Nucleoside analogues inhibiting PNP for the therapy of hyperuricemia in Lesch-Nyhan disease: preliminary in vitro studies

E. Baldini, G. Jacomelli, F. Corelli, V. Micheli

Dept. of Biotechnologies,Chemistry and Pharmacy - University of Siena- Italy

Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolytic cleavage of inosine (Ino), guanosine (Guo) and the respective deoxynucleosides into free bases and ribose/deoxyribose. PNP , widely distributed in most tissues, displays the highest expression in thymus and lymphoid cells, where it mainly removes purine deoxynucleosides generated from DNA breakdown. Nucleoside analogues inhibiting human (PNP), were initially developed as specific anticancer drugs that selectively suppress T cell proliferation in T cell malignancies. T cells are especially sensitive to the lack of PNP, as demonstrated by inherited deficiency, in which a severe combined immunodeficiency (SCID) occurs , due to dGTP cytotoxicity.

PNP inhibitors have more recently been successfully used in clinical trials to lower uric acid in gouty patients, in association or not with allopurinol.

Uric acid, produced by xanthine dehydrogenase (XD) in the final purine catabolism, is known to cause a number of problems including gout and renal failure when in excess, due to its low solubility. Severe hyperuricemia and hyperuricuria, together with hypoxanthine and xanthine accumulation, occur in hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency , a rare X-linked genetic defect. Virtually complete HPRT deficiency is found in Lesch-Nyhan Disease (LND), the most severe form, also manifesting with a devastating neurologic syndrome related to basal ganglia dysfunction, whose origin has not been completely clarified. Uric acid excess is very remarkable in these patients and is commonly managed by XD inhibitors (such as allopurinol), yielding in urate decrease but also in xanthine and hypoxanthine increase.

Present study is aimed at testing the reliability of PNP inhibitors as a therapy for urate, hypoxanthine and xanthine excess in LND patients. The rational for the use of such compounds is to obtain the effective reduction of uric acid production, by blocking hypoxanthine production upstream. Hypoxanthine accumulation has also been reported to have a role in some aspects of the neurological disorder in LND (1,2). On the other hand xanthine accumulation may yield in renal stones , even more difficult to be dissolved than urate ones. The therapeutic aim is to limit the administration of XD inhibitors to LND patients by supplying low doses of PNP inhibitors, reaching a suitable equilibrium with non-toxic d-nucleoside production .

We report the results of studies conducted in primary cultures of skin fibroblasts from control subjects and LND patients grown in the presence and absence of an analogue of Immucillin-G (3) to inhibit PNP activity . Cell viability, the amount of released oxpurines in the culture medium, and the endocellular nucleotide pattern have been monitored in different growth conditions (inhibitor concentration, time, added substrates). Our results demonstrate effective PNP inhibition by low concentration of inhibitor, with reduced hypoxanthine release, and no appreciable toxicity in control or patient cells, suggesting a possible new therapeutic strategy against oxipurine accumulation.

1. *Prior et al., Eur J Clin Invest, 37, 905–911(2007)*
2. *Bavaresco et al. Brain Res 1239, 198-206 (2008)*
3. *Semeraro et al. J. Med. Chem 49, 6037-6045 (2006)*