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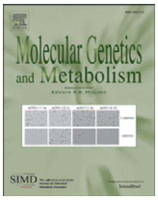

Minireview

Innovative therapy for Classic Galactosemia — Tale of two HTS

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ABSTRACT

Classic Galactosemia is an autosomal recessive disorder caused by the deficiency of galactose-1-phosphate uridylyltransferase (GALT), one of the key enzymes in the Leloir pathway of galactose metabolism. While the neonatal morbidity and mortality of the disease are now mostly prevented by newborn screening and galactose restriction, long-term outcome for older children and adults with this disorder remains unsatisfactory. The pathophysiology of Classic Galactosemia is complex, but there is convincing evidence that galactose-1-phosphate (gal-1P) accumulation is a major, if not the sole pathogenic factor. Galactokinase (GALK) inhibition will eliminate the accumulation of gal-1P from both dietary sources and endogenous production, and efforts toward identification of therapeutic small molecule GALK inhibitors are reviewed in detail. Experimental and computational high-throughput screenings of compound libraries to identify GALK inhibitors have been conducted, and subsequent studies aimed to characterize, prioritize, as well as to optimize the identified positives have been implemented to improve the potency of promising compounds. Although none of the identified GALK inhibitors inhibits glucokinase and hexokinase, some of them cross-inhibit other related enzymes in the GHMP small molecule kinase superfamily. While this finding may render the on-going hit-to-lead process more challenging, there is growing evidence that such cross-inhibition could also lead to advances in antimicrobial and anti-cancer therapies.

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1. Introduction

Galactose is the C-4 epimer of glucose, with an identical molecular formula, but a distinct structural formula. Despite its strong structural similarity to glucose, the conversion from galactose into glucose requires a few evolutionarily-conserved enzymatic steps, all residing in the cytoplasm, known as the Leloir pathway of galactose metabolism [1]. The main source of galactose in humans is dietary, mainly dairy products containing lactose, but other non-dairy foodstuffs can also contain galactose moieties [2,3]. In humans, galactose can also be produced endogenously, mostly through the enzymatic conversion between uridine diphosphate-glucose (UDP-glucose) and UDP-galactose, as well as the turnover of glycoprotein and glycolipids [4,5]. Upon entry to the Leloir pathway, galactose is first phosphorylated by galactokinase (GALK) to form galactose-1-phosphate (gal-1P) [6]. Together with the second substrate UDP-glucose, gal-1P is converted by galactose-1-phosphate uridylyltransferase (GALT) to form UDP-galactose and glucose-1-phosphate [7]. The Leloir pathway is completed by reversibly forming UDP-glucose from UDP-galactose by UDP-galactose-4-epimerase (GALE) [8,9] (see Fig. 1). Enzyme deficiencies in the Leloir pathway, caused by bi-allelic amorphic or hypomorphic mutations in any of the genes coding for the GAL enzymes have been described (see Refs. [10–14] for extensive reviews on this subject). Of these deficiencies, the most common disorder is Classic (Type I) Galactosemia, which is caused by bi-allelic amorphic mutations in the *GALT* gene, and is the main focus of this review. Infants born with Classic Galactosemia usually become ill within days after birth if exposed to breast milk or lactose-containing formula. Initially, the infant develops jaundice, and if lactose exposure continues, complications such as liver failure, *Escherichia coli* (*E. coli*) sepsis, coma, and death follow shortly after [13]. The main aspect of management is the replacement of lactose/galactose using soy-based formula, after which the infant usually recovers fairly quickly [13]. All 50 states in the U.S. and many developed countries have included Classic Galactosemia as one of the conditions screened for in the newborn period, ensuring that most infants survive without becoming ill [15].

Despite a galactose-restricted diet, most patients with Classic Galactosemia continue to accumulate significant amount of galactose, galactitol and gal-1P in their cells [13,16–18]. Further, it has become clear that even with early detection and (early) dietary intervention, there is still a significant burden of this disease due to chronic complications that arise in childhood and adulthood. The most common complications are speech dyspraxia, ataxia, and premature ovarian insufficiency [19,20]. To date, the pathophysiology of the acute toxicity syndrome and the chronic complications remains largely unknown, but it is reasonable to assume that any blockage in a metabolic pathway will lead to (i) accumulating precursor(s), (ii) alternate metabolites normally not encountered, or (iii) absent metabolites past the enzymatic block. Any, or a combination of these possibilities, could be responsible for the phenotypes associated with the enzymatic blockage. As to GALT-deficiency Classic Galactosemia, it is apparent that galactose and gal-1P accumulate in patients, with galactose being further metabolized through two alternative pathways to form galactitol and galactonate [17,18,21,22]. Among all the metabolites formed, gal-1P and galactitol have received most attention. But what are the potential toxicity targets of these toxic metabolites, and between gal-1P and galactitol, which plays a more important role in the pathophysiology of Classic Galactosemia?

Various reports suggested that gal-1P competitively inhibited UDP-glucose pyrophosphorylase [23–25], inositol monophosphatase [25–28], phosphoglucomutase [29], glycogen phosphorylase [30], or even glucose-6-phosphatase [31], although none of these *in vitro* findings has been fully substantiated in human patients *in vivo*. Nevertheless, if inhibition of UDP-glucose pyrophosphorylase occurs *in vivo*, it could potentially reduce UDP-glucose/galactose formation in the cells, and could cause aberrant glycosylation of proteins and lipids. In fact, there are reports showing abnormal circulating proteins, including transferrin and follicle-stimulating hormone (FSH) in the blood of GALT-deficient patients with elevated erythrocyte gal-1P [32–34]. Because of these findings, Classic Galactosemia has been broadly regarded as a congenital disorder of glycosylation. Recently, there is a renewed interest in the potential link between inositol metabolism and galactose metabolism [35,36]. If inhibition of inositol monophosphatases takes place *in vivo* as it was demonstrated in *in vitro* experiments [25–28], it could lead to reduced free inositol, and accumulation of inositol monophosphates in the cells. Indeed, decreased free and lipid-bound inositol in the tissues of both GALT-deficient patients and galactose-intoxicated rats has been reported [37,38].

Regarding the toxicity targets of galactitol, Berry and coworkers hypothesized that galactitol may also inhibit Na^+/*myo*-inositol transporter (SMIT1)-mediated *myo*-inositol transport *in vivo* by osmoregulatory control and jeopardize the availability of *myo*-inositol in the cellular level [35]. As excess galactose accumulates inside the GALT-deficient cells, it is reduced to galactitol by aldose reductase. Similar to sorbitol, the excess galactitol formed will cause osmotic imbalance inside the cells and through the action of TonEBP/OREBP and/or AP-1, lead to reduced transcription of the *SLC5A3* gene, which encodes the SMIT1 transporter [39–43]. One of the important roles of *myo*-inositol is to serve as the precursor of phosphatidylinositol-4,5-biphosphate (PtdIns-4,5-P_2), which is essential for numerous intracellular signal transduction pathways [44]. A severe deficiency of *myo*-inositol would therefore impair multiple signal transduction pathways such as the PI3K/AKT/mTOR cell growth pathway [45,46] (Fig. 2). In fact, it

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