Cancer Chemother Pharmacol (1985) 14:49–52

Cancer Chemotherapy and Pharmacology

Treatment of multiple myeloma with deoxycoformycin

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Summary. *A comparison of adenosine deaminase activity in intact human plasma cells and lymphocytes in vitro showed that plasma cells had at least as much activity of this enzyme as did T or non-T lymphocytes. This observation led us to examine the effectiveness of deoxycoformycin in the treatment of multiple myeloma. Thirteen patients with advanced refractory myeloma were treated with deoxycoformycin at 5 mg/m² daily for 3 days every 2 weeks until response or progression. Of the seven evaluable patients who received more than one cycle of therapy, two had a greater than 50% reduction in the level of myeloma protein and two had a demonstrable reduction in soft tissue disease. Toxicity consisted of marked nausea, anorexia lasting several days, and mild transient confusion in some patients. Plasma levels of deoxyadenosine and adenosine peaked on day 4 or 5 with average values of 1.9 and 0.6 μM, respectively. Red cell levels of dATP reached approximately 40% of ATP levels. The viability of plasma cells was shown to be greatly reduced in in vitro incubations with deoxycoformycin and low levels of deoxyadenosine (ID_{50} of 6 μM).*

Introduction

The use of melphalan and prednisone as the primary treatment for multiple myeloma results in a 50% or greater reduction in tumour size in approximately 70% of new patients, with a median survival for these responders of 40 months [2]. Alternative drug treatment for the initial nonresponders and the subsequently relapsing patients has been much less successful. In three recent studies with different types of combination chemotherapy the objective response rates recorded have been only 5%–15% in the initially nonresponding group and 15%–30% in relapsing group [3, 14; D. F. White et al. 1984, preliminary results]. Consequently, there is an obvious need for alternative modes of therapy for this disease.

Deoxycoformycin is a purine nucleoside analogue, produced by a streptomycete, whose principal biochemical effect is to inhibit the enzyme adenosine deaminase. Clinical interest in adenosine deaminase inhibitors was initially based on their potential to increase the therapeutic effectiveness of various adenosine analogues, such as arabinofuranosyladenine, which are rapidly deaminated to inactive derivatives. Later, Smyth et al. showed that deoxycoformycin alone had antitumor activity with their study of this drug in the treatment of acute lymphoblastic leukemia [21]. This initial report has stimulated several clinical studies on the activity of deoxycoformycin in lymphoproliferative diseases [18, 20]. In this report we present our initial results on the activity of this agent in multiple myeloma.

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Methods

Patients who were entered this study had been enrolled and had failed on the Canadian NCI multiple myeloma trial. This initial therapy consisted of oral melphalan (9 mg/m² daily × 4 days) and prednisone (50 mg b.i.d. × 4 days) every 28 days. Initial treatment failures and/or relapsing patients were treated with adriamycin (30 mg/m²), CCNU (20 mg/m²), vincristine (1.4 mg/m²), and prednisone (50 mg/m² b.i.d. × 4 days) every 28 days.

Pentostatin (2′-deoxycoformycin) was supplied in 10-mg ampules by the Investigational Drug Branch of the U.S. NCI (Bethesda). After reconstitution with normal saline, the deoxycoformycin was infused IV over 1–3 min at a dose of 5 mg/m² daily for 3 days every 2 weeks until response or progression. Since this agent has been associated with renal impairment, those patients with a creatinine clearance of less than 60 ml/min were excluded from this study. Allopurinol (100 mg b.i.d.) was given while patients were on this trial. If patients were hypercalcaemic at the time of entry into the study, the calcium levels were normalized with IV saline and steroids prior to deoxycoformycin therapy. No further steroids were given once deoxycoformycin therapy had been initiated.

A response was defined as a decrease of at least 50% in the level of the serum monoclonal protein. Progression was an increase in the myeloma protein by greater than 10 g/l, development of hypercalcaemia, or progressive soft tissue or skeletal lesions.

Plasma levels of deoxyadenosine and adenosine and the intracellular concentrations of ATP and dATP in red blood cells were determined as previously described by high-performance liquid chromatography of neutralized acid extracts on a reverse phase and an anion-exchange column, respectively [10, 19].

Adenosine deaminase activity was determined in intact lymphocytes and plasma cells which had been isolated from peripheral blood or bone marrow aspirations, respectively. Monocytes were removed by treatment with carbonyl iron and

the mononuclear cell fraction collected by centrifugation over 60% Percoll [1]. T lymphocytes were separated from the non-T lymphocytes by rosetting with neuraminidase-treated sheep erythrocytes [23]. Cells (5×10^6) were incubated for 30 min at 37° C with shaking in an air atmosphere in 0.1 ml RPMI 1640 medium prior to the addition of 0.1 m*M* [$^{8-14}$C]adenosine (50 mCi/mmol, Schwarz-Mann). Incubations were terminated by the addition of 5 µl cold 4.2 *M* perchloric acid and the extracts neutralized by treatment with alanine freon [10]. Purine bases, nucleosides, and nucleotides were separated by thin-layer chromatography and the radioactivity was measured in individual metabolites using a toluene-based scintillation fluid [10]. Deamination of adenosine was calculated as the sum of radioactivity in inosine and hypoxanthine; little or no xanthine and uric acid was formed.

The viability of plasma cells which had been incubated in vitro with deoxycoformycin and deoxyadenosine was monitored by vital staining with fluorescein diacetate. Cells were stained with fluorescein diacetate (5 µg/ml) for 14–30 min and the proportion of viable fluorescent cells was determined by fluorescence microscopy or flow cytometry using a Coulter Electronics Epics V system. In the flow cytometric analysis, excitation was at 488 nm and the emission between 530 and 590 nm was measured using the standard three log amplifiers on the Epics V system.

Results

Prior to starting the clinical study of the treatment of myeloma with deoxycoformycin, we carried out a comparative study of the level of adenosine deaminase activity in normal T and non-T lymphocytes and in myeloma plasma cells. This study utilized intact cells and the results are shown in Table 1. The plasma cell studies were carried out on the mononuclear fraction from marrow aspirates which contained at least 80% plasma cells. The average adenosine deaminase activities, expressed as nanomoles of adenosine deaminated per 10^6 cells per hour, for T lymphocytes, non-T lymphocytes, and plasma cells, were 0.49 ± 0.27, 0.69 ± 0.29, and 5.39 ± 2.23, respectively. These rates of deamination were obtained with 0.10 m*M* adenosine in the medium and were linear for at least 30 min. The increased volume of plasma cells relative to lymphocytes was not taken into account in expressing the levels of adenosine deaminase activity. With average literature values of 9 and 14 µ*M* for the diameters of lymphocytes and plasma cells, a ratio of plasma cell to lymphocyte volume of approximately 4 can be calculated. Therefore, on a volume basis plasma cells have 2–3 times as much adenosine deaminase activity as do peripheral blood lymphocytes. Addition of deoxycoformycin to these in vitro incubations resulted in inhibition of adenosine deamination with both normal lymphocytes and plasma cells. Inhibition of adenosine deamination was in excess of 80% and 98% with deoxycoformycin concentrations of 1 and 5 µ*M*, respectively.

The toxicity of the combination of deoxycoformycin and deoxyadenosine towards myeloma plasma cells from three patients was examined in vitro. The mononuclear cell fractions, containing greater than 70% plasma cells, from bone marrow aspirates were incubated in vitro in RPMI-1640 medium containing 10% fetal calf serum with deoxycoformycin (1 µg/ml) and varying concentrations of deoxyadenosine. Cell viability was monitored by vital staining with fluorescein diacetate for up to 4 days. The combination of deoxycoformycin and deoxyadenosine was toxic to plasma cells, as evidenced by the vital staining. The loss of viability was first observed about 20 h after the addition of deoxyadenosine, with cell lysis being observed between 48 and 72 h. The concentration of deoxyadenosine giving a 50% reduction in plasma cell viability by 72 h was 6 µ*M* (range 3–10 µ*M*). Neither deoxycoformycin nor deoxyadenosine alone had an effect on plasma cell viability.

The description of the 13 patients entered into the clinical study is shown in Table 2. The first 9 of the patients listed in Table 2 had not experienced an objective response to the initial drug therapy of melphalan and prednisone, although 4 of these latter patients had remained in a relatively stable state for 12–36 months (Mr MP, Mr SL, Mrs HS, Mr CS). Subsequent combination drug therapy had in all cases proven ineffective. The four remaining patients entered into this study had initially responded to melphalan and prednisone therapy but had

Table 1. Adenosine deaminase activity in intact lymphoid cells in vitro

Donor[a]	Enzyme activity[b]		
	T lymphocytes	Non-T lymphocytes	Plasma cells
LB	0.45	0.90	
BP	0.08	0.38	
EP	0.44	0.75	
PR	0.22	0.18	
JL	0.74	0.92	
GZ	0.45	0.93	
MB	0.92	0.91	
CP	0.59	0.54	
AH			5.26
SH			2.76
BS			5.45
NS			4.10
GD			5.34
ER			9.42

[a] T- and non-T lymphocytes were isolated from blood of normal individuals and plasma cells from myeloma marrow aspirates
[b] Nanomoles of adenosine deaminated per 10^6 cells per hour

Table 2. Characteristics of myeloma patients treated with deoxycoformycin

Patient	Age	Protein type	Cycles of deoxycoformycin	Entry status[a]
Mrs JM	45	IgG K	3	a, b
Mrs MS	62	IgG K	3	a
Mrs NM	53	IgG L	1	a, c
Mr JC	66	Kappa	1	a
Mr MP	63	IgG K	4	a
Mr SL	62	IgA K	7	–
Mr JB	45	Kappa	4	a, b
Mrs HS	56	IgG K	1	a, c
Mr CS	64	IgG K	1	a, c
Mr NW	63	IgA K	1	a, c
Mr EC	55	Kappa	5	a, b
Mr VB	71	IgG L	3	a
Mrs DS	47	IgG K	1	a, b

[a] At the time deoxycoformycin therapy was started patients presented with (a) progressive bone pain, (b) soft tissue disease, or (c) hypercalcaemia

subsequently relapsed and had all failed to respond to at least three courses of combination drug therapy.

At the time of entry into this study 4 of the 13 patients had hypercalcaemia and 4 had soft tissue involvement. These patients were all seriously ill, with Karnofsky status between 30% and 50%. All patients had increasing levels of myeloma protein.

The 13 patients entered into this study received 35 courses of deoxycoformycin therapy. Six patients were not considered evaluable as they died before receiving more than one course of deoxycoformycin therapy. While these patients died between 2 and 8 weeks after one course of therapy it was not possible to continue the treatment, due to extensive disease progression or patient refusal of further treatment. The causes of death in these six patients were related to debilitation and massive disease, and two of these patients had large malignant pleural effusions. In none of these cases was there any reduction of bone pain after deoxycoformycin treatment.

However, two patients (Mr MP, Mr SL) who had been primary treatment failures did have an objective response to this therapy, with reductions in myeloma protein of 39 to 17 gm/l and 81 to 40 gm/l after four and seven courses of deoxycoformycin, respectively. Prior to deoxycoformycin therapy these two patients had continued to progress through 13 and four cycles of secondary therapy, respectively. These responses were not maintained and the levels of myeloma protein in these two patients had returned to their pretreatment values after 7 and 10 months, respectively.

Two additional patients who had also been primary treatment failures had regression of soft tissue masses without alteration of their monoclonal protein levels. Follow-up mammography of Mrs JM 2 weeks after three cycles of deoxycoformycin showed a tumor reduction of over 50%. Similarly, after four courses of therapy there was a reduction of about 90% in an extensive soft tissue mass in the left supraclavicular region in Mr JB. Mr JB died of pneumonia 3 weeks after the last cycle of deoxycoformycin, and an autopsy proved residual myeloma in the left supraclavicular area.

The toxicity of this deoxycoformycin schedule was moderately severe in this patient population. Every patient experienced marked nausea and occasional vomiting, particularly by day 3. Prolonged anorexia lasting several days was also routinely encountered. Mild transient confusion occurred in three patients and was exhibited by altered personality, poor memory, and mild disorientation lasting general days. One patient who had received five cycles of this agent developed anosmia.

The absolute lymphocyte count routinely dropped to 20%–40% of the pretreatment levels by day 4 or 5. The neutrophil and platelet counts were not significantly altered and no red cell haemolysis was observed with this schedule of deoxycoformycin.

Pretreatment plasma levels of deoxyadenosine and adenosine averaged 0.24 μM (range 0.09–0.44) and 0.28 μM (range 0.08–0.56), respectively. Following deoxycoformycin treatment the plasma levels of deoxyadenosine and adenosine peaked on day 4 or 5 with average values of 0.90 μM (range 0.91–3.10) and 0.61 μM (range 0.36–0.81), respectively.

The increased plasma levels of deoxyadenosine were also reflected in increased levels of dATP which accumulated in red blood cells. The concentration of dATP in red cells peaked on day 5 or 6 with an average value of 32 nmol/10^9 cells (range 16–42), as against an average value of about 1 nmol/10^9 cells in patients prior to initiation of deoxycoformycin treatment. These peak levels of intracellular dATP were approximately 40% of the ATP concentration. In patients receiving multiple cycles of deoxycoformycin, the level of red cell dATP did not return to pretreatment levels within 14 days, as an average carry-over level of 7 nmol/10^9 cells (range 4–12) was observed. Peak levels of red cell dATP in subsequent cycles of therapy did not appear to differ from those observed in the initial cycle.

Discussion

The two observations which led to the testing of deoxycoformycin in various lymphoproliferative diseases were that the inherited deficiency of adenosine deaminase resulted in a severe combined immunodeficiency disease and that the lymphoid system had high levels of this enzyme [7, 21]. The preliminary work .to our clinical study demonstrated that myeloma plasma cells had an active adenosine deaminase activity and that deoxycoformycin was a very effective inhibitor of this activity in vitro. When the relative cell volumes of plasma cells and lymphocytes are considered, the deaminase activity in plasma cells is several-fold higher than that in T or non-T lymphocytes.

Adenosine deaminase activity of peripheral blood T lymphocytes has been reported to be from half as much as to 12 times greater than that of B lymphocytes [9, 16, 17, 22]. While there are many studies in the literature on the levels of adenosine deaminase in cell-free extracts, we are aware of only one study in which intact cells were used [17]. We have chosen to use intact cells as we believe this is a better model for determining the in vivo activity of adenosine deaminase and purine nucleoside phosphorylase.

Two recent studies have shown that in the presence of an adenosine deaminase inhibitor such as deoxycoformycin low concentrations of deoxyadenosine, but not of adenosine, will lead to loss of viability and cell lysis of human blood lymphocytes [5, 11]. These observations were very significant as they showed that mature cells of lymphoid origin were much more sensitive to deoxyadenosine than were actively growing cells in culture [6, 8, 15] or than stimulated human lymphocytes growing in colonies [4]. We have confirmed these observations and have shown that plasma cells are also sensitive to the combination of deoxycoformycin and deoxyadenosine in vitro. The concentrations of deoxyadenosine which will lead to a 50% reduction in viability within 48 h are 1 and 6 μM for T lymphocytes and plasma cells, respectively.

The observations that plasma cells contained an active adenosine deaminase that could readily be inhibited by deoxycoformycin and that plasma cells could be killed in vitro by combinations of deoxycoformycin and deoxyadenosine led us to believe that deoxycoformycin may have some clinical activity with myeloma as it does in other lymphoproliferative diseases.

All the patients entered into this study had failed to respond to several cycles of secondary combination therapy prior to the initiation of the deoxycoformycin therapy. Seven of the 13 patients entered into this study received more than one cycle of deoxycoformycin therapy. Of these patients, two showed an objective response with a greater than 50% drop in the serum level of the monoclonal protein and an improvement in bone pain, and two others showed a marked reduction in soft tissue masses but with no reduction in the level of the myeloma protein. No maintenance therapy was given to the two responding patients and they both relapsed with rising

levels of monoclonal protein 7 and 10 months after the discontinuation of the deoxycoformycin therapy. All four of the patients who showed some response to deoxycoformycin treatment had failed to exhibit an objective response to the melphalan and prednisone therapy and had continued to progress while receiving the secondary combination drug therapy. Six of the 13 patients entered on this study were not considered evaluable as they died before receiving more than one cycle of deoxycoformycin therapy.

In light of the poor results obtained with combination drug therapy in refractory multiple myeloma [3, 14], we believe that the results of this limited study indicate that additional studies of deoxycoformycin on this disease are warranted, particularly in the initially nonresponding patient group. It seems desirable to initiate deoxycoformycin treatment as soon as progression during melphalan and prednisone therapy is evident, as this would allow for more cycles of deoxycoformycin therapy before marked deterioration in the clinical status. While not life-threatening, the side-effects of deoxycoformycin treatment present a significant problem to patient acceptance in this patient group. Nausea and anorexia were seen in all cases, whereas conjunctivitis and transient neurological abnormalities were less common. Similar toxicities to deoxycoformycin have been observed in other patient populations [12, 18, 20]. The basis of the deoxycoformycin toxicity is currently not understood. There has been a recent report suggesting that tissue toxicity rarely occurred when the level of red cell dATP was less than that of the ATP [13].

The appearance of deoxyadenosine in the plasma together with the accumulation of dATP in the red blood cells of these deoxycoformycin treated patients indicate that deoxyadenosine is available at concentrations that are well within the range of those required to cause lymphocyte cell death in vitro. Whether these levels of deoxyadenosine are sufficient to cause plasma cell death is as yet uncertain. However, the target of chemotherapy in myeloma is unclear as it could be either plasma cells or some abnormal population of the B cell lineage.

The biochemical basis for deoxyadenosine toxicity in lymphoid cells is unclear but we have recently observed that DNA single-strand breaks appear a few hours after the addition of these two compounds to in vitro cultures of mature lymphocytes (L.W. Brox et al. 1984, Cancer Research, in press). These DNA lesions precede the loss of viability by 18–20 h. We have not yet attempted to correlate loss of plasma cell viability in vitro appearance of DNA lesions, and clinical outcome of deoxycoformycin treatment in myeloma patients.

Acknowledgements. We thank B. Pollock, J. LeFebvre, and C. Smith for their excellent technical assistance.

This work was supported by the NCI (Canada), the MRC (Canada), and the Alberta Heritage Fund: Applied Cancer Program.

References

1. Abo T, Yamaguchi T, Shimizu F, Kumagai K (1976) Studies of surface immunoglobins on human B lymphocytes. J Immunol 117: 1781
2. Bergsagel DE, Bailey AJ, Langley GR, MacDonald RN, White DF, Miller AB (1979) The chemotherapy of plasma cell myeloma and the incidence of acute leukemia. N Engl J Med 301: 743
3. Bonnet J, Alexanian R, Salmon S, Bottomley R, Amare M, Haut A, Dixon D (1982) Vincristine, BCNU, doxorubicin and prednisone (VBAP) combination in the treatment of relapsing or resistant multiple myeloma: a southwest oncology group study. Cancer Treat Rep 66: 1267
4. Brox LW, Pollock E, Belch A (1981) Adenosine and deoxyadenosine toxicity in colony assay systems for human T-lymphocytes, B-lymphocytes and granulocytes. Cancer Chemother Pharmacol 9: 49
5. Carson DA, Wasson DB, Lakow E, Kamatani N (1982) Possible metabolic basis for the different immunodeficient states associated with genetic deficiencies of adenosine deaminase and purine nucleoside phosphorylase. Proc Natl Acad Sci USA 79: 3848
6. Fox IH, Kelley WN (1978) The role of adenosine and 2′-deoxyadenosine in mammalian cells. Annu Rev Biochem 47: 655
7. Giblett ER, Anderson F, Cohen B, Pollara B, Meuwissen HJ (1972) Adenosine deaminase deficiency in two patients with severely impaired immunity. Lancet 2: 1067
8. Henderson JF, Scott FW (1980) Inhibition of animal and invertebrate cell growth by naturally occurring purine bases and ribonucleosides. Pharmacol Therap [A] 8: 539
9. Huang AT, Logue GL, Engelbracht HL (1976) Two biochemical markers in lymphocyte subpopulations. Br J Haematol 34: 631
10. Hunting D, Hordern J, Henderson JF (1981) Quantitative analysis of purine and pyrimidine metabolism in Chinese hamster ovary cells. Can J Biochem 59: 838
11. Kefford RF, Fox RM (1982) Deoxycoformycin-induced response in chronic lymphocytic leukaemia: deoxyadenosine toxicity in non-replicating lymphocytes. Br J Haematol 50: 627
12. Kefford RF, Fox RM (1983) Purinogenic lymphocytotoxicity: clues to a wider chemotherapeutic potential for the adenosine deaminase inhibitors. Cancer Chemother Pharmacol 10: 73
13. Koller CA, Mitchell BS (1981) 2′-Deoxycoformycin toxicity correlates with red cell dATP/ATP ratios. Proc Am Assoc Cancer Res 22: 220
14. Kyle RA, Pajak TF, Henderson ES, Nawabi IS, Brunner K, Henry PH, McIntyre OR, Holland JF (1982) Multiple myeloma resistant to melphalan: treatment with doxorubicin, cyclophosphamide, BCNU and prednisone. Cancer Treat Rep 66: 451
15. Lowe JK, Gowans B, Brox L (1977) Deoxyadenosine metabolism and toxicity in cultured L5178Y cells. Cancer Res 37: 3013
16. Macdermott RP, Tritsch GL, Formeister JF (1980) Adenosine deaminase and nucleoside phosphorylase activities in normal human blood mononuclear cell subpopulations. Clin Exp Immunol 42: 303
17. Nishida Y, Okudaira K, Tanimoto K, Akaota I (1980) The differences in purine metabolism between T and B lymphocytes. Exp Hematol 8: 593
18. Poplack DG, Sallan SE, Rivera G, Holcenberg J, Murphy SB, Blatt J, Lipton JM, Venner P, Glaubiger DL, Ungerleider R, Johns D (1981) Phase I study of 2′-deoxycoformycin in acute lymphoblastic leukemia. Cancer Res 41: 3343
19. Preiksaitis JK, Lank B, Ng PK, Brox L, LePage GA, Tyrrell DLJ (1981) Effect of liver disease on pharmacokinetics and toxicity of 9-β-D-arabinofuranosyladenine-5′-phosphate. J Infect Dis 144: 358
20. Prentice HG, Lee N, Blacklock H, Smyth JF, Russell NH, Ganeshaguru K, Piga A, Hoffbrand AV (1981) Therapeutic selectivity of and prediction of response to 2′-deoxycoformycin in acute leukemia. Lancet 2: 1250
21. Smyth JF (1979) Selective treatment of lymphoid malignancy with adenosine deaminase inhibitors. In: Enzyme defects and immune dysfunction. Excerpta Medica, Amsterdam, p 263 (Ciba Foundation symposium no 68)
22. Tung R, Silber R, Quagliata F, Conklyn M, Gottesman J, Hirschhorn R (1976) Adenosine deaminase activity in chronic lymphocytic leukemia. J Clin Invest 57: 756
23. Weiner MS, Bianco C, Nussenweig V (1973) Enhanced binding of neuraminidase-treated sheep erythrocytes to human T-lymphocytes. Blood 42: 939

Received May 15, 1984/Accepted June 14, 1984