EXTENDED REPORT

Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1): an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-h urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment

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ABSTRACT

Background Alkaptonuria (AKU) is a serious genetic disease characterised by premature spondyloarthropathy. Homogentisate-lowering therapy is being investigated for AKU. Nitisinone decreases homogentisic acid (HGA) in AKU but the dose-response relationship has not been previously studied.

Methods Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1) was an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study. The primary objective was to investigate the effect of different doses of nitisinone once daily on 24-h urinary HGA excretion (u-HGA_{24}) in patients with AKU after 4 weeks of treatment. Forty patients were randomised into five groups of eight patients each, with groups receiving no treatment or 1 mg, 2 mg, 4 mg and 8 mg of nitisinone.

Findings A clear dose-response relationship was observed between nitisinone and the urinary excretion of HGA. At 4 weeks, the adjusted geometric mean u-HGA_{24} was 31.53 mmol, 3.26 mmol, 1.44 mmol, 0.57 mmol and 0.15 mmol for the no treatment or 1 mg, 2 mg, 4 mg and 8 mg doses, respectively. For the most efficacious dose, 8 mg daily, this corresponds to a mean reduction of u-HGA_{24} of 98.8% compared with baseline. An increase in tyrosine levels was seen at all doses but the dose-response relationship was less clear than the effect on HGA. Despite tyrosinaemia, there were no safety concerns and no serious adverse events were reported over the 4 weeks of nitisinone therapy.

Conclusions In this study in patients with AKU, nitisinone therapy decreased urinary HGA excretion to low levels in a dose-dependent manner and was well tolerated within the studied dose range.

Trial registration number EudraCT number: 2012-005340-24. Registered at ClinicalTrials.gov: NCT01828463.

INTRODUCTION

Alkaptonuria (AKU) is a serious, autosomal recessive, multisystem disorder[1–3] affecting approximately 1 in every 250 000 people,[4] although some countries such as Slovakia and the Dominican Republic have a higher prevalence rate of around 1 in 19 000.[5 6] Morbidity in AKU is caused by increased levels of homogentisic acid (2,5-dihydroxyphenylacetic acid, HGA) due to a deficient enzyme, homogentisate 1,2-dioxygenase (HGD).[7] Despite efficient urinary excretion of HGA,[4] some of it is oxidised to a melanin-like polymeric pigment via benzoquinone acetic acid. This pigment polymer is deposited in connective tissues, particularly cartilage, a process termed ochronosis,[8] leading especially to severe premature arthritis with an early onset, affecting the spine and synovial joints, large and small.[3 4]

Current treatments are limited to palliative analgesia and arthroplasty.[9] Nitisinone, a competitive inhibitor of the enzyme 4-hydroxyphenyl-pyruvate dioxygenase (HPPD), decreases the formation of HGA.[10 11] Nitisinone has been used for the treatment of hereditary tyrosinaemia type 1 (HT-1) for more than 20 years.[10 11] HT-1 is due to accumulation of highly toxic metabolites (maleylacetoacetate and fumarylacetoacetate among others) further downstream from HGA, which leads to progressive liver and renal failure, and is fatal if not treated.[10 11]

It is hypothesised that if HGA levels are reduced before the onset of overt ochronosis, this might prevent the development of the debilitating features of AKU. Nitisinone has been shown to reduce plasma HGA levels and urinary excretion in humans,[12 13] and in a mouse model of AKU.[14] However, it has not undergone any formal clinical development for AKU, although three published investigator-initiated studies have been completed.[4 12 13] The 3-year study by Introne *et al* showed a significant reduction in urine HGA excretion by about 95% using a daily dose of 2 mg of nitisinone but no significant effect on clinical parameters.[13] One possible factor for the inconclusive effect on clinical parameters may be the use of a suboptimal dose.

AKU is a very slowly progressive disease with overt manifestations appearing after more than three decades of what is an inherited disease present from birth. The present study was only designed to investigate the metabolic effects of nitisinone, namely the relationship between different doses of nitisinone and 24-h urinary HGA excretion (u-HGA_{24}), and serum HGA (s-HGA) and tyrosine.

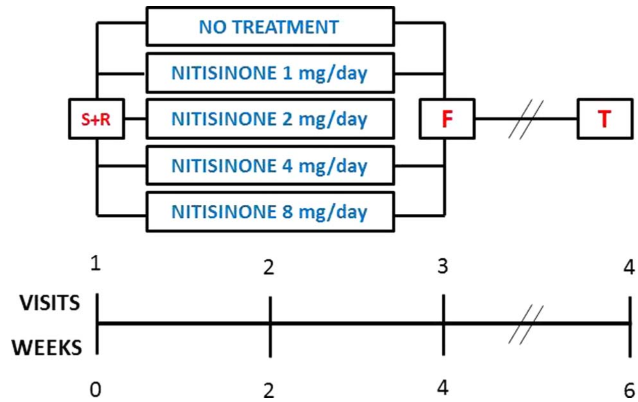

Figure 1 Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1) study design. The study consisted of two main periods: treatment and follow-up. After screening, patients were randomised at baseline (1:1:1:1:1) to no treatment (control), and oral daily doses of nitisinone of 1 mg, 2 mg, 4 mg and 8 mg. The treatment period consisted of 4 weeks, during which study drug was administered. At 6 weeks a follow-up telephone call concluded the study. (Abbreviations: S+R=screening, baseline and randomisation visit; F=final treatment visit; T=telephone follow-up visit).

METHODS

Patients

Patients with a well-documented AKU verified by increased urine HGA excretion and who were at least 18 years old were eligible for inclusion in the study. Details of inclusion and exclusion criteria are described in the supplementary appendix (table S1). In all patients, diagnosis of AKU was confirmed by *HGD* gene mutation identification performed during the study (data not shown).

Study design and intervention

The study was a randomised, open-label, parallel-group design with a no-treatment control group. Patients were randomised to receive either 1 mg, 2 mg, 4 mg or 8 mg nitisinone once daily or no treatment (control). Forty patients were randomised, equally distributed among the groups (eight patients per group). The treatment period consisted of 4 weeks, during which the study drug was administered. At 6 weeks a follow-up telephone call concluded the study. The study design is summarised in figure 1 and study procedures described in online supplementary table S2.

The study was open label, since it is not feasible to blind a study with HGA-lowering treatment in AKU. One of the cardinal signs of AKU is urine darkening on standing as HGA is oxidised. Patients could therefore easily know whether they were on nitisinone or not. Furthermore, any personnel involved at the investigative sites who were involved in the processing of urine samples would also be able to see this difference. However, the only subjective reporting in the study was that of adverse events (AEs). Patients were requested to maintain stable dietary habits during the 4-week study period in order not to change their dietary protein intake.

Altering the dietary protein intake could affect s-HGA concentrations and urine HGA excretion; the study was carried out in free-living patients with AKU with no intervention to change the diet in order to observe the effect of nitisinone alone on HGA and tyrosine not influenced by dietary change.

Rationale for dose selection

The choice of doses used in the present study was based on current knowledge regarding the HGA-lowering effect of nitisinone in AKU. In one previous study, mean u-HGA_{24} was reduced from 4 g/day to 230 mg/day using a dose of 2.1 mg of nitisinone daily.[12] In a 3-year study, using a dose of 2 mg daily, mean u-HGA_{24} decreased from 5.1 g/day to values ranging from 113 mg/day to 203 mg/day during the course of the study, which on average corresponded to a 95% decrease.[13] We wanted to investigate the effect of nitisinone on HGA at higher doses than the ones used in previous studies, and to find a dose that reduces HGA by close to 100%. At the same time, we were interested in determining the effect of nitisinone on serum tyrosine (s-Tyr) levels at a dose lower than 2 mg daily. Therefore, doses of 1 mg, 2 mg, 4 mg and 8 mg were used in this study.

The investigational medicinal product (IMP), which was a suspension of nitisinone (Orfadin) containing 4 mg/mL administered in the morning was used as it allowed easy administration of the selected doses. The daily dosing frequency in our study is based on the long half-life of nitisinone.[11]

Randomisation procedures

Patients were randomly assigned to one of five groups, in a 1:1:1:1:1 ratio stratified by study centre using randomly permuted blocks. Results for HGA and tyrosine were not accessible to the medical monitors, sponsor personnel or study site personnel until study completion.

Prior and concomitant therapy

Patients were allowed to continue on any chronic medication and any changes during the study were recorded, from the time of the screening and randomisation visit until the follow-up telephone call. Patients were not allowed to have used nitisinone within the 60 days prior to randomisation.

Treatment compliance

Product accountability records were kept by the pharmacy and investigator. All unused IMP was returned to the clinical study sites and measured. The amount consumed was compared with the expected consumption for the randomised dose.

Chemical measurements

Urine sample collection and handling

At baseline, and Weeks 2 and 4, urine was collected over 24 h into 2.5 L bottles containing 30 mL of 5N H_2SO_4 and stored away from bright light in cool conditions. The weight of the collected urine was recorded and used as the volume in the calculations of u-HGA_{24} assuming a density of 1 g/mL. An aliquot of the collected urine was frozen and kept at −20°C until analysis.

Serum sample collection and handling

Measurements of s-Tyr and s-HGA concentrations were performed at Weeks 0, 2 and 4. At each visit one sample was collected predose in fasting patients. Blood samples were collected in non-gel serum tubes. An aliquot of serum was immediately acidified using perchloric acid (10% v/v 5.8M), and kept frozen at −20°C until analysis.

Analyses of HGA and tyrosine

The concentrations of tyrosine and HGA in serum and urine were measured by liquid chromatography tandem mass spectrometry.[15] All analyses were performed on an Agilent 6490 Triple Quadrupole mass spectrometer with Jet-Stream electrospray ionisation coupled with an Agilent 1290 infinity Ultra High Performance Liquid Chromatography (UHPLC) pump and HTC autosampler. This method incorporates reverse-phase chromatographic separation on an Atlantis C18 column (100 mm×3.0 mm, 3 μm). Initial conditions of 80:20 water:methanol with 0.1% formic acid (v/v) increased linearly to 10:90 over 5 min. Matrix-matched calibration standards and quality controls were used with appropriate isotopically labelled internal standards. Quantitation was achieved in multiple reaction mode with two product ion transitions for tyrosine (positive ionisation) and HGA (negative ionisation). Samples were prepared by dilution in a combined internal standard solution (final concentrations of 0.4 μmol/L $^{13}C_6$-HGA and 2 μmol/L d_2-tyrosine in 0.1% formic acid (v/v) in deionised water). All serum and urine quantitation analyses were performed by the Department of Clinical Biochemistry and Metabolic Medicine at the Royal Liverpool University Hospital.

End points

The primary end point was the u-HGA_{24} in patients with AKU after 4 weeks of nitisinone treatment. Secondary end points supporting the primary objective included u-HGA_{24} after 2 weeks, as well as the urine HGA excretion adjusted per mol of urine creatinine ratio at Weeks 2 and 4.

Secondary end points included the predose s-HGA and s-Tyr concentrations at Weeks 2 and 4.

Safety assessment

At each visit, AEs and laboratory values were recorded. Routine laboratory processes at each clinical study site were employed to measure biochemistry and haematology profiles. At each visit a corneal slit lamp examination was performed to check for possible corneal toxicity. Tyrosine can occasionally cause idiosyncratic reversible corneal dendritiform keratopathy and skin rash with no consistent nitisinone dose or tyrosine concentration relationships.[16]

Statistical analysis

The primary variable, u-HGA_{24} at Week 4, was analysed using a mixed model for repeated measures (MMRM). The model included the study site, treatment group, visit, and the interaction between treatment group and visit as fixed factors and the baseline u-HGA_{24} as a covariate. Model-based least square means and associated 95% CIs for each treatment group were calculated. As the distribution of u-HGA_{24} was skewed, log-transformation was applied prior to analysis. The estimates were then back transformed to the original scale and thus the estimates correspond to adjusted geometric means. In addition, post hoc pairwise comparisons were performed with no adjustment for multiplicity. Linear contrasts from the MMRM model were used for these comparisons. The analyses were conducted on the full analyses set, using the statistical software SAS V.9.3.

Analysis of safety and tolerability data

Adverse events

All AEs during the study were coded using the Medical Dictionary for Regulatory Activities (MedDRA V.16.0). The incidence of AEs was summarised in frequency tables. The changes in safety laboratory parameters from baseline to all postbaseline visits were summarised by treatment group and visit using descriptive statistics.

Study funding and oversight

Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1) is part of the DevelopAKUre programme, which has received funding from the European Union Seventh Framework programme. The European Commission (EC) had no direct participation in any aspect of design and conduct of the study, drug supply or reporting. The objective of the clinical development programme is to investigate the possibility of an effective and safe treatment of AKU.

The study was conducted at two sites, Liverpool (UK) and Piešt'any (Slovakia) from May to October 2013. Data were recorded by investigators at each site, collected, and monitored by the Contract Research Organization PSR Group (Amsterdam, the Netherlands). The protocol and amendments were approved by the relevant ethics review boards and national regulatory authorities. Written informed consent was obtained from all patients before any study procedures. An external Data and Safety Monitoring Board was assigned to evaluate the safety data.

RESULTS

Patients and study treatment

Fifteen and 25 patients with AKU from the clinical study sites, in Liverpool and Piešt'any, respectively, were randomised into five groups (no treatment, 1 mg, 2 mg, 4 mg and 8 mg groups). All randomised patients completed the study. Patient demographics and baseline characteristics were similar across the five groups (table 1). The majority (67.5%) were male, and the mean age for all patients was 47.2 years ranging from 19 years to 63 years. Thirty-seven were Caucasian and three were Asian. Baseline s-HGA, s-Tyr and u-HGA_{24} are shown in table 2. No patient had abnormal renal function with decreased estimated glomerular filtration rate (eGFR). Serum creatinine and eGFR data are shown in table 1. There were no missing data for the primary or secondary variables. There were no obvious protocol deviations that affected the interpretation of the results.

All measurements of u-HGA¸ s-HGA and s-Tyr, performed with intra-assay and interassay coefficients of variation of less than 6% and 7%, respectively, across a large linear range of concentrations as shown in online supplementary table S4. The

Table 1 SONIA 1 Patient demographics, and related baseline data (mean (SD))*

	Untreated (N=8)	1 mg (N=8)	2 mg (N=8)	4 mg (N=8)	8 mg (N=8)	Total (N=40)
Age (years)	45.9 (15.3)	44.4 (10.9)	43.9 (13.7)	47.3 (10.7)	54.4 (7.3)	47.2 (11.9)
Body weight (kg)	71.0 (23.5)	86.9 (15.9)	74.6 (10.9)	76.9 (14.3)	81.1 (13.7)	78.1 (16.3)
Height (cm)	165.3 (12.1)	170.6 (7.1)	167.1 (9.4)	168.4 (5.9)	165.9 (6.7)	167.5 (8.3)
S-creatinine (mmol/L)	55.7 (13.5)	61.3 (10.2)	50.4 (10.7)	63.2 (13.3)	60.4 (11.2)	58.2 (12.2)
S-ALT/SGPT (μ/L)	34.0 (25.5)	27.6 (11.4)	35.6 (16.6)	30.9 (16.5)	26.8 (6.9)	31.0 (16.1)
eGFR (mL/min/m^2)*	127.4 (29.5)	140.6 (24.0)	156.8 (27.4)	119.3 (22.3)	132.4 (34.5)	135.3 (29.4)
Gender n (%)						
Female	4 (50.0)	1 (12.5)	3 (37.5)	3 (37.5)	2 (25.0)	13 (32.5)
Male	4 (50.0)	7 (87.5)	5 (62.5)	5 (62.5)	6 (75.0)	27 (67.5)
Race n (%)						
White	7 (875)	7 (875)	8 (100)	8 (100)	7 (875)	37 (925)
Asian	1 (125)	1 (125)	0 (00)	0 (00)	0 (00)	2 (5.0)
Others	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	1 (2.5)

*Data in parentheses are SD or %.
*Modification of Diet in Renal Disease calculation was employed.
S-ALT, serum alanine transaminase; SGPT, serum glutamate pyruvate transferase; eGFR, estimated glomerular filtration rate; SONIA 1, Suitability Of Nitisinone In Alkaptonuria 1.

measurement uncertainty data has been included in online supplementary appendix table S4b.

The daily HGA excretion in the no-treatment arm was not significantly different over the three visits suggesting that there was no major change in dietary habits in that group.

Primary end point

Urinary HGA excretion

Baseline u-HGA_{24} varied substantially between individuals and ranged from 14.4 mmol to 69.5 mmol (corresponding to 2.43–11.7 g) (table 2).

At Week 4, a clear dose-response relationship between nitisinone dose and u-HGA_{24} was observed (table 2). This is also illustrated in figure 2A (all patients) and in figure 2B (data at Week 2 and Week 4 for treated patients only). The adjusted geometric means and associated 95% CIs were 31.53 (27.19 to 36.57) mmol, 3.26 (2.27 to 4.69) mmol, 1.44 (1.00 to 2.06) mmol, 0.57 (0.39 to 0.81) mmol and 0.15 (0.11 to 0.22) mmol for the untreated, 1 mg, 2 mg, 4 mg and 8 mg doses, respectively. The greatest reduction in $uHGA_{24}$ was seen with the 8 mg dose.

From the MMRM, treatment group, visit, treatment group-visit interaction and baseline u-HGA_{24} were statistically significant (p values: <0.0001, 0.008, 0.020, 0.002, respectively) and site was not significant (p=0.523). All post hoc pairwise comparisons between doses at Week 4, were statistically significant (p=0.002 or lower in all cases). A similar pattern was observed for pairwise comparisons at 2 weeks.

The urine HGA excretion adjusted per mol of urine creatinine ratios at baseline and Week 4 are presented in table 2. They confirm the results seen for the u-HGA_{24} values without creatinine correction, and indicate acceptably complete 24-h urine collection.

Secondary end point

Serum HGA

s-HGA was quantifiable in all patients before starting nitisinone treatment. Supplementary data are shown for relationship between s-HGA and u-HGA_{24} at baseline. The correlation between s-HGA and u-HGA_{24} at baseline was weak (r=0.286,

Table 2 Mean (SD) and range* for u-HGA_{24}, u-HGA/creatinine, s-HGA and s-Tyr

	Untreated N=8	1 mg N=8	2 mg N=8	4 mg N=8	8 mg N=8
u-HGA_{24} (mmol)					
Baseline	29.8 (5.1) 22.3–38.8	36.7 (14.6) 20.7–69.5	31.4 (7.4) 20.0–40.7	35.4 (13.6) 14.4–55.4	27.3 (5.2) 17.1–32.9
Week 4	31.0 (4.6) 22.2–35.4	3.9 (1.7) 1.8–6.7	1.6 (0.8) 0.6–3.5	0.7 (0.4) 0.2–1.5	0.1 (0.05) 0.1–0.2
u-HGA/creatinine (mmol/mol)					
Baseline	2.8 (0.9) 2.0–4.3	2.3 (0.3) 1.9–2.8	3.1 (0.3) 2.5–3.6	2.8 (0.4) 2.3–3.3	2.7 (0.6) 2.0–3.7
Week 4	3.0 (0.9) 1.8–4.2	0.2 (0.1) 0.1–0.4	0.1 (0.04) 0.1–0.2	0.05 (0.03) 0.01–0.1	0.01 (0.005) 0.01–0.02
s-HGA (μmol/L)					
Baseline	27.5 (8.9) 14.6–45.5	28 (11.1) 5.8–41.4	30.3 (7.7) 20.6–41.9	32.1 (6.6) 22.2–41.4	28.3 (7.8) 15.6–37.6
Week 4	30.5 (12.4) 14.4–53.3	ND	ND	ND	ND
s-Tyr (μmol/L)					
Baseline	54 (15) 39–87	68 (20) 49–113	62 (10) 47–78	60 (9) 46–71	55 (5) 48–63
Week 4	56 (15) 42–91	653 (106) 450–806	715 (171) 506–965	803 (155) 657–1155	813 (145) 523–927

*Lower row in cell is range of data.
ND, not determined (below 3.1 μmol/L); s-HGA, serum HGA in acidified fasting sample; s-Tyr: serum tyrosine in fasting sample; u-HGA/creatinine: urine HGA excretion adjusted per mol of urine creatinine; u-HGA_{24}: urine HGA excretion over 24 h.

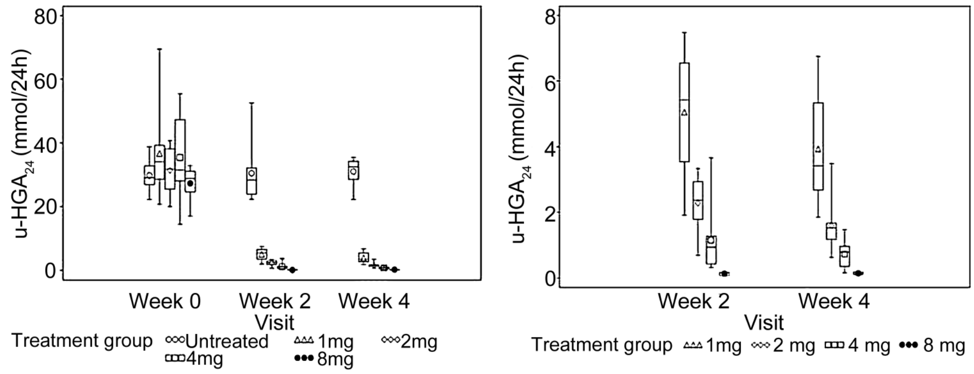

Figure 2 (A) Box plots of 24-h urinary excretion (μmol/24 h) of homogentisic acid (u-HGA_{24}) in untreated and nitisinone-treated patients with alkaptonuria (AKU) over time. (B) Box plots of u-HGA_{24} in nitisinone-treated patients with AKU at Weeks 2 and 4.

p=0.074) (see online supplementary figure S1). After treatment, s-HGA values were below the lower limit of quantification (3.1 μmol/L) in 56% of all samples collected in treated patients (table 2) (1 mg: 1 patient; 2 mg: 3 patients, 4 mg: 7 patients; 8 mg: 7 patients at Week 4). No calculation of descriptive statistics for s-HGA was therefore performed.

Serum tyrosine

The s-Tyr data, pretreatment and after 4 weeks of treatment, are presented in table 2 and figure 3. Mean s-Tyr increased with dose post nitisinone. However, as seen in figure 3, there was large interindividual variability in the data, and with few exceptions, all nitisinone-treated patients had levels above 500μmol/L, with the highest observation (1117 μmol/L) seen for a patient in the 4-mg group. The relationship between change in u-HGA_{24} and change in s-Tyr is described further in the supplementary data section (figure S2). There was no correlation between these two variables (r=−0.025, p=0.890).

Other safety results

No safety concerns were identified in this 4-week study. AEs are summarised in online supplementary table S3. There were no serious AEs. No event occurred in more than one patient. Back pain was reported by two patients in different dose groups. All events were considered mild, except for back pain in one patient in the 4-mg dose group. No abnormalities or changes in clinical chemistry or haematology laboratory data were observed. No patient experienced any corneal effects of elevated tyrosine.

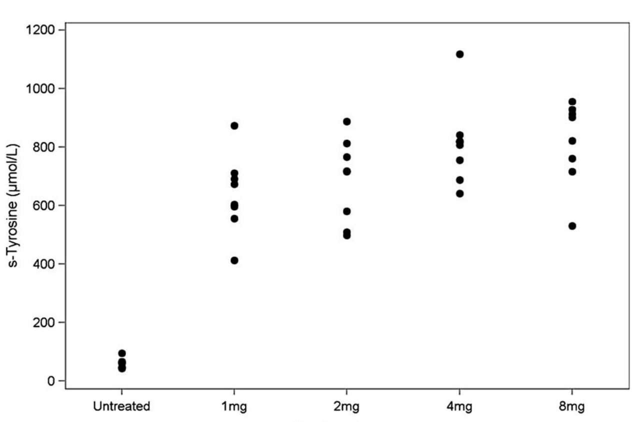

Figure 3 Fasting predose serum concentrations of tyrosine at Week 4 (all patients).

The use and effect of concomitant medication taken during the conduct of the study was reviewed. No known CYP3A4 inhibitors or inducers were used in the study.

DISCUSSION

A clear dose-response relationship was observed for the effect of nitisinone on the urinary excretion of HGA, with excretion decreasing consistently across the studied dose interval of 1–8 mg. Since the study used objective assessments of efficacy (changes in HGA levels), the open design was unlikely to have introduced bias. The 8-mg dose resulted in a mean reduction of u-HGA_{24} of 98.8% compared with baseline. Doses in the treatment of HT-1 are considerably higher than those used in this study.[11] But in that disease, the accumulating tyrosine metabolites are extremely toxic, and the disease is fatal if not treated. Thus, in HT-1 doses must be high enough to secure a near 100% inhibition of HPPD in all patients at all times, which is not considered necessary for AKU.

While it is not possible to guarantee full compliance, the data on the s-HGA, s-Tyr and u-HGA_{24} are sufficiently consistent for us to believe that compliance was not an issue during the study. The fact that each patient consumed at least 80% of the prescribed dose is supportive of this statement.

There is currently no approved pharmacological therapy for AKU. Treatment therefore relies on palliative analgesia and joint replacement surgery. Current experience with nitisinone is limited to three studies carried out at the National Institutes of Health (NIH), USA.[4 12 13] The last nitisinone study carried out by the NIH, a 3-year outcomes study, was inconclusive for the rheumatological end point (hip rotation).[13] One possible reason for the inconclusive NIH study is that an optimal dose may not have been used. We therefore investigated the HGA-lowering effect of different doses of nitisinone to find a dose that could lower u-HGA_{24} by close to 100%.

There is a lack of data correlating levels of serum and urine HGA with the evolution of AKU in patients. Therefore the level of HGA post nitisinone that would prevent ochronosis if treatment is started sufficiently early, or the level that would arrest or delay ochronosis in humans if treatment is started later in AKU, is currently unknown. Lifetime treatment of AKU mice with nitisinone resulted in an 88% plasma HGA reduction, down to less than 10 μmol/L, and completely prevented ochronosis.[14] Furthermore, in vitro evidence also indicates that 10μmol/L HGA is insufficient to cause ochronosis.[17] It is reasonable to assume that the low s-HGA achieved by nitisinone in this study will prevent ochronosis.

The effect of nitisinone on clinical symptoms and long-term safety needs to be further investigated. A longer study (SONIA 2) is underway to answer this question.

CONCLUSIONS

Treatment of patients with AKU with nitisinone at doses of 1–8 mg reduced u-HGA and s-HGA in a dose-dependent manner. No safety concerns were raised from this short-term study.

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Contributors LRR, NS, AKH, JR, JAG, JCJ were responsible for the conception of the trial. LRR, AH, JR, RI, CvK, BO, DL, MR,TFC, EEP, JS contributed to the study design and statistical analyses. LRR, NS, HA, OGT, JR, RI contributed to recruitment of patients. LRR, RF,MCB, HB, JR, RI undertook medical procedures. LRR, AMM, ATH, JJD, JAG, JCJ, JM, PC, GR provided methodological advice or undertook chemical analyses. AZ, MN were responsible for the HGD gene mutation identification. CJ, TK, AC, LS, AZ, MN, FG, K-HLQS, DB, AS contributed to project management. LRR drafted the first version of the report. All authors contributed to the interpretation of data, writing and revision of the manuscript. All authors approved the manuscript for publication.

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Supplementary Appendix

1.Inclusion and Exclusion criteria (Table S1)

2.Schedule of events (Table S2)

3.Summary of Adverse events (Table S3)

4. Summary of performance of assays for s-HGA, s-Tyr and u-HGA (Table S4a, b)

5. Fasting pre-dose serum concentrations of HGA against u-HGA_{24} at baseline (all patients) (Figure S1)

6. Fasting pre-dose serum concentrations of Tyrosine against u-HGA_{24} at Week 4 (nitisinone treated patients) (Figure S2)

7. Fasting pre-dose change in serum concentrations of Tyrosine against change in u-HGA_{24} at Week 4 from Baseline (nitisinone treated patients). (Figure S3)

Table S1. Inclusion and exclusion criteria in SONIA 1

Inclusion criteria

1. Diagnosis of AKU verified by documented elevated urinary homogentisic acid excretion.
2. Age ≥18 years.
3. Willing and able to visit the investigational site for study visits.
4. Signed written informed consent given

Exclusion criteria

The presence of any of the following will exclude a patient from inclusion in the study:

1. Currently pregnant or lactating.
2. Female patient of child-bearing potential not using a reliable method of contraception.
3. Known allergy to nitisinone or any of the constituents of the investigational product.
4. Current keratopathy or uncontrolled glaucoma.
5. Current malignancy.
6. Uncontrolled hypertension (blood pressure greater than 180 mmHg systolic or greater than 95 mmHg diastolic).
7. Unstable cardiovascular disease.
8. Serum potassium < 3.0 mmol/L.
9. eGFR < 60 mL/min (Modification of Diet in Renal Disease [MDRD])
10. ALT > 3 x upper limit of normal.
11. Hemoglobin < 10.0 g/dL.
12. Platelets < 100 x 10^9/L.
13. White blood count < 3.0 x 10^9/L.
14. History of alcohol or drug abuse.
15. Participation in another clinical study within 3 months of randomization.
16. Treatment with nitisinone within 60 days of randomization.
17. Psychiatric illness or neurological disease that interferes with compliance or communication with health care personnel.
18. Foreseeable inability to cooperate with given instructions or study procedures.
19. Any other medical condition which in the opinion of the investigator makes the patient unsuitable for inclusion.

Table S2. Schedule of events for SONIA 1

	Visit 1 Week 0	Visit 2 Week 2	Visit 3 Week 4	Follow up Week 6[1]
Informed consent	X			
Demographics	X			
Medical history	X			
Physical examination	X		X	
Inclusion/exclusion criteria				
Weight and height	X		X[2]	
Vital signs	X		X	
12-lead ECG	X			
Slit-lamp eye assessment	X	X	X	
Randomization	X			
Genetic profile	X			
Hematology	X	X	X	
Clinical chemistry	X	X	X	
Pregnancy test[3]	X			
24-hour urine for HGA	X	X	X	
Urine metabolomics	X	X	X	
Plasma metabolomics	X		X	
Urine biomarkers	X	X	X	
Serum biomarkers	X	X	X	
s-Tyr, s-HGA, fasting (predose)	X	X	X	
s-Tyr, s-HGA, 24-hour profiles	X		X	
s-nitisinone, fasting (predose)	X	X	X	
s-nitisinone, 24-hour PK profile			X	
Administration of nitisinone	←→ (Week 0 to Week 4)			
Prior and concomitant medication	←→ (Week 0 to Week 6)			
Adverse Events[4]	←→ (Week 0 to Week 6)			

1) Telephone contact only.
2) Weight only.
3) Women of childbearing potential.
4) All SAEs reported to Week 6 and thereafter only SAEs judged to be related to the IMP

Table S3.Number (%) of patients who had at least one adverse event (AE)

MedDRA Preferred Term	**Untreated (N=8)**	**1 mg (N=8)**	**2 mg (N=8)**	**4 mg (N=8)**	**8 mg (N=8)**
Patients with any AE	1(13%)	2(25%)	3(38%)	3(38%)	2(25%)
Abdominal pain lower	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)
Arthralgia	1(13%)	0(0%)	0(0%)	0(0%)	0(0%)
Back injury	0(0%)	0(0%)	1(13%)	0(0%)	0(0%)
Back pain	0(0%)	0(0%)	0(0%)	1(13%)	1(13%)
Diarrhoea	0(0%)	0(0%)	0(0%)	0(0%)	1(13%)
Dizziness	0(0%)	1(13%)	0(0%)	0(0%)	0(0%)
Erythema	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)
Eye pain	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)
Fatigue	0(0%)	0(0%)	1(13%)	0(0%)	0(0%)
Foreign body sensation in eyes	0(0%)	1(13%)	0(0%)	0(0%)	0(0%)
Haemoglobin decreased	0(0%)	1(13%)	0(0%)	0(0%)	0(0%)
Headache	0(0%)	1(13%)	0(0%)	0(0%)	0(0%)
Muscle injury	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)
Nasopharyngitis	0(0%)	0(0%)	0(0%)	0(0%)	1(13%)
Oral herpes	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)
Pain of skin	0(0%)	0(0%)	0(0%)	0(0%)	1(13%)
Rash	0(0%)	1(13%)	0(0%)	0(0%)	0(0%)
Vitreous floaters	0(0%)	0(0%)	1(13%)	0(0%)	0(0%)

Table S4. Summary of performance of assays for s-HGA, s-Tyr and u-HGA

	Urine HGA			**Serum HGA**			**Serum Tyrosine**	
Concentration (μmol/L)	**Intra-assay (n=6)**	**Inter-assay (n=15)**	**Concentration (μmol/L)**	**Intra-assay (n=6)**	**Inter-assay (n=20)**	**Concentration (μmol/L)**	**Intra-assay (n=6)**	**Inter-assay (n=20)**
30	3.7%	6.7%	3.2	8.6%	11.7%	24	5.3%	6.7%
110	1.9%	3.2%	20	3.6%	4.3%	60	2.3%	2.3%
718	2.9%	3.2%	145	5.6%	4.1%	810	3.6%	3.6%
12,000	2.5%	3.0%	400	3.1%	4.7%	1514	2.9%	2.9%

Data represented as % coefficient of variation

Table S4b: Uncertainty of measurement of u-HGA, s-HGA and s-Tyr

Urine HGA*		
Mean umol/L	Running SD	n=
85.7	5.8	60
3000	74	60
14,810	515	60
Serum HGA*		
26	2.1	62
198	9.5	62
417	17	62
Serum Tyrosine*		
30	1.7	62
612	25	62
1575	66	62

*Data represented as mean and current performance SD (95% confidence)

Figure S1: Fasting pre-dose serum concentrations of HGA against u-HGA$_{24}$ at baseline (all patients).

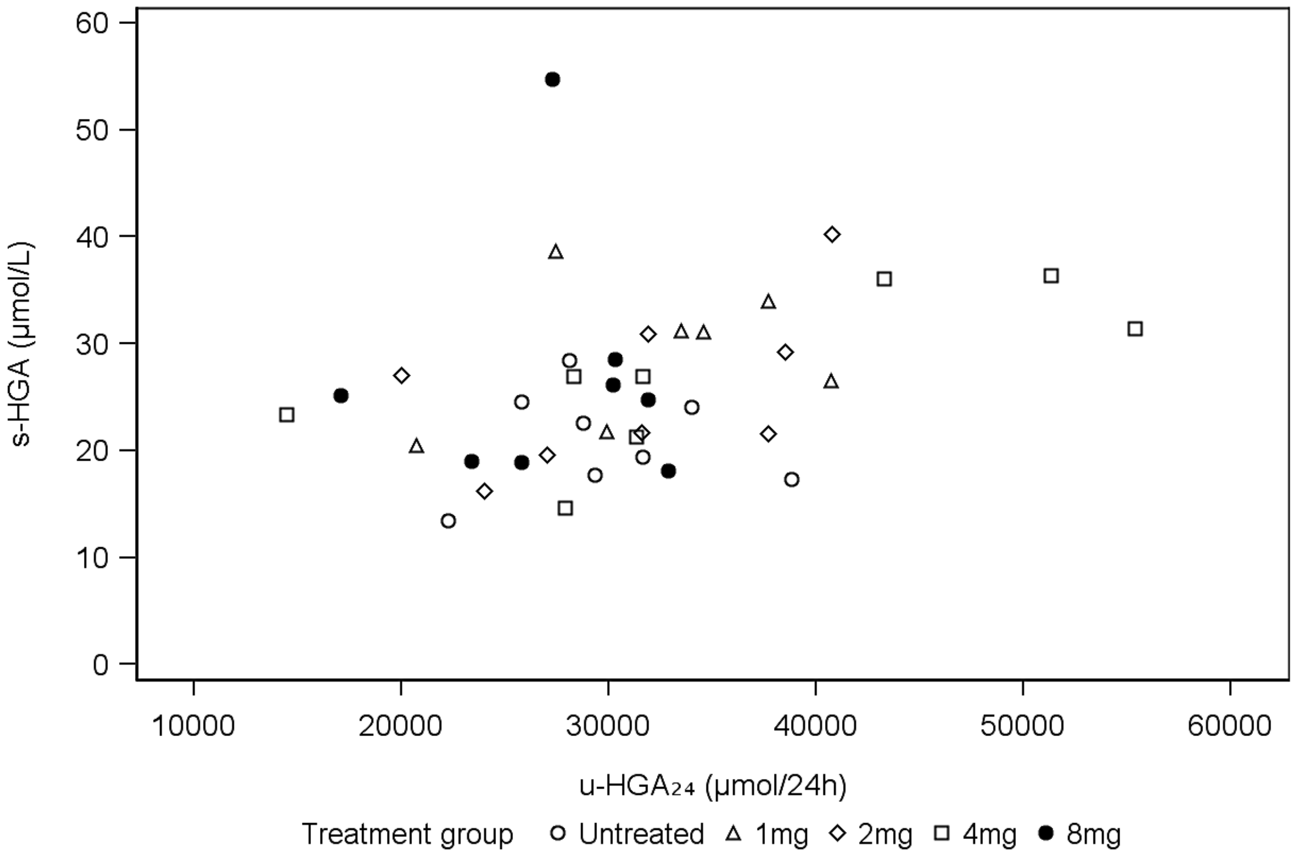

Figure S2: Fasting pre-dose serum concentrations of tyrosine against u-HGA_{24} at Week 4 (nitisinone treated patients).

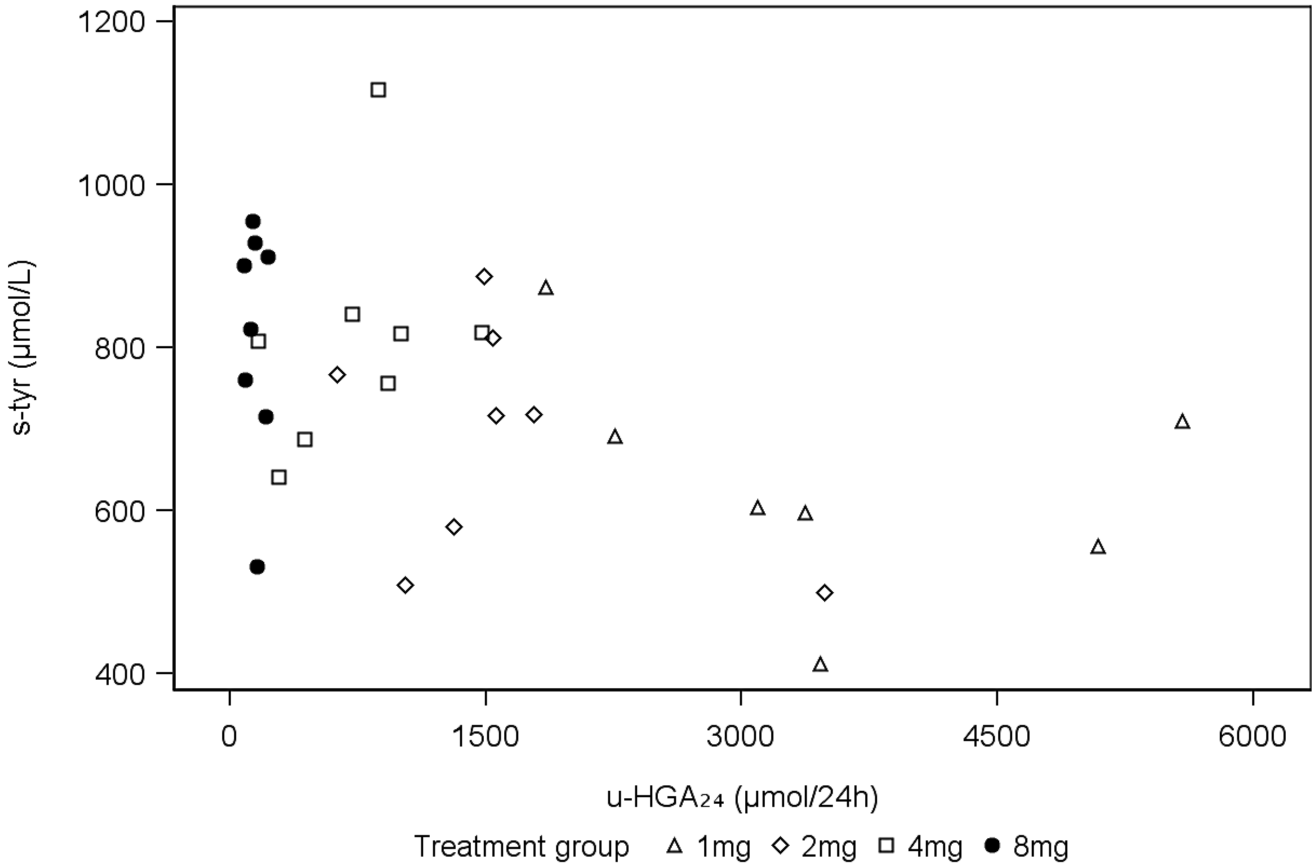

Figure S3: Fasting pre-dose change in serum concentrations of tyrosine against change in u-HGA$_{24}$ at Week 4 from Baseline (nitisinone treated patients).