Galactose-1-Phosphate Uridyl Transferase (GALT) and Galactosemia

A Review of GALT Function and Current Theories on Galactosemia Pathogenesis

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Abstract

All living organisms depend on the metabolism of carbohydrates for energy and the biosynthesis of necessary glycoconjugates. One of these carbohydrates is the monosaccharide galactose. Galactose is metabolized by humans through the Leloir pathway of galactose metabolism, which contains three enzymes to modify galactose so that it can be incorporated into glycolysis for the production of cellular energy. The middle enzyme of this pathway, galactose-1-phosphate uridyltransferase, produces uridine diphosphogalactose (UDP-gal) from galactose-1-phosphate (gal-1P), and a deficiency of this enzyme results in the human disease galactosemia. Galactosemia is diagnosed soon after an infant begins feeding, and although a galactose-restricted diet eliminates immediate acute symptoms, long-term complications typically persist. The exact mechanism of galactosemia pathogenesis is not currently understood, but research is being performed concerning the possible involvement of the unfolded protein response (UPR), human inositol monophosphatase (hIMPase), and abnormal glycan composition of enzymes, hormones, and growth factors. Future research of GALT and galactosemia will aim to better understand the pathogenesis of galactosemia with hopes of a more effective treatment to decrease long-term complications of the disease.

Introduction

All living organisms must utilize sugars, or carbohydrates, for the production of cellular energy and the synthesis of necessary cellular glycoconjugates. Humans not only consume dietary carbohydrates, but can also synthesize modified carbohydrate monomers using reversible metabolic pathways. Galactose is a carbohydrate monomer typically consumed as a part of the dissacharide lactose in the human diet. The human body metabolizes and utilizes galactose via the Leloir pathway of galactose metabolism. This pathway includes three main enzymes, each of which produces a different metabolic intermediate with the goal of incorporating galactose into glycolysis for energy production. The middle enzyme of the Leloir pathway is galactose-1-phosphate uridyl transferase (GALT), which produces uridine diphosphogalactose (UDP-gal) from galactose 1-phosphate (gal-1P). UDP-gal can continue in the Leloir pathway to eventually be used for energy production, but it is also used to synthesize numerous necessary glycoconjugates. GALT deficiency in humans results in galactosemia, a potentially fatal disease if not dealt with immediately from birth. The exact disease mechanism of galactosemia is not yet completely understood, but possible models of pathogenesis have been proposed and will be described in this review.

The Leloir Pathway

The Leloir pathway is the only mechanism of galactose metabolism in both humans and yeast, and has been extensively studied and characterized using both a yeast model and an *Escherichia coli* (*E. coli*) model (Grossiord, Luesink, Vaughan, Arnaud, & de Vos, 2003; Kalckar, Kurahashi, & Jordan, 1959; T. Slepak, Tang, Addo, & Lai, 2005; Yarmolinsky, Wiesmeyer, Kalckar, & Jordan, 1959). Kalckar, Kurahashi, & Jordan

named and characterized the Leloir pathway in 1959 while studying a strain of *E. coli* K-12 defective in galactose metabolism and unable to grow on galactose-containing media. Three enzymes, galactokinase (GALK), galactose-1-phosphate uridyl transferase (GALT), and galactose-4-epimerase (GALE), and their respective mechanisms were discovered and defined (Kalckar et al., 1959).

Upon entry into the cell, galactose is first phosphorylated by GALK to produce galactose-1-phosphate (gal-1P), which is one of the two substrates of GALT. GALT produces one uridine diphosphoglactose (UDP-gal) and one glucose-1-phosphate (glu-1P) from one uridine diphosphoglucose (UDP-glu) and one gal-1P. The desired product of GALT, UDP-gal, is the substrate of GALE. GALE epimerizes UDP-gal to produce UDP-glu, which can then be modified further for entry into glycolysis or used as UDP-glu to synthesize necessary glycoconjugates in the cell (Grossiord et al., 2003; Kalckar et al., 1959; Mollet & Pilloud, 1991). Further modification of UDP-glu involves the loss of uridine monophosphate to produce glu-1P, then the action of a mutase enzyme to produce glucose-6-phosphate, a glycolytic intermediate that enters glycolysis for energy production in the form of ATP (Grossiord et al., 2003). Not all UDP-gal produced is epimerized to UDP-glu by GALE. Because galactose is an essential component of many glycoconjugates, some UDP-gal is used for the synthesis of these sugar moieties. The UDP form of these sugars is necessary for use in glycoconjugate synthesis, highlighting the importance of GALT function in the metabolism and cellular use of galactose (Lai, Langley, Khwaja, Schmitt, & Elsas, 2003).

GALT Mechanism of Function

GALT, the second enzyme of the Leloir pathway, is a dimeric enzyme, consisting of two identical subunits and encoded for on chromosome 9 in humans. Each of these subunits is 379 amino acid residues in length and has one active site for catalytic activity. Both subunits function to carry out the same reaction simultaneously, allowing for more efficient production of UDP-gal.

GALT functions via the double-displacement mechanism of ping-pong kinetics. In this mechanism, the enzyme is first modified by one substrate to produce the first product before acting on the second substrate to return to its original state and produce the second, desired product. In the case of GALT, the first substrate is UDP-glu. The active site of GALT contains a histidine amino acid residue, found in humans as the 186th amino acid in the protein's primary sequence. UDP-glu binds to this histidine, resulting in the release of glu-1P and the modification of GALT with the addition of a UMP group to the histidine residue (uridine monophosphohistidine, or his-UMP). Gal-1P can then bind to the uridyl-enzyme intermediate, allowing the enzyme to transfer the UMP group from histidine to gal-1P and produce UDP-gal. The enzyme is then reset to its original state, and can repeat the reaction.

GALT Deficiency

GALT deficiency produces metabolic complications in almost all organisms that utilize the enzyme. Yarmolinsky et al. were among the first to study such deficiency, and did so using E. coli sensitive to the presence of galactose (Yarmolinsky et al., 1959). Results showed that of the three enzymes in the Leloir pathway, only a deficiency of GALT could produce the negative effects associated with galactose-sensitivity, namely

growth inhibition (Yarmolinsky et al., 1959). In humans, GALT deficiency typically results from a mutation in the gene encoding the GALT enzyme. The most common mutation in humans involves a point mutation at amino acid residue 188, causing a glutamine residue to be translated as an arginine residue (Tyfield et al., 1999). The displacement of glutamine, a moderately hydrophilic amino acid, with arginine, a basic, positively charged amino acid, results in a change in charge and therefore enzymatic conformation only two residues away from the active site of the enzyme, leading to inactivity of the enzyme. Individuals homozygous for this mutation or another mutation causing loss of enzyme activity have complete or almost complete loss of GALT activity, a condition known as galactosemia (Arn, 2003).

Galactosemia

Galactosemia is a human disease resulting from loss of GALT activity in galactose metabolism. The disease is typically diagnosed soon after birth, as breast-feeding infants are consuming lactose via breast milk or formula. Lactose, when metabolized, produces one glucose and one galactose monomer per lactose molecule. The glucose monomer is metabolized via glycolysis, but the galactose monomer, when fed through the galactose metabolic pathway, cannot be metabolized past gal-1P. Disease symptoms arise in infants soon after they begin feeding, and a diagnosis can be made. Pathogenesis of galactosemia is not completely understood, although several mechanisms of the disease processes have been proposed (Charlwood, Clayton, Keir, Mian, & Winchester, 1998; Lai & Elsas, 2000; Lai et al., 2003; Liu et al., 2012; Sturiale et al., 2005). Treatment currently involves management of diet, although some drug targets in the disease process have been proposed.

Diagnosis and Screening

Galactosemia occurs about one in every 30,000 to 60,000 babies in the US and is typically diagnosed using newborn screening or via the observation of disease symptoms in an infant ("Expanded Newborn Screening Using Tandem Mass Spectrometry Financial, Ethical, Legal and Social Issues (FELSI)," 2013). Newborn screening for galactosemia and other inherited genetic disorders is available in all of the states and provinces of the United States, due to the high death rate associated with untreated individuals. For galactosemia screening involves testing infant blood and urine samples for the presence of GALT and galactose metabolites (Freer, Ficicioglu, & Finegold, 2010). Samples are first tested for galactose concentration and GALT activity, and if galactose levels are high and/or GALT activity is low, the samples are also assayed for gal-1P and tested for some of the more common DNA mutations associated with galactosemia (Freer et al., 2010). GALT enzyme presence of less than 32 µmol/L (normal 150-500 µmol/L) is usually indicative of GALT-deficient galactosemia (Freer et al., 2010). Some states, such as Pennsylvania and New York, legally require all newborns to be screened for certain inherited genetic disorders, including galactosemia, within 24 to 48 hours after birth, with results being reported seven to 10 days after the blood is taken and no charge being administered for the screening ("Conditions Screened by State," 2013; "Pennsylvania Screening Services for Newborn Babies," 2012).

Newborn screening is essential in detecting and treating galactosemia efficiently and before physical and mental damage has been done that cannot be reversed. One study found that about 80% of children given newborn screening for galactosemia were diagnosed within two weeks of age, compared to 35% that were not screened. Only 20%

of the children screened, however, were free of GALT deficiency symptoms at the time of diagnosis (Waggoner, Buist, & Donnell, 1990). More efficient and timely screening methods are necessary to decrease the cases of infants who are already exhibiting disease symptoms at the time of diagnosis.

Description

Because newborn screening is typically not performed until at least 24 hours after an infant has begun feeding, galactosemic infants will consume galactose before diagnosis and will likely show disease symptoms almost immediately. Acute symptoms of galactosemia observed in newborns who have begun milk feeding include poor feeding, vomiting, liver dysfunction, hypotonia and lethargy, cataracts, and predisposition to sepsis (Coman et al., 2010). Though uncommon due to effective newborn screening, undiagnosed galactosemia can lead to liver cirrhosis, mental retardation, and even death (Antshel, Epstein, & Waisbren, 2004). Patients with galactosemia have been found to have higher rates of ovarian failure, cataracts, speech and language impairments, and developmental delays, even with a monitored dietary intake (Antshel et al., 2004). A long-term study in Ireland, where galactosemia is most prevalent, followed 130 Irish galactosemic patients, all but eight of which were diagnosed with newborn screening. Ninety-two percent of these individuals were homozygous for the Q188R mutation. Hypergonadotropic hypogonadism (HH), a condition of hypogonadism as a result of an impaired response of the gonads to sex hormones, was seen in 91.2% of the females, and IQ was less than 89 for almost all of the 85 individuals that received IQ testing (Coss et al., 2013; Friedman, Barrows, & Kim, 1983). Although acute symptoms at birth can be

managed with diet, long-term outcomes involving impaired sexual and mental function are still prevalent among galactosemic individuals.

A case report from 2012 described disease symptoms of a full-term male infant who was fed a lactose-containing formula from birth. After showing symptoms of lethargy and poor feeding around six days of life, newborn screening tests were performed on a dry blood spot from the second day of life, showing positive results for galactosemia at day eight of life. GALT activity in the infant was assayed and found to be absent, and galactose metabolites, such as galacticol and gal-1P, were found to be present in very high levels in the child. Galacticol accumulation in brain tissue led to acute encephalopathy, but with dietary treatment and fresh frozen plasma administration the encephalopathy cleared and the child was achieving normal growth and development at one year of age. Long-term follow-up with the child revealed delayed language acquisition, as well as speech defect and cognitive impairment (Berry, 2012). This study, like many others involving individual cases of galactosemia, describes the typical progression of disease for infants diagnosed with galactosemia in the first few days of life. What causes such complications and developmental impediments involves the pathogenesis of galactosemia, where much of the current research concerning this disease is taking place.

Pathogenesis

While the disease processes and mechanisms of galactosemia are not completely understood, numerous theories have been proposed. One of the most serious complications associated with galactosemia is neurological impairment, which can occur even in treated patients. This complication may result from either damage in utero,

damage that occurs before diagnosis and treatment, poor adherence to dietary restrictions, and/or accumulation of gal-1P even when adhering to dietary treatment (Antshel et al., 2004). Almost all proposed mechanisms of galactosemic disease processes involve the build-up of galactose metabolites, namely gal-1P, as well as significantly decreased levels of UDP-gal. High levels of gal-1P (up to 3.3 mM) are found almost exclusively in galactosemic individuals, leading to the assumption that gal-1P plays an essential role in the pathogenesis of galactosemia (Lai et al., 2003). Gal-1P has been proposed to interfere with cellular enzymes, as well as induce cellular responses normally reserved for cellular stress. The low levels of UDP-gal also play a crucial role in the cellular dysfunction observed in galactosemia, as UDP-gal is an essential component of many important cell signaling molecules and other cellular receptors. When UDP-gal levels are low, the cell is forced to utilize other available sugars, changing the chemical make-up of these signals and receptors and leading to a lack of proper cellular function.

Endoplasmic reticulum – the UPR. Slepak et al. conducted a study in 2009 to investigate the observation of induced endoplasmic reticulum stress in a human cell model of classic galactosemia (T. I. Slepak, Tang, Slepak, & Lai, 2007). Primary fibroblasts from three random galactosemic patients were used as the test environment, and three different growth conditions were tested. A hexose-free medium was used to rule out the possibility that glucose starvation caused the observed effects of GALT deficiency, and a hexose-free medium containing only 0.1% glucose was used to observe cell viability in the absence of galactose. Finally, cells were grown in hexose-free medium containing only 0.1% galactose to obtain a disease model with accumulation of the toxic metabolite gal-1P. Cells grown in the hexose-free medium alone did not grow

and detached from the plate surface on which they were initially attached, while cells grown in 0.1% glucose grew normally and were not affected by the GALT deficiency.

Cells grown in 0.1% galactose showed accumulation of gal-1P similar to that observed in the erythrocytes of untreated galactosemic patients (T. I. Slepak et al., 2007). Continued experiments were performed only with this hexose-free, galactose-containing media. GALT-deficient and GALT-positive (normal GALT expression) cells were compared for behavior in the presence of galactose, with a concentration on gene expression in each cell type. Results showed the many of the same genes involved in the unfolded protein response (UPR) were upregulated in the GALT-deficient cells. The UPR functions in mammalian cells to respond to stress encountered by the endoplasmic reticulum when unfolded or improperly folded proteins begin to accumulate inside the endoplasmic reticulum. Transcriptional activators of the genes involved in the UPR were upregulated in the GALT-deficient cells grown in galactose, including CHOP, ATF4, ATF3, all of which increase transcription of the genes encoding ER resident proteins, lectins, and foldases (Schroder & Kaufman, 2005). Protein chaperones, lectins, and foldases function in the UPR to help ease endoplasmic reticulum stress by aiding the protein folding process, and if these molecules cannot resolve the stress adequately the cell tries to degrade the unfolded proteins by increasing transcription and translation of endoplasmic reticulum resident proteases, or protein-degrading enzymes (Eriksson et al., 2004; Hosokawa, Wada, Natsuka, & Nagata, 2006). The master ER stress sensor protein, GRP78/BiP, was also found to be up-regulated in the GALT-deficient cells grown in galactose, and the amount of GRP78/BiP produced was proportional to the amount of galactose present in the medium. Because GRP78/BiP can also be induced in starvation,

GALT-deficient cells were also grown in medium containing galactose and glucose, which resulted in the same observation of GRP78/BiP up-regulation and showed that the up-regulation was a result of GALT-deficiency. Slepak et al. also investigated the observation that GALT-deficient cells grow slower than normal, even in the presence of glucose, by observing the levels of the fibroblast epidermal growth factor receptor (EGFR). EGFR was found to be present at much lower levels in GALT-deficient cells compared to normal fibroblasts, especially when the GALT-deficient cells were grown in galactose. EGFR protein synthesis and turnover are both related to the functional state of the ER, implying the role of ER stress in GALT-deficient cells in impeding proper growth factor function and therefore slowing cellular growth.

Typically, ER stress is related to calcium homeostasis in the ER, as a disturbance in calcium homeostasis initiates ER stress. Slepak et al. analyzed the role of GALT-deficiency in this process, and found that galactosemic cells experience much less free calcium release from the ER into the cytoplasm upon bradykinin addition (stimulates calcium release from the ER by activation of phospholipase C and IP_3 release (Hashii, Nozawa, & Higashida, 1993)) (T. I. Slepak et al., 2007). The IP_3 receptor presence was found to be unchanged, meaning the low levels of calcium release resulted from either altered levels of calcium in ER stores or lower levels of IP3 release due to a reduction of phosphatidylinositol diphosphate (PIP_2), which will be discussed later.

The connection between GALT-deficiency, galactose presence, and ER stress demonstrated by Slepak et al. was also explored in a study by De-Souza et. al. in 2014, which found that the UPR actually serves to protect galactosemic cells in the yeast model (De-Souza et al., 2014). Two different yeast models were utilized; one with a

dysfunctional copy of the GALT gene and the other treated with lithium and galactose. Lithium treatment with galactose growth conditions mimicked GALT-deficiency in galactosemic cells, as lithium inhibited phosphoglucomutase and allowed for the build-up of gal-1P. This study found first that gal-1P is required for the induction of the UPR observed in galactosemic cells. The GALK encoding gene of yeast was deleted in both models, resulting not only in the elimination of the unnecessary UPR activation, but also the disappearance of all toxic effects associated with galactosemic cells. The GALK-deficient cells did not have an increased tolerance to other known stressors of the ER, implying that the elimination of the UPR did not result from increased tolerance to ER stress no matter what the source.

Because the UPR is normally a protective response when cells undergo ER stress, De-Souza et al. investigated whether the UPR is also protective when activated in galactosemic cells. To test this, GALT-deficient yeast mutants were constructed that did not have the genes necessary for activating the UPR in yeast (*ire1*Δ) and were compared to GALT-deficient cells that were still able to activate the UPR. Results showed that *ire1*Δ GALT-deficient mutants were even more sensitive to galactose than UPR-positive GALT mutants, while levels of gal-1P accumulation did not significantly differ between strains, indicating that increased gal-1P is not responsible for the increased galactose sensitivity in the absence of a functional UPR. Implications of this study indicate the importance of the UPR in the adaptation of yeast cells for survival in galactosemic conditions, but when applied to human galactosemic cells shows an antagonistic effect. The UPR may protect galactosemic cells initially, but a prolonged response initiates

apoptosis that kills the cell and may contribute to observed disease symptoms (De-Souza et al., 2014).

While galactosemia-induced ER stress and the ensuing UPR are clearly affected by GALT-deficiency and gal-1P accumulation, the exact mechanism of the correlation remains unknown and requires further study. Implications made in the Slepak study indicate a possible interference of gal-1P with the normal function of human inositol monophosphatase (hIMPase) in IP_3 signaling, as well as the possibility that a disruption in cellular glycoconjugate composition leads to or is involved in the lack of ERGF secreted by GALT-deficient galactosemic human fibroblasts (T. I. Slepak et al., 2007). Both of these possible galactosemic disease mechanisms have been studied to increase the understanding of exactly how gal-1P accumulation leads to the disease symptoms of galactosemia.

Inositol monophosphatase disruption. hIMPase is an essential enzyme in the pathway of inositol synthesis from glucose-6-phosphate (glu-6P), directly converting glu-6P to the intermediate molecule myo-inositol-1-phosphate (myo-inositol-1P). hIMPase can also perform the reverse reaction and hydrolyze myo-inositol-1P, making it a crucial enzyme for maintaining appropriate cellular levels of myo-inositol (Hallcher & Sherman, 1980). Mehta et al. conducted a study in 1999 to observe the proposed relationship between gal-1P and hIMPase, specifically related to inositol mediated signaling in the brain and the possible correlation between this phenomenon and the neurological damage observed in galactosemic individuals (Mehta, Kabir, & Bhat, 1999). Using a yeast model expressing hIMPase, it was found that growing GALT-deficient yeast cells containing hIMPase on galactose-containing media allowed for cellular survival and growth on this

media, even though a more recent study has shown that GALT-deficient cells do not survive when grown on hexose-free media containing only galactose as the carbon source (T. I. Slepak et al., 2007). This positive growth was found to be a result of hIMPase presence and function, as addition of lithium, a known inhibitor of hIMPase, led to cessation of growth. Implications were that IMPase either reduces gal-1P accumulation or facilitates metabolism of galactose, or a combination of the two. Positive growth of GALT-deficient hIMPase containing yeast cells on media containing only galactose meant either galactose or gal-1P was being metabolized to lead to the elimination of galactose toxicity (Mehta et al., 1999). hIMPase expressing, GALT-deficient yeast cells lacking functional GALK were constructed to determine which metabolite the cells were utilizing. The GALK-deficient cells were unable to produce gal-1P, meaning if the cells grew on galactose-containing media then they were using galactose and not gal-1P. Results showed that the hIMPase GALT/GALK-deficient yeast cells were unable to grow on media containing only galactose, and gal-1P was inferred to be the metabolite allowing for growth of GALT-deficient cells containing hIMPase.

To investigate the exact metabolic pathway of gal-1P utilization in these cells, the few possible enzymatic mechanisms were considered for obtaining glu-1P from gal-1P in the absence of GALT. The most likely pathway involved UDP-galactose pyrophosphorylase (UGalP), an enzyme that can reversibly convert gal-1P to UDP-gal, and UDP-glucose pyrophosphorylase (UGluP), which reversibly converts UDP-glu to glu-1P. Gal-1P would have to first be converted to UDP-gal with UGalP, after which GALE, still present and functional as it would normally be, converts UDP-gal to UDP-glu, which is then converted to glu-1P by UGluP and metabolized via glycolysis. The

study confirmed that this pathway is utilized in yeast cells, and that overexpression of IMPase relieves galactose toxicity in GALT-deficient cells via modulation of UGlu/GalP activity. GALT-deficient cells with overexpressed hIMPase and grown in galactose had significantly greater UGalP activity compared to cells grown in glycerol and cells without overexpressed hIMPase, showing that hIMPase and galactose are independent positive modulators of UGalP activity (Mehta et al., 1999).

The results of this study imply a strong connection between hIMPase activity and the utilization of accumulated gal-1P in GALT-deficient cells. In 2003, Bhat investigated the nature of the relationship between hIMPase and gal-1P, proposing that IMPase is a cross link between galactose metabolism and the phosphotidylinositolbisphosphate (PIP2) signaling pathway, and that this connection is at least partially responsible for the long-term neurological impairments seen in many galactosemic patients (Bhat, 2003). Gal-1P is typically a normal intermediate of galactose metabolism, but when allowed to accumulate in galactosemic individuals it also has the opportunity to interfere with IMPase activity. In the PIP2 signaling pathway, IMPase converts multiple inositolbisphosphates to inositol. This is especially important in brain tissue, as PIP2 signaling is a vital second messenger for several neurotransmitters in the brain. PIP2 is hydrolyzed to produce diacylglycerol and inositol 1,4,5-triphosphate (IP3), two second messengers that activate protein kinase C and release internal stores of calcium. The rate of PIP2 signaling is dependent upon the regeneration of PIP2, and this synthesis relies directly on the amount of free inositol in the cell. The production of inositol by IMPase is a crucial part of driving the PIP2 signaling pathway, as free inositol is a necessary substrate of the pathway. Gal-1P interferes with inositol production by competing with

inositolbisphosphates as a substrate of IMPase, leading to decreased free inositol production and therefore less PIP2 signaling (Bhat, 2003; Parthasarathy, Parthasarathy, & Vadnal, 1997). Bhat also points out the possibility that the reversible function of UGalP is able to produce gal-1P in galactosemic individuals, even when the individual is on galactose-restricted diet and is consuming no dietary galactose. This production of gal-1P from a source other than galactose could explain the occurrence of long-term physical impairment, such as brain damage, in galactosemic patients on a galactose-restricted diet. Three specific enzymes – GALT, UGalP, and hIMPase – are proposed by Bhat to be primarily responsible for maintaining exact cellular concentrations of gal-1P in healthy individuals, and when one of these enzyme (GALT) is deficient, multiple cellular pathways are affected (Bhat, 2003).

Relating the studies with IMPase to future studies conducted concerning the UPR reveals that alteration of calcium release, part of PIP2 signaling, is a factor in both. Changes in cytoplasmic calcium levels resulting from calcium release from the ER after IP3 production from PIP2, stimulated by bradykinin, was found to decrease significantly in galactosemic cells grown in galactose (De-Souza et al., 2014). Current research would imply that the decrease of calcium release is an indirect result of gal-1P accumulation, as gal-1P competitively inhibits IMPase, therefore decreasing free inositol in the cell and resulting in slower regeneration of PIP2 for IP3 production (Bhat, 2003; De-Souza et al., 2014; Mehta et al., 1999).

Low UDP-gal and changes in glycoconjugate composition. A third topic for study concerning galactosemia pathogenesis involves UDP-gal, another of the metabolites of galactose metabolism. UDP-gal is a common component of the synthesis

of numerous glycoconjugates in the cell, including signal receptors and signal molecules like hormones and growth factors. One such growth factor, ERGF, was studied and discussed previously in connection with the possible role of the UPR in galactosemia pathogenesis, as ERGF has sugar moieties that contain multiple galactose monomers in healthy individuals but may have altered composition (and therefore altered function) in galactosemic cells (T. I. Slepak et al., 2007). Multiple studies have been conducted concerning the extensive effects a lack of UDP-gal may have on the synthesis of multiple galactose-containing glycoconjugates, many of these studies implying that a change in the normal sugar composition is at least partially responsible for the disease symptoms of galactosemia. One such study by Charlwood et al. in 1997 investigated abnormal glycosylation of transferrin from galactosemic individuals, showing that the N-linked glycans of serum transferrin from human galactosemic cells are shortened from their normal length and usually end with N-acetylglucosamine (Charlwood et al., 1998). The analysis of galactosemic transferrin glycosylation compared to normal transferrin glycosylation showed that galactosemic transferrin was not only more heterogeneous in glycosylation sequence, but the glycans were also truncated. The most commonly observed, biantennary glycan associated with transferrin was observed to account for 86% of all transferrin glycans in healthy individuals, but only 13% in galactosemic patients, the other 87% composed primarily of four different truncated glycans. Some of the galactosemic patients tested did show a significant increase in the common transferrin glycan (up to 82%) after a galactose-free diet was initiated, but others did not, leading to inconclusive data concerning galactosemia treatments involving diet in connection to normal glycosylation of cellular proteins.

Charlwood et al. also studied the specific structures of the truncated glycans found on galactosemic transferrin, finding that all four common truncated glycans observed consisted of the core pentasaccharide of most N-linked polysaccharides, three mannose residues and two N-acetylglucosamine residues, with slight modifications. N-acetylglucosamine was typically found in place of normal residues, such as fructose or galactose, at the terminal, non-reducing ends of glycan branches, making the glycoproteins more favorable for acceptance of galactose residues (in the form of UDP-gal) when available (Charlwood et al., 1998). No clear relationship was established between levels of gal-1P and normally-glycosylated transferrin, as dietary treatment decreased gal-1P concentration, but normal glycosylation percentages ranged from 18-82%. The primary conclusion of this study revealed that the truncation of transferrin glycans in untreated galactosemic individuals was consistent with a decreased ability of the cells to galactosylate protein glycans.

Stiurale et al. conducted a similar study in 2005 to further investigate hypogalactosylation of galactosemic cell glycoconjugates. They compare the abnormal isoform patterns of multiple enzymes and hormones associated with galactosemia to congenital disorders of glycosylation (CDG), which are genetic defects of glycan synthesis that affect either the assembly or processing of N-linked glycans (Sturiale et al., 2005). The goal of this study was to analyze the glycosylation of serum transferrin, like Charlwood et al., then compare the results to CDG-affected and healthy individuals. Results partially confirmed the Charlwood study, showing that transferrin was hypoglycosylated and had an abnormal increase in fucosylation and branching of N-linked glycans in galactosemic individuals. An interesting, somewhat conflicting result

compared to the Charlwood study was that abnormal N-glycan formation presented in the form of the loss of an entire N-linked oligosaccharide at one or both of the glycosylation sites on transferrin, rather than the loss or gain of an N-acetylglucosamine residue. Sturiale et al. also found that the extent of defective glycosylation is dependent on how long the galactosemic patient has been exposed to dietary galactose, and that a galactose-free diet in these individuals is sufficient to eliminate abnormal transferrin N-glycan isoforms and return glycan compositions to normal (Sturiale et al., 2005).

A study conducted by Lai et al. in 2003 sought to explain the glycosylation changes in galactosemic cells by studying the availability of UDP-gal in GALT-deficient human cells. Lai hypothesized that a deficiency of UDP-gal resulted from either a lack of cellular energy due to galactose not being metabolized, accumulated toxins like gal-1P, or a decreased production of essential UDP-hexoses, like UDP-gal (Lai et al., 2003). The study eliminated the first hypothesis, based on the finding that both normal cells and GALT-deficient cells were able to grow on hexose-free media supplemented only with fetal bovine serum (FBS). The second hypothesis, concerning gal-1P toxicity, was studied by comparing galactosemic cells with GALT-transfected cells, growing both in hexose-free media containing FBS and 0.1% galactose. Results showed that expression of GALT in GALT-deficient galactosemic cells allowed for growth on this media, while GALT-deficient cells were unable to grow, leading to the question of whether decreased presence of gal-1P or increased presence of UDP-gal was what led to viable growth of the GALT-deficient cells made to express GALT. UDP-hexose concentrations of GALT-deficient cells grown on a galactose-containing media were found to decrease substantially after only 24 hours, even though these levels started out below normal

before growth in galactose, and gal-1P levels in these cells increased markedly during growth on galactose. Lai observed that the addition of human UDP-glucose pyrophosphorylase (hUGP2), an enzyme that functions to produce UDP-glucose from glu-1P and UTP, to GALT-deficient cells aided in decreasing gal-1P concentrations and allowed for cell growth and viability on galactose (Lai & Elsas, 2000; Lai et al., 2003). hUGP2 was found to utilize gal-1P as a substrate to produce UDP-gal, but with a very high K_m, meaning hUGP2 will only act on gal-1P at high concentrations, otherwise preferably utilizing glu-1P as a substrate. GALT was also found to have a much higher capacity for UDP-gal synthesis from gal-1P (about 1000-times higher rate), making it by far the more effective enzyme for UDP-gal production compared to hUGP2; however, overexpression of hUGP2 in experimental cells may have aided in decreasing gal-1P toxicity by converting some gal-1P to UDP-gal (Lai et al., 2003). This would not necessarily be a wise option for treatment though, as gal-1P is a competitive inhibitor of hUGP2 and would impair UDP-glucose production, only creating further complications in galactosemic cells. Not only was UDP-gal production less than normal in GALT-deficient cells, but UDP-glu was also found to be present at levels as low as 25% of that in a healthy individual. Restoration of GALT activity in galactosemic cells was found to increase levels of UDP-gal and UDP-glu to that of a healthy individual and decrease the accumulation of gal-1P, leading to the conclusion that GALT-deficiency causes a decrease in UDP-hexose concentrations in affected cells. It was also concluded that gal-1P plays a vital role in the decrease of UDP-glu in galactosemic individuals, as it was discovered that gal-1P is a competitive inhibitor of hUGP2 and will stop the production

of UDP-glu by hUGP2 when present at high concentrations such as those found to be associated with galactosemia (Lai et al., 2003).

Broadening the study of abnormal galactosemic glycans, Liu et al. characterized both the N- and O-linked glycomes (all sugar moieties present) of plasma proteins from galactosemic individuals, ranging from neonatal age to children and adults with determined outcomes. Results showed that the composition of N- and O-linked glycans of older children and adults with galactosemia following a galactose-restricted diet were close to if not exactly that of healthy individuals, and there appeared to be no connection between glycosylation defects and severity of patient outcome, provided the patient followed a strict galactose-free diet.

Although the research with abnormal glycan composition in galactosemic individuals appears to show that diet can resolve these abnormalities, the question that remains to be investigated is whether abnormal glycans that have not yet been eliminated by dietary treatment in the galactosemic neonate can lead to the long-term negative outcomes of galactosemic patients, and whether time of galactose exposure affects outcome severity.

Treatment

Galactosemia, upon diagnosis soon after birth, is treated immediately by the removal of galactose from the diet. Although this eliminates the acute symptoms of the disease, many patients still develop long-term complications, including developmental delay, motor abnormalities, and, in women, ovarian failure. Though the exact molecular mechanisms causing these complications are not completely understood, ideas for

possible drug targets have been proposed, in addition to life-long dietary restriction of galactose.

Diet. The most common and currently most effective form of treatment for galactosemia is dietary restriction of galactose consumption. This is primarily manifested in the avoidance of lactose, as many foods ingested by humans that contain galactose do not contain galactose monomers but rather galactose-glucose disaccharides, or lactose. For infants this is especially important, since breast milk contains lactose, and galactosemic infants must consume galactose-free formula. Though it was typically believed that galactose was only found in the human diet in the form of lactose, there have been studies documenting the presence of free galactose in some fruits and vegetables. A study by Gross and Acosta in 1991 documented the monomeric galactose content of 45 different fruits and vegetables. Results showed that foods like artichoke, mushrooms, olives, and peanuts contained less than 0.1 mg of free galactose per 100 g of plant tissue, while persimmon, similar to tomato, contains up to 34.5 mg free galactose per 100 g plant tissue. Other fruits and vegetables, like dates, papaya, bell pepper, tomato, and watermelon were found to have upwards of 10 mg free galactose per 100 g plant tissue (Gross & Acosta, 1991). A second study by Gropper and colleagues revealed that other fruits and vegetables, such as pineapples, honeydew melon, and blueberries also contain significant amounts of free galactose (18.7 mg/100g, 26.7 mg/100g, and 26.2 mg/100g, respectively), and some baby foods are high in free galactose content and therefore unsuitable for a galactose-restricted diet (Gropper, Weese, West, & Gross, 2000). The maximum galactose content of foods in a galactose-restricted diet is 5

mg/100g, and any foods exceeding 20 mg/100g galactose are absolutely prohibited in the recommended galactosemic patient diet (Acosta & Ryan, 1997).

Conclusion

Galactose-1-phosphate uridyltransferase (GALT) is an enzyme in the Leloir pathway of galactose metabolism, and its function is to produce UDP-galactose from galactose-1-phosphate. UDP-gal can continue in galactose metabolism to eventually enter glycolysis and produce metabolic energy, or it can be used to synthesize necessary glycoconjugates, such as hormones and protein glycans. When cells are deficient in GALT, the result is the accumulation of its substrate, gal-1P. Numerous studies have shown gal-1P to be toxic when it accumulates to significantly above-normal concentrations, but the exact mechanism of gal-1P toxicity and the mechanism of galactosemia disease symptoms are not fully understood. Possibilities of pathogenic mechanisms in humans involve the UPR, hIMPase, and abnormal glycan composition. The UPR is regulated genetically, and galactosemic human cells have been shown to upregulate the genes involved in initiating the UPR, a response typically only utilized during ER stress due to an abundance of unfolded or improperly folded proteins in the ER lumen. hIMPase is an enzyme that plays a crucial role in the PIP2 signaling pathway, as it cleaves multiple inositol bisphosphates to produce inositol, a necessary molecule for the regeneration of PIP2 and the continuation of an efficient signal response. Studies have shown that glycan compositions of enzymes like transferrin and growth factors like ERGF are altered and usually truncated compared to the normal glycans in untreated galactosemic individuals. Dietary restriction of galactose has been shown to eliminate these glycan abnormalities, but there may still be a connection between abnormal glycans

before treatment and the presence of long-term complications in most galactosemic individuals. Current treatment for galactosemia involves strict dietary restriction of galactose, including all foods and drinks known to contain lactose. With continued study and investigation into the exact mechanisms of the pathogenesis of galactosemia, a more efficient treatment may be discovered to not only eliminate the acute symptoms of galactosemia, but also the long-term complications.

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