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REVIEW ARTICLE

Glutathione Synthetase Deficiency: An Inborn Error of the Gamma-Glutamyl Cycle

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Abstract:
Glutathione (GSH) is a tripetptide consisting of the amino acids glutamate, cysteine and glycine. It is ubiquitous in the eukaryotic organism and plays a role in many fundamental cellular processes. GSH is metabolized in the gamma-glutamyl cycle in which six enzymes take part in its synthesis and turnover. The most common disorder of the gamma-glutamyl cycle is glutathione synthetase (GSS) deficiency. About 70 patients have been described worldwide. GSS deficiency is inherited in an autosomal recessive manner resulting in decreased levels of cellular glutathione and subsequent overproduction of 5-oxoproline which accumulates in body fluids and is excreted in urine. GSS deficiency is a heterogeneous condition with varying clinical severity. Based on the severity of the clinical symptoms it is classified into three groups. The most severe form is mainly associated with metabolic acidosis, usually present in the neonatal period, haemolytic anemia, 5-oxoprolinuria and central nervous system (CNS) damage. Diagnosis is established by measurement of enzyme activity and mutation analysis. Antenatal diagnosis is possible. Treatment is symptomatic and aims at correction of metabolic acidosis, prevention of haemolysis and support of endogenous defence against reactive oxygen species (ROS). The prognosis is difficult to predict, as the number of patients is limited and the clinical condition varies widely.
Keywords: *gamma-glutamyl cycle, glutathione, haemolytic anemia, metabolic acidosis, 5-oxoprolinuria*
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Glutathione and its metabolism in the gamma-glutamyl cycle

Glutathione (GSH; L-gamma-glutamyl-L-cysteinylglycine) is a tripeptide composed of the amino acids acids glutamate, cysteine and glycine. GSH was recognized more than 100 years ago, and its structure was established in 1935 [1]. It is present in virtually all aerobic cells at millimolar concentrations [2,3,4] where it takes part in numerous fundamental processes. Apart from being one of the most important antioxidants in the eukaryotic organism, it participates in detoxification of certain drugs [5] or carcinogens, protection against lipid peroxidation and electrophiles [6], and has anti-viral effects [7]. Furthermore, it plays a role in the biosynthesis of DNA, proteins, and leukotrienes [8]. Beside this, the tripeptide is involved in cell proliferation, apoptosis [9] as well as in neurotransmission [10] and neuromodulation [11]. Decreased levels of GSH are found in several diseases such as liver cirrhosis, pulmonary disease, gastrointestinal or pancreatic inflammation, diabetes, HIV infection, and neurodegenerative diseases [12,13].

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GSH metabolism takes place in the gamma-glutamyl cycle which is catalysed by six enzymes [14]. GSH is synthesised from glutamate by the sequential actions of glutamate-cysteine ligase (GCL, formerly gamma-glutamylcysteine synthetase) and glutathione synthetase (GSS). Under physiologic conditions, GSH synthesis is regulated by negative feedback

inhibition of glutamate-cysteine ligase suggesting that the enzyme can respond rapidly to increased need for GSH [15].

Degradation of GSH involves four enzymes. Gamma-glutamyl transferase (GGT, formerly gamma-glutamyltranspeptidase) initiates the breakdown of GSH by catalysing the transfer of its gamma-glutamyl-group to acceptors (mostly cystine, but also other amino acids). The gamma-glutamyl residues are substrates of the gamma-glutamyl-cyclotransferase (GCT) which converts them to 5-oxoproline (L-pyroglutamate) and the corresponding amino acids. Conversion of 5-oxoproline to glutamate is catalysed by action of 5-oxoprolinase (5-OPase), the rate-limiting enzyme of the gamma-glutamyl cycle in many tissues. A dipeptidase splits cysteinylglycine, which is formed in the transpeptidation reaction, into glycine and cysteine.

Genetic defects in humans have been described in five of the six enzymes of the gamma-glutamyl cycle: gamma-glutamylcysteine synthetase deficiency (OMIM #230450), glutathione synthetase deficiency (GSS deficiency; OMIM #266130), gamma-glutamyl transpeptidase deficiency (OMIM #231950), 5-oxoprolinase (OMIM #260005), and dipeptidase.

Glutathione synthetase deficiency – the most common inborn error of the gamma-glutamyl cycle

Deficiency of GSS is the most common and the best-characterized of the inborn errors of GSH metabolism. The three dimensional structure of the human GSS enzyme, a homodimer with a subunit size of 52 kDa, has been revealed in 1999 [16]. As the enzyme catalyses the last step of GSH synthesis, its deficiency leads to low cellular concentrations of GSH and excessive production of gamma-glutamylcysteine, the metabolite before the enzyme defect. Reduced feedback inhibition of the gamma-glutamate-cysteine ligase leads to overproduction of gamma-glutamylcysteine which is converted into 5-oxoproline by action of gamma-glutamyl-cyclotransferase. The excessive formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase leading to accumulation of 5-oxoproline in body fluids causing metabolic acidosis and 5-oxoprolinuria. Gamma-glutamylcysteine contains both reactive groups of GSH (i.e. the gamma-glutamyl and the sulfhydryl residues). It accumulates in fibroblasts of patients with GSS deficiency in levels similar to that of GSH in control cells and may to some extent compensate for lack of GSH [17].

Clinical Features

The first patients with GSS deficiency were described in the 1970s. Jellum et al. discovered large amounts of 5-oxoproline in the urine of a mentally retarded patient with metabolic acidosis and named the disease pyroglutamic aciduria [18]. A lesion in the gamma-glutamyl cycle was supposed, but the exact location could not be determined. Hagenfeldt et al. reported a second patient with metabolic acidosis, haemolysis and 5-oxoprolinuria [19]. Indication of a defect of GSS was found through enzymatic studies in this patient and her sister who showed with similar symptoms [20]. Marstein et al. described a mentally retarded patient with ataxia and seizures in whom GSH could not be detected in erythrocytes and GSS activity was less than 2% of normal [21]. Autopsy of one of the first patients described with GSS deficiency who died at 28 years of age revealed atrophy of the granular cell layer of the cerebellum and focal lesions in the frontoparietal and visual cortex and the thalamus [22]. Boxer et al. reported frequent infections due to defective granulocyte function in a patient with GSS deficiency [23]. Spielberg et al. observed several episodes of neutropenia in a patient with GSS deficiency and found excessive accumulation of hydrogen peroxide, impaired protein iodination and bacterial killing in neutrophils to oxidative stress associated with phagocytosis [24]. Ophthalmological abnormalities, e.g. fundus lesions, retinal pigmentations, crystalline opacities in the lenses, poor adaptation to darkness and pathological electroretinograms have been described in some patients [25,26]. Retinal dystrophy has also been observed in adult patients [27]. Antenatal cerebral haemorrhage has been reported in a patient with moderate GSS deficiency [28], and two further cases of cerebral haemorrhages in two neonates were observed in post-mortem investigations [29]. Although the association between peripartal cerebral haemorrhage and GSS deficiency might be coincidental in these cases, in vitro studies suggest that platelet function might be altered in GSS deficiency [30,31,32].

Today, about 70 patients from 55 families have been reported worldwide, but the exact prevalence of GSS deficiency is still not known. The clinical

presentation is very heterogeneous and the severity varies widely. In the past, patients were classified as having a deficiency restricted to erythrocytes (McKusick 231900) or a generalised deficiency (McKusick 266130). Today, it is known that there is only one gene for GSH synthetase in the human genome, and the various clinical varieties most likely reflect different mutations or epigenetic effects. A new classification of GSS deficiency, based on the severity of the clinical signs, has been proposed [29]. According to the severity of their symptoms, patients are classified as mild, moderate or severe (Table 1). Patients with the mild phenotype show mild haemolytic anaemia as their only clinical symptom. Cellular levels of GSH are usually sufficient to prevent accumulation of 5-oxoproline in body fluids. Nonetheless, high urinary excretion of 5-oxoproline can be present in these patients. In the moderate form, patients usually present during the neonatal period with severe and chronic metabolic acidosis, mild to moderate haemolytic anaemia, jaundice and massive urinary excretion of 5-oxoproline. After the neonatal period, the condition usually stabilises, but patients may become critically ill during infections due to pronounced acidosis and electrolyte imbalances. Several patients died during such episodes. In addition to the symptoms mentioned above, severly affected patients develop progressive central nervous system (CNS) damage, e.g. mental retardation, seizures, spasticity, ataxia, and intention tremor. Some patients suffer from recurrent severe bacterial infections, probably due to defective granulocyte function. It is estimated that about 25% of all patients with GSS deficiency die early, often in the neonatal period, of electrolyte imbalances and/or infections [33].

The first pregnancy in a woman with moderate GSS deficiency has been reported to be uneventful resulting in a healthy infant with neither the pregnant woman nor the fetus being affected negatively [34].

Table 1: Clinical signs and symptoms in glutathione synthetase deficiency.

	Mildly affected patients	Moderately affected patients	Severely affected patients
Metabolic acidosis	None described	Almost always present at birth, stabilisation after neonatal period	Almost always present
Haemolytic anaemia	Always present	Always present at birth, stabilisation after neonatal period	Almost always present from birth
CNS symptoms	None described	None described	Progressively present (mental retardation, ataxia, spasticity, seizures)
Recurrent bacterial infections	None described	None described	May be present in some patients
5-oxoprolinuria	May be present	Always present	Always present

Diagnostic tests

Findings like haemolytic anaemia in combination with metabolic acidosis (without ketosis or hypoglycemia) may lead to the suspicion of GSS deficiency. Massive urinary excretion of 5-oxoproline (up to 1 g/kg body weight per day) occurs which can be determined by gas chromatography-mass spectrometry [35]. Although the terms 5-oxoprolinuria or pyroglutamic aciduria have been used synonymously to GSS deficiency, the biochemical finding of 5-oxoprolinuria is also present in several other inborn errors of metabolism not

involving the gamma-glutamyl cycle and other conditions which should be considered during diagnostic work [36]. Excessive formation of 5-oxoproline has been described e.g. in patients with homocystinuria [37]. In nephropatic cystinosis 5-oxoprolinuria may occur because of secondary impairment of the gamma-glutamyl cycle due to decreased availability of free cysteine and can be corrected through cysteamine therapy [38]. Transient 5-oxoprolinuria of unknown cause has been reported in very preterm infants [39,40]. Limited availability of glycine in malnutrition and pregnancy [41] as well as increased turnover of collagen, fibrinogen and other proteins containing considerable amounts of 5-oxoproline in patients with severe burns or Stevens-Johnson syndrome [42] may lead to 5-oxoprolinuria. In addition, certain drugs like paracetamol, vigabatrin or some antibiotics (flucloxacillin, netimicin) are known to induce 5-oxoprolinuria, probably through interaction with the gamma-glutamyl cycle [43,44,45]. Particular infant formulas and tomato juice may contain modified proteins with increased content of 5-oxoproline [46].

GSS deficient patients show increased levels of gamma-glutamylcysteine and cysteine in cultured fibroblasts and low GSH levels in erythrocytes or cultured fibroblasts. Enzymatic analysis of GSS in red blood cells and / or cultured fibroblasts has been described by Ristoff et al. [47]. In affected patients, enzyme activities of 1-30 % of healthy controls are found. Mutation analysis confirms the diagnosis. A study including 41 patients with GSS deficiency showed no significant differences among the clinical phenotypes with respect to enzyme activity, levels of GSH or gamma-glutamylcysteine in cultured fibroblasts.

One symptomatic patient with GSS deficiency has been identified through tandem mass spectrometry-based newborn screening [48]. Antenatal diagnosis can be performed by analysis of GSS activity in cultured amniocytes [49], or by measuring the levels of 5-oxoproline in amniotic fluid [50].

Genetics

GSS deficiency is inherited in an autosomal recessive manner. The GSS gene is localised on chromosome 20q11.2 [51] and consists of 13 exons distributed over 32 kb [52,53]. Mutation analysis of the GSS gene has been described, and nearly 30 different mutations in the GSS gene have been identified [54,55,56,57]. In seven patients with severely decreased GSS activities in cultured fibroblasts and undetectably low levels of GSS no disease causing mutations in the coding exons or intron-exon boundaries of the GSS gene could be identified [57]. Because of the high frequency of splice mutations (approximately 40%), it is recommended that mutation analysis at the genomic level should be completed with analyses of RNA transcripts. Heterozygous carriers of GSS deficiency are healthy and show an enzyme activity of 55% of the normal mean and normal levels of glutathione [58]. Although no definite correlation between genotype and phenotype could be established, mutations causing aberrant splicing, frameshift or premature stop codons seem to be associated with the moderate or severe clinical phenotypes, but additional genetic or epigenetic factors seem to alter the phenotypes [58]. The milder forms of the disease are usually caused by mutations mainly affecting the enzyme stability [59].

Clinical management and prognosis

The clinical management of GSS deficient patients is aimed at correction of acidosis, prevention of haemolytic crises and support of endogenous defence against reactive oxygen species (ROS). During the neonatal period, correction of metabolic acidosis and electrolyte imbalances as well as treatment of anaemia and excessive hyperbilirubinemia are of crucial importance.

Correction of acidosis can be reached through bicarbonate, citrate or trishydroxymethylamino–ethame (THAM). Doses of up to 10 mmol per kg body weight and day or even higher in episodes of acute infections may be required.

Repeated red blood cell transfusions might become necessary in patients with increased haemolysis. Drugs and foods known to cause haemolytic crises in patients with glucose-6-phosphatase dehydrogenase deficiency should be avoided. Treatment with erythropoietin was reported to lead to a good clinical and haematological response in one patient with GSS deficiency [60].

Boxer et al. reported as early as 1979 that treatment with vitamin E (alpha-tocopherol) at doses of 400 IU per day increased red blood cell survival, corrected the defective granulocyte function and eliminated

neutropenia during intercurrent illness [23]. Further evidence for the benefits of treatment with vitamin E in patients with GSS deficiency comes from the finding that vitamin E in vitro protects granule cells in the cerebellum of the rat brain [61]. As the neuropathological findings in GSS deficiency resemble those seen in Minamata disease, chronic mercury intoxification, it was suggested that further treatment with antioxidants might be beneficial [22]. Vitamin C (ascorbic acid) and GSH spare each other in a rodent model [62]. Short-term treatment with vitamin C lead to an increase in the levels of GSH in lymphocytes in a trial with GSS deficient patients [63]. Both vitamins E and C are thought to replenish the lack of GSH as a scavenger of free radicals. Supplementation of vitamin E at doses of 10 mg per kg body weight and day and vitamin C at doses of 100 mg per kg body weight and day is recommended [64]. A long-term follow-up study of 28 patients suggested that early supplementation with both vitamins may prevent CNS damage and improve the long-term clinical outcome in GSS deficient patients [29].

The value of N-acetylcysteine, which is known to protect cells from oxidative stress in vitro, in the treatment of GSS deficiency is controversial. It was suggested that the low intracellular glutathione concentrations and cysteine availabilty might be increased by N-acetylcysteine [65]. However, supplementation with N-acetylcysteine should not be generally recommended [17] because it was shown that patients with GSS deficiency accumulate cysteine which is known to be neurotoxic in excessive amounts [66]. A therapeutic trial with orally administered glutathione showed no lasting benefit in two patients with GSS deficiency [26]. Glutathione esters, lipid soluble preparations which are easily transported into cells where they are converted into GSH, have been tried in animal models of GSH deficiency and in two patients with GSS deficiency [67]. However, associated toxic effects due to production of alcohols as a by-product during hydrolysis to release GSH make them of limited use. In vitro studies showed that addition of S-acetylglutathione to the medium of cultured fibroblasts from patients with GSS deficiency normalized intracellular GSH content [68].

Due to the rarity of the disease and the heterogeneity of the clinical condition the prognosis for individual patients is difficult to predict. Early diagnosis, correction of acidosis and early supplementation with vitamin E and vitamin C appear to be the most important factors regarding the survival and the long-term outcome of patients [29].

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