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Comparative *in vitro* and *in vivo* activities of two 9-deazaguanine analog inhibitors of purine nucleoside phosphorylase, CI-972 and PD 141955

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Abstract—An in-parallel comparison is presented of the *in vitro* and *in vivo* properties of two 9-deazaguanine analog inhibitors of purine nucleoside phosphorylase (PNP), CI-972 [8-amino-9-deaza-9-(3-thienylmethyl)guanine] and PD 141955 [9-deaza-9-(3-thienylmethyl)guanine] (published K_i values of 0.83–8.0 and 0.08 μM, respectively). Despite structural similarities, PD 141955 was considerably more potent and active in all systems studied. The respective IC_{50} values for inhibition of MOLT-4 cell growth in the absence and presence of 10 μM 2′-deoxyguanosine (GdR) were > 50 and 5.06 μM for CI-972 and 15.4 and 0.061 μM for PD 141955. PD 141955 induced accumulation of dGTP in GdR-treated MOLT-4 and CEM cells at log-lower concentrations than were required of CI-972, and the magnitude of dGTP accumulation in PD 141955-treated T cell cultures was markedly greater (e.g. 366 vs 100 pmol/10^6 CEM cells at 10 μM). PD 141955 administered orally produced a dose-dependent elevation of plasma inosine and guanosine in rats over a broad concentration range. Mean plasma inosine concentrations following a 150 mg/kg p.o. dose peaked at 6.21 and 13.2 μM in CI-972 and PD 141955-treated rats, respectively. Low levels of inosine were detectable at 50 μg/kg following oral administration of PD 141955.

Purine nucleoside phosphorylase (PNP,* EC 2.4.2.1), a purine salvage enzyme, catalyzes the reversible phosphorolysis of guanine- and hypoxanthine-based (d)nucleosides to their respective purine bases and (d)ribose-1-phosphate [1, 2]. Patients with homozygous deficiency in PNP have markedly impaired T cell function with normal-to-elevated B cell function. Because of the sparing of B cell function in PNP deficiency, inhibitors of PNP have been suggested to have the potential to be T cell-selective immunosuppressive agents with application to a wide variety of clinical settings [3–5]. A large number of PNP inhibitors representing a variety of chemical classes has been synthesized [4–9; reviewed in 10, 11]. A few of these compounds have been evaluated for effects on lymphoblastoid cells *in vitro* and for the ability to modulate purine metabolism *in vivo* (e.g. elevate plasma inosine and guanosine) [12–14]. In our efforts to design PNP inhibitors with better physicochemical properties than those exhibited by purine-based inhibitors (e.g. PD 119229 [13]), a series of 9-deazaguanines† was synthesized and evaluated *in vitro* and *in vivo* [15–18]. One 9-deazaguanine compound, CI-972 [8-amino-9-deaza-9-(3-thienylmethyl)guanine; 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one), has been studied extensively [15–18] and appears to be the first compound to be evaluated in humans. Another compound in this series, 9-deaza-9-(3-thienylmethyl)guanine [PD 141955; 2-amino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one], differs from CI-972 only by the absence of the amino group at the 6 position of the pyrrolo[3,2-*d*]pyrimidine ring. Recently several compounds in the 9-deazaguanine series were described by Ealick *et al.* [19]. Those compounds were designed using the three-dimensional structure of PNP obtained by X-ray crystallography [19]. One of the most potent PNP inhibitors in that series was PD 141955‡ [19]. We find PD 141955 to be approximately 12-fold more potent than CI-972 using a human erythrocyte PNP enzyme assay (assay described in [20]) (respective K_i values of 0.067 μM (unpublished observation) and 0.83 μM [15, 17, 18])§. The increased PNP inhibition found for the desamino compound results from a better fit in the active site of the PNP enzyme due to reduced steric hindrance and more favorable environment from hydrophobicity and hydrogen-bonding perspectives [19]. We present here a comparison of additional *in vitro* and *in vivo* activities of CI-972 and PD 141955. The removal of the amino group at the 6 position of the pyrrolo[3,2-*d*]pyrimidine ring of CI-972, generating PD 141955, imparted not only a significant increase in PNP inhibitory potency to PD 141955, but also increased potency *in vitro*, using human lymphoid cell lines, and *in vivo*, in terms of plasma nucleoside elevation.

Materials and Methods

Chemicals. Purine bases, nucleosides and nucleotides were obtained from the Sigma Chemical Co. (St. Louis, MO). [^{3}H]Thymidine (TdR) was obtained from New England Nuclear (Boston, MA). PD 141955 and CI-972 were synthesized at Parke-Davis Pharmaceutical Research, Warner-Lambert Co.

Cell culture systems. The methods used have been described previously [3, 14, 21]. PNP inhibitors were added to microtiter plate wells containing MOLT-4 or CEM (T cells) or MGL-8 (B cell) lymphoblastoid cells at 5×10^5 per well. 2′-Deoxyguanosine (GdR) was co-added to augment the changes in PNP substrate flux that occur during inhibition of PNP. The effects of GdR on [^{3}H]TdR uptake were determined in parallel with cultures treated with PNP inhibitors only. The cells were cultured for 64 hr and labeled with [^{3}H]TdR for the final 16 hr of culture. Nucleotides were extracted from lymphoblastoid cells with cold 60% methanol after 16 hr of incubation and were analyzed by HPLC using a Whatman anion exchange column and a mobile phase of 0.45 M potassium buffer (pH 3.6) [22].

Nucleoside elevation in vivo. The methodology for assessing the effects of PNP inhibitors on plasma nucleosides

* Abbreviations: PNP, purine nucleoside phosphorylase; B, bone marrow-derived lymphocyte; CEM, human T cell lymphoblastoid cell line; GdR, 2′-deoxyguanosine; MGL-8, human B cell lymphoblastoid cell line; MOLT-4, human T cell lymphoblastoid cell line; T, thymus-derived lymphocyte; and TdR, thymidine.

† The chemical nomenclature for compounds with the trivial name of 9-deazaguanine is 2-amino-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one.

‡ Designated BCX-5 by BioCryst Pharmaceuticals, Inc.

§ 2-Amino-3,5-dihydro-7-(3-thienymethyl)-4*H*-pyrrolo-[3,2-*d*]pyrimidin-4-one is reported to be 100-fold more potent than 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one in a calf spleen PNP enzyme assay (respective IC_{50} values of 0.08 and 8.0 μM when determined in 50 mM phosphate [19]).

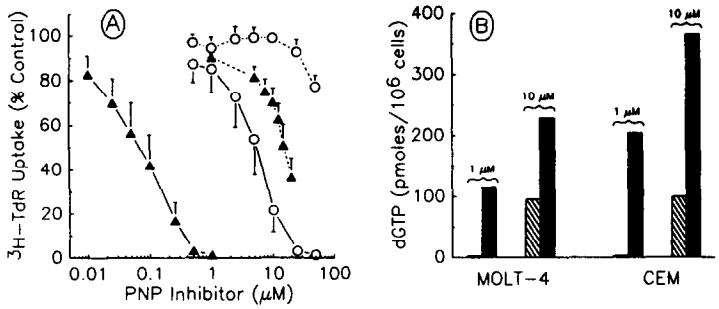

Fig 1. Effects of PNP inhibitors on human T cell lymphoblastoid cell lines. (A) PD 141955 (▲) or CI-972 (○) was added to MOLT-4 cultures at the concentrations indicated, with (——) or without (--) addition of 10 μM GdR, and incorporation of [^{3}H]TdR was determined after a 64-hr culture. Results are the means ± SEM of 8 experiments in which both PNP inhibitors were evaluated in parallel. The range for [^{3}H]TdR uptake in untreated MOLT-4 cultures was 174,400 to 248,400 cpm (mean ± SEM = 208,800 ± 6,000). (B) CI-972 (hatched bars) and PD 141955 (solid bars), at 1 or 10 μM, were studied for their effects on dGTP accumulation in MOLT-4 and CEM cells in the presence of 10 μM GdR. The limit of detection of the HPLC system was 2.8 pmol dGTP/10^6 cells.

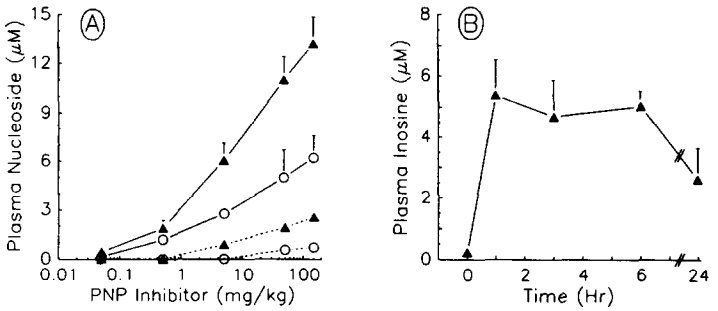

Fig. 2. Plasma nucleoside elevation in rats following oral administration of PNP inhibitors. (A) PD 141955 (▲) or CI-972 (○) was administered to rats (N = 8/group) at the doses indicated and the rats were exsanguinated 1 hr later. Plasma inosine (—) and guanosine (--) were quantified by HPLC (mean ± SEM). The limit of detection of the HPLC system was 0.02 μM. (B) Time-course study on the effects of PD 141955 (5 mg/kg) on plasma inosine in rats. Values are means ± SEM of 8 rats/group. Inosine was statistically significantly elevated through 24 hr post dosing ($P < 0.05$, Students' t-test). The duration of action for CI-972 in this test is reported to be ≃ 4 hr [16].

has been described previously [12–14]. Outbred male Wistar rats (Charles River, Wilmington, MA, N = 8/group), weighing 125–140 g, were given a single p.o. dose of PNP inhibitor dissolved or suspended in propylene glycol and were exsanguinated 1 hr (for dose-response studies) or at various times (for time-course studies) after dosing. Plasma nucleotides were analyzed by HPLC [13].

Results and Discussion

Effect on human lymphoblast growth and nucleotide pools. CI-972 and PD 141955 were compared in parallel for effects on MOLT-4 growth in eight assays (Fig. 1A). The IC_{50} values of CI-972 and PD 141955 without coaddition of GdR were > 50 and 15.4 μM, respectively. With coaddition of 10 μM GdR, the respective IC_{50} values were 5.06 μM and 61 nM. This differential in potency (≃ 80-fold) may reflect a combination of factors, not just the increased PNP inhibition by PD 141955. CI-972 is non-inhibitory to MGL-8 at concentrations up to 50 μM [15, 16], whereas PD 141955 had an IC_{50} of ≃13 μM for MGL-8 in the absence or presence of 10 μM GdR (data not shown). Inhibition of MOLT-4 and CEM lymphoblast growth, but not that of MGL-8 (data not shown), by PD 141955 plus GdR was associated with enhanced accumulation of dGTP (Fig. 1B). Accumulation of dGTP occurred at concentrations of PD 141955 that were substantially lower than those required for CI-972 to produce this effect, and the magnitude of dGTP accumulation was markedly greater in PD 141955-treated cultures (Fig. 1B).

Accumulation of plasma nucleosides. Patients with homozygous deficiency in PNP have elevated levels of PNP substrates in plasma and urine, e.g. plasma inosine levels range between 14 and 115 μM in PNP deficiency compared to undetectable levels in normals [2]. We have shown previously that administration of PNP inhibitors to rats causes elevation of PNP substrates in plasma [12–14], and reduced excretion of allantoin in urine following fructose infusion [11]. A single p.o. dose of PD 141955 caused linear, dose-dependent elevation of plasma inosine and guanosine over the concentration range of 0.5 to 150 mg/kg p.o. (Fig. 2A), with low levels of inosine being detectable in some rats at doses as small as 50 μg/kg. Mean plasma

inosine concentrations peaked at 13.2 μM, but some rats had inosine concentrations > 18 μM, within the range found for homozygous PNP-deficient patients. CI-972, tested in parallel, also produced marked elevation of plasma nucleosides, although CI-972 was less potent and apparently less active than PD 141955. The activity shown here for CI-972 was somewhat greater than reported previously [16]. In our time-course studies, PD 141955 produced elevation of plasma inosine that was statistically significant over the duration of the study (24 hr) following a single 5 mg/kg p.o. dose (Fig. 2B), while the duration of action of CI-972 in this test is reported to be about 4 hr [16].

PD 141955 thus represents a compound that is at least 10-fold more potent than CI-972 in PNP enzyme inhibition assays [17–19 and unpublished observations], and also produced a marked increase in GdR-dependent inhibition of human MOLT-4 and CEM lymphoblasts *in vitro*, compared to CI-972. *In vivo*, PD 141955 was up to 30-fold more potent than CI-972, although this relationship cannot be firmly established because of the non-parallel nature of the respective dose–response curves. Although potent inhibitors can be designed effectively using X-ray crystallography and PNP inhibition data, enhanced enzyme inhibition in a cell-free system is not sufficient to ensure that a compound will have improved activity in a living cell system, much less *in vivo*, where numerous factors relating to metabolic disposition have a significant bearing on pharmacologic effect. An example to support this statement is PD 119229 (CI-950), which has a PNP K_i equal to that of PD 141955, yet is considerably less active against human T lymphoblasts and in terms of inosine elevation [13, 22]. Removal of the amino group at the 6 position of the pyrrolo[3,2-*d*]pyrimidine ring of CI-972, generating PD 141955, imparted a significant increase in PNP inhibitory potency to PD 141955 compared with CI-972. The superior activity of PD 141955 when tested against human T cell lymphoblasts, and also *in vivo* as judged by inosine and guanosine elevation, are features of this molecule that could not be predicted solely on the basis of enzyme inhibition and X-ray crystallography data.

Departments of Immunopathology and †Chemistry
Parke-Davis Pharmaceutical Research
Warner-Lambert Co.
Ann Arbor, MI 48105
U.S.A.

RICHARD B. GILBERTSEN*
USHA JOSYULA†
JAGADISH C. SIRCAR†‡
MI K. DONG
WEN-SHEN WU
DAVID J. WILBURN
MARY CAROL CONROY

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* Corresponding author: Richard B. Gilbertsen, Ph.D., Parke-Davis Pharm. Res., 2800 Plymouth Road, Ann Arbor, MI 48105. Tel. (313) 996-7545; FAX (313) 996-4333.

‡ Current address: Biofor Ltd., P.O. Box 629, Waverly, PA 18471.

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