Forodesine – Preclinical Studies

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Forodesine – the drug

Chemically forodesine is [(1S)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol]. The agent is also known as immucillin hypoxanthine (immucillin H) or BCX-1777. This is a potent inhibitor of the enzyme purine nucleoside phosphorylase (PNP).

PNP – the enzyme

The enzyme PNP is responsible for phosphorolysis of (2'-deoxy)guanosine to the guanine nucleobase and (2'-deoxy)ribose-1-phosphate.[1] X-ray crystallographic analyses suggested that the mammalian enzyme is a trimeric structure that accepts only 6-oxopurine nucleosides such as (2'-deoxy)guanosine and inosine, but not (2'-deoxy)-adenosine or the pyrimidine (2'-deoxy)nucleosides as substrates.[2,3] In addition to this selectivity, the substrate preference of human and bovine PNP is high, with K_m values between 10 and 40 μM for inosine and dGuo, which results in high phosphorolysis efficiency ($V_{max}/K_m = 2.56$ for dGuo).[1,4-6]

Rationale for PNP inhibitors

The discovery that the rare genetic deficiency of PNP in children, due to mutations in the gene encoding for PNP, causes profound T-cell lymphopenia provided impetus for development of PNP inhibitors for T-cell diseases.[7-10] Consistent with the catabolic role of this enzyme on the substrate dGuo, biochemical investigations in pediatric patients with PNP deficiency revealed that there was an increase in the level of plasma dGuo.[11-14] Serum dGuo was maintained between 2 and 20 μM in these patients, compared with undetectable levels in healthy individuals. T-cell specificity was due to the inherently greater phosphorylation of dGuo and slower catabolism of the phosphorylated dGTP in T-cells. This, in turn, leads to dGTP-directed inhibition of DNA synthesis and cell death.[11-16] This knowledge provided the rationale for using PNP as a target for development of therapeutics that would be selective to T-cells. Attempts to achieve high levels of plasma dGuo by intravenous infusion of dGuo was hampered by its rapid degradation resulting from the high specific activity of PNP, ubiquitous in large body organs such as liver, spleen, kidney, and circulating lymphocytes and erythrocytes in blood.[8,17] Hence, pharmacologic inhibition of PNP would be required to increase plasma dGuo concentration.

Inhibitors of PNP

Several agents have been shown to inhibit PNP,[18] and pharmacokinetic studies have demonstrated that greater than 95% continuous inhibition of PNP is required to achieve significant reduction in T-cell levels.[19] Acyclovir, a potent inhibitor of *Herpes simplex* virus replication, also inhibits PNP, albeit to a lesser extent, making it unsuitable for clinical use.[20] Similarly, allopurinol, 6-mercaptopurine, and 6-methoxypurines inhibit PNP, but only at very high drug concentrations.[21] C-8 substituted analogs such as 8-iodoguanosine and 8-aminoguanosine have been used as inhibitors of PNP and resulted in T-cell selective cytotoxicity.[21,22] Additional PNP inhibitors include analogs of deoxyguanosine such as 8-amino-9-(2-thienylmethyl)guanine (PD119229)[23] and analogs of deazaguanine.[24,25] However, the inhibitory activity was not as potent as that observed with N7 substituted congeners.[4] For example, an N7 modified analog, BCX-34 (Peldesine) had an IC_{50} of 30 nM; however, when used in clinical trials to treat patients with psoriasis and cutaneous T-cell lymphomas, there was no significant clinical activity. Enzymatic studies indicated that BCX-34 had a rapid off rate and could not inhibit PNP completely and elevate the plasma dGuo levels necessary for T-cell suppression.[18,26]

Forodesine- PNP Inhibitor

Schramm's group used another strategy to design more potent PNP inhibitors by identification of the transition-state structure stabilized by the target enzyme.[27-29] Geometric and electrostatic properties of the transition-state of substrate were used as an atomic blueprint to design chemically stable isologues to act as analogs. Using inosine as a substrate for transition-state analysis, a series of 9-deazanucleoside analogs, termed immucillins, was designed to mimic the transition-state.[30-32] The immucillins have a carbon-carbon linkage between a cyclic amine moiety that replaces ribose, and either 9-deaza-hypoxanthine or 9-deaza-guanine (immucillin H (now forodesine) and immucillin G, respectively). These analogs inhibited PNP with high potency; the Ki values were in 20–80 pM range for human and bovine enzyme.[33]

Forodesine in cell lines

Forodesine in the presence of dGuo was tested on malignant T cell lines and on normal activated human peripheral T cells. These drugs inhibited the growth of malignant T cell leukemia lines with the induction of apoptosis. These actions were selective as no or minimal inhibition was observed in malignant B cells, several solid tumor cell lines, or normal human nonstimulated T cells. Phosphorylation of deoxyguanosine and the accumulation of dGTP, were essential for activity of the drugs.[34,35]

Forodesine in animal model systems

In vivo investigations in murine model systems suggested that BCX-1777 elevates dGuo levels, however, the drug was not effective because of low or no accumulation of dGTP in mouse T-cells. In contrast, in the human peripheral blood lymphocyte severe combined immunodeficiency mouse model, the drug was effective in prolonging life.[36] Furthermore, an oral formulation showed 63% bioavailability in mice[36] but much lower in primates.[37]

Forodesine in humans

The first phase I investigation[38] could be viewed as preclinical investigation as it was designed to test the hypothesis that forodesine would inhibit PNP *in vivo* resulting in biochemical sequelae that would increase levels of dGuo in plasma and dGTP in leukemic T-cells. Since prior investigations of another PNP inhibitor (BCX-34 or Peldesine) had failed to achieve this objective, this study was done only in 5 patients with several plasma and cellular pharmacokinetic and pharmacodynamic endpoints. Pharmacokinetic investigations of the parent drug in plasma showed that concentrations between 4–8 µM of forodesine were achieved with 40 mg/m² dosing.[38] This starting dose was thus likely sufficient to achieve an effective inhibitory level of forodesine in plasma, given that the concentration needed to inhibit the human PNP enzyme is in the picomolar range. Additionally, the observed peak level of forodesine (median 5.4 uM); and the long t½ (median 10 hours) in this study suggested that once daily dosing of 40 mg/m² might be sufficient to provide adequate and maintained drug exposure for inhibition of PNP. Consistent with these observations, infusions of forodesine for 30 minutes resulted in a rapid increase of both plasma dGuo (median 14 µM) and inosine, and a single dose produced a sustainable 24 hour dGuo response.

With these high and maintained plasma levels of dGuo, it was expected that circulating T-cells would also accumulate high levels of intracellular dGTP. In three of 5 patients, the leukemia cells accumulated concentrations of dGTP which were 40 to 60-fold greater than pretreatment levels. Sample from one patient had a 10-fold increase while leukemia cells from another patient had no augmentation in intracellular dGTP level. These data, albeit in a limited number of patients, strongly suggested heterogeneity regarding accumulation of dGTP which is not directly related to plasma levels of dGuo. In addition, a direct relationship was observed between leukemia cell reduction in peripheral blood and intracellular accumulation of dGTP. In the patient with progression of disease during therapy, no intracellular accumulation of dGTP was observed. In contrast, the other 4 patients had cytoreduction of disease which correlated with marked increases in intracellular dGTP. In summary, this was the first demonstration in human that forodesine is an effective inhibitor of PNP, resulting in increase in plasma dGuo and cellular dGTP. Ongoing phase II clinical trials are exploring the efficacy of protracted single daily dosing of intravenous forodesine while phase I studies with an oral formulation have just been initiated.

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