REVIEW

Clinical utility of nitisinone for the treatment of hereditary tyrosinemia type-1 (HT-1)

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Abstract: Medical therapy for hereditary hepatorenal tyrosinemia (hereditary tyrosinemia type 1, HT-1) with nitisinone was discovered incidentally, and is a by-product of agrochemistry. It blocks the catabolic pathway of tyrosine, thereby leading to a reduction in the accumulation of toxic metabolites in HT-1. It has to be combined with a low-protein diet supplemented with amino acid mixtures devoid of tyrosine and phenylalanine. This treatment option has completely changed the clinical course of patients suffering from HT-1 who used to die in the first few months to years of life from liver failure, renal dysfunction, and/or hepatocellular carcinoma (HCC). It is essential to start nitisinone therapy early in life to avoid sequelae; beginning treatment in the newborn period is ideal. As initial clinical symptoms of HT-1 are often atypical and because there is a clinically latent phase during the first few months of life in many patients, newborn screening is required to secure early diagnosis. Succinylacetone in blood is a reliable screening parameter whereas tyrosine is neither specific nor sensitive. Especially HCC, but also liver and kidney dysfunction, rickets, and neurological crises can be prevented in most patients if nitisinone therapy is started in the newborn period. It is essential to adhere to a low-protein diet to avoid tyrosine toxicity. Reversible eye symptoms may occur as a side-effect of nitisinone, but other side effects are rare. Neurocognitive development is impaired in some patients, and the reason for this is unclear. Metabolic monitoring includes measurement of tyrosine, succinylacetone, and nitisinone concentrations in blood.

Keywords: diet, hepatocellular carcinoma, hepatorenal tyrosinemia, nitisinone, newborn screening, succinylacetone

Introduction to the pathophysiology, clinical symptoms, and treatment of hereditary tyrosinemia type-1

Hereditary tyrosinemia type-1 (hepatorenal tyrosinemia, HT-1) is a rare inborn error of metabolism in the catabolism of the amino acid tyrosine (Figure 1). In Central Europe, the prevalence is 1:125,000, although much higher incidence rates are observed in other regions such as Turkey, Quebec, and India. HT-1 is inherited as an autosomal-recessive trait and is, thus, more common in populations with a high degree of consanguinity.

Biochemically, a deficiency of fumarylacetoacetase leads to the accumulation of not only tyrosine but also different metabolites such as maleylacetoacetate, fumary-lacetoacetate, and succinylacetone (SA) – the latter is commonly used as a surrogate parameter of toxicity in blood and/or urine (Figure 1).

Diagnosis is based on elevated SA levels in blood and/or urine, as tyrosine elevation is an unreliable marker. There are many false-positive and false-negative results when tyrosine is used as the only diagnostic parameter.[1]

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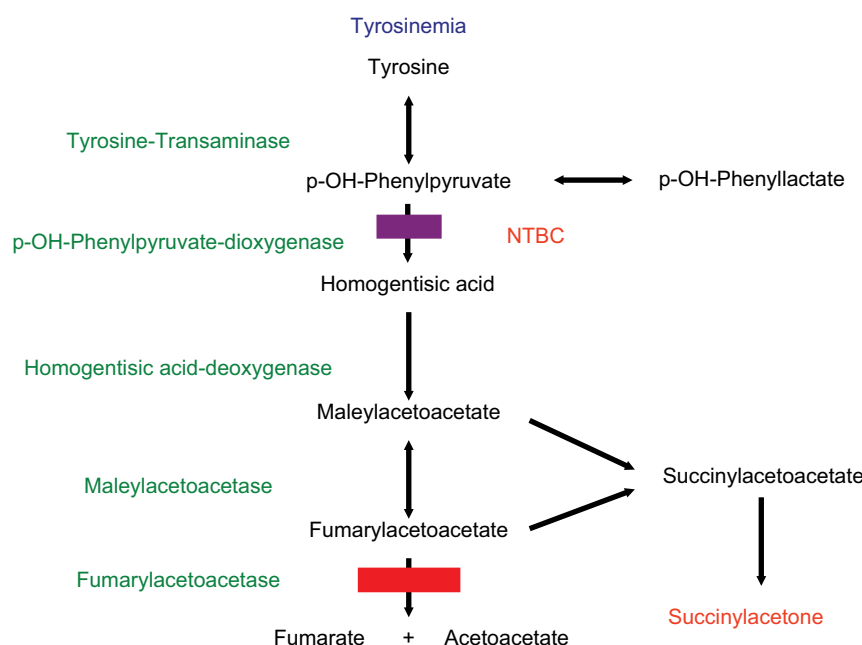
Figure 1 Degradative pathway of tyrosine. HT-1 is due to fumarylacetoacetase deficiency. NTBC (nitisinone) leads to proximal inhibition in the tyrosine pathway with reduction of toxic metabolites (surrogate parameter succinylacetone).

Clinical symptoms comprise liver dysfunction (sometimes culminating in liver failure), tubular dysfunction, rickets, and, sometimes, neurological symptoms. A long-term complication is hepatocellular carcinoma (HCC); neurocognitive deficits can be observed in some patients.[2–5]

Therapy consists of nitisinone (Orfadin®, 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane 1-,3-dione, NTBC) in order to decrease levels of toxic compounds (Figure 1). Nitisinone blocks the degradation of tyrosine. A low-protein diet supplemented with special amino acid mixtures devoid of tyrosine and its precursor phenylalanine is required to prevent excessive tyrosine levels. In patients in whom nitisinone therapy fails to prevent acute liver failure or in those who develop HCC, liver transplantation (LTx) is the therapy of choice.[4,5]

Nitisinone was approved by the European Medicine Agency (EMA) under exceptional circumstances in 2005. The first clinical use of nitisinone in HT-1 dates back to 1991. Originally, nitisinone was developed as a weed-killer by Zeneca Agrochemicals.[6] It was epidemiologically observed that the growth of plants and weeds was inhibited under the bottlebrush plant (*Callistemon citrinus*).[7] It became clear that neither the shade nor the litterfall of these plants were responsible for suppression of plant and weed growth. Rather, a substance – which was identified as leptospermone – in the soil under the bottlebrush plant was shown to have bleaching activity on the emerging plants.[8] The allelochemical leptospermone was extracted from the bottlebrush plant and

chemically characterized. Leptospermone belongs to the triketone family and inhibits chloroplast development due to a lack of plastoquinone secondary to hepatic 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition; thus, it served as a blueprint for the synthesis of nitisinone.[9]

Toxicology testing of nitisinone revealed that it was not acutely toxic, but eye lesions (keratopathy) could be observed in animals after prolonged treatment. Lesions were reversible upon cessation of exposure to the compound. Elevated tyrosine levels were found in the blood and urine after nitisinone exposure. Nitisinone was established as a potent inhibitor of rat hepatic HPPD at the Zeneca Central Toxicity Laboratories (Figure 1). Inhibition of human HPPD was demonstrated in human liver by Sven Lindstedt and his group at Gothenburg University (Sweden). The research was already ongoing for an inhibitor of HPPD to treat patients with the lethal disease HT-1. Zeneca Pharmaceuticals acquired the compound as a potential drug candidate, although with some reluctance, as only a limited number of patients with this rare disease would possibly benefit from this drug. The Swedish Medical Agency approved a clinical trial with nitisinone in HT-1 patients and, in February 1991, a critically ill 2-month-old baby became the first HT-1 patient to be treated with nitisinone in Gothenburg. SA quickly disappeared from the urine and the clinical state gradually improved. Subsequently, several HT-1 patients were successfully treated with nitisinone on a compassionate-use basis. In the following years, many patients were successfully

treated with nitisinone, culminating in the approval of nitisinone by the US Food and Drug Administration in 2002 and the European Medicines Agency (EMA) in 2005 under "exceptional circumstances" as large-scale field trials and real-world data in HT-1 patients were unavailable. The first sublicense-holder Swedish Orphan International was obliged to conduct postmarketing studies, which are now carried out by the present sublicense-holder Swedish Orphan Biovitrum (SoBi) in the framework of the "OPAL" study.

Review of pharmacology, mode of action, and pharmacokinetics of nitisinone

In HT-1, organ damage and dysfunction is mediated by toxic compounds like maleylacetoacetate, fumarylacetoacetate, and SA (Figure 1). The main organs affected are the liver and kidneys; rickets and neurological crises may occur; and HCC may be a long-term complication.[3] Nitisinone blocks the catabolic pathway of tyrosine proximal to the deficient enzyme fumarylacetoacetase at the level of 4-OH-phenylpyruvate-dioxygenase (HPPD), which catabolizes p-OH phenylpyruvate to homogentisic acid, thereby reducing the levels of toxic compounds (Figure 1). SA is used as a surrogate parameter for toxic compounds. As the block in tyrosine catabolism results in tyrosine accumulation, a protein-reduced diet supplemented with a special amino acid mixture devoid in phenylalanine (precursor of tyrosine) and tyrosine is required to prevent tyrosine toxicity. Calculation of tyrosine and phenylalanine intake is not required in most patients with HT-1.

In rat liver, enzyme kinetic studies revealed inhibition of HPPD by nitisinone in a dose- and time-dependent manner with a rate constant of $9.9 \times 10^{-5}\,\mathrm{s}^{-1}\,(\mathrm{nmol}\,\mathrm{L}^{-1})^{-1}$.[10] Nitisinone does not bind irreversibly to HPPD; the enzyme–inhibitor complex dissociates with a half-life of 63 hours in rats at 25°C. Tests in human adult volunteers revealed nitisinone had a half-life of 54 hours.[11] It is recommended that nitisinone be administered in a twice-daily dosage. However, based on the long half-life of nitisinone, once-daily dosing was advocated,[12] which seems to be adequate to maintain metabolic control. Moreover, once-daily dosing may improve adherence to pharmacological therapy.

The recommended dose of nitisinone is 1–2 mg/kg per day, in two divided doses. In a recent survey,[13] we found several HT-1 patients in whom nitisinone was titrated down without hampering metabolic control, as judged by the absence of SA, with doses as low as 0.3 mg/kg per day shown to be sufficient.
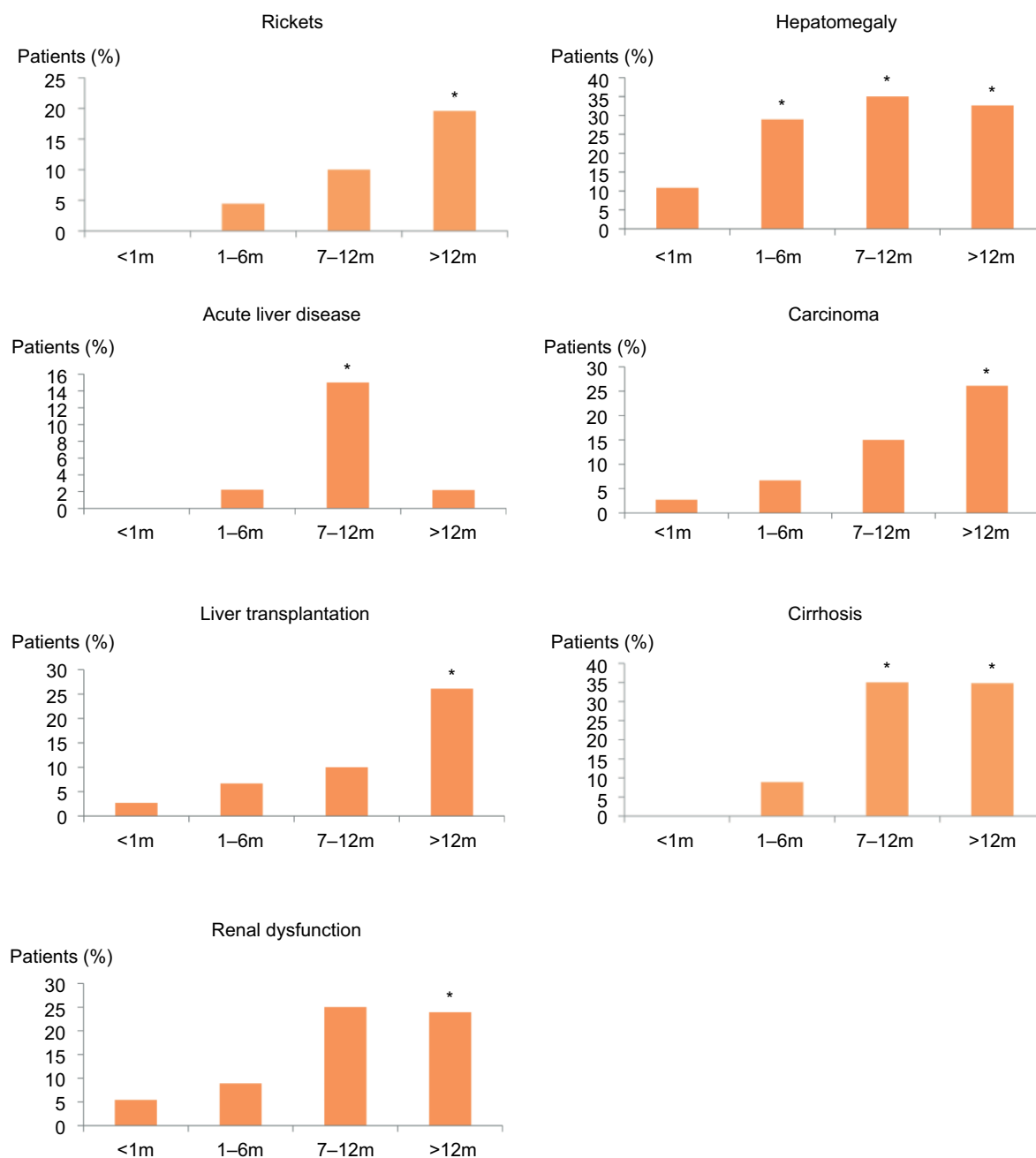
Efficacy studies

Although it is clear that nitisinone can reduce the levels of toxic compounds, as judged by the surrogate parameter SA, and secure survival, the impact on long-term clinical outcome is less clear. This is mainly due to the rarity of the disease. In the literature, a few regional studies can be found,[14–20] recommendations by a group of experienced clinicians were published,[21] and there is only one multinational cross-sectional study that included 168 patients from 21 centers.[13] This study showed a clear benefit of nitisinone treatment in combination with a low-protein diet supplemented by amino acid mixtures devoid of tyrosine and phenylalanine. If present, liver dysfunction/failure, renal dysfunction, tubulopathy, and rickets could be reversed by nitisinone treatment. Furthermore, HCC prevalence could be reduced by nitisinone treatment. Long-term complications, especially development of HCC, critically depend on early initiation of treatment, and, therefore, on early diagnosis. If treatment is started beyond the first year of life, the risk of HCC is 13 times higher as compared to patients for whom treatment is initiated in the neonatal period; furthermore, risk of liver cirrhosis, rickets, and tubular dysfunction is increased 40-, 19-, 4.3-fold, respectively (Figure 2).[13] Thus, early diagnosis in the newborn period is essential to secure a good long-term outcome.[15,18,22] Initial clinical symptoms (hepatomegaly, renal tubular dysfunction, rickets, etc.) are atypical, and often occur after a clinically latent phase. Therefore, it is difficult to make an early diagnosis based on clinical symptoms alone.[13] Neonatal mass screening is crucial to secure early diagnosis (and treatment) in the newborn period. Tyrosine as a screening parameter is not appropriate due to the high rate of false positives and false negatives; SA is the screening parameter of choice.[1,13,23,24]

It recently became evident that many patients with HT-1 suffer from neurocognitive deficits.[25–28] The etiology of these sequelae is unclear, although it may be attributed to tyrosine toxicity, phenylalanine deficiency, drug toxicity, or natural disease progression in long-term survivors with HT-1. Prospective clinical studies are necessary to solve this issue.

Nitisinone leads to the accumulation of tyrosine as it blocks tyrosine catabolism. Protein restriction is necessary to avoid tyrosine toxicity, and target values for plasma tyrosine levels have been tentatively set by consensus to below 400 µM.[13]

The incidence of acute or chronic liver failure and HCC has dropped enormously since nitisinone was approved for clinical use. However, a few patients may develop

Rickets

Hepatomegaly

Acute liver disease

Carcinoma

Liver transplantation

Cirrhosis

Renal dysfunction

Figure 2 Effect of age at treatment initiation with nitisinone and low-protein diet on development of clinical symptoms.
Note: *P<0.05 vs <1 month. Reproduced from Mayorandan S, Meyer U, Gokcay G, et al. Cross sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. *Orphanet J Rare Dis.* 2014;9:107.[13]

therapy-refractory liver failure. HCC is mostly due to late initiation of treatment in the absence of neonatal screening.

No alternative treatments for HT-1 are currently known. After the marketing exclusivity for Orfadin® expired, alternative nitisinone-containing medication was produced in Turkey and the United Kingdom at lower prices.

Safety and tolerability

Side effects in nitisinone-treated patients are rare and mostly reversible. Eye pain, eye itching/conjunctivitis, corneal crystals, and thrombocytopenia are the most common side effects reported;[13] these are mostly related to high tyrosine levels and are reversible when tyrosine levels are lowered by strict dietary compliance.[30]

Neurocognitive deficits are a problem in long-term management[25–29]; it is not clear whether these are direct side effects of nitisinone treatment, a result of high tyrosine or low phenylalanine levels, or part of natural disease progression. It seems reasonable to reduce nitisinone levels without compromising metabolic control (based on SA levels in urine

and/or blood). Therefore, nitisinone levels should be regularly determined in dried blood spots, together with SA.[31] In most cases, it is possible to titrate down the daily dose of nitisinone initially from 1 mg/kg per day without hampering metabolic control. The tentative therapeutic range of nitisinone concentration in dried blood is 20–40 μM.[13]

Patient-focused perspectives

Adherence to drug therapy with nitisinone is obviously not easy but, based on nitisinone levels in dried blood, most patients manage to take nitisinone regularly. Once-daily dosing may enhance adherence. Life-long adherence to a low-protein diet supplemented with an amino acid mixture is a challenge. Calculation of protein, phenylalanine, or tyrosine intake is not required in most cases and this facilitates adherence. Dietary noncompliance often leads to eye problems, which are reversible with reduced protein intake. Moreover, adherence to therapy depends on financial reimbursement at least in some patients. In most centers participating in our cross-sectional survey, costs for nitisinone treatment and special low-tyrosine amino acid mixtures are covered by health insurance or the government whereas patients were reimbursed for protein-restricted diet in only half of the centers.[13]

Regular outpatient visits are required for monitoring of diet and disease and are time-consuming as well as a financial burden. Sending dried blood for monitoring of SA, nitisinone, and tyrosine from home facilitates control of therapy.[31]

As female patients with HT-1 reach adulthood, "maternal" HT-1 becomes an issue. A few successful pregnancies of mothers with HT-1 have been reported where the use of nitisinone was not interrupted during pregnancy and lactation.[32,33]

After liver transplantation, SA is still excreted in urine by many patients as the enzyme defect is also expressed outside the liver. It is not clear whether nitisinone should be continued in these cases to avoid toxicity. The use of nitisinone implies introducing a low-protein diet supplemented with an amino acid mixture.

Conclusion

Nitisinone as a therapeutic option in HT-1 was discovered by chance and is a by-product of agrochemistry. Its use has substantially changed the outcome in HT-1 patients. Nitisinone treatment has to be combined with a low-protein diet supplemented with an amino acid mixture devoid of tyrosine and phenylalanine to avoid tyrosine toxicity. Based on the long half-life of nitisinone, once-daily dosing should be possible.

There are a few side effects, mostly related to the eye, which are reversible upon stricter protein restriction. In most HT-1 patients, there is an initial latent phase; early diagnosis based on clinical symptoms is not possible in most HT-1 patients, and newborn screening with SA as the screening parameter is essential. Early diagnosis and treatment leads to a much better outcome; especially, HCC incidence is low when treatment is started in the newborn period. In some patients, neurocognitive deficits occur.

Treatment monitoring includes tyrosine levels (therapeutic target, <400 μM), SA- (therapeutic target, negative), and nitisinone concentration.

Disclosure

The author reports no conflict of interest in this work.

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