Pregnancy During Nitisinone Treatment for Tyrosinaemia Type I: First Human Experience

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Abstract A 19 year old woman with tyrosinaemia type 1 gave birth to a healthy girl after 41 weeks of gestation. Nitisinone was continued throughout the pregnancy (maternal levels 68–96 $\mu\text{mol/l}$, target level 30–60 $\mu\text{mol/l}$).

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Tyrosine levels during pregnancy were between 500 and 693 $\mu\text{mol/l}$ (normal values 20–120 $\mu\text{mol/l}$) and phenylalanine levels between 8 and 39 $\mu\text{mol/l}$ (normal values 30–100 $\mu\text{mol/l}$). Nitisinone was measurable in neonatal blood immediately after birth, at a level comparable to the simultaneous level in the mother. Nitisinone half-life in the neonate was estimated to be 90 h. Tyrosine levels in the neonate decreased from 1,157 $\mu\text{mol/l}$ at birth (cord blood) to normal levels within 4 weeks. Phenylalanine levels in the neonate were normal from birth on. The child had a normal psychomotor development as assessed throughout the first year of life.

This is the first report worldwide of a pregnancy during treatment with nitisinone.

In this case, no adverse effects of nitisinone, maternal high tyrosine or low phenylalanine were detected in the child, so far. Long-term results in a larger cohort of pregnancies and births are needed to determine whether nitisinone can be administered safely during pregnancy.

Abbreviations

HT-1 Hepatorenal tyrosinaemia
Phe Phenylalanine
Tyr Tyrosine

Introduction

Tyrosinaemia type 1 (hepatorenal tyrosinaemia, HT-1, OMIM: 276700) is a rare, metabolic disorder affecting about one child in 100,000. It is a hereditary autosomal recessive disease caused by a deficiency in the enzyme fumarylacetoacetase (FAH). Patients often present with liver failure and gastrointestinal bleeding (Scott 2006).

Renal signs of HT-1 in infants include tubulopathy and Fanconi syndrome. Due to renal phosphate wasting, rickets can develop (Scott 2006; Kvittingen and Holme 2000).

Nitisinone (NTBC; 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione) reverses the lethal nature of the disease by preventing the accumulation of the toxic metabolites, maleylacetoacetate, and fumarylacetoacetate, hence allowing HT-1 patients to lead a normal life. Nitisinone prevents the accumulation of fumarylacetoacetate and, therefore, its conversion to succinylacetone (Grompe 2001).

Animal toxicity studies with nitisinone have shown teratogenicity. A dose 2.5-fold higher than the maximum recommended human dose (2 mg/kg/day) caused abdominal wall defects (omphalocele and gastroschisis) in rabbits. A second study, in mice, showed a statistically significant reduced survival and growth of the pups during the weaning period at dose levels of 125- and 25-fold, respectively, the maximum recommended human dose. Rats showed a reduced mean pup weight and corneal lesions due to exposure to nitisinone through breast milk (Summary of product characteristics Orfadin; <http://www.ema.europa.eu>).

Three pregnancies in two mothers with tyrosinaemia type 2 have been reported so far. Tyrosinaemia type II patients show high tyrosinaemia, as well, but do not show the succinylacetone production of HT-1 patients. The first patient presented at 34 weeks of gestation with a plasma tyrosine (Tyr) level of 1,302 $\mu\text{mol/l}$ and a phenylalanine (Phe) level of 37 $\mu\text{mol/l}$. The intake of protein ranged from 60 to 90 g per day. The first child was born at term and had a birth weight of 1.9 kg. He was evaluated at 1 year and 4 months and showed microcephaly and maxillary hypoplasia. Development testing showed a developmental quotient of 72 (Brunet–Lezine test). Her second child was evaluated at 12 months and also had microcephaly, was unable to walk, and had delayed speech development. Both children had normal Tyr levels at the time of evaluation (Cerone et al. 2002).

In the second report, pregnancy in a 25 year old woman with tyrosinaemia type II is described. A protein restricted diet supplemented with a Tyr/Phe free amino acid mixture was followed from week 5 of pregnancy. A restriction of natural protein intake to 0.16 g/kg/day in early pregnancy and 0.38 g/kg/day in the last trimester reduced Tyr levels to between 100 and 200 $\mu\text{mol/l}$. Plasma Phe levels stayed in the range of 20–40 $\mu\text{mol/l}$. There was a normal weight gain and fetal growth and the mother remained asymptomatic throughout pregnancy. The neonate was born at term with length, weight, and head circumference between the twenty-fifth and fiftieth percentiles (Francis et al. 1992).

Materials and Methods

Amino acids and succinylacetone on blood spots of the newborn were determined by tandem-MS. Amino acids in the cord blood of the newborn and in the mother were determined by ion-exchange chromatography.

Nitisinone levels in plasma were measured with an UPLC-MSMS-based technique. A nitisinone concentration between 30 and 60 $\mu\text{mol/l}$ is considered to be therapeutic and minimizes the production of succinylacetone in the urine (Davitt-Spraul et al. 2011).

The blood samples of the newborn taken on day 0 were from the umbilical cord. All other blood samples are capillary (blood spot).

Case Report

Personal and Family History

A girl born in 1990 from non-consanguineous parents was diagnosed with HT-1 at the age of 3 years when she presented with rickets, hepatosplenomegaly, phosphaturia, and tubulopathy (hyperaminoaciduria, hypercalciuria, and glucosuria). Liver biopsy showed micro-macronodular cirrhosis and kidney biopsy revealed tubular damage. An ultrasound showed enlarged kidneys. She was started on nitisinone in January 1994 and in February 1995 blood α -fetoprotein and succinylacetone in urine and blood had normalized.

Over the years, all biochemical parameters remained stable, there was no proteinuria, no urinary succinylacetone was detected since the start of nitisinone. Apart from her intake of nitisinone which was very accurate, the protein restricted diet, supplemented with Phe/Tyr free amino acid mixture, was followed correctly. On abdominal MRI, the liver margins were irregular, but there were no indications of cirrhosis or portal hypertension, there were no focal lesions. The kidneys show small cysts.

In October 2009, at age 19, the patient was transferred to the adult metabolic clinic. She had no focal liver lesions, no cognitive dysfunction and a normal bone densitometry. Dietary adherence was very good (nitisinone 0.5 mg/kg/day). She was taking an oral contraceptive (Mercilon®, Organon Belgium NV; Belgium) since March 2009.

Levels of Tyr and Phe during the 4 years before pregnancy are shown in Fig. 1. As seen here, her Phe levels remained stable and within normal range (30–100 $\mu\text{mol/l}$). Tyr levels were elevated due to the underlying disorder and nitisinone intake; normal range: 20–120 $\mu\text{mol/l}$.

Fig. 1 Tyrosine and phenylalanine levels in the mother prior to detection of pregnancy; last value = at time of detection of pregnancy, at 6 months of pregnancy; date of delivery = time 0. Normal values are depicted as the gray shaded area

Pregnancy Course

Pregnancy was detected in January 2010, at 27 weeks of amenorrhea in an otherwise healthy HT-1 patient. The oral contraceptive pill was discontinued and detailed fetal imaging studies were performed. Ultrasound and MRI of the fetus were reassuring. Growth was within normal range for gestational age (Fig. 2). Discontinuation of nitisinone treatment was discussed with all involved parties. Nitisinone treatment 0.5 mg/kg/day (3×10 mg per day) was ultimately continued because pregnancy had developed without apparent problems so far and, therefore, the risk for hepatic or renal failure due to discontinuation of therapy was estimated to be more important than the risks related to nitisinone exposure to the fetus during the remaining of pregnancy. Figure 3 shows nitisinone levels during pregnancy. No values immediately prior to pregnancy are available.

Tyr and Phe levels were monitored regularly from the moment the pregnancy was detected. Tyr and Phe levels from 3 months prior to delivery until 9 months after delivery are presented in Fig. 3. At 33 weeks of pregnancy, there was a decrease of Phe (and Tyr) in plasma, with Phe decreasing below normal values. Dietary changes were considered necessary. The dose of amino acid mixture was elevated to 4×19 g Tyr2 (Phe/Tyr free amino acid mixture from Nutricia NV; Belgium) per day. This intake was

spread over the day, three times with a meal and one intake before bedtime. Vitamin and mineral supplement (Omnibionta Pronatal, Merck Consumer Healthcare nv/sa; Belgium) was also introduced 2 months prior to delivery. Four protein free sandwiches per day were changed into two protein free sandwiches and two normal bread sandwiches, thus increasing the intake of natural protein. No more dietary changes were made during the last month of pregnancy.

Dietary details throughout the pregnancy are listed in Table 1.

Neonatal Course

At 41 weeks of gestation, labor was induced and a healthy girl was born after an uneventful delivery. Apgar score for breathing, pulse, and muscle tone were scored and resulted in 0-0-1 at birth, 9-9-9 after 1 min and 10-10-10 after 5 min. The birth weight was 2,950 g, length was 49 cm.

Tyr, Phe, and nitisinone levels are shown in Fig. 4. Tyr in the newborn normalized slowly. After 28 days, an almost normal Tyr level was reached (50–150 μmol/l). The highest Phe level was measured on the day of birth but was within normal range (40–140 μmol/l) and fell rapidly the following days.

Nitisinone in the umbilical cord blood was 69 μmol/l, while in the mother it was 94 μmol/l at delivery (last intake

Fig. 2 (a) Prenatal ultrasound at 28 weeks of gestation. (b) Fetal MRI of the fetal brain at 34 weeks of gestation. Both imaging modalities show normal cerebral anatomy, gyration, and migration for the

gestational age. (c) MRI scan of the fetus at 34 weeks of pregnancy shows normal development

of 10 mg of nitisinone 4 h prior to delivery). Five days after birth, the nitisinone level in the newborn was already under the target values and on day 28, no nitisinone could be detected (Fig. 4). There was no succinylacetone detected in the blood spots of the newborn at any time point. The calculated half-life (elimination half-life) is 90 h, this is based on two observations, thus it is an estimation. We presumed a single compartment model, with linear elimination ($y = 69 + 0.3833x$).

The mother was not keen on breastfeeding the child and this choice was supported. Data showed a reduced mean weight and corneal lesions in rat pups due to nitisinone exposure via milk (Summary of product characteristics Orfadin; <http://www.ema.europa.eu>).

Weight and length of the newborn were within normal standards for the Flemish population (SPE standards; Devlieger et al. 1996). Postnatal growth in the child follows the p50–75 (Percentile 50–75) and her head circumference follows the p10–25 up till 12 months of age (i.e., to date). Her mother, during childhood, followed p50–75 for weight and p25–50 for length and head circumference. Data of the father are not available. The child's hearing exam was normal at the age of 2 weeks.

At approximately 10 months, the child underwent an extensive neurological examination which showed no abnormalities. The mental development score was 97 and motor development score 86 (Bayley Scales of Infant Development) which is within normal range. In general,

Fig. 3 Tyrosine, phenylalanine, and nitisinone levels in the mother; * switch from 3 × 15 g Tyr 2 to 4 × 19 g Tyr 2; # starting two normal sandwiches; § date of delivery (time = 0). Normal values are depicted as the *gray* shaded areas

Table 1 Diet during pregnancy

Time of pregnancy	Energy intake (kcal/day)	Protein intake (g protein/kg bodyweight/day)	Natural protein intake (g protein/kg/day)	Protein intake from amino acid supplement (tyr 2) (g protein/kg/day)	Energy
Prior to pregnancy until 32 weeks of pregnancy	2,319	55.6 g P/day = 0.8 g P/day/kg	0.4	0.4	9.7% P32.4% F57.9% CH
33 weeks	2,524	71.1 g P/day = 0.9 g P/kg/day	0.3	0.6	11.5% P29.4% F59.1% CH
34 weeks	2,488	76 g P/day = 1 g P/kg/day	0.4	0.6	12.3% P30.1% F57.6% CH
41 weeks	2,971	75 g P/day = 1 g P/kg/day	0.4	0.6	10.2% P28.1% F61.7% CH

P = protein, F = fat, and CH = carbohydrate

Fig. 4 Tyrosine (1157 µmol/l at birth, cord blood), phenylalanine (80 µmol/l at birth, cord blood) and nitroisone (69 µmol/l at birth) levels in the newborn; § date of birth

the neurological development up till 12 months of age can be considered normal.

Discussion and Conclusions

We describe an adult female tyrosinaemia type I patient, who unintentionally became pregnant during adequate intake of oral contraceptive. She did not miss any pills, was not ill, did not experience any gastroenteritis episode with vomiting and/or diarrhea before becoming pregnant. There are no data on the metabolism of nitroisone and possible interactions with the metabolism of oral contra-

ceptives, so the possibility of a decreased efficacy of her oral contraceptive cannot be formally excluded.

As the patient was already 27 weeks pregnant when the pregnancy was detected, and as the fetal ultrasound and MRI were normal, it was decided that continuation of the treatment (diet, amino acid mixture, and nitroisone) was preferable to changing the treatment or cessation of nitroisone, which would create an unpredictable risk. This decision was thoroughly discussed with all involved parties. During pregnancy, no treatment changes were made, except for an increase in intake of amino acid mixture and natural protein. This adjustment is comparable to what is customary in case of pregnancy in a phenylketonuria mother, to

keep Phe levels within normal range and supply sufficient protein to allow normal growth of the fetus. In this case, natural protein intake was increased because Phe levels fell below normal values (Fig. 3). Further increase of natural protein intake was decided against, in view of the already high maternal Tyr levels.

Nitisinone in the umbilical cord blood was 69 $\mu\text{mol/l}$ at birth, while in the mother it was 94 $\mu\text{mol/l}$ at delivery (paired sampling, last intake of 10 mg of nitisinone 4 h prior to delivery) (Figs. 3 and 4). This indicates that nitisinone passes the placenta almost freely. Five days after birth, the nitisinone level in the newborn was already below the reference target treatment values and on day 28, no nitisinone could be detected anymore (Fig. 4). There was no detectable succinylacetone in the bloodspot of the newborn at any time point. Half-life of nitisinone in the neonate was calculated to be 90 h.

Phe levels at birth were normal in the fetus (80 $\mu\text{mol/l}$) and at the lower limit of normal values in the mother (30 $\mu\text{mol/l}$) (Figs. 3 and 4). The ratio between maternal and fetal Phe is comparable to what is described in pregnancies in phenylketonuria mothers and reflects active transplacental transport of Phe. Fetal plasma Phe is on average 1.5 times higher than maternal Phe (Schoonheydt et al. 1994). Tyr level in the neonate at birth was 1,157 $\mu\text{mol/l}$, while in the mother it was 434 $\mu\text{mol/l}$ (Figs. 3 and 4). The level of Tyr in the neonate probably reflects the fact that nitisinone also inhibits Tyr metabolism in the fetus, but could also partly be due to active transplacental transport. Indeed, fetal plasma Tyr is on average 2.2 ± 0.5 (mean $\pm$ 1 standard deviation) times that of the mother (Mitchell et al. 2001). In conclusion, in this first ever case of pregnancy under nitisinone therapy, with moderately elevated maternal Tyr and normal to decreased maternal Phe, outcome for the child seems acceptable so far, at the age of 12 months. Long-term results in a larger cohort of pregnancies and births have to be awaited.

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A Concise, 1 Sentence Take-Home Message

Normal 1-year development in a child born from a mother with tyrosinaemia type 1 taking nitisinone during pregnancy.

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