Fludarabine Monophosphate, an Effective New Agent in Leukaemias and Lymphomas

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Chronic lymphocytic leukaemia and low-grade non-Hodgkin’s lymphomas are diseases for which until recently no treatment leading to long lasting complete remission or cure existed. Fludarabine monophosphate, a purine analogue, was recently introduced into clinical trials for chronic lymphocytic leukaemia and low-grade non-Hodgkin’s lymphomas and found to be very effective. In this report we review the available data relating to fludarabine monophosphate and present our experience with fludarabine administration in refractory B-chronic lymphocytic leukaemia and low-grade lymphocytic lymphoma in Athens and Jerusalem.

Pharmacodynamics and Pharmacokinetics

After the success of cytarabine (ara-C), efforts have been made to identify other purine analogues with antineoplastic activity. The first of these nucleosides was ara-A which is inactivated by adenosine deaminase and therefore its bioavailability is limited [1]. After some structural modifications, the soluble and adenosine deaminase-resistant fluorinated purine analogue F-ara-A monophosphate (fludarabine) was developed by Montgomery and Hewson [2]. Its structural formula (9-B-D-arabinosyl-2-fluoroadenine) is presented in Figure 1.

Mechanism of Action

After 5 minutes of systemic administration fludarabine monophosphate is dephosphorylated to F-ara-A [3] phosphorylation to obtain cytotoxic activity. The enzyme responsible for this phosphorylation is intracellular deoxycytidine kinase [4,5], although adenosine kinase has been proposed as an alternative [6]. F-ara-A is thus converted to its active form F-ara-ATP.

Many models of the mechanisms of action have been proposed (Table 1). However, the major mechanism is inhibition of DNA synthesis in two ways: 1) F-ara-ATP directly competes with dATP for utilization by DNA polymerases [7], and 2) F-ara-ATP is incorporated into DNA [7,8], resulting in disturbance of the DNA replication machinery. Thus whenever F-ara-ATP is incorporated into DNA, DNA strand elongation is terminated at the incorporated sites. F-ara ATP acts as a DNA chain termination [7], may cause deletion of genetic material and may be responsible for mutagenicity and cytotoxicity [9].
Table 1. Mechanisms of action of fludarabine

1. Inhibition of DNA synthesis
   a. Competition with dATP for utilization by DNA polymerases
   b. Incorporation into DNA
2. Termination of RNA synthesis
3. Inhibition of DNA repair
4. Depletion of NAD pools
5. Inhibition of several enzymes: DNA polymerases, DNA primase, ribonucleoside diphosphate reductase, DNA ligase, topoisomerase II and deoxycytidine kinase

Clinical trials have demonstrated the therapeutic efficacy of fludarabine in B-chronic lymphocytic leukaemia (B-CLL). It is known that peripheral lymphocytes are not actively replicating so that inhibition of DNA synthesis may not be the major mechanism of fludarabine action in B-CLL and related disorders. Termination of RNA synthesis may in fact contribute to the cytotoxic effect of fludarabine in these malignancies. In this respect it has been shown that F-ara-ATP is also incorporated into RNA [8,10] - although not as strongly as into DNA - and might thereby interfere with the termination of protein translation [11]. Two other mechanisms have been postulated especially for lymphoid tissues. The first is inhibition of DNA repair [12]. Lymphoid tissues have the lowest levels of deoxynucleotide and the highest activity of deoxycytidine kinase whereas in other tissues the reverse is observed [13,14]. DNA repair directly correlates with the level of deoxynucleotide. The second possible mechanism is that F-ara-ATP may induce increased poly (ADP) ribosylation and depletion of NAD pools, which may lead to decreased cellular anergy and thereby to cell death [15]. Fludarabine also appears to inhibit the activity of several enzymes which interfere with DNA and RNA synthesis such as DNA polymerases and DNA primase and ribonucleoside diphosphate reductase [16]. Other possible enzyme targets are DNA ligase and topoisomerase II [17]. The above-mentioned increased deoxycytidine kinase activity in lymphoid tissues, which is of great importance for fludarabine-induced toxicity, may be due to a direct effect on the enzyme itself [18]. Recent studies by Robertson and Plunkett have shown that there is enhanced apoptosis in B-CLL cells when fludarabine is used. Thus cell death due to apoptosis is yet another possible mechanism for the effect of fludarabine in CLL [18].

Fludarabine acts as a modulator of the metabolism of ara-C and other purine analogues such as 2-CdA. It has been shown that there is a two-fold increase in the rate of intracellular ara-CTP accumulation if fludarabine is administered before incubation of human lymphocytes with ara-C [19]. It has been demonstrated that in vitro synergy takes place between fludarabine and other chemotherapeutic agents such as gallium nitrate, cytarabine plus cisplatinum and mitoxantrone. Pre-exposure of human leukaemic cells to fludarabine, followed by cytarabine, resulted in a 2.2 times higher accumulation of cytarabine triphosphate. The simultaneous incubation of B-CLL cells with fludarabine and mitoxantrone resulted in additive cytotoxicity. Dose and schedule-dependent radiosensitization has also been proved with fludarabine administered 1 hour before irradiation.

Competitive intracellular interaction takes place between the natural and fraudulent nucleotides [4]. Decrease of the cellular pools of dATP is crucial for the development of combination chemotherapy regimens containing fludarabine [20].

Effects of Fludarabine on Healthy Cells

Treatment with fludarabine results in reversible lymphocytopenia [22]. In fact, B cell counts increased after two treatment cycles. Lymphocytopenia developed rapidly within 6 days and was generally reversible within 3 weeks. A decrease of only 18% was observed in absolute neutrophil counts. A relation between white blood cell toxicity and fludarabine dosage has not been demonstrated. Potential mechanisms of fludarabine toxicity in leukaemic lymphocytes versus healthy tissues are more efficient intracellular transport and enhanced phosphorylation of F-ara-A [23]. The effect on CD4+ cells is quite strong and results in relatively long lasting immunosuppression with a tendency to infections which is troublesome [23].