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Effect of nitisinone (NTBC) treatment on the clinical course of hepatorenal tyrosinemia in Québec

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ABSTRACT

Background: Hepatorenal tyrosinemia (HT1, fumarylacetoacetate hydrolase deficiency, MIM 276700) can cause severe hepatic, renal and peripheral nerve damage. In Québec, HT1 is frequent and neonatal HT1 screening is practiced. Nitisinone (NTBC, Orfadin ®) inhibits tyrosine degradation prior to the formation of toxic metabolites like succinylacetone and has been offered to HT1 patients in Québec since 1994.

Methods: We recorded the clinical course of 78 Québec HT1 patients born between 1984 and 2004. There were three groups: those who never received nitisinone (28 patients), those who were first treated after 1 month of age (26 patients) and those treated before 1 month (24 patients). Retrospective chart review was performed for events before 1994, when nitisinone treatment began, and prospective data collection thereafter.

Findings: No hospitalizations for acute complications of HT1 occurred during 5731 months of nitisinone treatment, versus 184 during 1312 months without treatment ($p<0.001$). Liver transplantation was performed in 20 non-nitisinone-treated patients (71%) at a median age of 26 months, versus 7 late-treated patients (26%, $p<0.001$), and no early-treated patient ($p<0.001$). No early-treated patient has developed detectable liver disease after more than 5 years. Ten deaths occurred in non-nitisinone treated patients versus two in treated patients ($p<0.01$). Both of the latter deaths were from complications of transplantation unrelated to HT1. One probable nitisinone-related event occurred, transient corneal crystals with photophobia.

Interpretation: Nitisinone treatment abolishes the acute complications of HT1. Some patients with established liver disease before nitisinone treatment eventually require hepatic transplantation. Patients who receive nitisinone treatment before 1 month had no detectable liver disease after more than 5 years.

1. Introduction

We report the outcome of treatment of hepatorenal tyrosinemia (HT1, MIM 276700) [1], a severe hereditary metabolic disorder of childhood, with nitisinone, which specifically inhibits an early step of tyrosine degradation (Fig. 1) [2]. The setting is the province of Québec, Canada, which is suited for clinical studies of HT1 because the disease is frequent due to high prevalence of a founder mutation, IVS12 + 5G > A [3], in the French-Canadian population [4,5] and because of a universal newborn screening program which refers all

Abbreviations: AFP, alpha-foetoprotein; ALA, delta-aminolevulinic acid; ALT, alanine aminotransferase; FAH, fumarylacetoacetate hydrolase; HT1, hereditary tyrosinemia, type 1; 4HPL, 4-hydroxyphenyllactate; 4HPP, 4-hydroxyphenylpyruvate; PBG, porphobilinogen; SA, succinylacetone; SEM, standard error of the mean.

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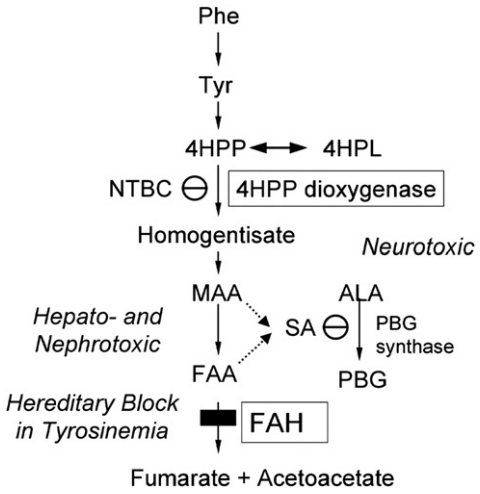

Fig. 1. Nitisinone and tyrosine metabolism. The amino acids phenylalanine (Phe) and tyrosine (Tyr) are degraded as shown. HT1 results from deficiency of the last enzyme, fumarylacetoacetate hydrolase (FAH). Metabolites immediately upstream of FAH, fumarylacetoacetate (FAA) and possibly maleylacetoacetate (MAA), are felt to cause succinylacetone (SA) is a stable derivative of FAA. Elevated levels of SA are pathognomonic for HT1. SA strongly inhibits porphobilinogen synthase, causing secondary accumulation of delta-aminolevulinic acid (ALA) and neurologic crises [8]. Nitisinone (NTBC) potently inhibits 4-hydroxyphenylpyruvic acid (4HPP) dioxygenase and restricts the production of toxic metabolite downstream from this point. Other abbreviation, 4HPL, 4-hydroxyphenyllactic acid.

patients to a small number of physicians, allowing for treatment to be started before the development of clinical symptoms.

HT1 patients typically present in infancy with acute liver failure, cirrhosis, neurologic crises with pain and paralysis [1] and renal tubular dysfunction with hypophosphatemic rickets. Interpatient variability is great. With age, there is increasing risk of hepatocellular carcinoma [6]. Patients who survive beyond infancy may develop chronic renal failure. We compare the outcome of children born during the first ten years that nitisinone was available in Québec with that of patients born in the preceding decade, during which all current treatment options except nitisinone were available, including newborn screening, diet therapy and liver transplantation.

2. Material and methods

2.1. Patient groups

All known HT1 patients in Québec born between February 1984 and February 2004 (Fig. 2) were identified. In all patients, the diagnosis of HT1 was confirmed by the presence of elevated levels of succinylacetone in blood or urine. The clinical course of patients was recorded until hepatic transplantation, death, or August 1, 2009, whichever came first. Data for events before 1994 were obtained from retrospective chart review; subsequent data, by prospective recording. Three patient groups were studied: N, never-nitisinone-treated; L, late treatment (composed of patients who started nitisinone after 30 days of age) and E, early treatment (started on or before 30 days of age). Criteria for inclusion in a nitisinone treatment group included: [1] having received nitisinone for at least 2 weeks and [2] lack of documented noncompliance, defined as admitted refusal of nitisinone, plus having documented, inappropriately low plasma nitisinone levels.

The data of late-treated patients consisted of an initial non-nitisinone-treated period and a later nitisinone-treated period. Each period was analyzed separately when evaluating possibly recurrent HT1-related events.

Since 1994, nitisinone treatment has been offered to and accepted by all nontransplanted HT1 patients in Québec. Three patients who received nitisinone were not included in the nitisinone-treated groups. [1] Patient N28, a non-French-Canadian patient, did not have newborn screening detection. She presented with cirrhosis and hepatocellular carcinoma, and liver transplantation was performed. She is included in the never-NTBC-treated group because she received NTBC for only 1 week. [2] A HT1 patient born outside of Québec was diagnosed at age 5 years by family screening, when typical neurological crises lead to the diagnosis of HT1 in a sibling. He had three neurological crises before receiving nitisinone at the age of 10 years. Later, he repeatedly refused to take nitisinone, had nearly undetectable plasma nitisinone levels and developed neurological crises. [3] This patient, who had a chronic course, was excluded because she was born before 1984.

2.2. Treatment protocol

Participants were enrolled in the ongoing International NTBC Study administered in Gothenburg [6]. Doses of nitisinone were initially fixed at 0.6 or 1.0 mg/kg daily in two daily oral doses. For the first 2 years of the study, patients received a recrystallized preparation of NTBC supplied by S Lindstedt and E Holme. Thereafter, they received commercially-produced nitisinone. After 1999, nitisinone dose was titrated in order to minimize urine SA levels [7]. Dietary restriction of phenylalanine and tyrosine was prescribed, aiming to maintain plasma tyrosine at 200–400 μmol/L.

Pretreatment samples were obtained for assay of plasma tyrosine, phenylalanine and alpha-fetoprotein (AFP) and of urine 4-hydroxyphenylpyruvate (4HPP), 4-hydroxyphenyllactate (4HPL), succinylacetone (SA) and delta-aminolevulinic acid (ALA). Two 12-hour urine collections were performed, starting immediately after the first dose of nitisinone, and 24-hour collections on days 2 and 3. Blood and 24 hour urine samples were obtained on days 7 and 14, at 1, 2, 3, 4 and 6 months, and every 3 months thereafter, for assay of plasma and urinary SA, urinary ALA, plasma amino acids, and plasma nitisinone levels. Collaborating physicians completed physical examination forms and provided results of complete blood count and plasma alpha-foetoprotein (AFP), alanine and aspartate aminotransferases, albumin, protein, gamma-glutamyltransferase, bilirubin, creatinine, urea, electrolytes, blood gases, calcium, phosphate and alkaline phosphatase. Patients were genotyped for the common IVS12 + 5G > A mutation [3]. Abdominal imaging included ultrasound every 6 months and annual computerized tomography or magnetic resonance imaging.

The treatment protocol was approved by the Ethics Committee of CHU Sainte-Justine. Informed consent was obtained before enrollment.

3. Definition of variables and analysis

All hospitalizations related to the acute complications of HT1 were noted, including hospitalizations for preventive treatment and observation during infections. Hospitalizations with neurologic crises, defined as in [8], were noted separately. For descriptive purposes, the total length of hospital stays and the total length of time studied were noted. For comparative statistics, the course of each patient was divided into calendar months. Each month was classified as to whether the patient had received nitisinone during that month, and whether an acute event occurred during the month, i.e., neurological crisis or hospitalization for HT1-related reasons other than a neurological crisis. The dates of liver transplantations and deaths were recorded. The first month of life, during which no HT1-related complication was observed in any patient, was excluded from the calculations. Groups were compared by the Chi square test.

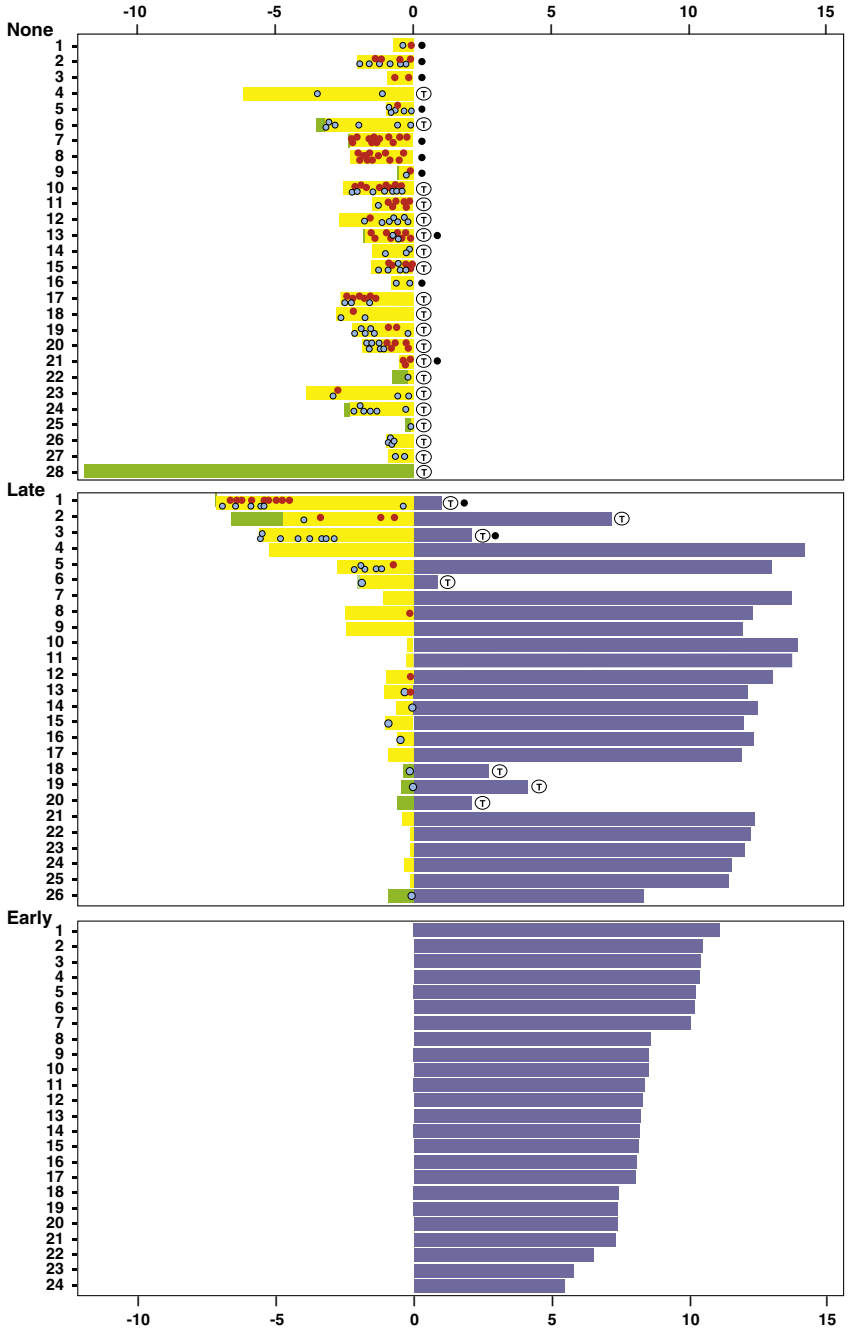

Fig. 2. HT1 in Québec, 1984–2009. The three treatment groups are shown: (N) non-nitisinone, top panel; (L), late nitisinone, middle panel and (E) early nitisinone, lower panel. Each horizontal bar represents one patient. Fill colors represent treatments as follows: green, none; yellow, diet and supportive treatment; violet, diet plus nitisinone. Dots represent HT1-related hospitalizations: red dots, neurological crises; blue dots, hospitalizations for other reasons. Hepatic transplantation is indicated by an encircled letter T, and death by a black oval.

4. Results

4.1. Comparison of groups

4.1.1. Patient characteristics

Fig. 2 summarizes the clinical course of the 78 HT1 patients. Twenty-eight patients never received nitisinone, 26 were first treated after 30 days of age and 24, before 30 days. A total of 1312 patient-months without nitisinone treatment were registered (777 months from the non-nitisinone group and 535, late-treated) and 5731 months with treatment (3138, late-treated group; 2593, early-treated group).

4.1.2. Clinical course

Acute complications of HT1 were frequent before nitisinone treatment (Fig. 2, Table 1). Patients in the pre-nitisinone group spent a total of 56/784 months (7.1% of their lives) in the hospital, including 29 months (3.7% of their lives) in active neurological crises. Conversely, no patient developed an acute decompensation while treated with nitisinone. Among non-nitisinone-treated patients, 8/28 (29%) died before transplantation, at a mean age of 16 months, versus no deaths before transplantation in nitisinone-treated patients ($p<0.01$).

Liver transplantation was performed in 20 (71%) of non-nitisinone treated patients. The indications were cirrhosis or cancer (13 patients),

Table 1
Effect of NTBC treatment on hospitalizations for acute HT1-related reasons.

Group	Total non-NTBC	N (never-treated)	L (late-treated)		E (early-treated)	Total NTBC
Subgroups	[N + L (pre)]		Pre-NTBC	NTBC		[L(NTBC) + E]
Patients-months	1312	777	535	3138	2593	5731
Months with tyrosinemia-related hospitalization (neurological crises included)	184	141	43^{***}	$0^{\ddagger\ddagger\ddagger}$	$0^{***,\ddagger\ddagger\ddagger}$	$0^{***,\S\S\S}$
Months with neurological crises	88	71	17^{***}	$0^{\ddagger\ddagger\ddagger}$	$0^{***,\ddagger\ddagger\ddagger}$	$0^{***,\S\S\S}$

Comparisons with the following groups are indicated as follows: N, *; L (pre), ‡; N + L (pre), §. The number of repetitions of symbols indicates the level of significance. e.g., for asterisks: *, <0.05; **, <0.01; ***, <0.001.

acute hepatic failure (2 patients) and previous neurological crises (5 patients) (Table 2). The median age at transplantation was 26 months (range, 5 months to 12 years, Fig. 2). Seven late-treated patients (Patients L1, L2, L3, L6, L18, L19, L20), all of whom had chronic liver abnormalities before receiving nitisinone, were transplanted because abdominal imaging suggested macronodular cirrhosis. Cirrhosis was confirmed pathologically in each case. Hepatocellular carcinoma was present in two livers and the other five had dysplastic foci.

Following liver transplantation, there were two deaths each in the never-treated (2/20, 10%) and late-treated groups (2/7, 28%; NS). Both deaths of late-treated patients were due to complications unrelated to HT1.

Since 1994, six HT1 patients in Québec were not detected by screening, because of birth outside of Québec or screening failure (Table 3). Four required liver transplantation.

Between the birth of the last patient included in this series and the date of analysis (1 August, 2009), 22 new patients were identified in Québec. Their clinical courses have been uneventful. They were excluded from statistical analysis because their short follow-up periods were insufficient for analysis of long-term complications, and their inclusion could falsely bias the analysis of long term complications in favor of treatment.

4.1.3. Liver function and imaging

Liver function abnormalities were common before nitisinone treatment: in the late-treated group, pretreatment factor VII levels were <50% of normal in 17/27 (63%); international normalized ratio >1.3, 14/26 (54%, range, 1.39–8.55); activated partial thromboplastin time (>40 s), 9/25 (36%); factor V levels<50% of normal mean, 2/17 (11.8%). Alanine aminotransferase (ALT) levels >60 IU/L were initially observed in 3/26 cases (11.5%). All normalized by 4 months of treatment, most within 1 month.

Before treatment, all patients had elevated AFP levels for age, of highly variable degree (18 to >300,000 μg/L; normal after 12 months, <10 μg/L), which typically normalized during the second year of treatment (Fig. 3e). Plasma AFP values declined slowly in Patients L3 and L20 (both >100 μg/L at 15 months); each subsequently developed cirrhosis and underwent transplantation. Patient L2 had normal AFP levels for 7 years, and then showed a steady slow increase, accompanied by the appearance of findings consistent with cirrhosis on abdominal imaging; hepatocarcinoma was found at transplantation. Patient L10 has maintained AFP levels of ~40 μg/L for over 10 years, with normal imaging results, and has not been transplanted.

4.1.4. Kidney function

No patient developed renal failure, hypophosphatemia, glucosuria or generalized aminoaciduria during treatment. Patient L26, who presented with hypophosphatemic rickets, showed rapid improvement in renal tubular function following nitisinone treatment.

4.1.5. Genotype

IVS12 + 5G > A accounted for 85/100 (85%) of the mutant alleles in nitisinone-treated patients. In the late-treated group, IVS12 + 5G > A homozygotes were observed that presented with acute, chronic or mild liver disease or with neurological crises.

4.1.6. Metabolites

As expected, levels of metabolites downstream from the nitisinone-induced block decreased, whereas tyrosine increased following nitisinone administration (Fig. 3). Metabolite levels stabilized by 3 months of treatment (Fig. 3). In the 12-hour urine sample immediately following the first dose of NTBC, mean urine SA and ALA levels decreased 7.3-fold and 2.4-fold, respectively (Figs. 3a,b). After 1 week of nitisinone treatment, their levels were not significantly different from those ≥3 months later.

"Upstream" metabolites increased, including tyrosine (Fig. 3d) and 4-hydroxyphenyl compounds (not shown). There was marked intra-patient variation, presumably reflecting differences in dietary intake, catabolic events and possibly in the completeness of nitisinone-induced inhibition. Measurements of urinary 4HPP and 4HPL were extremely variable and were abandoned for clinical use. Plasma phenylalanine levels were 32 ± 19 μmol/L (mean ± SD, n = 431), below the 10th centile for age (39 μmol/L in infants [9]). Initial plasma methionine

Table 2
Effect of NTBC Treatment on liver transplantation and death in hepatorenal tyrosinemia in Québec.

Group	N (never-treated)	L + E (treated at any time)	L (late-treated)	E (early-treated)
Patients (n)	28	50	26	24
Transplantation	20	7^{***}	7^{***}	$0^{***,\dagger\dagger\dagger}$
Death	10	2^{***}	2^{**}	$0^{***,\dagger}$
Death before transplantation	8	0	0	0
Death after transplantation	2	2	2	0

Comparisons with the following groups are as indicated: N, *; L, †. The number of repetitions indicates the level of significance. e.g., for asterisks: *, <0.05; **, <0.01; *** <0.001.

Table 3
HT1 patients in Québec not identified by newborn screening, 1994–2004.

Patients	Age at presentation	Clinical presentation	Clinical outcome	NTBC (duration)[a]
N28	10 years	Liver cancer	Transplantation	1 week
L18	3 months	Hepatic failure	Transplantation	37 months
L19	5 months	Hepatic failure	Transplantation	52 months
L20	7 months	Hepatomegaly	Transplantation	27 months
L24	2 months	Hepatomegaly	Resolution	15 years
L26	11 months	Hypophosphatemic rickets	Resolution	10 years

Patients were not detected by screening either because of birth outside of Québec or because of screening failure. All patients received NTBC immediately after diagnosis except N28.

[a] Duration of NTBC treatment until transplantation or date of analysis (1 August, 2009).

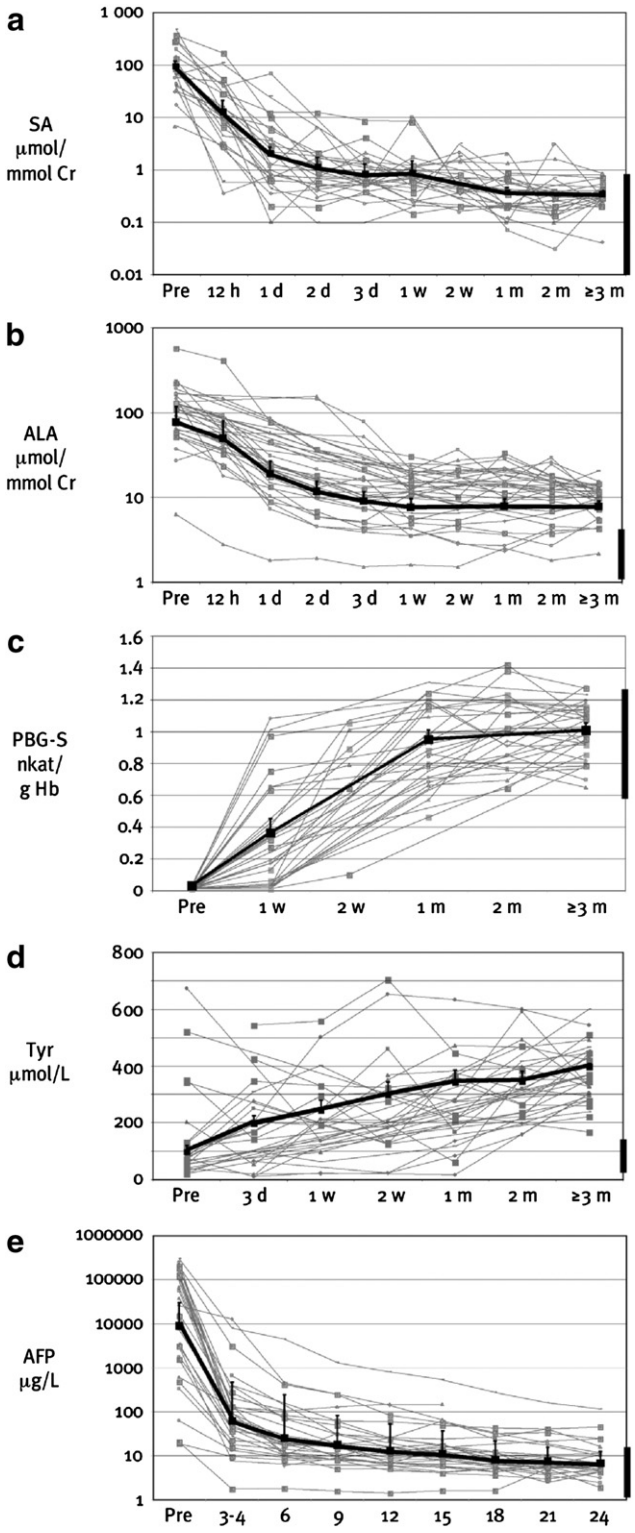

Fig. 3. Metabolite and alpha-fetoprotein values before and during nitisinone treatment. The courses of individual patients are shown by gray lines. Means (in panels with an arithmetic scale) or geometric means (logarithmic scale) are shown by black lines, calculated from the patients for whom results were available at each indicated time point. (a) Urinary SA (n = 17 patients); (b) urinary ALA (n = 12); (c) erythrocyte porphobilinogen synthase (n = 16); (d) plasma tyrosine, (n = 12); (e) plasma AFP (n = 20).

level was >60 µmol/L (90th centile for age, 38 µmol/L [9]) in 5/18 (28%) of late-treated patients.

Hypermethioninemia was roughly proportional to the clinical severity of acute liver dysfunction and it resolved in all cases by 6 months.

4.2. Adverse events during nitisinone treatment

4.2.1. Ocular crystals

After 8 months of treatment, Patient L3 developed photophobia and corneal crystals, which disappeared within 24 h of strict dietary restriction.

4.2.2. Hypoglycemia

Patient E4 had recurrent unexplained episodes of asymptomatic fasting ketotic hypoglycemia during the first 2 years of life, but had normal growth and development.

4.2.3. Other parameters

Twelve episodes of asymptomatic elevations of ALT level ≥60 IU/L occurred after three or more months of treatment (mean, 89 international units/L; range 60–580). They resolved spontaneously without changes in nitisinone dose. Complete blood counts, immunoglobulins, thyroid function, bilirubin, alkaline phosphatase, calcium, phosphate, amylase, cholesterol, triglycerides, electrolytes, blood gases, chest radiographs, echo- and electrocardiograms showed no consistent or sustained abnormality.

5. Discussion

Nitisinone treatment dramatically improved the course of HT1. Before the availability of nitisinone, patients and their families lived in uncertainty because of the unpredictable but frequent and often-fatal acute neurologic crises and episodes of acute hepatic failure (Fig. 2, Table 1). Conversely, none of 24 patients treated from the neonatal period and followed for over 5 years developed detectable liver disease, while in patients not treated with nitisinone, 20/28 (71%) required transplantation, at a median age of 26 months (Fig. 2).

Adverse effects of nitisinone treatment were minor. Transient ocular crystals were observed in Patient L3 and are reported in other nitisinone-treated patients [1] and in hypertyrosinemic rats [1,10]. The crystals may be composed of tyrosine, a relatively insoluble amino acid [1]. A contribution of nitisinone or its metabolites to the crystals cannot be formally eliminated. We obtain an urgent slit lamp examination if patients complain of photophobia, but no other ocular crystals were documented in the study period. It is unclear whether the episodes of asymptomatic hypertransaminasemia were related to nitisinone treatment. They resolved despite continuation of nitisinone, and hence may have been related to intercurrent events such as unidentified infections.

Patients acutely ill from acute complications of HT1 derive benefit rapidly from nitisinone treatment. In HT1 patients, acute hepatic dysfunction can rapidly lead to liver failure and neurologic crises [1], with a high risk of mortality. No new acute hepatic or neurologic events occurred after the first dose of nitisinone. Also, levels of SA and ALA, the clinically-measurable compounds most closely linked to hepatic and neurological toxicity, decrease within hours after the first dose of nitisinone (Fig. 3).

In Québec HT1 patients, chronic hepatic and renal injury is slowed or possibly prevented by nitisinone. However, nitisinone treatment will not reverse established hepatic lesions like oncogenic mutations or fibrosis, and is not expected to directly influence the speed of recuperation from acute hepatic or paralytic crises except to prevent further crises. Late-treated HT1 patients can develop cirrhosis and hepatocarcinoma (Fig. 2; references [11,12]). From our data, we cannot resolve whether this reflects the natural course of preexisting liver damage or in part represents disease progression under treatment. A practical answer may be provided by the long-term course of compliant patients treated from the neonatal period. Even in HT1 patients with indication for liver transplantation at diagnosis, a period of nutritional and other stabilization with nitisinone treatment is a consideration before surgery.

Currently the main indication for liver transplantation in nitisinone-treated HT1 patients in Québec is persistent evidence of cirrhosis on imaging. Because neurologic crises are abolished by adequate nitisinone therapy, a history of neurological crisis is no longer a valid indication for transplantation. An isolated increase of AFP, especially if progressive, calls for close surveillance by imaging and liver biopsy. In this series, identification of even a single persistent nodule on imaging has been associated with cirrhosis. Furthermore, all patients with cirrhosis showed at least one area of high grade dysplasia or hepatocarcinoma.

Do the conclusions of this study apply outside of Québec? Two potential criticisms of the data are that unrecognized factors unrelated to nitisinone may have reduced the severity of HT1, and that the Québec cohort is not representative because of its high prevalence of one mutation. Strongly against the first idea are the lack of other changes in diagnosis and treatment over the years covered by the study, and the clinical course of patients in Québec since 1994 that were not identified by screening (Table 3). The outcomes of these patients resemble those of patients reported elsewhere who are treated with nitisinone after being detected clinically [13]. Regarding the common Québec mutation, IVS12 + 5G > A [3], it is frequent elsewhere [14,15]. Furthermore, the phenotypes of IVS12 + 5G > A homozygotes span the clinical spectrum of HT1. Also, the early-treated cohort contains 6 patients with mutations other than IVS12 + 5G > A on one or both alleles, who like IVS12 + 5G > A homozygotes, remain asymptomatic. Although our data cannot exclude genotype–phenotype effects in HT1, they provide no compelling evidence in favor of an effect of FAH genotype upon nitisinone responsiveness.

Together, the data strongly suggest that early nitisinone treatment was the key factor in the unprecedentedly good outcome of the neonatally-treated patients, that a combination of neonatal screening and early nitisinone treatment is the medical treatment of choice for HT1, and that these conclusions are applicable to HT1 patients elsewhere.

HT1 is listed in the primary newborn screening panel of the American College of Medical Genetics [16]. The use of blood tyrosine level for newborn screening of HT1 lacks both sensitivity and specificity [1]. In contrast, blood succinylacetone is an excellent marker for HT1 [1] and tandem MS-based methods are being developed for its detection [17–19]. Their application to newborn screening could radically change the course of tyrosinemia outside of Québec.

Important questions remain about long-term nitisinone treatment. What plasma levels of nitisinone, phenylalanine and tyrosine are optimal? Because neurologic abnormalities are reported in other forms of hypertyrosinemia [1], we control plasma tyrosine levels with a low-protein diet. Long-term developmental assessment of HT1 patients will be important. Although in theory it may reduce dietary tolerance for natural proteins, phenylalanine supplementation is recommended by some groups [20].

Will hepatocyte replacement or gene therapy supersede nitisinone treatment? Promising results have been obtained in FAH-deficient mice [21]. However, because damage in HT1 is cell-autonomous [1], effective treatment would require replacement of all hepatocytes, without the development of benign regenerative nodules that are currently difficult to distinguish noninvasively from malignancy. Unless major advances occur in the efficiency of gene- or cell-replacement technology, nitisinone will be essential for the optimal medical management of hepatorenal tyrosinemia in the foreseeable future.

6. Conclusions

Nitisinone treatment effectively prevents acute hepatic and neurologic crises in compliant HT1 patients. The reductions of succinylacetone and ALA excretion within 12 h following the first dose of nitisinone are consistent with a near-immediate therapeutic effect. In this series, the liver function of all patients markedly improved on nitisinone treatment, but some late-treated patients developed cirrhosis with high grade dysplasia or hepatocarcinoma. In contrast, all patients detected by newborn screening and started on treatment within a month of birth have normal liver function. The combination of neonatal screening plus early nitisinone treatment is currently the medical treatment of choice for HT1.

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