

Nitisinone in the Treatment of Hereditary Tyrosinaemia Type 1

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Abstract

Hereditary tyrosinaemia type 1 (HT-1) is a rare genetic disease caused by mutations in the gene for the enzyme fumarylacetoacetase. It usually presents with liver failure but can be manifest as chronic liver disease. Rarely, it may present with nonhepatic manifestations such as renal dysfunction, porphyria-like illness or cardiomyopathy. There is a high lifetime risk of developing hepatocellular carcinoma (HCC). Prior to the development of liver transplantation, most patients died in childhood.

The clinical manifestations stem from the cytotoxicity of tyrosine metabolites accumulating proximal to the metabolic defect. Nitisinone acts on tyrosine metabolism upstream of the defect to prevent the production of these metabolites. Nitisinone is used in combination with a tyrosine- and phenylalanine-restricted diet.

Nitisinone has transformed the natural history of tyrosinaemia. Liver failure is controlled in 90% of patients, those with chronic liver disease improve and nonhepatic manifestations are abolished. Nitisinone is well tolerated and has few adverse effects other than a predictable rise in plasma tyrosine levels.

Nitisinone provides protection against HCC if it is started in infancy, but if commenced after the age of 2 years, a significant risk of HCC remains. Furthermore, where nitisinone is used pre-emptively, liver disease appears to be prevented, suggesting the importance of neonatal screening for tyrosinaemia where possible. Nitisinone is indicated for all children with HT-1, and liver transplantation is only indicated where nitisinone fails, or where the development of HCC is likely or suspected.

The development of nitisinone has created a new era in the management of hereditary tyrosinaemia type 1 (HT-1). This article outlines the clinical features and pathophysiology of tyrosinaemia and the rationale for, and the results of, nitisinone therapy in this disorder.

1. Hereditary Tyrosinaemia Type 1 (HT-1)

HT-1 is a heterogeneous multisystemic genetic disease caused by deficiency of fumarylacetoacetase (FAH), the terminal enzyme in tyrosine metabolism (figure 1). FAH is highly expressed in hepatocytes

and renal tubular cells. The underlying defect is a mutation in the gene for FAH.^[1] A large number of causative mutations have been identified,^[2] but there is no obvious genotype-phenotype correlation. It has a worldwide distribution and an overall incidence of 1/100 000, except in the Saguenay-Lac St-Jean region of Quebec where, because of a founder effect, 1 in 20 of the population are carriers.^[3]

Liver disease is the dominant clinical manifestation of HT-1. Approximately 75% of individuals present with acute infantile liver failure, with most others presenting with chronic liver disease.^[4] All

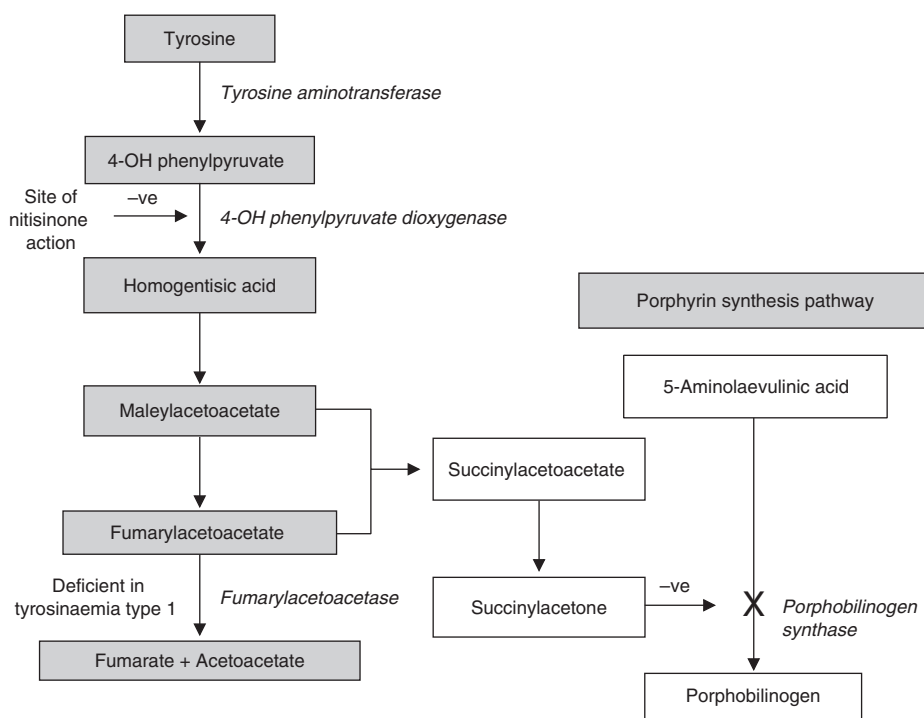


Fig. 1. Tyrosine catabolic pathway. Fumarylacetoacetase is deficient in patients with tyrosinaemia type 1. Toxic metabolic metabolites accumulate proximal to the block and cause local and systemic toxic effects. Nitisinone inhibits 4-OH phenylpyruvate dioxygenase and prevents formation of toxic metabolites distal to this point.

survivors are at an extremely high lifetime risk of developing hepatocellular carcinoma (HCC). Other clinical manifestations of the disease include renal tubular dysfunction, hypophosphataemic rickets, porphyria-like neurological crises, hypoglycaemia as a result of islet cell hyperplasia, and cardiomyopathy.^[3]

Diagnostic findings include mildly elevated plasma bilirubin and hepatic transaminases, raised plasma tyrosine and methionine, grossly raised plasma alpha fetoprotein (AFP) and significant coagulopathy. The diagnosis is usually reached by urine organic acid analysis. The detection of urinary succinylacetone (SA) is pathognomonic. The diagnosis is confirmed by measuring FAH activity in fibroblasts or lymphocytes, or by demonstrating the presence of pathogenic mutations in FAH.^[3]

Prognosis is related to age at presentation and clinical phenotype. First-year survival with dietary treatment is <40% in those presenting clinically in the first 2 months of life, 77% in those presenting

between 2 and 6 months and 96% in those presenting after 6 months of age. Mortality in childhood was usually as a result of liver failure, with HCC becoming an important cause of mortality in the second decade.^[4] The current outcome has been transformed by availability of liver transplantation and the use of nitisinone.^[5]

1.1 Pathogenic Mechanisms

The mechanism of liver and renal disease in humans with HT-1 is unknown, but animal and cell experiments have provided useful insights into this. Organ dysfunction appears to result from the toxicity of metabolites accumulating immediately proximal to the metabolic block in hepatocytes and proximal renal tubular cells. These toxins are fumarylacetoacetate (FAA) and maleylacetoacetate (MAA) and their metabolites SA and succinylacetoacetate (SAA) [figure 1]. FAA and MAA only exist intracellularly, while SA and SAA are released and have a systemic effect.

FAA is known in animal studies to cause apoptosis,^[6,7] to be mutagenic^[8] and pro-oxidant.^[3] Abnormal hepatic gene expression occurs, some of which may be adaptive, and there is a high level of chromosomal breakage and instability.^[9] Some of this is caused by inhibition of DNA-ligase activity and subsequent interference with DNA replication and repair.^[10]

These endogenous toxins are also produced in the renal tubular cell, but in addition, circulating SA is toxic to the renal tubule and inhibits cell growth.^[11] SA also has a systemic effect as a potent inhibitor of porphobilinogen (PBG) synthase, which causes the porphyria-like neurological crises. This effect is manifest by raised urinary 5-aminolevulinic acid (ALA).

There is evidence that more proximal metabolites do not account for the cellular damage in HT-1. Raised levels of tyrosine itself and its more proximal metabolites do not cause any liver disease in humans with tyrosinaemia types 2 and 3 or alkaptonuria.^[3] Mutant mice completely deficient in both FAH and 4-hydroxyphenylpyruvate dioxygenase (HPPD) do not develop liver disease or HCC, suggesting that only metabolites produced distal to HPPD are cytotoxic.^[12,13]

How this translates into differing clinical phenotypes is as yet unclear. There is no genotype-phenotype correlation, and cases of differing phenotypes within the same family are well recognised.^[14,15] Animal models suggest that where apoptosis becomes dominant, liver failure develops, whereas if an adaptive abnormal gene expression results, there is sufficient cellular protection for survival, resulting in a chronic phenotype. This latter short-term survival benefit is at the cost of increased risk from mutagenesis and HCC.^[16] The factors determining which cell fate predominates are not at all clear, but are likely to include individual genetic, antioxidant, inflammatory and biochemical circumstances.^[17]

Another factor contributing to phenotypic variability is the phenomenon of genetic reversion. Here, reversion of any mutant allele to the wild type occurs in one allele.^[18] These reverted cells therefore gain a survival advantage and clonally expand so that the liver becomes a mosaic of FAH-negative and -positive nodules. This results in some (incomplete) phenotypic correction but it does not protect

against the development of HCC.^[18-20] This correction does appear to be functionally important, as the extent appears to be inversely proportional to the severity of clinical disease.^[19]

1.2 Treatment of HT-1

Dietary treatment consists of a normocaloric, phenylalanine- and tyrosine-restricted diet. This results in improved renal function and may have a role in preventing liver dysfunction, but does not reverse established liver failure or prevent the development of HCC.^[3] Children presenting before 6 months of age given dietary treatment had a 1-year survival of only 51%.^[4]

Liver transplantation transformed the management of HT-1, offering a functional metabolic 'cure' with 85% long-term survival. Prior to the introduction of nitisinone, transplantation was the treatment of choice, with the only debate surrounding the timing of surgery.^[5,21]

2. Nitisinone

Nitisinone [2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione] is a triketone initially developed as a herbicide. In early toxicology tests, exposed rats developed corneal lesions. Investigations showed that these were due to an acquired tyrosinaemia resulting from inhibition of HPPD.^[22] Lindstedt et al.^[23] anticipated that inhibiting this enzyme would prevent the production of FAA and MAA in tyrosinaemia type 1. In a truly courageous act of faith, they gave this compound to a severely affected infant. This had a remarkable and dramatic clinical effect. Its effectiveness was shortly confirmed in four further patients and the nitisinone era had arrived. This initial experience led to the establishment of a multicentre study of nitisinone based in Gothenburg, Sweden, which continues to date and has recruited >300 children worldwide.^[24] Since 1996, nitisinone has been marketed by Swedish Orphan AB. It received US FDA approval in January 2002 and formal European Union drug agency approval in February 2005.

2.1 Pharmacokinetics and Metabolic Effects

Nitisinone is efficiently and rapidly absorbed and taken up by liver, kidney and lacrimal glands. Nitisi-

none is hydroxylated in the liver, and excretion occurs in equal amounts in urine and faeces.^[22,25,26] In human volunteers, the serum half-life is >50 hours.^[27]

Nitisinone therapy should be commenced at 1mg/kg/day as a single daily dose. There is a rapid metabolic effect; plasma tyrosine levels rise within hours, urinary SA disappears within days.^[28] Plasma SA is protein bound and hence takes longer to disappear. Erythrocyte PBG synthase is a biomarker for SA activity, which should normalise within 1 month.^[28] AFP is almost invariably grossly raised, and the normal response to nitisinone is a logarithmic decrease. Urinary ALA is usually grossly raised at presentation and falls rapidly with nitisinone treatment, but does not always normalise.^[24]

2.2 Clinical Effects of Nitisinone

2.2.1 Liver Failure

Infants with liver failure are often desperately ill with a severe coagulopathy and ascites. Nitisinone administration usually results in a remarkable visible clinical improvement within days. At least 90% will respond completely.^[5,29-31] Where the coagulation profile improves within 1 week, recovery can be assumed.^[5] If the coagulation profile has not improved within 1 week, the nitisinone dosage should be increased and liver transplantation considered. The majority of infants who present clinically will already have established cirrhosis and treatment converts them into a stable chronic liver disease phenotype.

2.2.2 Chronic Liver Disease

Chronic liver disease is a less common and more heterogeneous presentation. Nitisinone has been associated with consistent improvement in clinical and nutritional status; established evidence of portal hypertension has resolved and established hepatic nodules may disappear.^[3,23,32,33] In the nitisinone era, it is extremely rare to require liver transplantation for complications of progressive liver disease.

2.2.3 Nonhepatic Manifestations of HT-1

Renal tubular function shows a rapid improvement and usually normalises. Tubular function remains normal in those treated prospectively from infancy.^[34] Glomerular function appears to remain

stable on long-term follow up, but there are few prospective data available.^[24]

Nitisinone treatment in the first 3 months of life seems to prevent the development of cardiomyopathy.^[35] Where cardiomyopathy is established, the natural history is one of improvement, irrespective of the mode of treatment.^[35]

Nitisinone affords complete protection against porphyria-like neurological crises, and if present, they respond dramatically to the introduction of nitisinone.^[36]

2.3 Pre-Emptive Treatment

It appears intuitive that nitisinone should ideally be used prior to onset of disease and in particular before the development of cirrhosis. This can only be achieved if there is an efficient neonatal screening programme in combination with selective screening of at-risk siblings. In our unit, we have experience of six infants diagnosed in this manner. Nitisinone was commenced at a mean age of 4 weeks, and all are clinically normal with normal liver function and liver ultrasound appearance after mean follow-up of 3 years. In Quebec, there has been an efficient neonatal screening programme since 1970. Recently, all those detected by neonatal screening have started nitisinone and dietary treatment immediately. The early outcome of this group is extremely encouraging, with normal physical growth and developmental progress and without evidence of liver dysfunction.^[34] This experience clearly mandates the development of neonatal screening programmes. The technique used is likely to differ between regions and countries depending on the timing of screening and the likely mutation profile in the community.

Prenatal diagnosis is possible by chorionic villi sampling, or if the mutation is unknown, by measurement of SA in amniotic fluid.^[3] The availability of rapid postnatal diagnosis in siblings and the excellent outcome of pre-emptive treatment has decreased the need for prenatal diagnosis.

2.4 Monitoring of Treatment

2.4.1 Dietary Control

Nitisinone therapy must be combined with a tyrosine- and phenylalanine-restricted diet. This is a

restrictive diet but should not be viewed as a sentence. With dietetic support, encouragement and imagination, the diet can be made varied, interesting and culturally relevant. The efficacy of this treatment is judged by both growth and amino acid control. Tyrosine levels should be maintained at $<500 \mu\text{mol/L}$. This level has been taken because there are some concerns that higher levels may be toxic to the brain, as in tyrosinaemia type 3, or to the eye and skin, as in tyrosinaemia type 2.^[3] Higher tyrosine intake will also increase the flux through the catabolic pathway, which, at least in the mouse model, is associated with an increased risk of liver disease.^[13] In our practice, all parents are trained in home capillary blood sampling; the samples are then posted to the laboratory for regular amino acid monitoring.

2.4.2 Metabolic Control

In our clinic, we check plasma and urinary SA and erythrocyte PBG synthase monthly for the first 3 months and subsequently every third month. PBG synthase should be maintained at the best 'normal' level achieved for each patient and SA should be undetectable. Initial dose adjustment of nitisinone should be made monthly on a per-kilogram basis during rapid growth in infancy. Subsequent adjustments are needed infrequently and should be made to maintain metabolic control as above. At present, there are insufficient data to recommend a target nitisinone level, but with a dose of 1mg/kg/day , most patients achieve a plasma level of $>30 \mu\text{mol/L}$.^[28]

2.4.3 Detection of Hepatocellular Carcinoma

Detection of HCC relies on combined radiological and biochemical monitoring. We recommend carrying out an abdominal ultrasound every 6 months and hepatic magnetic resonance imaging (MRI) annually. If a suspicious nodule develops, this will usually be an indication for transplantation, but other radiological modalities such as contrast ultrasound or MRI may be useful to help clarify the pathology.

AFP should be checked every third month. Any rise in AFP, or a failure to fall, is a cause for concern. Total AFP is sensitive but not specific for HCC.^[24] There has been limited but encouraging experience with the use of the lectin-reactive AFP

fraction, which appears to be more specific for HCC.^[33,37]

2.4.4 Developmental Assessment

Developmental delay is not a recognised consequence of HT-1.^[3,17] However, treatment with nitisinone causes a tyrosinaemia type 3 phenotype to develop. The natural form of this disorder, genetic HPPD deficiency, is rare and poorly described, but developmental delay is a well recognised component.^[38,39] In view of this, it appears prudent to recommend regular developmental follow-up in children with HT-1 being treated with nitisinone.

2.5 Adverse Effects

Nitisinone appears to be extremely well tolerated. There is a predictable increase in tyrosine levels to $1300\text{--}1500 \mu\text{mol/L}$ unless it is combined with a phenylalanine- and tyrosine-restricted diet. Ocular toxicity due to deposition of tyrosine crystals is a theoretical possibility, but in practice is rare. There is no need to screen for ocular signs as they will be symptomatic if present^[40] and will respond to dietary restriction. There have been occasional reports of transient neutropenia or thrombocytopenia,^[24] which is more likely to be related to underlying liver disease than to the drug.

However, the total experience with nitisinone remains small in absolute terms. It is important that all centres collaborate to maintain a database of treated patients and that all are treated as part of a structured protocol that incorporates developmental monitoring. This is particularly important given the known neurological involvement found in tyrosinaemia type 3, which is the result of a naturally occurring deficiency of HPPD.^[3]

2.6 Does Nitisinone Prevent the Development of Hepatocellular Carcinoma?

This is the most important and complex question surrounding the long-term use of nitisinone. We do not completely understand the mechanistic sequence of carcinogenesis in HT-1, but we should distinguish between those at risk from HCC and those where HCC has already developed. Historical data suggest that HCC rarely develops before 2 years of age^[4,41] and only exceptionally before 1 year.^[24,42,43]

It seems logical that early treatment, with its profound effect on metabolic control and toxin suppression, should reduce the risk of subsequently developing HCC. The evidence from the Gothenburg study strongly supports this.^[24] Discounting one child who had already developed HCC at the start of treatment,^[42] only one child treated before the age of 1 year has been reported to have developed HCC.^[43] Moreover, the biological behaviour of this tumour was very atypical for HCC; it presented very early with metastases, the primary tumour shrank and pulmonary metastases resolved with chemotherapy alone. This behaviour is much more typical of hepatoblastoma than HCC, and a question must remain about the true diagnosis in this case. Of children treated between 1 and 2 years of age in the Gothenburg study, two had HCC at presentation and only one has developed late HCC. This latter child developed HCC after 11 years of nitisinone treatment (personal observation). This is much less common than the 18–37% incidence reported in the pre-nitisinone era.^[41,44]

However, there is no room for complacency in this group. Nitisinone does not correct the abnormal hepatic gene expression,^[9] fibrogenic markers^[45,46] or hepatic dysplasia^[5] seen in humans with HT-1. In the mouse model, HCC develops despite lifelong treatment,^[47] even when high doses are used in combination with a strict tyrosine-restricted diet.^[13] Interestingly, mutant mice completely deficient in both FAH and HPPD do not develop liver disease or HCC, suggesting that, at least in the currently used dosages, nitisinone does not completely inhibit HPPD.^[12,13]

The situation is not so optimistic where nitisinone treatment starts after the age of 2 years. In the long-term, the incidence of HCC (or where HCC is suspected strongly enough to indicate liver transplantation) in this group is approximately 25%. In these patients, HCC has developed as late as 6 years after the introduction of treatment.^[24,48] On a positive note, all reported cases of HCC developing during nitisinone treatment have developed in hepatic nodules and have been associated with a high AFP level.

2.7 Who Should Have a Liver Transplant for HT-1 in the Nitisinone Era?

In acute liver failure, transplantation should be considered if the coagulation profile has not improved after 1 week of treatment.

In chronic liver disease, indications for transplant relate to suspected HCC rather than progressive liver disease. This may be indicated by the development of a new hepatic nodule or a rise in AFP levels. The situation is less clear where the liver is already nodular before nitisinone treatment has started. Here, it may be worth a trial of nitisinone, but where nodules persist it will be impossible to completely exclude the development of HCC and transplantation should be considered.

Those aged >2 years when nitisinone is commenced are at higher risk from HCC. Whether to offer elective transplantation to this group or to delay listing until there is evidence of HCC is moot. This recommendation will have to be individualised; incorporating family preference, local liver transplant results, waiting list dynamics and whether or not living-related transplantation is an option.

3. Conclusion

The introduction of nitisinone has brought a new era in the management of HT-1. Nitisinone is indicated for all patients with HT-1 and transplantation is only indicated where nitisinone has failed or been started too late. This development mandates neonatal screening for HT-1 where feasible.

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