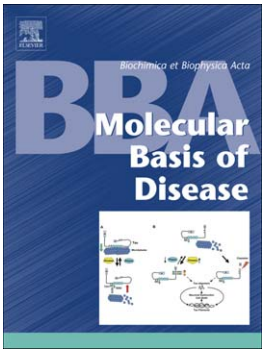

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Effects of alanine:glyoxylate aminotransferase variants and pyridoxine sensitivity on oxalate metabolism in a cell-based cytotoxicity assay *

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ABSTRACT

The hereditary kidney stone disease primary hyperoxaluria type 1 (PH1) is caused by a functional deficiency of the liver-specific, peroxisomal, pyridoxal-phosphate-dependent enzyme, alanine:glyoxylate aminotransferase (AGT). One third of PH1 patients, particularly those expressing the p.[(Pro11Leu; Gly170Arg; Ile340Met)] mutant allele, respond clinically to pharmacological doses of pyridoxine. To gain further insight into the metabolic effects of AGT dysfunction in PH1 and the effect of pyridoxine, we established an "indirect" glycolate cytotoxicity assay using CHO cells expressing glycolate oxidase (GO) and various normal and mutant forms of AGT. In cells expressing GO the great majority of glycolate was converted to oxalate and glyoxylate, with the latter causing the greater decrease in cell survival. Co-expression of normal AGTs and some, but not all, mutant AGT variants partially counteracted this cytotoxicity and led to decreased synthesis of oxalate and glyoxylate. Increasing the extracellular pyridoxine up to 0.3 μM led to an increased metabolic effectiveness of normal AGTs and the AGT-Gly170Arg variant. The increased survival seen with AGT-Gly170Arg was paralleled by a 40% decrease in oxalate and glyoxylate levels. These data support the suggestion that the effectiveness of pharmacological doses of pyridoxine results from an improved metabolic effectiveness of AGT; that is the increased rate of transamination of glyoxylate to glycine. The indirect glycolate toxicity assay used in the present study has potential to be used in cell-based drug screening protocols to identify chemotherapeutics that might enhance or decrease the activity and metabolic effectiveness of AGT and GO, respectively, and be useful in the treatment of PH1.

1. Introduction

The hereditary kidney stone disease primary hyperoxaluria type 1 (PH1, OMIM 259900) is caused by a functional deficiency of the liver-specific peroxisomal pyridoxal-phosphate-dependent enzyme alanine:glyoxylate aminotransferase (AGT) (1). Failure to transaminate intraperoxisomal glyoxylate to glycine in PH1 leads to its oxidation by cytosolic lactate dehydrogenase (LDH) to the metabolic end-product oxalate. The elevated synthesis and excretion of oxalate can lead to the deposition of insoluble calcium oxalate in the kidney and urinary tract, and eventually renal failure. More than 170 mutations have been identified in the gene encoding AGT (*i.e. AGXT*), which lead to a wide variety of molecular and cellular phenotypes, including accelerated AGT degradation, aggregation, loss of catalytic activity, and peroxisome-to-mitochondrion mistargeting (2–4).

Abbreviations: AGT, alanine:glyoxylate amino transferase; COM, calcium monohydrate crystals; GO, glycolate oxidase, PH1, primary hyperoxaluria type 1; PLP, pyridoxal-5'-phosphate

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