Preclinical testing of PNP inhibitors analogues of Immucillin-G for the therapy of Lesch-Nyhan disease: preliminary in vitro studies

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Lesch-Nyhan Disease (LND) is a rare X-linked genetic disease characterized by hyperuricaemia, gout and severe neurological syndrome. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency occurs causing uric acid, hypoxanthine and xanthine accumulation. Uric acid excess is commonly managed by xanthine oxidase inhibitors, yielding in xanthine and hypoxanthine production. The biochemical basis of the neurological pathology is still unclear, and no effective therapy is available. Deazaguanine derivatives inhibiting human purine nucleoside phosphorylase (PNP) were used in clinical trials to lower uric acid in gouty patients. This study is aimed at testing the reliability of PNP inhibitors as a therapy for LND urate and hypoxanthine excess.

A 9-deazaguanosine analogue of Imucillin-G (Semeraro et al. J. Med. Chem 2006; compound 1a) was used as PNP inhibitor. PNP inhibition and apparent kinetic constants were measured in crude lysates from normal erythrocytes and fibroblasts by HPLC-linked methods (Micheli et al. BBA 2002). Nanomolar 1a concentrations markedly decreased Vmax not affecting Km_{ino} suggesting non-competitive inhibition. Primary cultures of control subjects skin fibroblasts were grown with DMEM plus 10% h.i. FCS, 1% penicillin/ streptomycin, at 37°C and 5 % CO_2, in the presence or absence of 1a (10 nM and 1 μM) and of 20 μM inosine for 24, 48 and 72 h. Perchloric extracts obtained from medium and cells were then analysed by HPLC. No significant reduction of viable cells was demonstrated by MTT test and no remarkable alteration in cell nucleotide pattern was found after any incubation condition, while 1 μM 1a caused hypoxanthine and xanthine decrease together with inosine and guanosine increase in the culture medium.

Present data demonstrate effective PNP inhibition by low 1a concentration, with no appreciable toxicity. Studies are in progress in HPRT-deficient cells, in view of a new therapeutic strategy against oxipurine accumulation.

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