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Use of nitisinone in patients with alkaptonuria

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Abstract

Alkaptonuria, a rare autosomal recessive disorder caused by mutations in the HGD gene and deficiency of homogentisate 1,2 dioxygenase, is characterized by ochronosis, arthritis, and daily excretion of gram quantities of homogentisic acid (HGA). Nitisinone, an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, can drastically reduce urinary excretion of HGA in individuals with alkaptonuria. We investigated the safety and the HGA-depleting efficacy of nitisinone in an open-label, single-center study of 9 alkaptonuria patients (5 women, 4 men; 35-69 years of age) over the course of 3 to 4 months. Each patient received nitisinone in incremental doses, 0.35 mg bid followed by 1.05 mg bid, and remained on this dosage and a regular diet for 3 months. Nitisinone reduced urinary HGA levels from an average of 4.0 ± 1.8 (SD) g/day to 0.2 ± 0.2 g/day ($P < .001$). The average plasma tyrosine concentration, initially 68 ± 18 μmol/L, rose to 760 ± 181 μmol/L ($P < .001$). During the final week of the study, 5 patients adhered to a protein-restricted diet (40 g/day), and their mean plasma tyrosine level fell from 755 ± 167 to 603 ± 114 μmol/L. Six of the 7 patients who received nitisinone for more than 1 week reported decreased pain in their affected joints. Weekly ophthalmologic examinations showed no signs of corneal toxicity. Adverse events included the passing of kidney stones, the recognition of symptoms related to aortic stenosis, and elevation of liver transaminase levels. We conclude that low-dose nitisinone effectively reduced urinary HGA levels in patients with alkaptonuria. Future long-term clinical trials are planned to determine the benefits of nitisinone in preventing joint deterioration and providing pain relief, and its long-term side effects.

1. Introduction

Alkaptonuria, an autosomal recessive disorder with an incidence of approximately 1 in a million live births [1,2], results from mutations in the gene HGD, which codes for an essential hepatic enzyme, homogentisate 1,2-dioxygenase [3-5]. This enzyme in the tyrosine degradation pathway converts homogentisic acid (HGA) to maleylacetoacetic acid; the enzyme deficiency results in massive urinary excretion of HGA and accumulation of this compound in the tissue of affected individuals. The urine turns dark upon standing due to oxidation of HGA to benzoquinones, which form melanin-like polymers. These compounds bind preferentially to connective tissue, causing ochronosis, with its darkening of ear cartilage, sclera, and bone, its arthritis and joint destruction, and its cardiac valve disease [1,2]. Treatment with vitamin C to enhance HGA degradation has not proven helpful [6]. Dietary therapy restricting phenylalanine and tyrosine is difficult to maintain and has had no demonstrable efficacy in improving the symptoms of alkaptonuria, although long-term controlled clinical trials have not been performed [1].

Another possible approach to reducing HGA production involves the administration of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3cyclohexanedione, or nitisinone [7], a compound that inhibits 4-hydroxyphenylpyruvate dioxygenase

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(4-HPPD), the enzyme that produces HGA (Fig. 1). Nitisinone is currently approved by the Food and Drug Administration for the treatment of tyrosinemia type I, a rare disease caused by deficiency of fumarylacetoacetate hydrolase and resulting in liver failure, hepatocellular carcinoma, and renal tubular dysfunction in infants and children [8]. Nitisinone decreases the formation of toxic, oxidizing metabolites in tyrosinemia type I children and constitutes the treatment of choice for this otherwise fatal disease [9,10]. In 2 patients with alkaptonuria, low-dose nitisinone reduced the urinary excretion of HGA by 69% or more [2]. However, plasma tyrosine concentrations rose significantly, creating a risk of corneal crystal formation, corneal epithelial damage, and photophobia.

In the present study, we examined the safety of administering nitisinone at a dosage adequate to reduce urinary HGA excretion, typically 2 to 9 g/day in alkaptonuria patients [1,2], to 0.5 g/day or less. We hypothesized that such a significant reduction, if maintained for years, might retard the progression of joint disease in alkaptonuria patients. We also incorporated a week of reduced dietary protein into our protocol, to determine whether restriction of tyrosine and phenylalanine would lower plasma tyrosine levels in nitisinone-treated patients. These investigations were performed to provide a basis for future clinical trials of nitisinone for the joint disease of alkaptonuria.

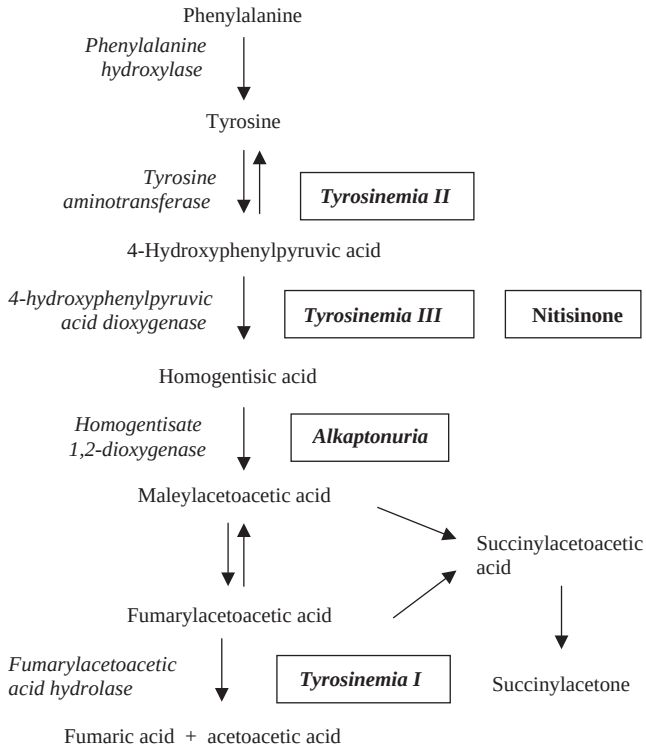

Fig. 1. The tyrosine degradation pathway. The biochemical defect in alkaptonuria is deficiency of homogentisate 1,2-dioxygenase. Nitisinone inhibits 4-HPPD, blocking the formation of homogentisic acid. Tyrosinemia type I is due to deficiency of fumarylacetoacetic acid hydrolase. Tyrosinemia type II results from deficiency of tyrosine aminotransferase. Deficiency of 4-HPPD results in tyrosinemia type III.

2. Patients and methods

2.1. Patients

Nine patients (4 male and 5 female; aged 35-69 years) were enrolled in an open-label clinical protocol to investigate the safety and HGA-reducing efficacy of oral nitisinone in alkaptonuria patients. The protocol was approved by the Institutional Review Board of the National Human Genome Research Institute, and all patients gave written informed consent. All patients were previously enrolled in a clinical protocol to study the natural history of alkaptonuria. The diagnosis of alkaptonuria in our 9 patients was based upon the finding of urinary HGA excretion in the range of 1.9 to 9.0 g/day (normal, 10-100 mg/day).

2.2. Exclusion criteria

Patients with keratopathy, current contact lens use, or uncontrolled glaucoma were excluded from the study. Additional exclusion criteria included a history of myocardial infarction or electrocardiographic evidence of myocardial infarction, arrhythmia, tachycardia, bradycardia, left bundle branch block, and chest radiographic abnormalities. Patients with history of pulmonary insufficiency, psychiatric illness that interfered with compliance or communication, current malignancy, open skin lesions, and uncontrolled hypertension were not eligible. Also excluded were patients with significant anemia, thrombocytopenia or leucopenia, hypokalemia, elevated serum creatinine, transaminase, or creatine kinase levels, an increased erythrocyte sedimentation rate, abnormal thyroid function tests, and plasma tyrosine levels greater than 150 μmol/L.

2.3. Clinical studies

Individuals eligible for this study were admitted to the Warren G. Magnuson Clinical Center at the National Institutes of Health for an initial period of up to 4 weeks. At the beginning of this period, baseline laboratory values, electrocardiogram, echocardiogram, renal ultrasound, magnetic resonance imaging of the brain, and evaluations by physical medicine and ophthalmology were obtained. Nitisinone, provided by Swedish Orphan International AB under Investigational New Drug exemption #46865, was initiated at an oral dose of 0.35 mg bid (approximately 0.01 mg/kg per day) for 8 days. If the day-7 urinary HGA excretion were less than 0.5 g/day, the patient would be discharged on that dose; otherwise, the nitisinone dose was increased to 1.05 mg bid (approximately 0.03 mg/kg per day). If the day-15 HGA excretion were less than 0.5 g/day, no change in the dose would be made; otherwise, the dose would be increased to a dose of 4 mg bid (0.1 mg/kg per day). Once an individual reached his or her maintenance dose of nitisinone, he or she was discharged to maintain that dosage for up to 15 weeks; during the last week of treatment, patients were placed on a low protein diet of 40 g/day. Patients returned to the NIH Clinical Center for weekly ophthalmologic examinations,

24-hour urine collections for HGA measurement, and serial blood collections for laboratory analyses, including a complete blood count, erythrocyte sedimentation rate, serum electrolytes and creatinine, liver function and mineral panels, plasma amino acids, and nitisinone levels.

This protocol initially called for cessation of the clinical trial if 3 serious adverse events occurred, regardless of whether these events were considered related to nitisinone use. A serious adverse event was defined by medical and laboratory parameters determined a priori, including corneal pain that interfered with daily living, new neurological or cardiac involvement, a severe skin rash, serum ALT greater than 70 U/L, hemoglobin less than 10 g/dL, white blood cell count less than 2.5 kg/μL, or serum potassium less than 3.0 or greater than 5.4 mEq/L. The investigators met weekly with a medical monitor not involved in the study to discuss safety issues and help determine what should be considered a serious adverse event.

2.4. Rehabilitation analyses and measurements

All patients received a systematic musculoskeletal examination, consisting of joint and soft tissue assessment. Axial and large joints were palpated for pain, standard range of motion (ROM), and presence of effusion. Quantitative spine flexion (Schober's test) [11,12] was performed on all patients. Small joints of hands and feet were evaluated by inspection and palpation. Joint alignment abnormalities were noted.

Each patient was issued a set of self-administered questionnaires including the SF-36 (a general health survey) [13,14], the Human Activity Profile, the Health Assessment Questionnaire, and the Fatigue Assessment Instrument. A careful musculoskeletal history was obtained documenting specifics of work and leisure activity, as well as injuries and surgical procedures.

2.5. Homogentisic acid assay

Urinary HGA levels were measured according to the spectrophotometric method of Lustberg et al [15], with linearity in the range of 6 to 60 μmol/L. Plasma HGA was measured using an HPLC method developed for Swedish Orphan International AB by AAI Deutschland GmbH & Co KG, Neu-Ulm, Germany. Briefly, 200 μL of plasma, 20 μL of 85% phosphoric acid, and 50 μL of internal standard (3,4-dihydroxyphenylacetic acid, 10 μg/mL) were mixed by vortexing and 20 μL of 60% perchloric acid was added. After 15 minutes at 10 °C, the sample was centrifuged and 10 μL of 2 mol/L ascorbic acid was added to 100 μL of the supernatant. The extract was injected onto an HPLC Ultrasphere ODS column (Beckman, Fullerton, Calif) attached to an SPD-6A ultraviolet detector (Shimadzu, Kyoto, Japan). Absorbance, measured at 286 nm, was linear with HGA concentration between 0.25 and 50.0 μg/mL.

2.6. Plasma amino acid assay

Plasma amino acid analyses were carried out using a Biochrom 30 (Biochrom Ltd, Cambridge, England) cation-exchange amino acid analyzer with post–column ninhydrin detection [16].

2.7. Plasma nitisinone assay

Plasma nitisinone was quantified by liquid chromatography–electrospray ionization–tandem mass spectrometry as previously described [17], with linearity in the range of 0 to 152 μmol/L. These analyses were performed by the Biochemical Genetics Laboratory, Children's Hospital and Regional Medical Center, Seattle, Wash.

2.8. Dietary analyses

Subjects were mailed detailed instructions to keep baseline 3-day food records before their initial protocol admission. Throughout the 3- to 4-month treatment phase, subjects continued to keep 2-day food records weekly while they remained on a regular, ad libitum diet. A trained dietitian reviewed all food records weekly for accuracy and completeness, in person with each subject. For the last week of the study, subjects were placed on a protein-restricted diet (40 g/day); during this time, daily food records were collected. Food records (ie, those collected at baseline, 6 selected days during the treatment phase while on nitisinone 1.05 mg bid, and 3 days during the protein-restricted week) were analyzed using the Nutrition Data System for Research software version 4.06/34, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, Minn [18].

3. Results

3.1. Patients

At baseline, the 9 patients under investigation had elevated urinary HGA excretion, normal plasma tyrosine concentrations, and variable joint and aortic valve involvement (Table 1). All patients continued to take medications prescribed for preexisting conditions in addition to nitisinone. Of the 9 patients, 4 exited the study before its conclusion, after 71, 5, 5, and 32 days, and 5 completed the entire study, including the final week of a low-protein diet (Fig. 2). All patients required the first increment in nitisinone dosing (from 0.35 to 1.05 mg bid), but no patient required a second increment to reduce urinary HGA excretion below 0.5 g/day. The occurrence of 3 serious adverse events in the first 5 patients, regardless of whether or not they were related to nitisione use, necessitated halting the study, in accordance with study requirements established a priori. After a 9-week hiatus to allow a review of these complications, enrollment was reinitiated for patients 6 through 9.

3.2. Urinary homogentisic acid excretion

Baseline daily urinary HGA excretion before treatment with nitisinone averaged 4.0 $\pm$ 1.8 (SD) g/day (normal, 10-100 mg/day). After receiving nitisinone at a dose of

Table 1
Baseline findings in 9 alkaptonuria patients treated with nitisinone

Patient	Sex/age (y)	Clinical involvement[a]	Urine HGA (g/d)	Plasma tyrosine (μmol/L)	Protein intake (g/d)
1	F/53	Back, shoulder, knee; renal calculi; aortic valve stenosis	3.47	62	47
2	F/50	Back, knee; aortic valve calcification	3.69	63	48
3	F/69	Back, hip, knee, shoulder; severe aortic valve stenosis, mitral valve calcification	2.56	65	51
4	M/46	Back, knee; prostate calcification	3.38	73	77
5	M/41	Back, knee	2.81	63	99
6	F/64	Back, neck, shoulders (replaced), knees (replaced); mild aortic valve stenosis; coronary artery calcification	3.90	66	78
7	M/35	Back, knee, hip, shoulder	7.85	95	309
8	F/55	Back, neck, knee, hip	2.52	34	42
9	M/44	Back, knee, hip, shoulder; calcification in right kidney	5.99	93	69
Normal values			0.01-0.10	35-90	Males 71-101, females 55-62

[a] All patients had ear cartilage pigmentation and all except patient 7 had scleral pigment. Patient 6 noted dark urine, and patient 4 had hand and face ochronosis.

0.35 mg bid for 7 days, 7 patients exhibited a mean urinary HGA excretion of 1.4 ± 1.0 g/day (Fig. 3). In addition to these 7 individuals, patients 4 and 5 also received the lowest dose of nitisinone, but for only 5 days. In patient 4, this produced a 62% reduction in urinary HGA excretion, from 3.4 to 1.3 g/day. In patient 5, it resulted in a 43% reduction in urinary HGA, from 2.8 to 1.6 g/day. All 9 patients maintained a level of daily HGA excretion above 0.5 g/day while receiving 0.35 mg of nitisinone twice daily. For 7 patients, the nitisinone dosage was increased on day 8 to 1.05 mg bid; the mean urinary excretion of HGA on day 15 was 0.23 ± 0.09 g (Fig. 3). The 7 patients were maintained on the 1.05-mg bid dosage of nitisinone for 25 to 94 days. During this time, the overall urinary HGA levels averaged 223 ± 238 mg/day on a regular diet (Fig. 3). Five patients participated in the week-long low-protein diet, and all 5 exhibited lower urinary HGA levels during this period, with a mean of 124 ± 42 mg/day.

3.3. Plasma HGA results

Plasma HGA levels were assayed in patients 1 to 3, 6, 8, and 9. In patients 1 and 3, the plasma HGA level was below the limit of detection (0.25 μg/mL) even at baseline. All the remaining patients had detectable concentrations of plasma HGA at baseline (ie, 0.47, 2.62, 1.25, 2.53 μg/mL in patients 2, 6, 8, and 9, respectively). The plasma HGA subsequently decreased below the detection limit in patients 2 and 6 during the maintenance period as well as on the low-protein diet. In patient 8, the plasma HGA also decreased from baseline, to below the detection limit during the maintenance phase and to 0.39 μg/mL during the low-protein diet. In patient 9, the plasma HGA decreased to 0.44 μg/mL during the maintenance period and to below the detection limit while on the low protein diet.

3.4. Plasma nitisinone

The mean plasma nitisinone level during the first week of treatment on 0.35 mg bid was 0.19 ± 0.09 (SD) μmol/L (n = 18). On a dose of 1.05 mg of nitisinone twice daily, the mean plasma nitisinone level was 1.39 ± 0.57 μmol/L (n = 34, $P < .0001$) and did not increase significantly after the first week of this treatment. The plasma was obtained at times that were not fixed relative to the administration of nitisinone.

3.5. Plasma tyrosine

The mean plasma tyrosine level at baseline on a regular diet was 68 ± 18 (SD) μmol/L (normal, 35-90 μmol/L), but these levels increased rapidly with nitisinone treatment (Fig. 3). Despite receiving only 5 days of nitisinone at

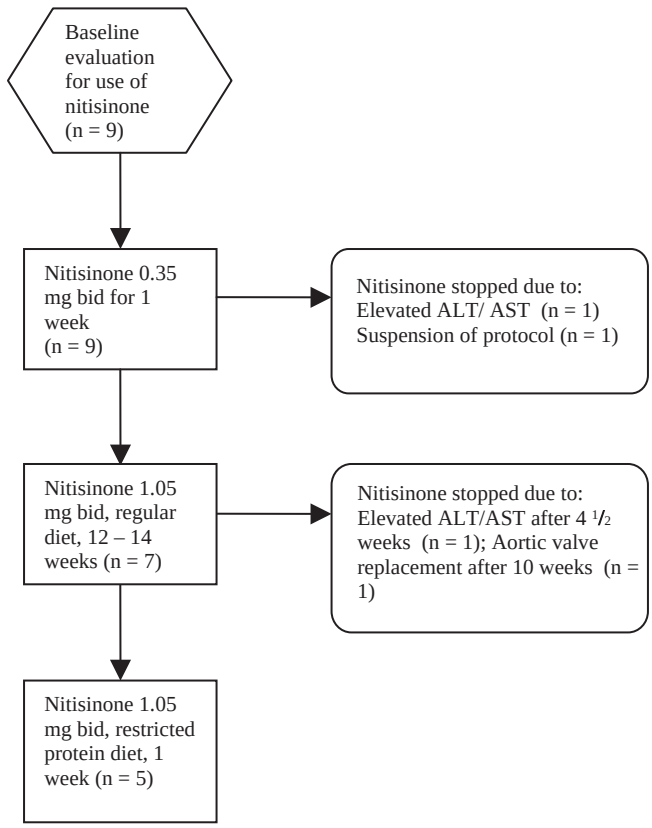

Fig. 2. Flow chart showing the course of nitisinone treatment in 9 patients with alkaptonuria. ALT indicates alanine transaminase; AST, aspartate transaminase.

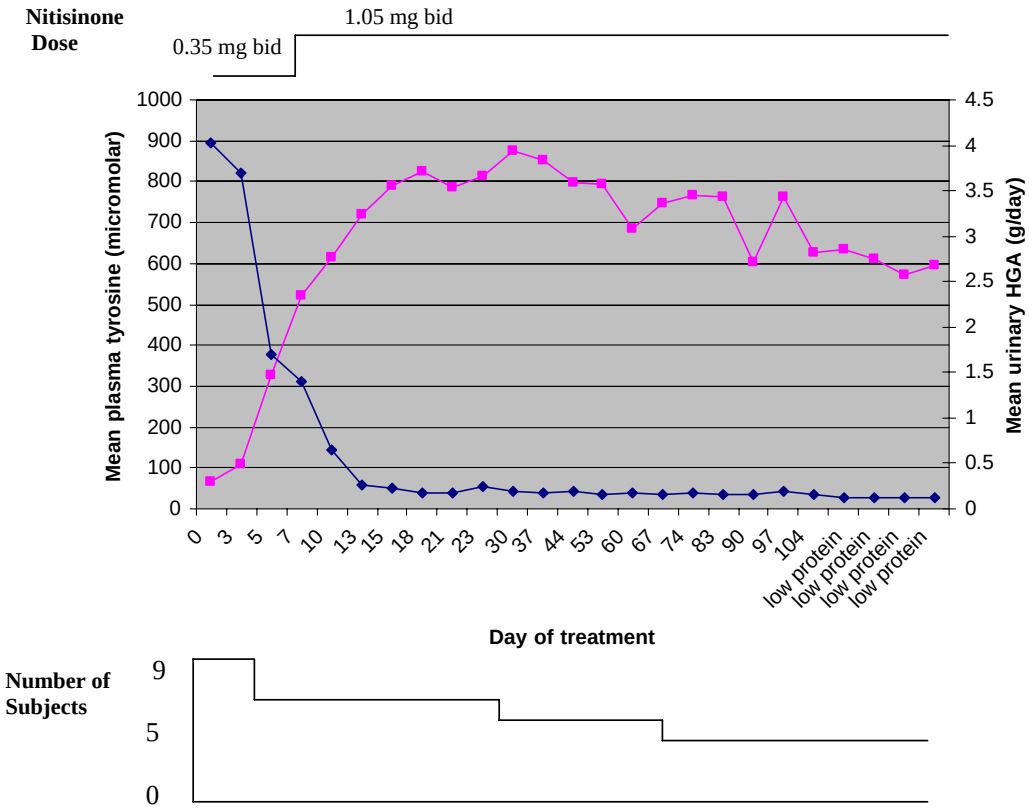

Fig. 3. Mean plasma tyrosine and urinary homogentisic acid levels in patients with alkaptonuria treated with nitisinone. Note the significant decrease in mean urinary homogentisic acid after week 2, accompanied by a rise in mean plasma tyrosine concentration.

0.35 mg bid, patients 4 and 5 displayed elevations in plasma tyrosine concentrations to 407 and 250 μmol/L, respectively. At a nitisinone dose of 1.05 mg bid, 7 patients had a mean plasma tyrosine levels of 760 ± 181 μmol/L (Fig. 3). While on a diet restricted to less than 40 g of protein per day during the last week of nitisinone treatment, all 5 remaining patients exhibited a lowering of their plasma tyrosine levels, with reductions ranging from 52 to 216 μmol/L (Table 2). The mean plasma tyrosine concentration was 603 ± 114 μmol/L on the protein-restricted diet and 755 ± 167 μmol/L on a regular diet (Table 2).

Table 2
Protein intake and plasma tyrosine levels of 5 nitisinone-treated alkaptonuria patients on regular and protein-restricted diets[a]

Patient	Protein intake (g/d)		Plasma tyrosine (mean ± SD) (μmol/L)	
	Regular diet[b]	Restricted diet[c]	Regular diet[b]	Restricted diet[c]
1	54.0	20.0	762 ± 137	564 ± 61
2	53.6	29.2	845 ± 89	672 ± 61
6	60.2	40.1	901 ± 92	685 ± 133
8	46.4	39.7	794 ± 131	675 ± 50
9	73.9	36.3	472 ± 82	420 ± 36
Mean	57.6 ± 10.3	33.1 ± 8.5	755 ± 167	603 ± 114*

[a] Nitisinone dosage was 1.05 mg bid.
[b] Regular diet was for 10 to 13 weeks.
[c] Restricted diet was for 1 week.
* $P > .05$.

3.6. Clinical and laboratory safety parameters

Clinical and laboratory findings at baseline and at specific times after starting nitisinone treatment are shown in Table 3. No signs of ocular toxicity were noted on weekly ophthalmologic examinations in any of the patients. Neurological examinations were normal throughout the treatment protocol, and MRI scans of the brain before and after treatment were normal. Laboratory measures of hematological, renal, hepatic, endocrine, and muscle function remained normal throughout the study. The high plasma tyrosine concentrations resulting from nitisinone treatment were not associated with changes in blood pressure or heart rate (Table 3). Of the 7 patients receiving nitisinone at 1.05 mg bid, all noticed that their urine no longer darkened on standing.

3.7. Adverse events

Four serious adverse events occurred and are described below.

1. On day 38 of treatment, patient 1 experienced vomiting and right flank pain. Repeated abdominal CT scans over the next 3 days revealed right and left hydroureter and hydronephrosis, and the patient passed several black kidney stones. A larger stone was removed from the left ureter under cystoscopy with lithotripsy on day 76. A temporary stent was

Table 3
Clinical and laboratory parameters for alkaptonuria patients at baseline and after nitisinone treatment[a]

Parameter	Baseline (n = 9)	Day 7 (n = 7)	Day 18 (n = 7)	Day 30 (n = 7)	Day 60 (n = 6)	Day 90 (n = 4)	Diet week[b] (n = 5)
Corneal toxicity	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Brain MRI	Normal						Normal
Weight (kg)	75.0 (60-111)			78.8 (62-110)	75.6 (63-109)		74.8 (62-108)
Systolic BP (mm Hg)	116 (103-145)	112 (98-128)	118 (92-134)	114 (95-146)	113 (90-146)	121 (98-134)	122 (94-127)
Diastolic BP (mm Hg)	69 (48-86)	68 (45-88)	68 (49-86)	67 (53-87)	66 (53-82)	70 (62-78)	71 (63-82)
Heart rate (beats/min)	78 (61-98)	78 (64-90)	83 (70-92)	83 (63-98)	82 (64-92)	87 (84-88)	88 (80-95)
Hemoglobin (g/dL)	13.3 (11.5-15.1)	12.3 (10.8-13.6)	12.2 (11-13.3)	11.7 (10.7-13.2)	12.2 (11.1-14)	12.0 (10.4-13.6)	12.7 (11.4-13.9)
White blood cell (kg/μL)	5.6 (4.5-6.5)	6.0 (4.8-7.2)	6.6 (5.7-7.9)	6.3 (4.9-7.7)	6.8 (4.2-9.8)	5.7 (4.7-6.7)	6.1 (3.5-8.1)
Platelets (kg/mm^3)	216 (112-322)	246 (167-399)	248 (160-371)	238 (161-384)	262 (177-498)	257 (166-396)	214 (157-241)
ESR (mm/h)	20 (2-51)	29 (11-50)	31 (13-56)	29 (14-45)	26 (13-41)	27 (14-39)	28 (13-38)
Sodium (mmol/L)	139 (137-140)	139 (137-140)	138 (136-140)	138 (137-139)	139 (135-141)	140 (139-140)	138 (137-139)
Potassium (mmol/L)	4.2 (3.9-4.7)	4.3 (3.9-4.7)	4.4 (3.8-5.1)	4.2 (3.4-4.7)	4.3 (3.9-4.8)	4.3 (4-4.9)	4.1 (3.5-4.8)
Total CO_2 (mmol/L)	27 (25-31)	28 (27-30)	28 (24-30)	27 (25-30)	29 (26-30)	29 (27-32)	29 (25-30)
Creatinine (mg/dL)	0.8 (0.6-0.8)	0.7 (0.6-0.8)	0.7 (0.6-0.8)	0.7 (0.6-0.9)	0.75 (0.6-0.9)	0.8 (0.7-0.8)	0.7 (0.7-0.8)
ALT (U/L)	20 (10-39)	27 (11-38)	30 (23-40)	46 (23-144)	27 (19-41)	29 (20-34)	28 (24-31)
AST (U/L)	22 (10-36)	25 (16-37)	25 (17-37)	29 (19-58)	22 (19-30)	25 (21-27	23 (16-29)
Total bilirubin (mg/dL)	0.9 (0.5-2.3)	0.6 (0.3-1.5)	0.7 (0.4-1.1)	0.7 (0.3-0.8)	0.7 (0.3-1.7)	0.5 (0.4-0.7)	0.6 (0.4-0.9)
Calcium (mmol/L)	2.27 (2.16-2.41)	2.28 (2.19-2.37)	2.26 (2.17-2.33)	2.21 (2.14-2.33)	2.26 (2.12-2.35)	2.27 (2.21-2.34)	2.25 (2.12-2.39)
Phosphorous (mg/dL)	3.9 (3.4-4.8)	4.1 (3.4-4.8)	4.4 (3.3-5.1)	4.1 (2.9-4.5)	4.1 (3.4-4.5)	4.3 (3.2-4.8)	4.3 (3.6-5.1)
Creatine kinase (U/L)	99 (34-183)	72 (29-125)	80 (27-121)	96 (43-155)	63 (22-99)	97 (57-133)	76 (25-113)

[a] Values are means with ranges in parentheses.
[b] Protein restricted to 40 g/day.

placed. With ongoing review of this case by the medical monitor and the IRB, the patient was maintained on nitisinone treatment throughout the course of these events.

2. Patient 3 had a history of long-standing aortic stenosis as a complication of her alkaptonuria. During the tenth week of nitisinone treatment, she complained of decreased activity tolerance and presyncopy. An echocardiogram on day 74 of treatment showed critical aortic stenosis with calcification of the aortic valve and the mitral annulus. However, these results were essentially unchanged compared with her echocardiographic findings upon enrollment. Her electrocardiogram was also unchanged, and no orthostatic alterations in blood pressure were apparent. Nevertheless, based upon the subjective findings, cardiac catheterization was performed and the patient's aortic valve was repaired. Coronary artery bypass grafting was performed at the same time. The operation necessitated discontinuation of nitisinone after 10 weeks of treatment.
3. Patient 4 had normal liver function tests upon enrollment, with a serum AST of 31 U/L and ALT of 34 U/L. Two days after starting nitisinone (0.35 mg bid), his AST was 49 U/L and his ALT 46 U/L. By day 5, his AST was 75 U/L and ALT 95 U/L. The bilirubin and alkaline phosphatase, as well as all other blood studies and the physical examination, remained unchanged. Because of protocol specifications classifying an ALT greater than 70 U/L as a severe adverse event, the patient was terminated from the protocol. Serum liver function values peaked on day 8 (ALT 117 U/L, AST 94 U/L) and returned to normal 3 weeks later. Infectious causes of hepatitis were excluded, and the serum creatine kinase, erythrocyte sedimentation rate, and C-reactive protein remained normal. The serum gamma-glutamyltranspeptidase was mildly elevated at 95 U/L (normal, 11-52). The patient had a history of heavy alcohol use in the past. Although he denied current alcohol use, he was receiving several other potentially hepatotoxic medications including lovastatin, ibuprofen, omeprazole, and oxycodone/acetaminophen.
4. Patient 7 entered the nitisinone protocol with an ALT of 25 U/L and an AST of 16 U/L. Serum liver enzymes remained low until day 32, when the ALT was 144 U/L and the AST 58 U/L. The serum gamma–glutamyltranspeptidase and alkaline phosphatase were never elevated, and other laboratory studies remained normal. Nitisinone was discontinued on day 32 because the protocol then considered an ALT greater than 100 U/L to represent severe toxicity. ALT levels returned to normal within 1 week after stopping the medication. The patient had a past history of heavy alcohol and drug use. He was also receiving a variety of medications at the time of the adverse event, including oxycodone/acetaminophen, docusate sodium, choline magnesium trisalicylate, fluticasone propionate/salmeterol inhalation powder, fluticasone nasal spray, and albuterol. He had completed a course of azithromycin for an episode of acute bronchitis 2 days before the elevation in transaminases.

3.8. Dietary analysis

Mean dietary protein at baseline and during the maintenance phase of the study while patients had an

unrestricted diet was 80.2 g/day; the median intake was 62.6 g/day (n = 7). All patients ingested more than 40 g of protein daily. The subset of patients who adhered to a restricted protein diet for the last week of the study had a mean protein intake of 57.6 g/day while on a regular diet and 33 g/day while on the low-protein diet (Table 3). The average intakes of phenylalanine and tyrosine were 3.5 and 2.9 g/day, respectively, on a regular diet and 1.4 and 1.2 g/day, respectively, on the restricted protein diet.

3.9. Individual patients

Patient 1, a 53-year-old woman, passed bilateral renal stones after 5 weeks of treatment (see adverse event 1) and developed transient mild elevations in hepatic transaminase levels (ALT 60 U/L, normal 6-41; AST 46 U/L, normal 9-34) after 10 weeks of treatment with nitisinone (1.05 mg bid). The transaminase values returned to normal without intervention. Despite the renal lithiasis, this patient continued to receive nitisinone for 13 weeks, including the final week on a protein restricted diet. During the study, this patient reported a generalized improvement in ROM and a decrease in joint pain. Quantitative spine flexion (Schober's test) showed an improvement in ROM from 10.5 to 11.4 cm. On musculoskeletal examination, the patient showed an increase in shoulder external rotation on the right from 62° to 74° and on the left from 56° to 72°. These increases are clinically significant and likely to result in functional improvement.

Patient 2, a 50-year-old woman, noticed that her skin was drier than usual, although her physical examination was normal. She also noticed decreased pigmentation of her cerumen after 3 weeks of high dose nitisinone treatment. This patient completed 14 weeks of nitisinone, including a final week of dietary protein restriction. She experienced decreased pain in her back and decreased pain medication requirements. She also reported improved energy levels while receiving nitisinone.

Patient 3, a 69-year-old woman, underwent aortic valve repair during the course of the protocol (see adverse event 2), causing discontinuation of nitisinone after 10 weeks of treatment. She complained of dry, flaky skin, and physical examination revealed several erythematous scaling lesions which improved with moisturizing lotion. She also reported flatulence and occasional loose stools that improved after stopping nitisinone. Liver transaminase levels were mildly and transiently elevated (ALT 48 U/L; AST 45 U/L). This patient reported improved ROM and decreased pain levels. She also had decreased pain medication requirements and reported improved energy levels.

Patient 4, a 46-year-old man, developed elevated transaminase levels (ALT 95 U/L, AST 75 U/L) after receiving nitisinone, 0.35 mg bid, for 5 days (see adverse event 3), and nitisinone was discontinued immediately.

Patient 5, a 41-year-old man, was enrolled at the same time as patient 4. He did not develop an adverse event related to nitisinone. However, because of the occurrence of the study's third adverse event in patient 4, the protocol was temporarily suspended and patient 5 was required to stop nitisinone treatment. After amendments were made to the protocol, this patient was not able to return to the NIH to resume the study.

Patient 6, a 64-year-old woman, developed mild anemia in the middle of the study and responded well to iron supplementation. She completed 13 weeks of nitisinone therapy, including a final week on a protein restricted diet. This patient reported decreased pain and improved ROM in her back and major joints. She also had decreased pain medication requirements. Schober's test showed an improvement in the range of spine flexion from 13.0 to 13.3 cm. On musculoskeletal examination, the patient showed an increase in knee flexion ROM from 105° to 112° on the right and from 100° to 113° on the left. The patient's left knee flexion contracture also decreased from 7° to 0°. The increase in knee extension is clinically significant.

Patient 7, a 35-year-old man, developed elevated transaminase levels on day 32 of treatment, at which point nitisinone was discontinued and not restarted (see adverse event 4). He reported decreased levels of pain throughout his back, hips, and knees, and improved ROM along with decreased pain medication requirements. Schober's test showed an improvement in the ROM from 12.4 to 13.4 cm. On musculoskeletal examination, the patient showed an increase in shoulder internal rotation from 48° bilaterally to full ROM on the right and 67° on the left, and an increase in shoulder external rotation from 62° to 74° on the right and from 56° to 72° on the left. However, the patient also showed a clinically significant decrease in knee flexion from 130° bilaterally to 92° on the right and 105° on the left.

Patient 8, a 55-year-old woman, developed mild anemia after approximately 1 month on study. The anemia improved with iron supplementation. She also had elevated transaminase levels after 8 weeks of nitisinone, with peak levels at 11 weeks (ALT 55 U/L; AST 43 U/L). This patient completed 15 weeks of nitisinone, including the final week on a protein restricted diet. She reported decreased pain in her large joints, lower back, and cervical area during treatment with nitisinone.

Patient 9, a 44-year-old man, completed 15 weeks of nitisinone including a final week on a protein-restricted diet. He reported no change in his level of pain or mobility during nitisinone treatment. Schober's test showed a decrease in the range of spine flexion from 13.2 to 12.2 cm. The patient had an epidural lumbar spine injection of cortisone because of a history of chronic, diffuse thoracic, and lumbosacral spine pain. On musculoskeletal examination, the patient showed a clinically significant increase in shoulder external rotation from 70° to 90° bilaterally.

4. Discussion

The devastating clinical repercussions of alkaptonuria include the destruction of vertebral disks, major joints, and cardiac valves, the formation of renal and prostatic stones,

and calcification of the coronary arteries [1]. Progressive degenerative arthritis (ochronosis) of the spine and large appendicular joints often leads to progressive impairments, such as kyphosis, arthritis, and loss of motion often requiring joint replacement. The combination of aging and these impairments frequently lead to progressive functional decline in daily routines and ambulation difficulties [19,20]. Disability, especially in those who are in the sixth decade, often necessitates a change in vocational status and avocational activity.

For these complications, which require 5 to 6 decades to develop [2], the only available treatments are symptomatic (ie, joint and valve replacements and surgical removal of stones). No therapy directed at the basic metabolic defect, whether ascorbic acid or dietary protein restriction, has proven beneficial in controlling the insidious accumulation of HGA and its oxidation byproducts within the tissues of alkaptonuria patients.

Theoretically, nitisinone administration should address the basic problem in alkaptonuria by inhibiting the production of HGA via the tyrosine degradation pathway (Fig. 1). Practically, nitisinone must first be shown safe and effective in this regard, and an appropriate dosage regimen must be determined. Fortunately, substantial experience exists with nitisinone use, albeit largely in children with tyrosinemia type I, a fatal liver disease due to deficiency of an enzyme in the distal portion of the tyrosine degradation pathway (Fig. 1).

Since 1992, nitisinone has proven well-tolerated for short- and long-term use in tyrosinemia type I at a dosage of approximately 1 mg/kg per day [9]. However, nitisinone operates several steps before the basic defect in tyrosinemia type I. Because nitisinone inhibits the very reaction that produces HGA, a lower dosage may suffice. In fact, our incremental dosing scheme revealed that as little as 0.03 mg/kg per day (2.1 mg/day) uniformly and reproducibly reduced urinary HGA by 94% to approximately 220 mg/day (Fig. 3). At the same time, plasma HGA fell below the limit of detectability in all 4 patients who had detectable levels at baseline. If high HGA concentrations in alkaptonuric joints are achieved by high circulating HGA levels, the lowering of plasma HGA levels by nitisinone provides critical support for the value of this drug in treating alkaptonuric joint disease. Furthermore, the salutary effects of treatment were achieved at plasma nitisinone levels of 1.4 μmol/L compared with the levels of 50 to 60 μmol/L achieved in tyrosinemia type I patients [17]. We employed twice daily dosing because that regimen was used in tyrosinemia type I, but a single daily dose should be sufficient. Single-dose half-time data obtained in normal adult volunteers have indicated prolonged effects of the drug, with a $t_{1/2}$ of 54 hours [21], and plasma nitisinone concentrations plateaued in our patients between weeks 1 and 12 on 1.05 mg bid.

A difficult question arose regarding whether to restrict the protein intake of alkaptonuria patients receiving nitisinone. Patients with tyrosinemia type I observe a phenylalanine and tyrosine-restricted diet during nitisinone treatment to minimize the elevation of plasma tyrosine, with a target plasma tyrosine concentration less than 400 μmol/L [8]. Complying with this restricted diet is extremely difficult, especially when started in adulthood. Nevertheless, we wanted to know how much a mild protein restriction would lower plasma tyrosine levels in our alkaptonuria patients, in case such a regimen would be useful in the future. In 5 alkaptonuria patients treated with nitisinone, protein restriction during the last week of treatment did reduce plasma tyrosine levels, from 755 to 603 μmol/L. Although all patients showed a reduction in their tyrosinemia, only patient 9 achieved plasma tyrosine levels in the 400 μmol/L range (Table 3). Furthermore, most of the patients had reported some difficulty adhering to the restricted diet.

A rare but important complication of nitisinone use is ophthalmic irritation. Affected patients experience itching, burning, photophobia, corneal damage, and, rarely, corneal crystal formation. These symptoms resolve within a few days of strict limitation of phenylalanine and tyrosine intake [22]. The ophthalmic involvement is considered related to elevated plasma tyrosine levels, based upon experience in other hypertyrosinemic states. A keratopathy accompanies elevated plasma tyrosine levels in humans with tyrosinemia type II, due to tyrosine aminotransferase deficiency (Fig. 1). In such patients, with initial tyrosine concentrations between 734 and 2826 μmol/L, ophthalmic findings resolved with dietary restriction of tyrosine sufficient to reduce tyrosine levels to 280 to 879 μmol/L [23]. On the other hand, some tyrosinemic states lack ophthalmic involvement. Persons with tyrosinemia type III, which results from 4-HPPD deficiency (Fig. 1), have plasma tyrosine levels of 500 to 1300 μmol/L but no ophthalmic complications [24]. In a series of 11 patients with tyrosinemia type I, treated with nitisinone and a low tyrosine diet, median plasma tyrosine levels ranged between 206 and 900 μmol/L; the highest level was 1410 μmol/L. In this group, no patient developed ocular symptoms and ophthalmic examinations were normal [22].

In our series of patients with alkaptonuria, treated with nitisinone for 3 to 4 months, there were no ocular complications observed on weekly ophthalmic examinations. This was true despite elevated mean plasma tyrosine levels of 472 to 901 μmol/L (Table 2) and no dietary restriction.

The absence of ocular complications in our patients could occur for several reasons. A possible explanation is that we did not study enough patients for a long enough time to see the toxicity of hypertyrosinemia. In addition, the plasma tyrosine levels in our patients may not have been as high, chronically, as in tyrosinemia type I patients experiencing corneal irritation. Another possibility is that the low dose of nitisinone we employed, and the low plasma levels achieved, may have reflected low concentrations of nitisinone in corneal epithelial cells, where local tyrosine production could potentially cause the keratopathy. Finally, our adult patients may be less susceptible than children to keratopathies related to high plasma tyrosine levels.

Another potential side effect of nitisinone consists of the development of neurological problems related to hypertyrosinemia. This has been proposed because nitisinone blocks 4-HPPD, deficiency of which results in tyrosinemia type III, a disorder possibly associated with neurological problems [8]. However, none of our patients developed any neurological complications, and MRI scans of the brain before and after the treatment regimen were normal (Table 3). In addition, there were no clinical or laboratory abnormalities attributable to nitisinone therapy.

Although there appeared to be no serious hypertyrosinemia-related side effects in this study, there were 4 severe adverse events. These bore different degrees of relatedness to the nitisinone treatment. We think that the passing of renal stones in patient 1, already present as a complication of alkaptonuria itself, could have been related to nitisinone treatment. It is possible that the decrease in urinary HGA concentration attendant to nitisinone treatment led to the dissolution of already present calculi and resulted in their passage. This could be considered both an adverse event and a long-term benefit of nitisinone therapy.

The worsening of aortic stenosis symptoms in patient 3 is unlikely to be related to nitisinone treatment. No objective signs of disease progression were appreciated, and the patient's presence in a hospital setting, with attention to medical complications, may have prompted this patient to recognize her long-standing symptoms.

We cannot eliminate the possibility that the elevations in serum transaminases seen in our patients were related to nitisinone treatment. Two patients, 4 and 7, had approximately 2- to 3-fold elevations, consistent with the range for "transaminitis" [25,26]; both had a strong history of alcohol use in the past. Both were also receiving multiple medications, some of which have known or potential hepatotoxicity. Several other patients reported mild alcohol intake during the course of treatment and were on similar types of medications, including statins. None of these patients had severe elevations of ALT or AST. In addition, 3 patients (1, 3, and 8) developed mild transient elevations of ALT and AST. We consider that the transaminase elevations seen in our patients were likely multifactorial effects related to prior liver damage and concomitant medications use, exacerbated by nitisinone.

Mild adverse events may or may not have been related to nitisinone use. These included anemia which resolved with iron supplementation and the development of dry skin with areas of scaling. We note that individuals with tyrosinemia type II, in addition to ocular findings, have skin manifestations. These include painful hyperkeratotic plaques on the palms, soles, and plantar surface of the digits [8]. The lesions in our patient were not painful and were not localized to the hands and feet, suggesting that they were likely unrelated to the hypertyrosinemia.

Subjective salutary effects included decreased joint pain and medication requirements and improved mobility in 6 patients. Incidental findings were recognition of a normal colored urine on standing and lighter cerumen in 7 patients.

In conclusion, nitisinone at a dosage of 1.05 mg bid reduced by 94% the urinary homogentisic acid excretion of patients with alkaptonuria. Four of 4 patients with detectable plasma HGA at baseline had undetectable HGA after nitisinone treatment; this effect was associated with increased plasma tyrosine levels, but minimal toxic effects. Specifically, ophthalmic and neurological complications did not occur during short-term therapy without dietary protein restriction. However, 2 patients with other risk factors for hepatotoxicity exhibited transiently elevated serum transaminase levels. It remains to be determined whether reducing daily HGA production to approximately 200 mg is sufficient to retard progression of ochronosis in alkaptonuria patients. Long-term clinical studies are planned to delineate the benefits of nitisinone in reducing arthritis and other complications of alkaptonuria. Close monitoring of treated individuals, with special attention to hepatotoxicity, will be critical to determine the full spectrum of side effects.

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