Biochemical Pharmacology, Vol. 46, No. 6, pp. 1071–1075, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00
Pergamon Press Ltd

SHORT COMMUNICATIONS

Prevention of insulin-dependent diabetes mellitus by 2'-deoxycoformycin in the BB Wistar rat

(*Received* 26 *February* 1993; *accepted* 14 *June* 1993)

Abstract—The effect of the adenosine deaminase (ADA) inhibitor 2'-deoxycoformycin (dCF) on the development of insulin-dependent diabetes mellitus (IDDM) was assessed in the BB Wistar rat. Sixty-one male rats were treated from days 30 to 120 with 0, 0.5, 1.0 or 1.5 mg dCF/kg/week. The incidence of IDDM was 78% in the controls and was significantly ($P < 0.01$) decreased in rats receiving 1.5 mg dCF/kg/week (32%), but not in rats receiving lower doses of the drug. However, for those rats that became diabetic the mean time to the development of IDDM was unchanged in animals receiving dCF compared with control. dCF treatment did not produce significant weight loss in the animals or gross changes in the thymus, spleen or kidneys. Although the protective effect of dCF against IDDM was likely produced by immunosuppression, the different dCF dosages had similar effects on ADA suppression in spleen or thymus and on dATP accumulation in these organs.

There is compelling evidence that insulin-dependent diabetes mellitus (IDDM*) is caused by autoimmune destruction of the pancreatic islet cells with the primary mechanism being cell mediated [1]. In the genetically predisposed BB Wistar rat, an authentic model of IDDM, treatment with the immunosuppressive agent cyclosporin A (CSA) completely prevents the development of diabetes [2]. In patients, the process leading to islet cell destruction is insidious and is usually not complete at the onset of clinical symptoms [1]. Based on these considerations, patients have been treated with CSA soon after IDDM diagnosis to prevent further islet cell destruction. With this approach, CSA produces a 5-fold decreased chance of being insulin dependent after 1 year of therapy, and remissions occur more frequently if treatment is begun within 6 weeks of diagnosis [3]. Unfortunately, CSA produces a number of side-effects, including nephrotoxocity, and relapses occur following discontinuation of therapy [3, 4]. Other immunosuppressive therapies are of unproven benefit and have also been associated with significant side-effects [1].

The adenosine deaminase (ADA) 2'-deoxycoformycin (dCF) is a novel drug that was developed following the observation that congenital ADA deficiency is associated with severe lymphopenia and profound impairment of both humoral and cellular immune responses [5]. dCF has been used extensively in the treatment of lymphoid malignancies, and in low doses is particularly effective in the therapy of hairy cell leukemia, which is a chronic B cell disorder [6]. In addition to affecting B cells, dCF produces profound suppression of the T cell count, with equivalent reduction in both CD4 and CD8 cells, and this effect persists for many months after dCF has been discontinued [7]. In low doses, dCF is relatively well-tolerated, with nausea/vomiting and lethargy being the major toxicities [5, 6]. The mechanism whereby dCF produces lymphocytolysis in these disorders is unclear, but presumably is related to the accumulation of deoxyadenosine and adenosine following ADA inhibition [5, 8]. Deoxyadenosine is preferentially converted to dATP in lymphoid cells, and this deoxynucleotide can produce cell death through a variety of mechanisms [9, 10].

The immunosuppressive properties of dCF have been evaluated less extensively. Most studies have been carried out in the mouse or rat, where 0.25 mg/kg dCF produces greater than 90% inhibition of ADA in the spleen and thymus [11, 12]. Daily intraperitoneal injections of 0.25 to 1.0 mg dCF/kg body weight for 4 days produces a significant reduction in circulating lymphocytes at 24–72 hr, with suppression of the lymphoproliferative responses to T cell mitogens [12]. Additionally, the antibody response to immunization with sheep red cells is suppressed [12]. The immunosuppressive activity of dCF has also been demonstrated by allograft acceptance of LSTRA tumor cells across the H2 histocompatibility barrier [13]. A single dose of 15 mg dCF/kg, administered 24 hr before the transplant, resulted in a 69% allograft acceptance [13].

In the present study we have examined whether the immunosuppressive properties of dCF might protect against the development of IDDM in the BB Wistar rat model.

Materials and Methods

Efficacy of 2'-deoxycoformycin in preventing IDDM. Male BB Wistar rat pups were obtained from the Animal Resources Division of the Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. These rats have excellent breeding records and a predictable incidence of diabetes mellitus of 60–75% by 90 days of life [2]. The rats were fed *ad lib.* under controlled lighting conditions (12 hr of light alternating with 12 hr of darkness). At 30 days, the animals were randomized to receive 0 ($N = 9$), 0.5 ($N = 16$), 1.0 ($N = 17$), or 1.5 ($N = 18$) mg/kg dCF, administered by subcutaneous injection weekly. The rats were tested for glycosuria by "Tes-tape" three times per week from 30 to 60 days, and daily thereafter. When glycosuria was detected, the animals were treated with beef or pork protamine-zinc insulin (Connaught Laboratories, Toronto, Canada). Insulin was administered by subcutaneous injection to prevent mortality, and the dose was adjusted (2–4 units/day) on the basis of daily urine glucose testing.

At 120 days, 24-hr urine collections were obtained, the animals were anesthetized with sodium pentobarbital, and blood was collected by cardiac puncture. Liver function [serum aspartate transaminase (AST), alanine transaminase

* Abbreviations: ADA, adenosine deaminase; dCF, 2'-deoxycoformycin; IDDM, insulin-dependent diabetes mellitus; CSA, cyclosporin A; AST, serum aspartate transaminase; ALT, alanine transaminase; and GGT, γ-glutamate transaminase.

Table 1. Efficacy of 2′-deoxycoformycin in preventing diabetes mellitus

Group	Dose of dCF (mg/kg/week)	Total number	Non-IDDM	IDDM	Onset of IDDM (days)	
					Median	Mean ± SEM (N)*
1	0	9	22%	78%	81	91.0 ± 5.0 (7)
2	0.5	16	6%	94%	100	93.8 ± 3.4 (15)
3	1.0	17	24%	76%	95	98.2 ± 2.3 (13)
4	1.5	19	68%	32%†	95	91.0 ± 5.4 (6)

* Mean ± SEM of (N) determinations.
† $P < 0.01$, Group 4 vs all other groups.

Table 2. Effect of 2′-deoxycoformycin on organ weight

Group	Dose of dCF (mg/kg/week)	Total number	Kidney* (g)	Spleen* (g)	Thymus* (g)
1	0.5	6	1.81 ± 0.24	0.87 ± 0.09	0.39 ± 0.15
2	1.0	7	1.64 ± 0.09	0.79 ± 0.08	0.38 ± 0.05
3	1.5	9	1.61 ± 0.23	0.88 ± 0.25	0.53 ± 0.05†

* Values are means ± SEM of the number of determinations indicated.
† $P < 0.05$, Group 3 vs all other groups.

(ALT), γ-glutamate transaminase (GGT) and total bilirubin] and kidney function [serum and urine creatinine and creatinine clearance] were evaluated. The spleen, thymus and kidneys were collected, weighed and processed for light microscopic evaluation.

Effects of 2′-deoxycoformycin on ADA and dATP. Male rat pups were treated at 30 days of age with a single dose of 1.0 or 1.5 mg dCF/kg, and the effects of treatment on spleen and thymus ADA activities and dATP levels were assessed at 1, 24, 96 and 168 hr. At each time point three rats in each group were anesthetized with sodium pentobarbital and euthanized, and their thymuses and spleens were removed and weighed. The thymuses and spleens of six control rats at 30 days of age, who had not received dCF, were similarly obtained. DeoxyATP was extracted immediately from the tissues with 0.4 M perchloric acid and, following neutralization with 2 vol. of 0.5 M trioctylamine in trifluorotrichloroethane, was measured using a DNA polymerase assay [6]. DeoxyATP levels were expressed as picomoles per milligram of tissue. Portions of the tissues were stored at −80° for several weeks prior to ADA measurements. Tissue samples were weighed and reconstituted in phosphate-buffered saline, titrated to pH 6.8, freeze-thawed three times, and centrifuged at 15,000 *g* for 5 min. Aliquots of supernatant were then assayed for ADA activity as measured by the capacity to convert adenosine to inosine, using thin-layer chromatography, as we have described previously [8]. The ADA activity was expressed as nanomoles adenosine per hour per milligram of tissue.

Statistical methods. The time to develop IDDM for groups receiving 0, 0.5, 1.0 or 1.5 mg dCF/kg/week was determined using the log rank test. Individual groups were compared for differences in the incidence of IDDM, in overall and specific organ weights, and in metabolic parameters (creatinine, creatinine clearance, bilirubin, GGT, AST, ALT) using Fisher's exact test. IDDM and non-IDDM groups were similarly compared. The effect of dCF dose on the incidence of IDDM was also evaluated using the Mantel Haenzel trend test. dCF dose, creatinine clearance and GGT were tested as possible covariates for the development of IDDM using a logistic regression model.

Results and Discussion

The results of this study demonstrated that dCF can decrease the incidence of IDDM in the BB Wistar rat and this effect is dependent on the dCF dosage. Table 1 shows the incidence of IDDM and the time to onset in the four groups of rats receiving 0, 0.5, 1.0 or 1.5 mg/dCF/kg/week. Seventy-eight percent of the control rats developed IDDM with the median and mean times until the development of diabetes being 81 and 91.0 ± 5.0 days, respectively. Those rats receiving the highest dose of dCF (1.5 mg/kg/week) had a significantly ($P < 0.01$) reduced incidence of IDDM (32%); however, the time to onset for those animals that did develop diabetes was similar in all groups. No statistical difference in the incidence of IDDM between rats treated with 0.5 or 1.0 mg dCF/kg/week and controls could be demonstrated.

As the ability of dCF to protect against IDDM is likely related to its toxic effects on lymphoid tissue, it was predicted that the protective effect of dCF against IDDM would correlate with a decrease in spleen and thymus weight. However, there were no differences in the absolute and adjusted weights of the spleens obtained from rats treated with different concentrations of dCF. In contrast, the absolute mean weight of the thymus at 120 days was greater in the group receiving 1.5 mg dCF/kg/week compared with the groups receiving 0.5 or 1.0 mg dCF/kg/week ($P < 0.05$) (Table 2), and this was also true when the thymic weight was adjusted for body weight. As IDDM is itself immunosuppressive, it was possible that the difference in thymus weights could be explained by the greater incidence of diabetes in the groups receiving 0.5 or 1.0 mg dCF/kg/week. However, there was no difference in thymic weights between the IDDM group compared with the non-IDDM group when adjusted for body weight. The reason for the increased thymic weight in rats treated with 1.5 mg/kg/week is unknown. Morphological studies indicated no

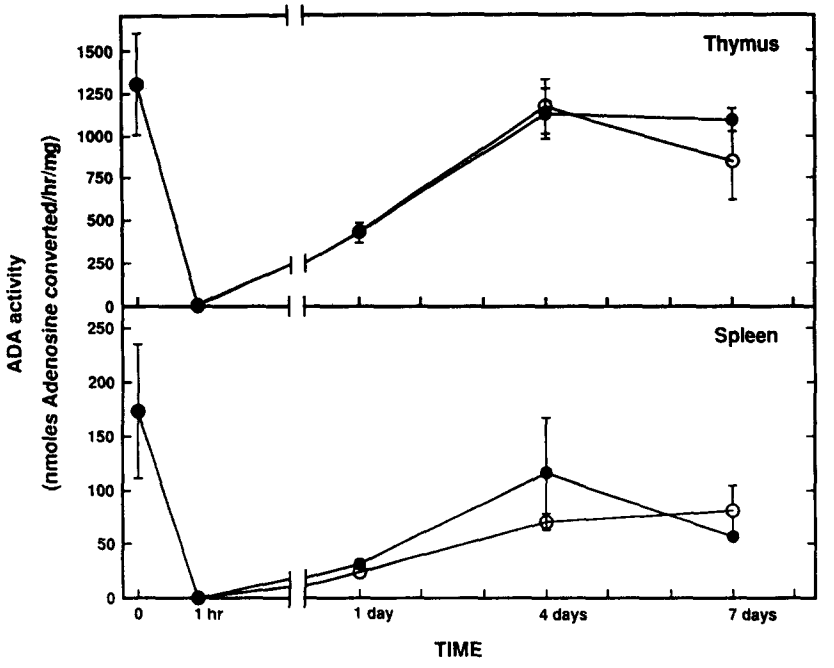

Fig. 1. Effect of dCF on ADA activities in the thymus and spleen of rats treated with 1.0 mg (●) or 1.5 mg (○) dCF/kg. At the indicated times, three rats for each drug group were killed, and the ADA levels were measured in the spleen and thymus as described in Materials and Methods. Values are the means ± SEM of 3–6 measurements.

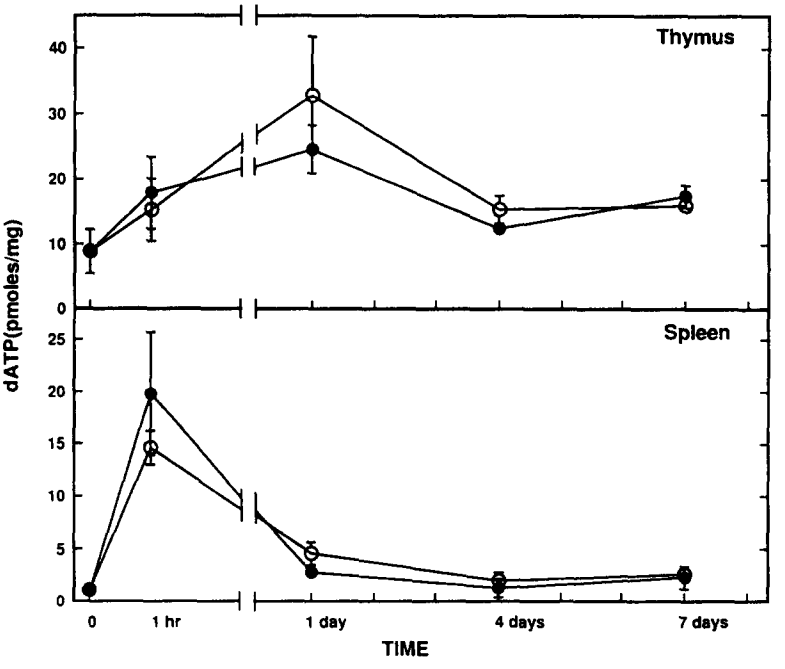

Fig. 2. Effect of dCF on dATP levels in the thymus and spleen of rats treated with 1.0 mg (●) or 1.5 mg (○) dCF/kg. At the indicated times, three rats for each drug group were killed, and the dATP activities were measured in the spleen and thymus, as described in Materials and Methods. Values are the means ± SEM of 3–6 measurements.

differences in the histology of thymuses and spleens in rats treated with different concentrations of dCF. Thus, if the protective effect of dCF against IDDM is immune mediated, this may involve alterations in lymphocyte subpopulations rather than gross changes in spleen or thymus.

The lymphocytolytic effect of dCF has been related to its ability to inhibit ADA with the subsequent accumulation of dATP in lymphoid tissue [5, 8–10]. Thus, the effects of 1.0 and 1.5 mg dCF/kg on ADA activities (Fig. 1) and dATP levels (Fig. 2) in the spleen and thymus were examined. As previously shown [11], cellular ADA activity in thymus (776 ± 188 nmol adenosine/hr/mg tissue) was approximately 10-fold greater than in spleen (118 ± 25 nmol adenosine/hr/mg tissue), and in both organs 1.0 or 1.5 mg dCF/kg inhibited enzyme activity by >99% at 1 hr. With both drug doses, the ADA activities in spleen and thymus had begun to return by 24 hr and returned to normal by days 4–7 after dCF treatment. The baseline dATP levels were 1.1 ± 0.3 and 8.95 ± 3.4 pmol/mg tissue in spleen and thymus, respectively. The dATP levels in the thymus increased 3-fold 24 hr following either 1.0 or 1.5 mg dCF/kg, and the levels decreased to pretreatment levels by day 4. There was also a slight increase in dATP levels in the spleen at 1 hr with both drug doses but, in contrast to the thymus, the dATP levels rapidly returned to normal.

Although dCF likely produces its preventive effects in IDDM by immunosuppression, the studies above indicate that the protective effects of the different doses of dCF do not correlate with gross morphological or biochemical changes in either spleen or thymus. In patients treated with dCF, the peripheral T-helper and -suppressor cell numbers are reduced to an equivalent degree [7]. However, a difference in the sensitivity of different T cell populations to dCF *in vitro* has been demonstrated previously, with T-suppressor cells being more sensitive to the cytotoxic effect of dCF and T-helper cells being more sensitive to inhibition of function [14, 15]. In the rat model it is possible that the specific lymphocyte populations responsible for IDDM may be selectively sensitive to the higher dose of dCF. To date, the effect of dCF on lymphocyte subsets has not been evaluated and will be examined in future studies. If depletion of a specific lymphocyte population correlates with the prevention of IDDM, this marker may be used as an endpoint for optimizing drug dosage and scheduling to prevent IDDM in the rat model and in subsequent clinical trials.

With the dosage scheduling used in this study, no apparent toxicity was observed with dCF. None of the rats died as a result of dCF and weight gain was not influenced by drug treatment. At 120 days, the mean ± SEM weights of the rats treated with 0 (358.6 ± 44.6 g), 0.5 (381.7 ± 46.1 g), 1.0 (364.1 ± 33.1 g) and 1.5 (370.8 ± 42.6 g) mg dCF/kg/week were not significantly different. However, when diabetic rats were compared with non-diabetic rats, there was a significant ($P < 0.05$) decrease in weights in the diabetic group at 120 days (377 ± 36 vs 412 ± 26 g), suggesting that part of the maintenance of weight in the 1.5 mg dCF/kg group was related to the fact that most rats were not diabetic. In the clinical situation, high doses of dCF have produced toxicity in the neurological system, kidneys and liver [16, 17]. However, in the present study no differences in serum creatinine, bilirubin, AST, ALT, GGT and creatinine clearance between groups receiving different dCF doses were observed. Likewise, there were no histological changes in the kidneys and livers in the rats treated with dCF, and the absolute and adjusted weights of the kidneys were not significantly different between the groups (Table 2). However, metabolic differences indicating renal dysfunction were observed between IDDM and non-IDDM rats, and the urine volume was significantly ($P < 0.01$) greater in diabetic rats (58.7 ± 42.2 mL/24 hr) compared with non-diabetic rats (8.38 ± 4.3 mL/24 hr). Similarly, the creatinine clearance was increased in diabetic compared with non-diabetic rats (1.42 ± 0.48 mL/min vs 0.97 ± 0.34 mL/min, $P < 0.05$). In summary, no structural or functional changes were noted as a consequence of dCF treatment. All functional changes could be ascribed to diabetes mellitus.

This study indicates that dCF may be a useful agent in preventing the development of IDDM, and further studies are required in the BB Wistar rat model to determine if higher doses of dCF are more effective in preventing IDDM without producing drug toxicity. In addition, other drug schedules, including more frequent dCF administration, need to be investigated to determine the optimum schedule for IDDM prevention. Since the suppressive effects of dCF on T cells have been shown to persist in patients for prolonged periods following treatment [5], it should be determined whether the protective effect of dCF against IDDM persists after the drug is discontinued. If this is the case, dCF might be a more effective agent than CSA for the prevention of clinical diabetes mellitus. In addition, newer nucleoside analogs, e.g. 2-chlorodeoxyadenosine and fludarabine, should also be evaluated for their ability to prevent IDDM, as they are also lymphotoxic but less likely than dCF to cause nausea/vomiting [18, 19].

Acknowledgements—The authors thank Dr. P. Thibert of the Health Protection Branch, Health & Welfare Canada for supplying the BB Wistar rats and Ms. J. Powell for typing this manuscript. This work was supported by grants from The Children's Hospital of Winnipeg Research Foundation and The National Cancer Institute of Canada.

*Departments of *Anatomy, ‡Internal Medicine, §Pharmacology and Therapeutics, and ||Pediatrics and Child Health*
University of Manitoba, and ¶Manitoba Institute of Cell Biology Manitoba Cancer Treatment and Research Foundation
Winnipeg, Manitoba, Canada R3E OV3

JAMES A. THLIVERIS*†
ASHAR BEGLEITER‡§¶
NATHAN L. KOBRINSKY||
LINDA VERBURG‡¶
HEATHER J. DEAN||
JAMES B. JOHNSTON‡¶**

REFERENCES

1. Wilson K and Eisenbarth GS, Immunopathogenesis and immunotherapy of type I diabetes. *Annu Rev Med* **41**: 497–508, 1990.
2. Laupacis A, Stiller CR, Gardell C, Keon P, Dupre J, Wallace AC and Thibert P, Cyclosporin prevents diabetes in BB Wistar rats. *Lancet* **1**: 10–11, 1983.
3. The Canadian-European Randomized Control Trial Group, Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* **37**: 1574–1582, 1988.
4. Martin S, Schernthaner G, Nerup J, Gries FA, Koivisto VA, Dupre J, Standl E, Hamet P, McArthur R, Tan MH, Dawson K, Mehta AE, Van Vliet S, von Graffenried B, Stiller C and Kolb H, Follow-up of cyclosporin A treatment in Type 1 (insulin-dependent) diabetes mellitus: Lack of long-term effects. *Diabetologia* **34**: 429–434, 1991.

† Corresponding author: Dr. James A. Thliveris, Department of Anatomy, University of Manitoba, 730 Williams Ave., Winnipeg, Manitoba, Canada R3E 0V3. Tel. (204) 789-3652; FAX (204) 772-0622.

** Dr. J. B. Johnston is a Portney Research Scholar of the Manitoba Cancer Treatment and Research Foundation.

5. O'Dwyer PJ, Wagner B, Leyland-Jones B, Wittes RE, Cheson BD and Hoth DF, 2'-Deoxycoformycin (Pentostatin) for lymphoid malignancies. *Ann Intern Med* **108**: 733–743, 1988.
6. Johnston JB, Eisenhauer E, Corbett WEN, Scott JG and Zaentz SD, Efficacy of 2'-deoxycoformycin in hairy-cell leukemia. A study of the National Cancer Institute of Canada Clinical Trials Group. *J Natl Cancer Inst* **80**: 765–769, 1988.
7. Kraut EH, Neff JC, Bouroncle BA, Gochnour D and Grever MR, Immunosuppressive effects of Pentostatin. *J Clin Oncol* **8**: 848–855, 1990.
8. Begleiter A, Glazer RI, Israels LG, Pugh L and Johnston JB, Induction of DNA strand breaks in chronic lymphocytic leukemia following treatment with 2'-deoxycoformycin *in vivo* and *in vitro*. *Cancer Res* **47**: 2498–2503, 1987.
9. Carrera CJ, Terai C, Lotz M, Curd JG, Piro LD, Beutler E and Carson DA, Potent toxicity of 2-chlorodeoxyadenosine toward human monocytes *in vitro* and *in vivo*. *J Clin Invest* **86**: 1480–1488, 1990.
10. Johnston JB, Leer K, Verburg L, Blondal J, Mowat MRA, Israels LG and Begleiter A, Induction of apoptosis in CD4+ prolymphocytic leukemia by deoxyadenosine and 2'-deoxycoformycin. *Leuk Res* **16**: 781–788, 1992.
11. Barton RW, The effects of an induced adenosine deaminase deficiency on T-cell differentiation in the rat. *Cell Immunol* **95**: 297–310, 1985.
12. Luebke RW, Lawson LD, Rogers RR, Riddle MM, Rowe DG and Smialowicz RJ, Selective immunotoxic effects in mice treated with the adenosine deaminase inhibitor 2'-deoxycoformycin. *Immunopharmacology* **13**: 25–35, 1987.
13. Chassin MM, Chirigos MA, Johns DG and Adamson RH, Adenosine deaminase inhibition for immunosuppression (letter). *N Engl J Med* **296**: 1232, 1977.
14. Callard RE, Ewing TM and Fox RM, Relative sensitivity of human T cell subsets to deoxyadenosine toxicity. *Clin Exp Immunol* **558**: 136–144, 1984.
15. Cohen AH, Bluestein HG and Redelman D, Deoxyadenosine modulates human suppressor T cell function and B cell differentiation stimulated by *Staphylococcus Aureus* protein A. *J Immunol* **132**: 1761–1766, 1984.
16. Major PP, Agarwal RP and Kufe DW, Clinical pharmacology of deoxycoformycin. *Blood* **58**: 91–96, 1981.
17. Poplack DG, Sullan SE, Rivera G, Holcenberg J, Murphy SB, Blatt J, Lipton JM, Venner P, Glaubiger DL, Ungerleider R and Johns D, Phase I study of deoxycoformycin in acute lymphoblastic leukemia. *Cancer Res* **41**: 3343–3346, 1981.
18. Piro LD, 2-Chlorodeoxyadenosine treatment of lymphoid malignancies. *Blood* **79**: 843–845, 1992.
19. Chun HG, Leyland-Jones B and Cheson BD, Fludarabine phosphate: A synthetic purine antimetabolite with significant activity against lymphoid malignancies. *J Clin Oncol* **9**: 175–188, 1991.