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RESEARCH REPORT

Relationship Between Serum Concentrations of Nitisinone and Its Effect on Homogentisic Acid and Tyrosine in Patients with Alkaptonuria

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Abstract *Background*: Alkaptonuria (AKU) is a serious genetic disease due to a defect in tyrosine metabolism, leading to increased serum levels of homogentisic acid (HGA). Nitisinone decreases HGA in AKU, but the concentration–response relationship has not been previously reported.

Objectives: To determine the relationship between serum concentrations of nitisinone and the effect on both HGA and tyrosine; secondly to determine steady-state pharmacokinetics of nitisinone in AKU patients.

Method: Thirty-two patients with AKU received either 1, 2, 4, or 8 mg nitisinone daily. Urine and serum HGA and serum tyrosine and nitisinone were measured during 24 h at baseline (before first dose) and after 4 weeks of treatment.

Results: Nitisinone pharmacokinetics (area under the curve [AUC] and maximum concentrations [C_{max}]) were dose proportional. The median oral clearance determined in all patients, irrespective of dose, was 3.18 mL/h·kg (range 1.6–6.7).

Nitisinone decreased urinary excretion of HGA in a concentration-dependent manner, with a maximum effect seen at average nitisinone concentrations of 3 μmol/L. The association between nitisinone and tyrosine concentrations was less pronounced. Serum levels of HGA at Week 4 were below the limit of quantitation in 65% of samples, which prevented determination of the relationship with nitisinone concentrations.

Conclusion: Nitisinone exhibits dose-proportional pharmacokinetics in the studied dosage interval. Urinary excretion of HGA decreases in a concentration-dependent manner, while the increase in tyrosine is less clearly related to nitisinone concentrations.

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Introduction

Alkaptonuria (AKU, OMIM reference 203500) is a rare autosomal recessive disorder caused by a deficiency in homogentisate 1,2-dioxygenase (HGD, EC 1.13.11.5), the third enzyme in the tyrosine metabolism pathway (Fig. 1). It converts homogentisic acid (HGA, 2,5-dihydroxyphenylacetic acid) to 4-maleylacetoacetic acid. Inability to metabolize HGA leads to urinary excretion of at least 90% of the compound, or 3–6 g per day, in patients with AKU (Garrod

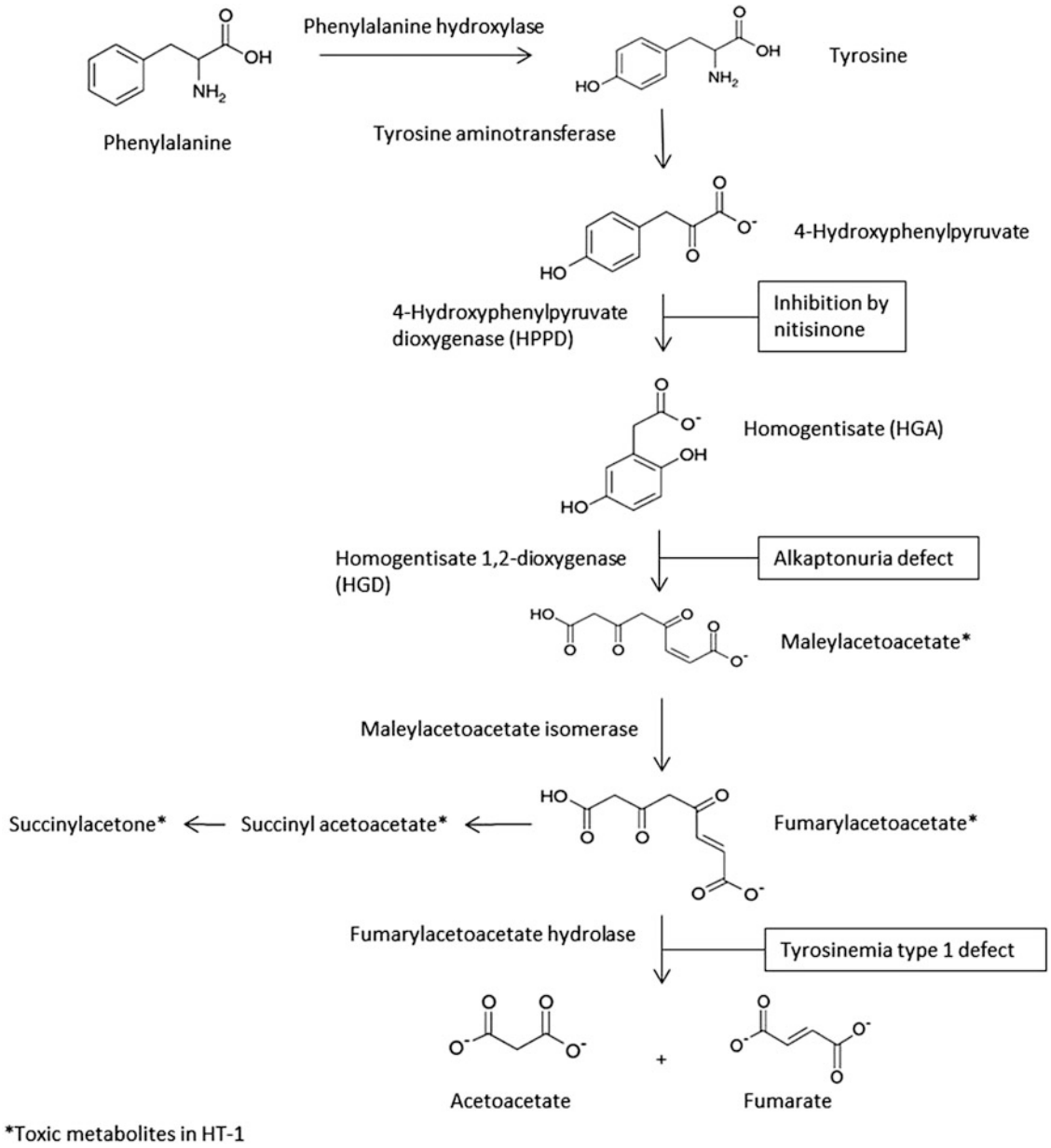

Fig. 1 Schematic presentation of tyrosine metabolism, with AKU and HT-1 defects, and nitisinone site of action

1902; Lustberg et al. 1969; Introne et al. 2011). Despite this pronounced renal elimination, some HGA is oxidized to a melanin-like polymeric pigment via benzoquinone acetic acid. The pigment is deposited in connective tissue, especially in cartilage, a process called ochronosis (Zannoni et al. 1969). This leads to early onset arthritis of the spine and synovial joints and other debilitating symptoms (O'Brien et al. 1963; Ranganath et al. 2013). There is currently no approved pharmacological treatment for AKU.

Nitisinone (also known as NTBC, an abbreviation of its full chemical name), a potent competitive inhibitor of the enzyme 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) (Fig. 1), has been proposed as a treatment for AKU (Anikster et al. 1998; Lock et al. 2014). This drug is registered in several countries for the treatment of hereditary tyrosinemia type I (HT-1, OMIM reference 276700) which is caused by a defect further downstream in the tyrosine metabolism pathway compared to AKU. In HT-1, nitisinone prevents the formation of the highly toxic metabolites maleylacetoacetate, fumarylacetoacetate, and succinylacetone (Lindstedt et al. 1992), and in combination with a tyrosine-restricted diet, it serves as a successful therapeutic intervention. In AKU, nitisinone can effectively reduce HGA and prevent ochronosis in mice (Suzuki et al.

1999; Preston et al. 2013) and reduce HGA in patients (Introne et al. 2011).

A 4-week dose–response study ("SONIA 1") in patients with AKU to investigate the effect of nitisinone on 24-hour urinary excretion of HGA (u-HGA_{24}), and serum concentrations of HGA and tyrosine, as well as the safety of this treatment has been performed and recently reported (Ranganath et al. 2014). In short, u-HGA_{24} decreased in a dose-dependent manner, with the highest dose (8 mg) reducing u-HGA by more than 98%. The fasting predose serum concentrations of HGA (s-HGA) also decreased, and predose serum tyrosine increased with increasing doses. All doses were well tolerated by the patients.

Previous studies with nitisinone have not determined its pharmacokinetics (PK) after repeated dosing nor the relationship between serum exposure and pharmacological effect. Therefore, the SONIA 1 study also aimed at determining nitisinone PK at steady state and to test for PK dose proportionality, as well as describing the relationship between serum concentrations and the effect on HGA and tyrosine in patients with AKU.

Materials and Methods

Patients

Patients with AKU were verified by increased urine HGA excretion and *HGD* gene mutation identification and were eligible for participation. Detailed inclusion and exclusion criteria have been presented earlier (Ranganath et al. 2014).

Study Design and Treatments

SONIA 1 was an international, multicenter, open-label, parallel-group, randomized study in 40 AKU patients, of which 32 (8 per dose group) received nitisinone in doses of either 1, 2, 4, or 8 mg nitisinone once daily for 4 weeks, administered as an oral suspension containing 4 mg nitisinone per milliliter. Another eight patients served as an untreated control group (data not included).

Measurements

Measurement of serum nitisinone, s-HGA, and tyrosine was performed over 24 h after 4 weeks of treatment (Week 4). The first sample was collected after breakfast, just prior to administration of the last dose of nitisinone. Subsequent postdose samples at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, and 24 h were taken. Full s-HGA and tyrosine profiles were also determined at baseline, before administration of the first dose of study medication.

Measurement of u-HGA_{24} was performed at baseline and at Weeks 2 and 4 (see details in Ranganath et al. 2014). Only baseline and Week 4 data are discussed here, since no 24-hour serum profiles were collected at Week 2.

The urine and serum samples were analyzed according to the liquid chromatography–mass spectrometry methods described by Hughes et al. (2014, 2015).

Calculations and Statistics

Based on nitisinone serum concentrations, the following PK variables were calculated using Phoenix WinNonlin 6.3 (Certara L.P., St. Louis, MO, USA): maximum and minimum concentrations (C_{max}, C_{min}), the area under the 24-hour concentration vs. time curve (AUC_{24}, calculated by the linear trapezoidal rule), and oral clearance (CL/F, calculated as dose/AUC_{24}). The same software was used to determine C_{max} and AUC_{24} for s-HGA and serum tyrosine.

The average concentrations during 24 h (C_{av}) were calculated as $AUC_{24}/24$ for nitisinone, s-HGA, and tyrosine.

The dose proportionality of nitisinone AUC_{24} and C_{max}, respectively, was evaluated using a power model (Gough et al. 1995).

Results

Demographics

Demographics for all 40 patients in SONIA 1 have previously been presented (Ranganath et al. 2014). The 32 patients who were treated with nitisinone, and are included in this report of the exposure–response relationships, had a mean age of 47.5 years (range 19–62 years), mean body weight of 79.9 kg (59–112 kg), and mean height of 168.0 cm (164–180 cm). Twenty-three of these 32 patients (72%) were male.

Nitisinone Pharmacokinetics

The AUC_{24} and C_{max} data indicate that exposure to nitisinone is dose proportional within the studied dose range, as indicated by the 95% confidence intervals for the regression coefficients, 0.90–1.26 for AUC_{24} and 0.85–1.21 for C_{max} (Table 1). The oral clearance was similar across the dose groups and ranged from 1.6 to 6.7 mL/h·kg with an overall median of 3.18 mL/h·kg.

The median C_{max}/C_{min} ratio for all patients was 1.80, ranging from 1.3 to 3.8 in individual patients (data not shown). Mean serum concentration profiles for the four doses are shown in Supplementary Figure S1.

Table 1 Dose proportionality of nitisinone PK parameters

	Estimate (beta)	95% CI lower	95% CI upper
AUC_{24} (μmol · h/L)	1.08	0.90	1.26
C_{max} (μmol/L)	1.03	0.85	1.21

AUC_{24} area under the 24-hour serum concentration profile, *CI* confidence interval, *C_{max}* maximum serum concentration

Relationship Between Nitisinone Exposure and the Effect on HGA and Tyrosine

At baseline, u-HGA_{24} ranged from 14 400 to 69 500 μmol across all dose groups (Ranganath et al. 2014). At Week 4, u-HGA_{24} decreased in relation to nitisinone concentrations up to a C_{av} of about 3 μmol/L, with no further decrease in u-HGA_{24} above this level (Fig. 2). In the seven patients with concentrations above 3 μmol/L (all receiving 8 mg nitisinone), median u-HGA_{24} was 135.7 μmol (range 83–213 μmol). The change in u-HGA_{24} from baseline was 99.4–99.7% in these patients.

Serum concentrations of HGA at Week 4 were below the lower limit of quantification (LLOQ, 3.1 μmol/L) in 65% of all samples from treated patients, and only four patients (three on 1 mg and one on 2 mg nitisinone) had HGA above the LLOQ in all samples. The number of patients with s-HGA below the LLOQ increased with increasing nitisinone dose, and therefore, no analysis of individual s-HGA data vs. nitisinone concentrations has been performed. However, for all patients on 1 mg, C_{av} could be reasonably well estimated, and a comparison of the median results at baseline and Week 4 (Table 2) indicates that a dose of 1 mg nitisinone decreased s-HGA by approximately 88%.

Median C_{av} values for serum tyrosine increased in relation to dose (Table 2), but no clear relationship between nitisinone exposure and individual tyrosine data could be seen (Fig. 2). At the highest nitisinone serum concentrations, above 5 μmol/L, daily average tyrosine concentrations were in the range of 800–1,000 μmol/L. However, all treated patients had daily tyrosine averages above 500 μmol/L, and values above 800 μmol/L were seen at nitisinone concentrations as low as 0.7 μmol/L and a dose of 1 mg.

Similar profiles were seen for serum tyrosine and HGA at baseline. After intake of breakfast (time = 0), mean serum tyrosine concentrations decreased from 58 μmol/L right after breakfast to 50 μmol/L at 4 h, thereafter to increase to a maximum of 65 μmol/L at 12 h. The s-HGA profile followed the tyrosine profile, except that it showed a slight increase between 0 and 2 h. Mean s-HGA concentrations after breakfast ($t = 0$) were 29 μmol/L, and maximum concentrations at 12 h were 47 mmol/L (Supplementary Figure S2).

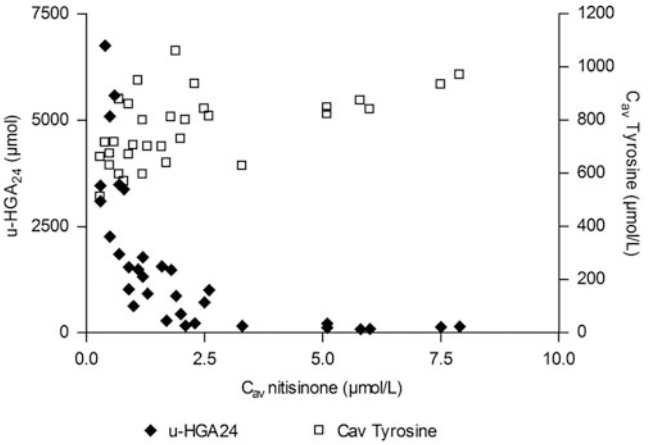

Fig. 2 Individual urinary excretion of HGA and daily average serum tyrosine concentrations vs. daily average serum nitisinone concentrations at Week 4 in AKU patients treated with nitisinone ($N = 32$)

Discussion

The nitisinone serum concentrations increased in proportion to dose, as shown by the power model analysis of AUC and C_{max} data and supported by the similar clearance in all dose groups The median oral clearance in all 32 patients, 3.2 mL/h·kg, is in reasonable accordance with the data in HT-1 patients; 4.0 mL/h·kg (0.0956 L/kg · day). The fluctuation in nitisinone serum concentrations (C_{max}/C_{min} ratio) was relatively low, supporting previous reports of a long half-life (Hall et al. 2001).

We found that nitisinone decreased urinary excretion of HGA in a concentration-dependent manner, up to nitisinone serum concentrations of about 3 μmol/L. This concentration was reached with a dose of 8 mg daily in seven of the eight patients in that dose group, and these had a reduction in u-HGA_{24} from baseline of at least 99.4%. Despite this near 100% decrease in u-HGA, the amount excreted at the highest nitisinone concentrations, or dose, is still about 50 times higher than the highest amount (2.92 μmol/24 h) found in a recent study in 22 normal subjects, using the same analytical method as in our study (Davison et al. 2014). It is therefore possible that even higher doses and concentrations of nitisinone could lead to a complete normalization, which would correspond to a decrease in u-HGA from baseline of 99.98–100.00% in individual patients.

Assuming that the reduction in u-HGA reflects the degree of inhibition of HPPD, the results indicate that the enzyme is almost completely inhibited at a nitisinone concentration of 3 μmol/L. Nitisinone is extensively bound to serum albumin with a free fraction of about 4.5% at

Table 2 Serum concentration data (median and range) for HGA, tyrosine, and nitisinone in patients with AKU treated with nitisinone ($N = 8$ per dose group)

	Time	1 mg	2 mg	4 mg	8 mg
Nitisinone					
C_{av} (μmol/L)	Week 4	0.47 (0.3–0.8)	1.04 (0.7–1.6)	1.94 (1.3–2.6)	5.49 (2.3–7.9)
C_{min} (μmol/L)	Week 4	0.30 (<0.2–0.5)	0.70 (0.5–1.2)	1.40 (1.0–2.3)	4.55 (1.5–6.2)
C_{max} (μmol/L)	Week 4	0.70 (0.4–1.1)	1.55 (0.9–1.9)	2.50 (1.9–3.0)	7.30 (3.3–9.7)
CL/F (mL/h·kg)	Week 4	2.80 (1.8–6.7)	3.49 (1.7–4.6)	3.49 (1.9–5.2)	2.27 (1.6–5.0)
HGA					
C_{av} (μmol/L)	Baseline	32.96 (22.5–44.1)	34.21 (25.1–29.1)	37.69 (28.9–49.9)	38.61 (25.2–47.7)
	Week 4	3.93 (0.5–7.3)	3.32 (<3.1–5.8)	<1.3 (<1.3–1.3)	<0.4 (<0.4–0.4)
C_{max} (μmol/L)	Baseline	51.30 (40.8–69.9)	52.85 (39.8–72.9)	61.70 (48.4–88.3)	57.05 (38.8–78.2)
	Week 4	6.60 (4.3–10.7)	5.10 (<3.1–7.5)	3.40 (<3.1–4.7)	<3.1 (<3.1–3.2)
Tyrosine					
C_{av} (μmol/L)	Baseline	56.0 (50–61)	62.6 (46–79)	58.0 (47–75)	59.3 (50–82)
	Week 4	667.3 (511–879)	702.1 (596–948)	805.9 (639–1,059)	860.0 (627–970)
C_{max} (μmol/L)	Baseline	71.0 (57–113)	77.5 (59–100)	80.5 (57–114)	75.0 (62–120)
	Week 4	721.0 (594–978)	835.5 (662–1,010)	889.0 (696–1,155)	984.0 (715–1,066)

C_{av} average serum concentration during the 24-hour dosing interval ($C_{av} = AUC_{24}/24$), C_{min} minimum serum concentration, C_{max} maximum serum concentration, *CL/F* oral clearance

normal serum albumin concentrations (unpublished data). Thus, a total concentration of 3 μmol/L corresponds to an unbound concentration of approximately 135 nmol/L. This is in agreement with the maximum inhibition of HPPD by nitisinone seen in vitro (Ellis et al. 1995).

The results indicate a dose–response relationship between s-HGA and nitisinone, as the number of patients with s-HGA below the LLOQ increased with dose. An analysis of the relationship between C_{av} for s-HGA and nitisinone concentrations could not be performed due to the nonquantifiable s-HGA values. The decrease in s-HGA, from baseline to Week 4, which could be estimated for the 1-mg dose (approximately 88%), is in line with the previously reported decrease in u-HGA_{24} for that dose (Ranganath et al. 2014).

The s-HGA profile at baseline shows the expected increase with feeding due to a continuous supply of tyrosine. The circulating concentrations of both HGA and tyrosine show a fall after the last meal and true fasting late in the evening and night, reaching a nadir prior to feeding in the morning.

At baseline, serum tyrosine was normal in the AKU patients, despite the defect in HGD. Normal, non-AKU subjects had fasting serum tyrosine concentrations up to 88 μmol/L (Davison et al. 2014). The maximum daily average in AKU patients at baseline was 82 μmol/L.

In patients with AKU, renal clearance of HGA is 4–5 times creatinine clearance suggesting active secretion of HGA (Lustberg et al. 1969). An ongoing study of proximal tyrosine metabolites in samples from SONIA 1 may throw more light on this issue, but such data are as yet unavailable. The importance of a normal renal function has also been illustrated in an AKU patient with renal failure. He had exacerbated ochronosis and s-HGA levels about twice those of other AKU patients. After a renal transplant, the levels decreased to those normally seen in AKU, and u-HGA increased by 2–3 g per day (Introne et al. 2002).

Inhibiting HPPD leads to pronounced tyrosinemia even at low nitisinone doses. High serum concentrations of tyrosine are known to cause eye lesions in some patients due to high concentrations in the aqueous humor (Hanhart 1947; Lock et al. 2014). In the treatment of HT-1 with nitisinone, it is therefore recommended that serum tyrosine be kept below 400–500 μmol/L, by using a diet low in tyrosine and phenylalanine (Mayorandan et al. 2014; SmPC for Orfadin). Even the lowest dose in our study, 1 mg daily, resulted in tyrosine levels above this limit in every patient, indicating that diet restrictions may be required also if treating AKU patients with nitisinone, at least if such patients would develop eye symptoms due to tyrosinemia.

In conclusion, nitisinone exhibits dose-proportional pharmacokinetics in the studied dosage interval. Urinary excretion of HGA decreases in a concentration-dependent manner, while the increase in tyrosine is less clearly related to nitisinone concentrations.

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Synopsis (Take-Home Message)

In patients with alkaptonuria, nitisinone decreases urinary excretion of HGA in a concentration-dependent manner, while the increase in serum tyrosine is less clearly related to nitisinone concentrations.

Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (6).

Informed consent was obtained from all patients before being included in the study.

Animal rights: Not applicable for this clinical study.

The study EudraCT number is 2012-005340-24 and is registered at ClinicalTrials.gov with number NCTO1828463 and the title "Dose Response Study of Nitisinone in Alkaptonuria (SONIA1)."

Conflict of Interest

B Olsson and J Szamosi are Sobi employees and shareholders. All other authors declare that they have no conflict of interest.

Contributors

BO, LRR, AKH, TFC, JR contributed to the study design.

LRR, JR undertook medical procedures.

ATH, AMA developed analytical methods and analyzed study samples.

TFC, EEP, JS, BO contributed to the statistical analyses including PK calculations.

BO drafted the first version of the manuscript.

All authors contributed to the interpretation of data and writing and revision of the manuscript.

All authors approved the manuscript for publication.

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