CONCISE PUBLICATIONS

Orotic Aciduria in Two Unrelated Patients with Inherited Deficiencies of Purine Nucleoside Phosphorylase

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ABSTRACT The urines of two unrelated children with inherited deficiencies of purine nucleoside phosphorylase have been found to contain significant quantities of orotic acid in addition to the previously reported purine nucleosides. The data are consistent with some cell types of these immunodeficient patients being deplete of pyrophosphoribosylphosphate, a precursor of both purine, and pyrimidine nucleosides. It is suggested that the pyrophosphoribosylphosphate-depleted cells may be some component of the thymus-dependent immune system.

INTRODUCTION

The inherited deficiency of purine nucleoside phosphorylase in humans is associated with a severe deficiency of thymus-derived lymphocytes or T cells but with minimally impaired or normal function of the bone marrow-derived or B lymphocytes. The deficiency of purine nucleoside phosphorylase seems to be causally related to the immunodeficiency since the inherited deficiency of adenosine deaminase, an enzyme which functionally precedes purine nucleoside phosphorylase, is also associated with a T-cell dysfunction and a variably impaired B-cell function. There have been several hypotheses proposed to explain the immune dysfunction in these enzyme-deficient disorders. The two prominent hypotheses are a cyclic AMP-mediated lymphotoxicity (1), and a pyrimidine starvation as originally proposed by Green and Chan (2). In a cell culture model system using pharmacological simulation of adenosine deaminase deficiency we have eliminated the hypothesis that cyclic AMP mediates the toxic effects of adenosine deaminase deficiency. We have also demonstrated the ability of the pyrimidine nucleoside, uridine, to reverse the cytotoxic effects of the simulated adenosine deaminase deficiency, and of simulated purine nucleoside phosphorylase deficiency (3).

In two independent laboratories we have characterized the disordered purine metabolism in two unrelated patients deficient in purine nucleoside phosphorylase (4, 5). Both patients excrete in their urine large quantities of inosine and guanosine and their respective 2'-deoxyribonucleosides. During our initial characterizations we attempted to determine whether the urines of these patients contained orotic acid. This intermediate in pyrimidine biosynthesis might be expected to accumulate if the inhibition of pyrimidine nucleotide synthesis occurred at the level of phosphoribosylation. Ishii and Green (6) and Snyder and Seegmiller (7) have independently reported the accumulation of orotic acid in cultured cells treated with adenosine. Because of the large quantities of purine nucleosides in the urines we were not able to

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assay for orotic acid, with the desired sensitivity. Subsequently we employed a radiochemical method using isotope dilution techniques to determine the concentration of orotic acid in the urine of one purine nucleoside phosphorylase-deficient patient. Having demonstrated orotic aciduria in that patient, we then developed a high pressure liquid chromatographic system for the detection of orotic acid in the presence of high concentrations of purine nucleosides. By the latter technique we have demonstrated orotic aciduria in the two patients with purine nucleoside phosphorylase deficiency. The administration of oral uridine to one patient diminished the excretion of orotic acid. These observations provide considerable insight as to the site of the disordered nucleotide metabolism.

METHODS

Patient A (8, 5), and patient B (4) have been described previously and were not receiving drugs other than prophylactic antibiotics at the times of study. The urine from the Dutch child (patient B) was lyophilized and sent at ambient temperature from the Netherlands to San Francisco where the analyses were done. The [*carboxyl*-^{14}C]orotic acid was purchased from New England Nuclear, (Boston, Mass.), and the orotate phosphoribosyltransferase contaminated with orotidylate decarboxylase was purchased from P-L Biochemicals, Inc., (Milwaukee, Wis.). All other purine and pyrimidine compounds and other reagents utilized were of the highest grades commercially available.

Isotope dilution assay. Orotic acid in the urine was assayed by an isotope dilution technique. Aliquots of urine were incubated at 37°C for 30 min with 0.12 nmol of [*carboxyl*-^{14}C]orotic acid (50 mCi/mmol), 0.6 nmol of pyrophosphorylribosylphosphate (PPriboseP)[1] in 0.6 ml in the presence of an excess (0.2 mg/ml) of orotate phosphoribosyltransferase–orotidylate decarboxylase. The released [^{14}C]carbon dioxide was trapped and counted as previously described (9).

Chromatography. High pressure liquid chromatographic assays of orotic acid in the urine were performed on an ALTEX high pressure system with a 152 dual wavelength ultraviolet detector. The samples (25 μl) were applied to a Partisil 10-SAX column (H. Reeve Angel & Co., Inc., Clifton, N. J.) and eluted at a flow rate of 2 ml/min with 7 mM potassium phosphate, pH 2.8. The eluates were monitored at 254 and 280 nm. Standard solutions of orotic acid and uridine monophosphate were employed as markers.

RESULTS

The isotope dilution technique for determining orotic acid in the urine samples provided a technique sufficiently sensitive to detect 5 μM orotic acid even in the presence of 10 mM inosine, as depicted in Fig. 1. The urine sample from purine nucleoside phosphorylase-deficient patient A contained approximately 60 μM orotic acid in addition to the purine nucleoside components previously reported (5).

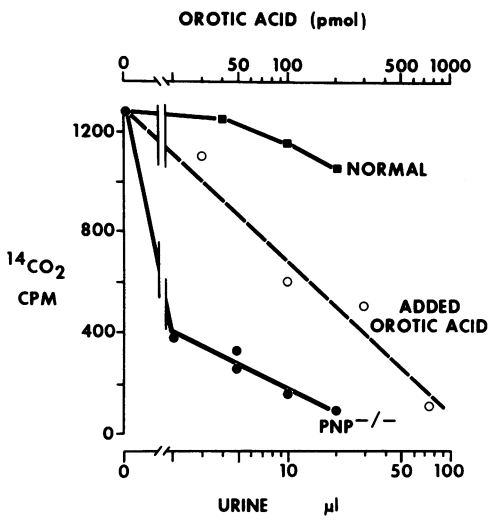

FIGURE 1 Isotope dilution assays of orotic acid in urines of purine nucleoside phosphorylase-deficient patient A and normal controls. The method and materials are described in the text. Urine from a normal child, ■; urine from patient A, ●; known quantities (picomoles) of orotic acid standard, ○. PNP, purine nucleoside phosphorylase; CPM, count per minute.

The high pressure liquid chromatographic system developed for these studies is also capable of detecting orotic acid at the 5 μM level. The tracings from a normal urine and the urines of the purine nucleoside phosphorylase-deficient patients are shown in Fig. 2 with the elution position of orotic acid indicated by the arrow. From standards the concentrations of orotic acid were calculated and are depicted in Table I along with the values for four age-matched normal controls. The treatment of the patients' urines with PPriboseP, and orotate phosphoribosyltransferase contaminated with orotidylate decarboxylase eliminated the presumed orotic acid peak with the appearance of a new uridine monophosphate peak, thus confirming the identity of the material as orotic acid.

During the treatment of patient A with oral uridine (100 mg/kg per day) the amount of orotic acid in her urine was reduced (Table I). A similar orotic acid reduction during uridine treatment has been reported in primary orotic aciduria (10).

Orotidine was absent (<5 μM) in all urines examined. Extracts of the erythrocytes of patient A were not detectably deficient in the conversion of [*carboxyl*-^{14}C]orotic acid to [^{14}C]O_2 in the presence of an excess (1 mM) PPriboseP.

DISCUSSION

Orotic aciduria is recognized in several inherited diseases and reflects the limited conversion of orotic

[1] *Abbreviation used in this paper*: PPriboseP, pyrophosphorylribosylphosphate.

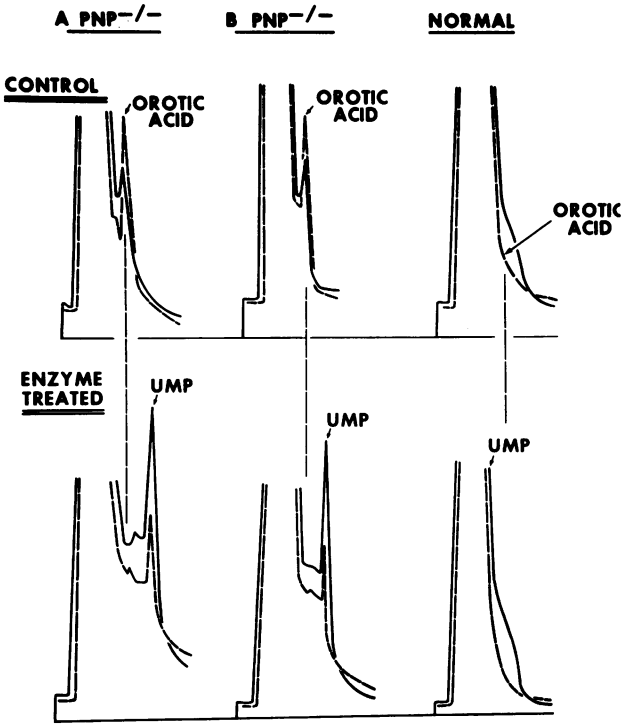

FIGURE 2 High pressure liquid chromatography of urine samples from purine nucleoside phosphorylase-deficient patients A and B and normal controls. Upper panel, no treatment; lower panel, after incubation of urine for 30 min at 37°C with 0.2 mg/ml orotate phosphoribosyltransferase-orotidylate decarboxylase in the presence of 1 mm PPribose P. Absorbance at 254 nm (——); absorbance at 280 nm (– – –). PNP, purine nucleoside phosphorylase; UMP, uridine 5'-monophosphate. For additional details see Methods.

acid to orotidylate. The limiting factor is usually a relative or absolute deficiency of orotate phosphoribosyltransferase even though in some cases the primary defects are in urea metabolism (11). The purine nucleoside phosphorylase-deficient patients do not have a disorder of urea metabolism as evidenced by their tolerance of high protein diets, and their degree of orotic aciduria is much less than that present in patients deficient in ornithine transcarbamoylase and in patients with type I orotic aciduria. Low intracellular PPribose P, the other substrate for orotate phosphoribosyltransferase, also results in the accumulation of orotic acid (12). We have previously demonstrated that the erythrocytes of both purine nucleoside phosphorylase-deficient patients have increased quantities of PPriboseP, but the cultured fibroblasts from patient A do not have elevated PPriboseP, and do not overproduce purines *de novo* (5). Clearly in vivo the patients are not generally deplete of PPriboseP, but the presence of orotic aciduria suggests that in the phosphorylase-deficient patients some cell types may be PPriboseP deplete. Diet does not affect the urinary excretion of orotic acid in normal persons or those heterozygous for orotic aciduria type I (13).

TABLE I
Urinary Orotic Acid in Purine Nucleoside Phosphorylase-Deficient Patients and in Normal Controls

Source	Urinary orotic acid	
	μM	*μg/mg creatinine*
Normals (n = 5)	<5	<4
Patient A	74	27
Patient B	52	34
Patient A during uridine treatment	<5	<4

Data are determined from high pressure liquid chromatography of urine as described in Methods. The urinary excretion of creatinine by patient A was 360 mg/24 h and that by patient B was estimated to be 175 mg/24 h.

Skaper et al. (14) have demonstrated that under conditions of glutamine deprivation cultured human fibroblasts are dependent upon exogenous inosine for the maintenance of PPriboseP levels. The generation of PPriboseP from inosine is almost certainly dependent upon its phosphorolysis to ribose-1-phosphate, and the subsequent conversion of the latter compound to ribose-5-phosphate, the immediate precursor of PPriboseP. Were a specific cell type dependent upon inosine for its source of PPriboseP, then the deficiency of purine nucleoside phosphorylase would have detrimental effects upon the ability of the cells of that type to divide and perhaps even to survive. It is possible that during the ontogeny of the immune system T cells or perhaps precursors of T cells are dependent upon inosine as a source of PPriboseP, perhaps due to an inactive hexose monophosphate shunt. The deficiency of purine nucleoside phosphorylase could thereby prevent the normal development of the immune system. This hypothesis is testable.

Another mechanism by which the purine nucleoside phosphorylase deficiency might result in orotic aciduria would be the inhibition of orotate phosphoribosyltransferase by an abnormal metabolite accumulated in the absence of the phosphorylase. Inosine at concentrations as high as 10 mM does not inhibit orotate phosphoribosyltransferase in vitro.

Whether purine nucleoside phosphorylase immunodeficiency and adenosine deaminase immunodeficiency results from a similar mechanism remains to be determined. One report states that orotic acid was not found in the urine of an adenosine deaminase-deficient patient, but the methods employed and sensitivities thereof were not described (15). Orotic acid does appear as an intracellular metabolite in cultured

cells in which adenosine deaminase has been pharmacologically simulated (6, 7).[2]

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