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Adenosine Deaminase Inhibitors: Their Role in Chemotherapy and Immunosuppression

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Introduction

The search for inhibitors of adenosine deaminase (ADA) is an area of drug development that has been actively pursued for the past 15 years. The development of the antibiotics coformycin and dCF, which are stoichiometric tight-binding inhibitors, has sparked new interest in this area. These drugs have greatly improved the therapeutic activity, and in some instances also the therapeutic index, of anticancer agents which are adenosine analogs. dCF also possesses lymphocytopenic and immunosuppressive activities. Because of its unusual combination of therapeutic activities, there is presently intense clinical interest in dCF both as a single agent and in combination with antitumor and antiviral adenosine analogs. On the other hand, aliphatic alcohol analogs of adenine, such as EHNA, possess intrinisic antiviral activity as well as potentiating the anticancer activity of adenosine analogs.

The recent proliferation of reports concerned with the use of ADA inhibitors in combination with antitumor agents, as well as with adenosine and 2′-deoxyadenosine, has prompted a need for the collation and comparative assessment of these data. In this review I have concentrated upon the pharmacological properties of ADA inhibitors, their use in studying the mechanisms of cytotoxicity of adenosine analogs and metabolites, and the preliminary pharmacokinetic and clinical findings of these drugs.

Abbreviations: ADA, adenosine deaminase; dCF, 2′-deoxycoformycin; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; ID_{50}, dose that inhibits 50%; SAH, S-adenosyl-homocysteine

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Structure-Activity and Kinetic Characteristics

Initially, synthetic analogs of adenosine were found to be weak competitive inhibitors of ADA with an ID_{50} in the range of 10^{-5} to 10^{-4} *M* [22, 27, 70, 93]. The structure-activity requirements optimal for activity were 6-amino and 9-ribosyl or 9-(2-hydroxyalkyl) functionalities. It was not until the rational design and synthesis of EHNA (Fig. 1) with an ID_{50} of 10^{-8} *M* that a relatively tight-binding competitive inhibitor became available [69].

The most potent inhibitors of ADA have evolved from the isolation of natural products. The purification of the antibiotics coformycin and dCF (Fig. 1) by Umezawa's laboratory [56, 68] and by Parke-Davis investigators [92], respectively, have provided prototypes of unique tight-binding transition state inhibitors with an ID_{50} of 10^{-8} to 10^{-9} *M* for coformycin [6, 14] and 10^{-9} to 10^{-11} *M* for dCF [4, 7, 21, 40], depending on the molecular species of ADA and the amount of enzyme present in the assay.

The tetrahedral configuration of the 8-hydroxy of the diazepine ring moiety of the coformycins resembles the transition state intermediate in the hydrolytic deamination of adenosine (Fig. 2). This unique structure enables them to function as stoichiometric transition state inhibitors, with the ribose and 2′-deoxyribose moieties providing added stabilization of the ADA-coformycin complex [91].

The first clue that the coformycin-type of ADA inhibitors were of the tight-binding class was provided by Sawa et al. [68]. The rate of inhibition by coformycin was found to increase with the length of preincubation to produce a mixed type of inhibition. These effects were confirmed and extended in elegant kinetic studies [14, 15], where it was shown that the extent and rate of inhibition was a dual function of enzyme concentration and length of incubation. The calculated K_i for coformycin with erythrocytic ADA

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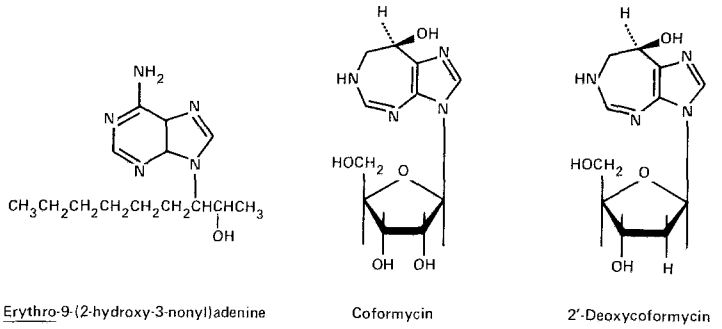

Fig 1. Structures of EHNA, coformycin and 2'-deoxycoformycin

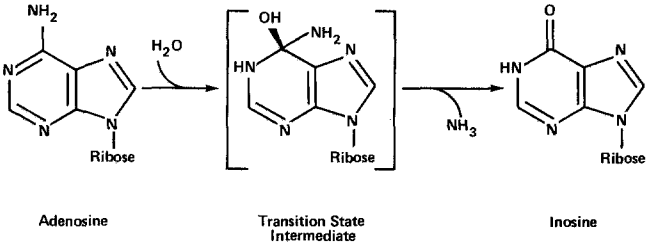

Fig. 2. Deamination of adenosine by adenosine deaminase, showing the transition state intermediate

was 2.2×10^{-10} *M*. Similar kinetic features were evident in subsequent studies with dCF where the K_i was calculated from the association and dissociation rate constants to be 2.5×10^{-12} *M* [4]. In contrast, the K_i for EHNA was 1.6×10^{-9} *M* [4]. The 100- and 1,000-fold lower K_i for coformycin and dCF, respectively, vs EHNA is a direct quantitative result of their correspondingly lower dissociation rate constants. A factor that must also be considered is the recently discovered differential sensitivity of various molecular species of ADA to dCF and EHNA [21]. It was found that the small-molecular-weight form of calf intestinal ADA was equally sensitive to both dCF and EHNA ($ID_{50} = 2 \times 10^{-11}$ *M*), whereas the large-molecular-weight species or the crude mixture of ADA was less sensitive to EHNA vs dCF ($ID_{50} = 1 \times 10^{-7}$ *M* and 2×10^{-8} *M* for EHNA and dCF, respectively, for the large-molecular-weight species, and 6×10^{-7} *M* and 2×10^{-8} *M*, respectively, for the crude enzyme mixture). Striking differences in the sensitivity of ADA from various mouse and rat tissues to EHNA in vitro have also been noted; however, the reason for this variation was not elucidated with respect to the molecular species of ADA affected [19, 84].

In vivo studies examining the effect of continuous IV infusion of EHNA [19] and dCF [82] in mice have yielded striking results. EHNA infused at a dose of 4–40 mg/kg per day for 5 days increased ADA activity in the lung, colon, small intestine, stomach, spleen, liver and kidney, but decreased ADA activity in the thymus. In contrast, dCF infused at a dose of 0.4 mg/kg per day for 5 days severely inhibited ADA activity in the thymus, colon, lung, liver, and kidney, while not markedly affecting ADA activity in the spleen and small intestine, and elevating ADA activity in the stomach [82]. Interestingly, mice bearing a SC transplanted colon tumor showed a greater susceptibiltiy to inhibition of ADA activity by dCF in the spleen, colon, and small intestine [82]. Thus, these results point to the necessity of slowly escalating the dose of dCF in patients with cancer, as well as the possible differences in response of reticuloendothelial tissues in normal vs cancer patients to dCF.

The kinetic parameters for inhibition by dCF in intact cells are quite different from cell-free or purified preparations of ADA. The association rate constant for intact erythrocytes or sarcoma 180 cells was 300- to 500-fold lower vs cell-free preparations [66]. This effect is probably the main determinant of the reduced ID_{50} of dCF for intracellular ADA, i. e., 10^{-8} to 10^{-7} *M* [33, 35]. On the other hand, the slower dissociation of the dCF-ADA complex in intact cells vs cell-free preparations [66] is undoubtedly a function of the rate of turnover of ADA in different cell types. This has recently been suggested by in vivo studies with dCF where the 48-h reactivation of ADA from inhibition by dCF was only 13% in erythrocytes which are anucleate and 80% in L1210 cells which are actively synthesizing mRNA [3, 19]. Additional considerations for the slow reactivation of intracellular ADA from inhibition by dCF are the varying sensitivities of different molecular species of

ADA [21, 71] and the presence of stabilizing or converting factors associated with ADA [23, 83].

The specificity of action of dCF and EHNA was studied in mouse lymphoma cells, where it was found that high concentrations (10^{-5} to 10^{-4} *M* range) of each drug affected all pathways of purine ribonucleotide synthesis [35]. Adenylate deaminase was also inhibited to a greater extent by dCF ($ID_{50} = 10^{-5}$ *M*) than by EHNA. Agarwal and Parks [5] found that coformycin and dCF, but not EHNA, inhibited muscle adenylate deaminase with a K_i of about 5×10^{-8} *M* and 2×10^{-8} *M*, respectively [5]. Recently, it was reported that the synthetic nucleotide analog of dCF, dCF-5′-monophosphate inhibited muscle adenylate deaminase with an ID_{50} of 2×10^{-7} *M* [28].

Potentiation of 2′-Deoxyadenosine and Adenosine Toxicity

One of the unique features of dCF is its high specificity for inhibiting only ADA with little if any effect on other cellular enzymes at optimal concentrations for inhibiting ADA [21]. Several laboratories have taken advantage of this unique pharmacological activity to study the cytotoxicological and biochemical effects of adenosine and 2′-deoxyadenosine when their deamination was prevented by the presence of dCF or another inhibitor of ADA.

The first indication that inhibition of deamination by adenosine could profoundly modify an intracellular process was reported by Wolberg et al. [90]. In the presence of EHNA and exogenous adenosine, the levels of cyclic AMP rose markedly in peritoneal lymphocytes sensitized to mouse ascites leukemia cells. Moreover, cytolysis of ascites cells by the sensitized lymphocytes was blocked by adenosine and this effect was enhanced 2- to 3-fold by the presence of EHNA. Increased elevations of cAMP in the presence of norepinephrine and dCF were also reported to occur in cerebral cortex slices in vitro [74].

The effects of ADA blockade on lymphocyte blastogenesis have been actively pursued since the study by Wolberg [90], in an attempt to assess the function of ADA in immunodeficiency. Since ADA activity does not increase during lymphocyte transformation [34, 65], its role is presumably a passive and protective one. It is known that most patients with combined immunodeficiency disease lack or contain low levels of ADA [20, 38, 71, 87]. This recessive trait is expressed by levels of 2′-dATP in lymphocytes and erythrocytes of these patients that are 5- to 100-fold higher than normal [20, 26]. This genetic error has been mimicked pharmacologically with inhibitors of ADA. EHNA in the presence of adenosine inhibited concanavalin A-mediated stimulation of [^{3}H]leucine incorporation in human peripheral lymphocytes by 50%, but 500 times more EHNA was required to produce the same amount of inhibition of [^{3}H]thymidine incorporation [11]. In contrast, 2′-deoxyadenosine but not adenosine in the presence of 1×10^{-6} *M* dCF inhibited [^{3}]leucine incorporation during the first day of blastogenesis in phytohemagglutinin-stimulated lymphocytes [85]. dCF alone had only a marginal inhibitory effect on [^{3}H]leucine incorporation [85]. These results suggest that a pre-S-phase event is associated with 2′-deoxyadenosine toxicity and not simply inhibition of DNA synthesis, presumably via inhibition of ribonucleoside diphosphate reductase. Conversely, Harrap and Paine [34] reported that only DNA synthesis in mitogen-stimulated lymphocytes was affected by adenosine and coformycin, while DNA and protein synthesis in L1210 cells were equally affected by the same regimen. Nevertheless, the common denominator in these studies is that the cytotoxicity of adenosine or 2′-deoxyadenosine on lymphocyte blastogenesis or tumor cell growth is markedly potentiated by EHNA [11, 45, 73], coformycin [34], or dCF [45, 85], and that greater inhibition of lymphocyte transformation is produced by 2′-deoxyadenosine vs adenosine in the presence of dCF [45, 85] or EHNA [73].

Ullman et al. [86] showed that in a mouse lymphoma line lacking 2′-deoxyadenosine kinase, the sensitivity of these cells to EHNA was greatly diminished. This suggested that phosphorylation of 2′-deoxyadenosine is required for producing cytotoxicity, as found in the wild-type lymphoma cells which produced more than a 10-fold elevation in 2′-dATP in the presence of 10^{-5} *M* EHNA. In contrast, Hershfield et al. [37] reported that adenosine kinase-deficient human lymphoblasts remained as sensitive as the parent cell line to growth inhibition by adenosine in the presence of EHNA. This apparent divergence in mechanism of action between adenosine and 2′-deoxyadenosine indicates that adenosine-mediated cytotoxicity may be produced via an adenine nucleotide-independent process. The validity of this suggestion was shown in studies where the addition of exogenous adenosine in the presence of coformycin or dCF did not significantly elevate ATP levels in mouse leukemia cells [34, 50, 55] and mitogen-stimulated lymphocytes [34], but that 2′-deoxyadenosine in the presence of EHNA [86], dCF [50] or coformycin [34] produced moderate to high elevations of 2′-dATP.

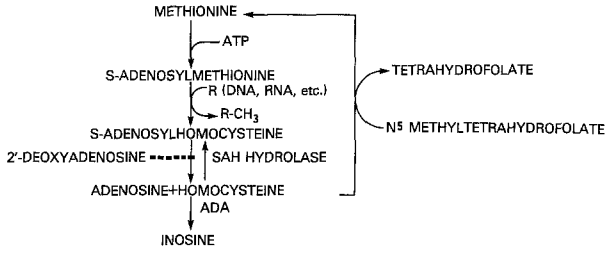

Fig. 3. Pathways for metabolism of S-adenosyl-L-methionine. Interference with hydrolysis of SAH via SAH hydrolase is denoted by the *dashed line* (– – –)

One essential metabolic process that may be directly affected by adenosine is methylation via S-adenosyl-L-methionine (Fig. 3). Kredich and Martin [43] and Kredich and Hershfield [42] showed that in the presence of EHNA and L-homocysteine thiolactone, adenosine produced marked toxicity to lymphoma cells and its effect correlated with the elevation of SAH and inhibiton of DNA and RNA methylation. This mechanism for adenosine toxicity is particularly intriguing, since the reversibility of SAH hydrolase has been demonstrated in vivo [39] and SAH is a potent inhibitor of mRNA methylation and viral multiplication [63, 64]. Interestingly, the adenosine alalog cordycepin (3′-deoxyadenosine) inhibits nuclear RNA methylation quite effectively [31] and may function via its conversion to 3′-deoxySAH [32], which is an effective inhibitor of 5′-cap methylation of mRNA [63, 64]. Zimmerman et al. [94] recently reported that human erythrocytes were capable of metabolizing cordycepin to an SAH-like metabolite in the presence of EHNA and homocysteine. Of equal importance was their observation that adenosine and homocysteine in the presence of EHNA could not only elevate SAH levels in lymphocytes but also increase the concentration of cyclic AMP [95]. This is particularly important, since both elevated levels of cyclic AMP and SAH could potentially inhibit, perhaps synergistically, normal lymphocyte function.

Alternatively, 2′-deoxyadenosine and adenine arabinoside have recently been reported to function as a tight-binding suicide inhibitor of SAH hydrolase causing irreversible inactivation of catalytic activity [36]. Such a phenomenon, if active in vivo, could indirectly elevate the intracellular concentrations of SAH, and thus produce impairment of methylation processes critical for cell growth und viability without invoking the necessity for the conversion of 2′-deoxyadenosine to its nucleotide form (Fig. 3). In recently completed studies, we have not found any evidence that 2′-deoxyadenosine in the presence of dCF can affect the intracellular concentration of SAH in L1210 cells, although adenosine, homocysteine and dCF markedly elevated SAH levels and arrested the methylation of nuclear RNA [29a].

Potentiation of Adenosine Analogs

The expected synergism of ADA inhibitors with adenosine analogs possessing antitumor activity has been demonstrated in a number of tumor systems. When adenosine analogs were combined with EHNA, marked synergism with cordycepin and adenine arabinoside was seen in L cells in tissue culture and with the latter drug in vivo with Ehrlich ascites cells [60]. More recent experiments with dCF demonstrated marked potentiation by dCF of the cytotoxic effects of cordycepin, xylosyladenine, adenine arabinoside and other adenosine analogs in mouse lymphoid leukemia cells both in vitro and in vivo [1, 10–12, 40, 46, 49]. It is significant that the combination of dCF and adenine arabinoside prolonged the life-span of mice bearing intracerebrally implanted L1210 cells by 50%–90%, whereas either drug alone was inactive [10, 46]. dCF alone did not possess any antitumor activity.

It is presumed that the mechanism of this synergism is the sustained formation of high levels of the active nucleotide metabolite derived from the parent adenosine analog. This phenomenon has been corroborated by chromatography studies employing dCF and adenine arabinoside or xylosyladenine, where the 5′-triphosphate accumulates in the presence of dCF [16, 55, 59, 67]. High levels of the 5′-triphosphate of cordycepin and xylosyladenine would be expected to be markedly cytotoxic, and in fact are inhibitory to nuclear RNA synthesis in L1210 cells [33, 58]. The latter effects also quantitatively correlate with the degree of synergism seen with this two-drug combination and the inhibition of cell growth of mouse leukemia cells in vitro or the prolongation of life-span of mice bearing P388 leukemia [1, 40]. Interestingly, the effect of dCF on the inhibitory action of cordycepin on nRNA synthesis in regenerating rat liver, a rapid-dividing tissue, is only marginal due to the low level of ADA in this tissue [29]. Plunkett et al. [61] have recently reported that the main determinant of the effect of adenine arabinoside on DNA synthesis in mice bearing P388 leukemia and treated in addition with either EHNA or dCF is the retention of the active metabolite, AraATP, in the tumor cells. This effect occurred despite the more rapid excretion of EHNA and the less sustained inhibition of ADA by this drug in P388 cells in comparison to dCF [62]. In addition, the rephosphorylation and half-life of AraATP in CHO

cells was not influenced by the presence of either dCF or EHNA [72]. These data support the conclusion that the primary factor influencing the therapeutic activity of adenosine analogs such as adenine arabinoside when inhibitors of ADA are co-administered is the initial concentration of the active antitumor metabolite, and not the rate of excretion of the ADA inhibitor nor the absolute degree and length of inhibition of ADA in the tumor cell. This hypothesis is supported by the equal synergism that 1×10^{-6} *M* dCF or EHNA exert on the cytotoxicity of adenine arabinoside 5′-monophosphate in L1210 cells in culture [11].

Combinations of ADA inhibitors and adenine arabinoside also show pronounced antiviral activity. In initial investigations, it was reported that dCF and adenine arabinoside markedly inhibited the plaque-forming ability of herpes simplex I and II [8, 76, 89]. Similar effects were noted with the combination of adenine arabinoside with coformycin [77] and EHNA [57]. It is of interest that, whereas dCF alone had no antiviral activity [57, 76], EHNA inhibited the plaque-forming ability of herpes virus by 50% at 10^{-6} *M* [57]. The potentiation of the antiviral activity of cordycepin by EHNA also showed a direct correlation with inhibition of total RNA synthesis in the infected HeLa cells [57], a relationship that also parallels the effect that cordycepin and either dCF [32] or EHNA [59] have on mouse ascites tumor and fibroblast cells, respectively.

The antiviral activity of EHNA appears to be a characteristic of adenine analogs with aliphatic alcohol substituents at the 9-position [70]. It has recently been reported that 9-(2,3-dihydroxypropyl)adenine, an inhibitor of ADA, strongly inhibits herpes simplex I and II, as well as the RNA viruses that produce vesicular stomatitis and measles [25]. Other aliphatic alcohol analogs of adenine and guanine also possess antiviral activity [25], and it will be interesting to see whether these newer analogs also have potent inhibitory effects on ADA.

An interesting activity of the adenosine analogs cordycepin and xylosyladenine that is potentiated by dCF, is their ability to directly competitively inhibit the phosphorylation of nuclear proteins via cyclic nucleotide-independent protein kinases [30, 47, 48]. dCF potentiates the inhibitory effect of xylosyladenine on the phosphorylation of nonhistone chromosomal proteins in L1210 cells to a greater extent than cordycepin [48], a result quite opposite to the synergism seen with these drug combinations on RNA synthesis [33, 58]. Since the phosphorylation of nuclear proteins is believed to play an active role in regulating transcription [80] this synergistic effect of dCF undoubtedly contributes to the overall interference by these drugs with transcription.

Immunosuppressive Activity

As EHNA can be distinguished from dCF by its intrinsic antiviral activity, so can dCF be differentiated by its highly selective immunosuppressive activity, which EHNA possesses to a lesser degree. Initial studies of the immunosuppressive effects of dCF used single doses of 0.5–1.0 mg/kg daily for 4 days, which inhibited ADA 80%–100% [9]. Inhibition of ADA in vivo under these conditions did not impair the mitogen-stimulated blastogenic response in vitro of thymocyte or spleen cells from these animals. However, this negative finding can be explained by the dilution of viable cells inhibited by dCF upon subculturing. When dCF is preincubated with lymphocyte cultures for 1–3 days before addition of the mitogen, it inhibits concanavalin A, phytohemagglutinin, and pokeweed mitogen-mediated blastogenesis in human peripheral lymphocytes with an ID_{50} of approximately 1×10^{-6} *M* [18]. In the latter experiments, blastogenesis was measured by [^{3}H]thymidine incorporation 3 days after the addition of mitogen. In studies where dCF was added simultaneously with the mitogen to lymphocyte cultures, and blastogenesis was assessed by [^{3}H]leucine incorporation at 1 day after addition of mitogen, only 15% inhibition was produced by 1×10^{-6} *M* dCF [85]. These studies suggest that the immunosuppressive action of dCF is associated with DNA synthesis or at least some biosynthetic event occurring during S-phase.

Immunosuppressive activity by dCF and to a lesser extent by EHNA was demonstrated by allograft acceptance of LSTRA tumor cells across the H-2 histocompatability locus [2, 17]. A single dose of 7.5–15 mg dCF/kg administered 24 h before the transplant resulted in 53% on 68% allograft acceptance vs 19% acceptance by a similar regimen of 200 mg EHNA/kg. Divided doses of 0.25–0.5 mg dCF/kg or 150 mg EHNA/kg daily for 6 days only produced 20% and 10% acceptance, respectively. EHNA administered at 10 mg/kg daily for 14 days produced a 60% acceptance rate of mouse pancreatic islet allografts across a non-H-2 histocompatability barrier [52]. Skin graft survival in mice across a non-H-2 histocompatability barrier was also markedly prolonged (73%–83% graft survival) by 18 daily doses of 3.3 and 50 mg EHNA/kg [51]. Thus, dCF appears to be a more potent immunosuppressive drug against a strong antigenic barrier, whereas EHNA although

not devoid of immunosuppressive activity appears to be a less effective agent.

The selectivity of dCF for lymphoid cells in mice [78] has also been confirmed in cancer patients where predominantly lymphocytopenia has been observed [41, 79].

The biochemical effects of EHNA on nucleotide and RNA synthesis have been examined in phytohemagglutin-stimulated lymphocytes [75]. At concentrations of 1×10^{-5} *M*, which completely inhibit ADA, EHNA inhibited the phosphoribosylpyrophosphate-dependent formation of ATP and ITP from adenine and hypoxanthine, respectively. Ribonucleotide pool sizes and RNA synthesis were not affected except at exceedingly high concentrations of 5×10^{-4} *M*. These results are consonant with the study by Henderson et al. [35], who also found that high concentrations of EHNA, as well as dCF, inhibited ribonucleotide biosynthesis in mouse lymphoma cells. It should be noted that these ancillary activities are achieved at concentrations of inhibitor that are 10- to 100-fold greater than necessary to inhibit ADA. They may be related, however, to the immunosuppressive effects of these drugs, which are produced only at dosages much greater than that required for inhibiting only ADA.

Disposition, Excretion and Metabolism

The ADA inhibitor most extensively studied pharmacokinetically has been dCF. The tight-binding characteristics of dCF have permitted the development of a highly specific enzymatic assay, which measures the inhibition of ADA in the presence of biological fluids. With these techniques, it has been shown that the distribution of dCF closely follows the concentration of ADA in the various tissues of all species of animals studied thus far [7, 19, 53].

The plasma $t_{1/2}$ for the dCF between rodents and dogs is similar (Table 1). dCF is rapidly cleared in the urine, but the rate appears to be much slower in man [79]. The latter effect is explained by the longer plasma $t_{1/2}$ in man which was not predicted by the preclinical data. The recent reports by Suling et al. [81] and Plunkett et al. [62] suggest that the duration of action of EHNA is approximately 50% of dCF as judged by their respective effects on increasing the $t_{1/2}$ of adenine arabinoside in the plasma [81] and ascites fluid [62] in mice. Approximately 50% of an IV dose of 5 mg EHNA/kg was cleared from the plasma in 1 h in the Rhesus monkey [54].

The total cumulative excretion of dCF in the urine and feces was 90%–100% of the administered dose of dCF [7, 19, 53]. Recently, studies of the metablism of dCF in L1210 cells in vitro have demonstrated that only 10%–15% of the drug (10^{-6} to 10^{-4} *M*) is converted to the nucleoside monophosphate in 1–2 h, but not further anabolized [88]. This is in contrast to the report by Muller et al. [55] that in mouse L5178Y leukemia cells in culture, 54% of 1×10^{-6} *M* coformycin is recovered as mono-, di- and triphosphates upon exposure to the drug for 24 h. EHNA is almost completely metabolized in mice with numerous hydroxylated metabolites of the aliphatic hydrocarbon moiety being present in the urine after IP administration [44]. This finding has been confirmed in the Rhesus monkey, where 70% of the administered IV dose was eliminated in the urine after 6 h, almost entirely as four metabolites [54].

Table 1. Urinary excretion and plasma $t_{1/2}$ for dCF

Species	Plasma $t_{1/2}$ (min)		% Recovered unchanged in urine in 24 h	Reference
	α phase	β phase		
Mouse	17–19	64–104	100	[53]
Rat	–	–	80	[7]
Dog	12–15	90–120	77	[19]
Man	45–90	240–360	34	[79]

Conclusions

This review has emphasized the pharmacological activities of ADA inhibitors, both as single agents and in combination chemotherapy. The two most studied drugs, EHNA and dCF, each have some unique attributes that should allow for them to be further developed on their own merits for clinical trials. Both drugs produce little or no effect on other processes associated with de novo purine synthesis or adenine nucleotide catabolism at concentrations that completely inhibit ADA; however, at 100-fold higher concentrations, EHNA appears less specific with respect to its site of action than dCF [35].

EHNA possesses antiviral activity, which dCF does not, and thus would be expected to be more efficacious in combination with antiviral adenosine analogs in the chemotherapy of acute viral infections. The development of closely related aliphatic alcohol analogs of adenine with potent antiviral activity attests to the unique properties of this structural class of drugs. On the other hand, dCF would be more advantageous for the chemotherapeutic treatment of cancer in combination with adenosine analogs, or quite possibly as a single agent in lymphoid leukemias. The longer half-life of dCF and its more

prolonged and potent inhibitory effects on ADA vs EHNA would be a particularly favorable pharmacological property for the chronic treatment of cancer patients. The instrinsic immunosuppressive activity of dCF at higher doses is an added feature of this drug. It may serve as a prototype for a new class of immunosuppressive agents lacking many of the unfavorable side effects of the current modalities of immunosuppressive therapy.

From an experimentalist's perspective, the use of ADA inhibitors has helped enormously in understanding the mechanism of action of adenosine metabolites and analogs whose catabolism was heretofore neglected with respect to their specificities of action. ADA inhibitors have also enabled us to understand the regulatory processes associated with immunodeficiencies characterized by a lack of ADA, and to further understand the maturation of the immune response.

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