF (100 mg/kg i.p.) obtained from the Drug Research and Development Branch, National Cancer Institute. Ten control mice were injected with an equivalent volume of isotonic saline. The animals were allowed food and water ad libitum, and were weighed daily for 10 days. Half of the treated and control mice were bled by venepuncture

Reprint requests should be addressed to: Dr. J. F. Smyth, Division of Medicine, Institute of Cancer Research, Sutton, Surrey, UK

0344-5704/78/0001/0049/$ 01.00

of the tail vein on days 1 (pretreatment), 6, and 10, for analysis of their total and differential blood counts. On day 10 all surviving animals were sacrificed by cervical dislocation, and autopsies performed with subsequent histopathological examination of the tissues.

Results

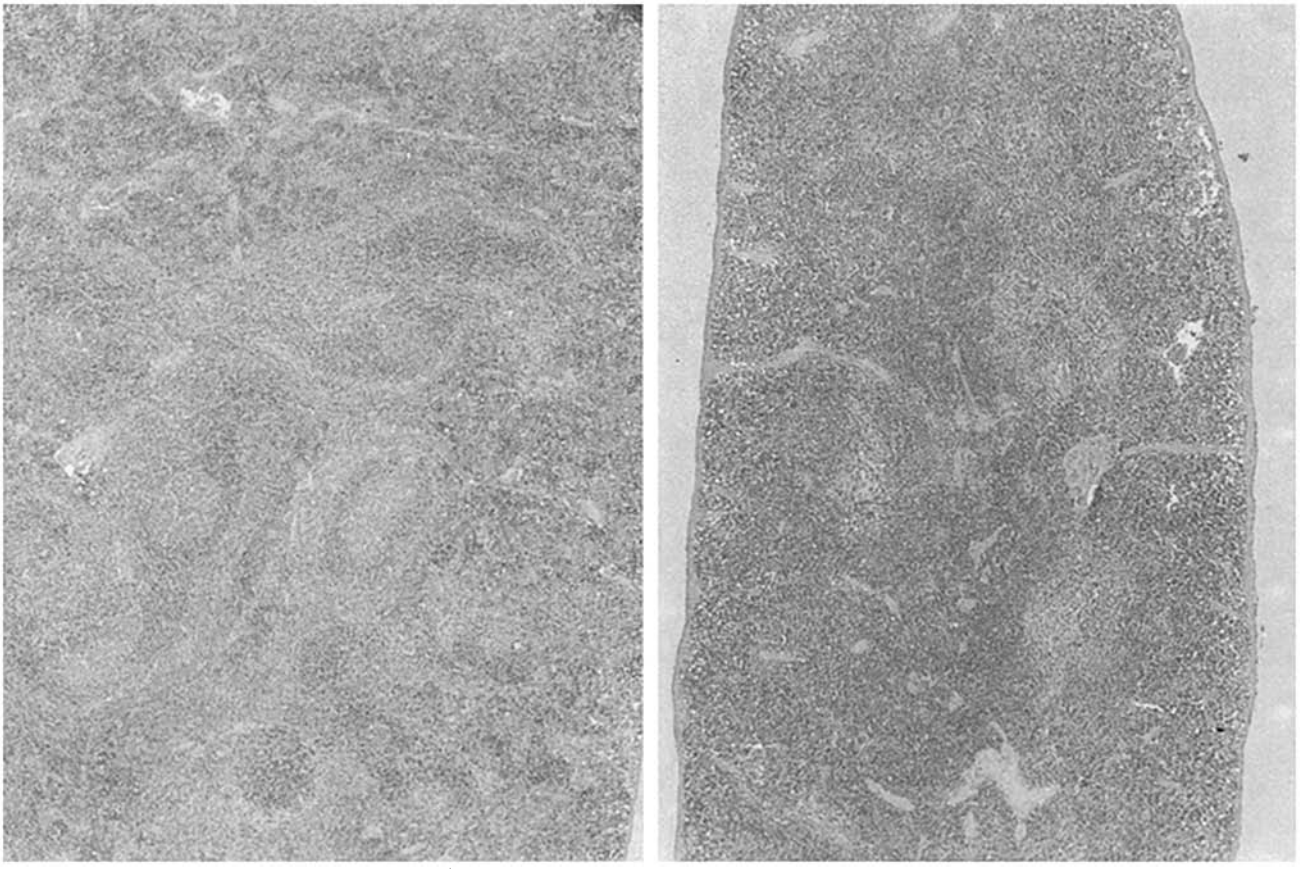
All animals treated with the ADA inhibitor lost weight progressively during the course of the observation period, and four mice died on days 5, 6, 8, and 9 respectively. The mean weight of treated animals killed on day 10 was 15 g (± 1.7 g) compared with 23 g (± 1.8 g) for control mice. At autopsy, the most striking finding was that of the small size of the spleens in treated animals (Table 1). The mean weight of spleens from treated mice was 3.3 mg/g total body weight (± 0.9 mg) in comparison with 4.6 mg (± 0.7 mg) for control spleens. This represents a 28% diminution in splenic weight.

Table 1. Effect of 2′-deoxycoformycin (100 mg/kg i.p.) on BDF_1 mice killed 10 days following treatment

	Weight[a]		
	Control (g)	Treated (g)	Change (%)
Whole animal	23.0 ± 1.8[a]	15.0 ± 1.7	−35
Spleen[b]	4.6	3.3 ± 0.9	−28
Liver[b]	51.0 ± 3.3	49.0 ± 7.0	− 4
Kidney[b]	7.4 ± 0.6	9.1 ± 2.3	+23
Lungs[b]	9.3 ± 1.5	13.5 ± 2.8	+45

[a] Mean ± standard deviation
[b] mg tissue per g total body weight

Histopathological examination of the spleens revealed marked lymphoid depletion with focal necrosis of lymphoid and reticuloendothelial cells. In spite of the severe lymphoid depletion observed in the spleen, occasional areas exhibited reticuloendothelial hyperplasia in areas of pre-existing lymphoid follicles (see Fig. 1). The total leucocyte count in treated animals increased by day 6 to 126% of controls, but decreased to 79% of control value by day 10. No significant alteration in differential leucocyte or platelet count was observed. Prior to treatment 84% of the cells were lymphocytes and 16% were polymorphonuclear leucocytes. Ten days after DCF treatment the differential count was 80% lymphocytes and 19% polymorphs. The mean platelet count of 72,000/μl (± 17,000) in treated animals on day 10 represents a 59% increase over control



A B

Fig. 1. A Normal mouse spleen. **B** Spleen from mouse treated 10 days earlier with 2′-deoxycoformycin (100 mg/kg i.p.); the spleen is atrophic and shows lymphoid depletion with focal necrosis of lymphoid cells. Both photographs taken at magnification × 55

mice with a platelet count of 45,500/μl (± 2,400). No toxicity to gastrointestinal tissues was observed. While there was no apparent alteration in leucocyte counts or differential, the animals died of widespread infection with histopathologically confirmed suppurative bronchopneumonia in the lungs, and suppurative pyelonephritis with microabscess formation in the kidneys.

Discussion

These results indicate that in mice a major effect of the ADA inhibitor 2′-deoxycoformycin is a toxicity to lymphoid cells, particularly dramatic in the spleen. No toxicity to any other organ system was observed other than that resulting from infection in the lungs and renal tract. The normal polymorphonuclear leucocyte counts in these mice suggest that these overwhelming infections may be in part related to consistent immunoincompetance stemming from the observed lymphocytotoxicity.

Inhibition of ADA in vitro has previously been shown to prevent the normal response of human lymphocytes to lectin mitogens (Hovi et al., 1976). Le Page et al. (1976) have reported that in mice treated with 2′-deoxycoformycin in vivo at doses as low as 0.5 mg/kg, an inhibition of the ADA activity greater than 80% occurs and persists for periods in excess of 72 h. These investigators also noted profound weight loss and death in their animals resulting from repeated exposure to DCF. Unfortunately no histopathologic details of toxicity were reported. In this study we have demonstrated for the first time, that inhibition of ADA in vivo produces a lymphocytotoxic effect.

Decrease in splenic size alone can be attributed to a number of nonspecific toxic stimuli, and the results of this preliminary investigation must not be overinterpreted. Nevertheless, the findings are entirely consistent with the hypothesis that inhibition of ADA can result in a selective lymphocytotoxic effect. The failure to see any change in peripheral lymphocyte count could be accounted for if, as might be expected, inhibition of ADA by DCF prevents the generation of new lymphoid cells, rather than affecting the numbers of existing mature lymphocytes in the circulation. In further studies it would be desirable to follow the peripheral lymphocyte count beyond the time course of the present experiment, to observe the eventual sequelae of the observed focal necrosis of lymphoid follicles in the spleen.

The specific metabolic requirement of lymphocytes for ADA indicated by the severe immunodeficiency that results from the genetic absence of this enzyme, and the marked elevation of ADA in malignant lymphocytes, suggests a possible specific and selective approach to lymphocytotoxic chemotherapy. 2′-deoxycoformycin is capable of inhibiting ADA in lymphocytes in vitro and in vivo and may prove to be a specific and therefore relatively nontoxic antilymphocytic compound of clinical value for immunosuppression and the treatment of lymphoid malignancies.

References

Brady, T. G.: Adenosine deaminase. Biochem. J. **36,** 478 (1942)

Brady, T. G., O'Donovan, C. I.: A study of tissue distribution of adenosine deaminase in six mammal species. Comp. Biochem. Physiol. **14,** 101 (1965)

Cohen, F.: Clinical features. In: Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency, p. 245. New York: Academic Press 1975

Conway, E. J., Cook, R.: The deaminases of adenosine and adenilic acid in blood and tissues. Biochem. J. **33,** 479 (1939)

Dissing, J., Knudsen, B.: Adenosine deaminase deficiency and combined immunodeficiency syndrome. Lancet **II,** 1316 (1972)

Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B., Meuwissen, H. J.: Adenosine deaminase deficiency in two patients with severe impaired cellular immunity. Lancet **II,** 1067 (1972)

Hall, J. G.: Adenosine deaminase activity in lymphoid cells during antibody production. Aust. J. Exp. Biol. **41,** 93 (1963)

Hovi, T., Smyth, J. F., Allison, A. C., Williams, S. C.: Role of adenosine deaminase in lymphocyte proliferation. Clin. Exp. Immunol. **23,** 395 (1976)

Johns, D. G., Adamson, R. H.: Enhancement of the biological activity of Cordycepin (3′-deoxyadenosine) by the adenosine deaminase inhibitor 2′-deoxycoformycin. Biochem. Pharmacol. **25,** 1441 (1976)

LePage, G. A., Worth, L. S., Kimball, A. P.: Enhancement of the antitumour activity of arabinofuranosyladenine by 2′-deoxycoformycin. Cancer Res. **36,** 1481 (1976)

Smyth, J. F.: The significance of adenosine deaminase activity in leukaemia. Proc. Am. Ass. Cancer Res. **12,** 235 (1976)

Smyth, J. F., Harrap, K. R.: Adenosine deaminase activity in leukaemia. Br. J. Cancer **31,** 544 (1975)

Received August 16, 1977/Accepted September 29, 1977