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Annotation

Galactosaemia: pathogenesis and treatment

J. B. HOLTON
Department of Child Health, Royal Hospital for Sick Children, St Michael's Hill, Bristol, BS2 8BJ, UK

Many inherited metabolic diseases have a poor prognosis in spite of attempts to control the metabolic disorder (Holton, 1995a). Confidence in the dietary treatment of classical galactosaemia, galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.10) deficiency (McKusick 230400), remained for many years after the first signs that there were long-term complications (Anon, 1984). No doubt this was because of the dramatic and almost complete reversal of the acute neonatal presenting features when milk and milk products were removed from the diet. However, it is now well established that learning difficulties, a speech disorder and gonadal failure in females are all very common in older patients. A severe ataxic condition has been found in a small proportion of galactosaemics, although in a milder form this too may be relatively common (Waggoner and Buist, 1993). The treatment of galactosaemia has remained essentially the same since it was first introduced (Mason and Turner 1935) and it is clear that other methods of treatment are necessary. These could emerge when there is a complete understanding of the pathogenesis of the long-term complications and the time at which damage occurs.

Garrod (1908) proposed that the pathogenesis of an inherited metabolic disease was related primarily to the metabolic abnormality. Galactosaemic patients on a normal milk intake accumulate large amounts of galactose and the first major product of its metabolism, galactose-1-phosphate (gal-1-P), in body tissue and fluids. In addition, galactitol and galactonate, products of normally minor pathways of galactose, are present in significant amounts (Segal and Berry 1995). It may be very relevant to the causation of the long-term complications of the disease that these abnormalities are not completely resolved when the diet free of milk and milk products is introduced. There have been two explanations for this: first that it is due to some galactose remaining in the diet, and second, that the galactose and gal-1-P are synthesized endogenously from glucose-1-phosphate (glc-1-P). Recent work seems to confirm that the latter mechanism is the most significant by far (Berry et al 1995).

A great deal of controversy has surrounded claims that the product of GALT, uridine diphosphate galactose (UDPGal), is deficient in galactosaemic patients to a degree suggesting pathological significance (Holton et al 1993). Ng et al (1989) published work based on enzymatic analysis, which showed that in the majority of patients with galactosaemia who had no GALT activity there was a clear-cut deficiency of UDPGal in red cells, skin fibroblasts and liver compared to normal subjects. A small proportion of patients had

very low, but measurable, GALT activity and UDPGal concentrations within the normal range.

Subsequent studies using HPLC to measure red cell nucleotide sugars (Berry et al 1992; Keevill et al 1993) found statistically significant reductions in UDPGal in galactosaemic patients, but with a very large overlap of levels in patient and normal populations. In addition, no reduction could be demonstrated in UDPGal concentrations in skin fibroblasts and leukocytes in patients with galactosaemia (Keevill et al 1994).

Doubt has been cast on the accuracy of the enzyme method used by Ng et al to measure nucleotide sugars (Kirkman 1995), although it is now claimed that the essential findings which instigated the controversy can be reproduced using an HPLC method to measure sugar nucleotides (Xu et al 1995). It should be pointed out that in all studies the concentration of UDPGlc has been found to be normal and the most significant observation is a reduction in the UDPGal/UDPGlc ratio in galactosaemia. In normal subjects this ratio is believed to be maintained by the activity of UDPGal-4-epimerase and the findings in galactosaemia may be explained by the fact that this enzyme is compromised in some way, and not by a reduction in UDPGal synthesis.

The most convincing hypothesis regarding a pathogenic mechanism in GALT deficiency involves the accumulation of galactitol. Clinical and experimental evidence suggests that in the acute toxic state galactitol accumulation and osmotic swelling in the lens of the eye is the primary cause of the cataract formation which occurs in all forms of galactose metabolism disorders, namely the deficiencies of GALT, galactokinase (EC 2.7.1.6; McKusick 230400) and uridine diphosphate galactose-4′-epimerase (EC 5.1.3.2; McKusick 230350). It is probable, also, that galactitol accumulation in the brain is the cause of pseudo-tumour cerebri, a rare complication of GALT and galactokinase deficiencies (Welch and Milligan 1987). The precise links between galactitol accumulation and cataract formation and whether galactitol may play a part in long-term complications of GALT deficiency continue to be subject of much speculation at the present time (Berry 1995).

Considerable attention has focused on gal-1-P as the cause of many of the acute and long-term features of galactosaemia. This is because of the apparent association of high gal-1-P levels with these complications in GALT deficiency and the absence of this biochemical abnormality and the clinical abnormalities, apart from cataracts, in galactokinase deficiency. There has been much experimental work which demonstrates an inhibitory effect of gal-1-P in many metabolic pathways of carbohydrate metabolism, and the possibility that it is involved in a futile phosphorylation cycle, trapping ATP (Gitzelmann 1995). Although these experiments have been extremely interesting, there has been no confirmation that they are relevant to the clinical problems.

More recently, interest has returned to the possibility of a galactosylation defect as the cause of some features of galactosaemia, suggested initially by Haberland et al (1971) after finding an abnormal pattern of glycoprotein in the brain of a galactosaemic patient. Observations in galactosaemic fibroblasts (Dobbie et al 1990; Ornstein et al 1992) point to a defect in galactose incorporation into glycoproteins, as does the work of Jaeken and colleagues (1992) showing abnormal electrophoretic isoforms of plasma transferrins in galactosaemic infants receiving milk. These isoforms probably lack galactosyl side-chains.

Two possible causes of a galactosylation defect have been proposed. Ng et al (1989) postulated that this was due to a deficiency of UDPGal, the galactosyl donor in the

galactosylation reaction. The controversy concerning whether a biologically significant deficiency of UDPGal occurs has been alluded to earlier. Alternatively, experimental work has indicated that gal-1-P may inhibit the UDPGal galactosyltransferases (Roth et al 1971). A rather interesting piece of work reported by Bonham and colleagues (1994), showing a very significant correlation between urinary transferrin/creatinine ratio and erythrocyte gal-1-P levels in galactosaemic patients on diet, could suggest that gal-1-P is interfering with galactosylation *in vivo*.

One final question which is essential in considering the pathogenesis of galactosaemia is that of when the damage to the organs occurs. It has been demonstrated that the key enzymes of galactose metabolism are present in the fetus as early as 10 weeks' gestation, often in activities higher than those found postnatally (Holton 1995b). One can postulate, therefore, that these enzymes are essential to normal development and that GALT deficiency may be harmful *in utero*.

Extremely high levels of gal-1-P have been found in galactosaemic fetal blood at 20 weeks' gestation, and of galactose, gal-1-P and galactitol in liver at the same gestational age (Ng et al 1977; Allen et al 1980). Evidence for fetal metabolite accumulation in GALT deficiency may be shown, also, by increased galactitol concentrations in amniotic fluid, which has been observed as early as 12 weeks (JT Allen, personal communication). It is well established that the accumulation of galactitol in amniotic fluid is unaffected by maternal restriction of galactose during the pregnancy (Jakobs et al 1988) and, likewise, several groups have shown that cord-blood gal-1-P concentrations remain high in spite of this treatment (Holton 1995b). Unfortunately, maternal galactose restriction during pregnancy also appears ineffective in improving long-term prognosis in galactosaemic children (Waggoner et al 1990).

The only real evidence that damage may have its origins *in utero* is the finding of a cataract in a galactosaemic child at birth (Donnell et al 1969) and in a 20-week-old affected fetus (Vannas et al 1975). Histological studies of liver cirrhosis in patients dying of galactosaemia have concluded that the pathology has originated *in utero*. In addition, all the available evidence points to the conclusion that ovarian damage also occurs *in utero* (Gibson 1995).

Only two possible new therapeutic approaches have emerged as the result of the considerable volume of studies directed towards understanding the pathogenesis of galactosaemia. First, the administration of uridine to patients increases the levels of sugar nucleotides in the red cells, including UDPGal, and it is hypothesized that this could correct problems due to reduced galactosylation (Ng et al 1989). Unfortunately, trials of uridine use have produced no convincing evidence of therapeutic value. Second, animal studies have indicated that the balance of tissue polyols could be restored by the use of aldose reductase inhibitors to block production of galactitol from galactose (Berry 1995). This approach has not been used in clinical trials and, in fact, it is not clear that there is any problem due to polyol levels when a galactose-restricted diet is used. In the absence of new therapies emerging from pathogenetic studies, attempts to enhance GALT activities may prove of value (Segal 1995).

REFERENCES

Allen JT, Gillett M, Holton JB, King GS, Pettit BR (1980) Evidence of galactosaemia *in utero*. *Lancet* **2**: 603.

Anon (1984) Clouds over galactosaemia [Editorial]. *Lancet* **2**: 1379–1380.

Berry GT (1995) The role of polyols in the pathophysiology of galactosemia. *Eur J Pediatr* **154** (Supplement 2): 40–44.

Berry GT, Palmieri MJ, Heales S, Leonard JV, Segal S (1992) Red blood cell uridine sugar nucleotide levels in patients with classical galactosemia and other disorders. *Metabolism* **41**: 783–787.

Berry GT, Nissim I, Lin Z, Mazur AT, Gibson JB, Segal S (1995) Evidence for endogenous synthesis of galactose in normal man and patients with galactosemia. *Lancet* **346**: 1073–1074.

Bonham JR, Allen JT, Chapman C (1994) Increased urinary transferrin excretion in galactosaemia: a possible indicator of control. In: *Abstracts of the 32nd Annual Symposium of the Society for the Study of Inborn Errors of Metabolism*. Sheffield, UK: SSIEM, P136.

Dobbie JA, Holton JB, Clamp JR (1990) Defective galactosylation of proteins in cultured skin fibroblasts from galactosaemic patients. *Ann Clin Biochem* **27**: 274–275.

Donnell GN, Koch R, Bergren WR (1969) Observations on results of management of galactosemic patients. In Hsia DY-Y, ed. *Galactosemia*. Springfield, IL: CC Thomas 247–268.

Garrod AE (1908) Inborn errors of metabolism: Croonian Lectures. *Lancet* **2**: 1–7, 73–79, 142–148, 214–220.

Gibson JB (1995) Gonadal function in galactosemics and galactose-intoxicated animals. *Eur J Pediatr* **154** (Supplement 2): 14–20.

Gitzelmann R (1995) Galactose-1-phosphate in the pathophysiology of galactosemia. *Eur J Pediatr* **154** (Supplement 2): 45–49.

Haberland C, Peron M, Brunngraber EG (1971) The neuropathology of galactosemia: a histopathological and biochemical study. *J Neuropathol Exp Neurol* **30**: 431–437.

Holton JB (1995a) Long term results of treatment of some inherited metabolic diseases. *Paediatr Rev Commun* **8**: 139–157.

Holton JB (1995b) Effects of galactosaemia *in utero*. *Eur J Pediatr* 154 (Supplement 2): 77–81.

Holton JB, de la Cruz F, Levy HL (1993) Galactosemia: the uridine diphosphate galactose deficiency – uridine treatment controversy. *J Pediatr* **123**: 1009–1014.

Jaeken J, Kint J, Spaaken L (1992) Serum lysosomal enzyme abnormalities in galactosaemia. *Lancet* **340**: 1472–1473.

Jakobs C, Kleijer WJ, Bakker HD, van Gennip AH, Przyrembel H, Niermeijer MF (1988) Dietary restriction of maternal lactose intake does not prevent accumulation of galactitol in fetuses affected with galactosaemia. *Prenat Diagn* **8**: 641–645.

Keevill NJ, Holton JB, Allen JT (1993) The investigation of UDPGlucose and UDPGalactose concentrations in red blood cells in patients with classical galactosemia. *Clin Chim Acta* **221**: 135–142.

Keevill NJ, Holton JB, Allen JT (1994) UDP-Glucose and UDP-Galactose concentrations in cultured skin fibroblasts of patients with classical galactosaemia. *J Inher Metab Dis* **17**: 23–26.

Kirkman HN (1995) Measurement of uridine diphosphate glucose and uridine diphosphate galactose — an appraisal. *Eur J Pediatr* **154** (Supplement 2): 72–74.

Mason HH, Turner ME (1935) Chronic galactosemia. *Am J Dis Child* **50**: 359–374.

Ng WG, Donnell GN, Bergren WR, Alfi O, Golbus MS (1977) Prenatal diagnosis of galactosaemia. *Clin Chim Acta* **74**: 227–235.

Ng WG, Xu YK, Kaufman FR, Donnell GN (1989) Deficit of uridine diphosphate galactose in galactosaemia. *J Inher Metab Dis* **12**: 257–266.

Ornstein KS, McGuire EJ, Berry GT, Roth S, Segal S (1992) Abnormal galactosylation of complex carbohydrates in cultured fibrobasts in patients with galactose-1-phosphate uridyltransferase deficiency. *Pediatr Res* **31**: 508–511.

Roth S, McGuire EJ, Roseman S (1971) Evidence for cell surface glycosyltransferases. *J Cell Biol* **51**: 536–547.

Segal S (1995) Galactosaemia unsolved. *Eur J Pediatr* **154** (Supplement 2): 97–102.
Segal S, Berry GT (1995) Disorders of galactose metabolism. In Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*, vol. 1, 7th edn. New York: McGraw-Hill, 967–1000.
Vannas A, Hogan MJ, Golbus MS, Wood I (1975) Lens changes in a galactosemic fetus. *Am J Ophthalmol* **80**: 726–733.
Waggoner DD, Buist NRM (1993) Long-term complications in treated galactosaemia: 175 US cases. *Int Pediatr* **8**: 97–100.
Waggoner DD, Buist NRM, Donnell GN (1990) Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inher Metab Dis* **13**: 802–818.
Welch RJ, Milligan DWA (1987) Cerebral oedema and galactosaemia. *Paediatrics* **80**: 587–589.
Xu YK, Kaufman FR, Donnell GN, Giudici T, Alfi O, Ng WG (1995) HPLC analysis of uridine diphosphate sugars: decreased concentrations of uridine diphosphate galactose in erythrocytes and cultured skin fibroblasts from classical galactosemia patients. *Clin Chim Acta* **240**: 21–33.

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