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A 3-year randomized therapeutic trial of nitisinone in alkaptonuria

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ABSTRACT

Alkaptonuria is a rare, autosomal recessive disorder of tyrosine degradation due to deficiency of the third enzyme in the catabolic pathway. As a result, homogentisic acid (HGA) accumulates and is excreted in gram quantities in the urine, which turns dark upon alkalization. The first symptoms, occurring in early adulthood, involve a painful, progressively debilitating arthritis of the spine and large joints. Cardiac valvular disease and renal and prostate stones occur later. Previously suggested therapies have failed to show benefit, and management remains symptomatic. Nitisinone, a potent inhibitor of the second enzyme in the tyrosine catabolic pathway, is considered a potential therapy; proof-of-principle studies showed 95% reduction in urinary HGA. Based on those findings, a prospective, randomized clinical trial was initiated in 2005 to evaluate 40 patients over a 36-month period. The primary outcome parameter was hip total range of motion with measures of musculoskeletal function serving as secondary parameters. Biochemically, this study consistently demonstrated 95% reduction of HGA in urine and plasma over the course of 3 years. Clinically, primary and secondary parameters did not prove benefit from the medication. Side effects were infrequent. This trial illustrates the remarkable tolerability of nitisinone, its biochemical efficacy, and the need to investigate its use in younger individuals prior to development of debilitating arthritis.

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1. Introduction

Sir Archibald Edward Garrod first described alkaptonuria (AKU, MIM 203500) in 1902 and coined the term "inborn error of metabolism" [1–3]. Today, alkaptonuria is widely recognized as an autosomal recessive disorder that results from deficiency of homogentisic acid dioxygenase, the third enzyme in the tyrosine degradation pathway (Fig. 1); consequently, homogentisic acid (HGA) accumulates in urine, plasma, cartilage and connective tissues [4–6]. In fact, alkaptonuria is named for the massive amount of HGA that accumulates in the urine, causing it to darken upon standing or upon exposure to alkaline conditions. Through ancillary pathways, HGA is oxidized to benzoquinones, which polymerize and bind to cartilage and connective tissues [7–11]. This causes an early-onset destructive arthritis of the spine and large joints, as well as valvular heart disease [5,12]. The orthopedic complications of alkaptonuria begin with vertebral disk narrowing in the thirties and progress to large joint destruction in the forties and fifties [13]. Kidney stones and, in men prostate stones also occur [5,13].

While the clinical features of alkaptonuria continue to be elucidated, there has been little advance in therapy; the mainstay of treatment remains symptomatic. In 1998, nitisinone (NTBC; 2-(2-nitro-4-fluromethylbenzoyl)-1, 3-cyclohexanedione), a potent inhibitor of para-hydroxyphenylpyruvic acid oxygenase, the second enzyme in the tyrosine catabolic pathway, was suggested as a potential treatment to block production of the offending HGA molecule (Fig. 1) [14]. Indeed, after receiving new drug approval for the treatment of hereditary tyrosinemia in 2002, nitisinone (Orfadin^{R}) was given to two alkaptonuria patients to establish proof of this principle [13]. In these two women, urinary HGA load was reduced by 95%, preparing the way

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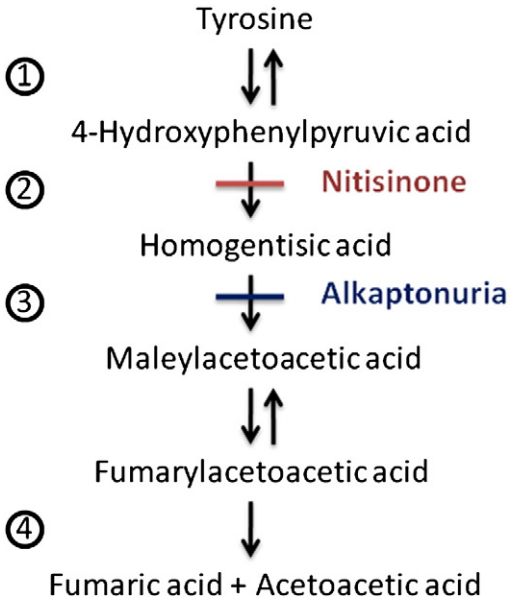

Fig 1. Tyrosine catabolic pathway. Designated enzymes: 1 = Tyrosine aminotransferase, 2 = 4-Hydroxyphenylpyruvic acid dioxygenase, 3 = Homogentisate 1,2-dioxygenase, 4 = Fumarylacetoacetic acid hydrolase. Nitisinone inhibits the 2nd enzyme, 4-hydroxyphenylpyruvic acid dioxygenase. Deficiency of the 3rd enzyme, homogentisate 1,2-dioxygenase causes alkaptonuria.

for additional clinical trials. A second study established appropriate dosing guidelines and evaluated safety [15]. These studies provided the foundation for a 3-year trial designed to confirm biochemical efficacy, investigate clinical outcomes, and further evaluate the safety of nitisinone in alkaptonuria patients.

2. Methods

2.1. Patient evaluations

Forty patients were enrolled in a prospective, randomized, IRB-approved protocol (clinicaltrials.gov # NCT00107783) between April, 2005 and April, 2009. Written, informed consent was obtained. Study length was 36 months from the time the fortieth patient was enrolled; since the enrollment period was 11 months, some patients participated for nearly 4 years. Twenty patients were randomized to the control group, receiving neither placebo nor surrogate medication. Twenty patients were randomized to the treatment group and received nitisinone 2 mg orally, once daily. Nitisinone (Orfadin®) is manufactured by Swedish Orphan Pharmaceuticals and was supplied to the patients through IND #71,780. The patients could not be masked to their treatment because their urine color revealed whether or not they were receiving nitisinone.

Participants were between the ages of 38 and 68 years at the study onset. Individuals were excluded if both hips had been replaced or they had significant, uncontrolled medical issues, a history of substance abuse, or psychiatric disease that would interfere with patient compliance.

All patients were evaluated initially and returned to the National Institutes of Health Clinical Center every 4 months for follow-up evaluation. Comprehensive blood and urine testing was performed at the initial visit and all subsequent visits. Urine studies included microscopic urinalysis and 24-hour collections for quantitative homogentisic acid, creatinine clearance, urine protein, glucose, minerals and electrolytes. Blood studies included plasma amino acids, plasma homogentisic acid and nitisinone levels, complete blood counts with differential and platelets, chemistry-20 panel, lipid panel, prothrombin and partial thromboplastin times, thyroid function testing, erythrocyte sedimentation rate, high sensitivity CRP, sex hormones, osteocalcin, calcitonin, and parathyroid hormone.

Initial evaluation included plain radiographs of the spine, chest, shoulders, hips and knees. A renal ultrasound and non-contrast CT of the abdomen and pelvis was performed to detect genitourinary stones. A baseline MRI/MRA of the brain was obtained. Bone densitometry using DXA (Hologic QDR4500, Bedford, MA) was performed to measure the central vertebral body bone mineral density. The values were compared to the Hologic reference data. An electrocardiogram and transthoracic echocardiogram assessed baseline cardiac function and extent of valvular disease. Baseline pulmonary function testing was performed. Plain radiographs, renal ultrasound, CT of the abdomen and pelvis, electrocardiogram and echocardiogram were repeated annually to monitor for adverse events. Magnetic resonance imaging and MRA of the brain, bone densitometry, and pulmonary function tests were repeated at the 3-year visit also to monitor for safety. These parameters were not primary or secondary outcome parameters. Echocardiograms and bone densitometry results are reported in the exploratory outcomes section.

An extensive musculoskeletal evaluation was performed at the initial visit and each subsequent visit. Evaluations included measurement of joint total range of motion (ROM) using goniometry of the shoulders, hips, and knees, Schober's test of spine flexion, functional reach assessment, timed get up and go measurement and 6 min walk tests. Full ophthalmologic evaluations were completed at each visit. Pain management was reviewed by the pain and palliative care service during each admission and was tailored to each individual's needs.

Upon enrollment, and annually thereafter, patients recorded 3-day food diaries that were reviewed by a registered dietician, coded, and analyzed using Nutrition Data System for Research software version 2008 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, to quantify total protein, phenylalanine, and tyrosine intake. Averages of each category were calculated for the control and nitisinone treatment groups at baseline. Collective averages from all subsequent visits for each group were also calculated.

A major concern related to nitisinone use is the occurrence of a corneal keratopathy. Children with hepatorenal tyrosinemia treated with nitisinone are routinely placed on a protein-restricted diet in an attempt to prevent corneal changes; compliance is variable. For our protocol, participants were allowed to continue their regular diet. There was no protein restriction for either the control or nitisinone treated groups, since adult compliance with such a diet was considered unlikely, published reports show no correlation between plasma tyrosine concentrations and the occurrence of corneal changes [16–18], and every case of nitisinone-related keratopathy was reversible upon stopping nitisinone treatment [16,17].

2.2. Outcome parameters and statistical considerations

The primary outcome parameter was total (internal + external) hip range of motion in the worse hip, i.e., the hip with the greatest loss of rotation at baseline. Secondary outcome parameters included Schober's test of spinal flexion, functional reach, timed get up and go, and 6 min walk tests.

Primary and secondary data were analyzed using repeated measures with random coefficients. The model contains terms for treatment*time effects, along with baseline age*time and baseline worse hip rotation*time effects, complete with random intercepts and slopes. One individual died 1 month after enrolling in the study, and two individuals had their second hip replaced during the course of the study. For statistical considerations, hip range of motion following death or hip replacement was recorded as zero. Two individuals receiving nitisinone were removed from treatment during the course of the study. Both individuals' data were analyzed in the treatment group under an intent-to-treat approach.