Asymptomatic Corneal Keratopathy Secondary to Hypertyrosinaemia Following Low Dose Nitisinone and a Literature Review of Tyrosine Keratopathy in Alkaptonuria

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Abstract Nitisinone, although unapproved for use in alkaptonuria (AKU), is currently the only homogentisic acid lowering therapy with a potential to modify disease progression in AKU. Therefore, safe use of nitisinone off-label requires identifying and managing tyrosine keratopathy. A 22-year-old male with AKU commenced 2 mg daily nitisinone after full assessment. He was issued an alert card explaining potential ocular symptoms such as red eye,

tearing, ocular pain and visual impairment and how to manage them. On his first and second annual follow-up visits to the National Alkaptonuria Centre (NAC), there was no corneal keratopathy on slit lamp examination. On his third follow-up annual visit to the NAC, he was found to have typical dendritiform corneal keratopathy in both eyes which was asymptomatic. Nitisinone was suspended until a repeat slit lamp examination, 2 weeks later, confirmed that the keratopathy had resolved. He recommenced nitisinone 2 mg daily with a stricter low protein diet. On his fourth annual follow-up visit to the NAC, a routine slit lamp examination showed mild corneal keratopathy in the left eye. This is despite him reporting no ocular symptoms. This case highlights the fact that corneal keratopathy can occur without symptoms and any monitoring plan with off-label use of nitisinone in AKU will need to take this possibility into account. This is also the first time that typical corneal keratopathy has been described with the use of low dose nitisinone in AKU without symptoms.

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Introduction

Alkaptonuria (OMIM#203500) is a progressive severe osteo-articular disease with no approved disease modifying therapy to date (Ranganath et al. 2015). Approaches to management are currently symptomatic and palliative and employ ineffective analgesia and surgery, including spinal surgery and joint replacements. The only current hope in terms of disease modification is a drug called nitisinone (Ranganath et al. 2013). Early nitisinone therapy may prevent morbidity; and if started later, it has the potential to slow or arrest disease progression. Nitisinone is not yet

licensed for AKU and despite considerable morbidity, AKU is characterised by a relatively normal lifespan. Therefore, safety is an important issue and potential adverse effects are of interest to those involved in the management of metabolic disorders.

AKU is inherited in an autosomal recessive fashion. It is characterised by high circulating homogentisic acid (HGA) due to a genetic defect in the enzyme homogentisate dioxygenase (HGD, EC 1.13.11.5) (Phornphutkul et al. 2002). Ochronosis is the main pathogenetic event in AKU and it results from the conversion of HGA to a polymeric melanin-like pigment that has affinity to connective tissues, especially cartilage (Zannoni et al. 1969). Ochronosis leads to arthritis, valvular heart disease, nephrolithiasis and tendon ruptures (O'Brien et al. 1963).

Nitisinone inhibits p-hydroxyphenyl pyruvate dioxygenase and decreases HGA (Lock et al. 1998). In keeping with the mode of action of nitisinone, circulating tyrosine increases. The tyrosinaemia that occurs during nitisinone treatment resembles hereditary tyrosinaemia type 3. Adverse effects known to be associated with tyrosinaemia include corneal and dermal toxicity (Meissner et al. 2008).

Tyrosinaemia related corneal lesions are reported to be less than 9% in children with hereditary tyrosinaemia type 1 (HT-1) who are treated with nitisinone (Holme and Lindstedt 1998; Gissen et al. 2003). Schauwvlieghe et al. (2013) have reported tyrosine keratopathy in a 16-year-old male who received nitisinone for HT-1. Although corneal symptoms resolved after stopping nitisinone, tyrosine crystals were still detectable in the corneal epithelium using confocal microscopy and slit lamp examination.

Nitisinone has been shown to reduce plasma HGA levels and decrease urinary HGA excretion by greater than 95% in humans (Introne et al. 2011; Ranganath et al. 2016; Milan et al. 2017) and to completely prevent ochronosis in a mouse model of AKU (Preston et al. 2014; Keenan et al. 2015). Since 2012, low dose nitisinone has been used off-label in the NHS England designated National Alkaptonuria Centre (NAC), at the Royal Liverpool University Hospital. Safety monitoring, including annual elective slit lamp examination, is part of the standardised care.

Case Report

A 22-year-old man presented to the NAC in 2012. He had increased circulating and urinary HGA (16.3 $\mu\text{mol/L}$ and 8,416 $\mu\text{mol/L}$, respectively); as well as two genetic mutations in the HGD loci, consistent with the diagnosis of AKU. Apart from asymptomatic arthropathy of his ankles and feet, he had no other clinical features of AKU. He was also known to have unexplained mild splenomegaly and stable persistent thrombocytopenia. At baseline (V1), a slit

lamp examination of the eye including the cornea was carried out and found to be normal. The visual acuity was 6/4.8 in both eyes. The rest of the physical examination was normal. He was then commenced on nitisinone 2 mg alternate days for 3 months, and then increased to 2 mg daily from month 3 onwards. He was counselled on how to control dietary protein to minimise the rise in serum tyrosine associated with nitisinone treatment. He was advised on an initial 1.0 g/kg protein daily intake to maintain his weight and prevent catabolism. At his visit to the NAC one (V2) and 2 years (V3) after beginning nitisinone 2 mg daily, visual acuity was 6/4.8 in both eyes. Slit lamp examinations were carried out electively and there was no corneal keratopathy. At his third annual visit to the NAC (V4), slit lamp ocular examination revealed typical dendritiform corneal keratopathy in both eyes which was asymptomatic. Visual acuity was 6/5 in the right eye and 6/6 in the left eye. The anterior chamber, iris and lens were all normal. On further questioning, he reported no eye symptoms. Dietary assessment revealed that his dietary protein intake had increased as a result of moving from a predominantly vegetarian diet to relying on take away meals. Nitisinone was stopped and he was given dietary counselling to reduce his protein intake. After 2 weeks, resolution of the dendritic lesion was confirmed on slit lamp examination and nitisinone was restarted at 2 mg daily.

At the annual follow-up visit a year later, namely 4 years after commencing nitisinone (V5), the slit lamp examination showed mild corneal keratopathy in the left eye. Visual acuity was 6/4.8 in each eye; the iris, lens and anterior chamber were all normal. Subjectively, he reported some eye dryness but no eye pain, redness, tearing or visual impairment. The patient also admitted to relapsing in terms of his diet. He was again advised to stop taking nitisinone for 2 months and slit lamp examination was arranged. Table 1 summarises the results of serum tyrosine (sTYR), serum HGA (sHGA), 24 h urinary HGA excretion (uHGA24), weight and dietary protein intake. Figure 1 shows eye photos taken during visits 2–5.

Discussion

To our knowledge, this is the first case describing asymptomatic and in particular painless tyrosine keratopathy in a patient receiving just 2 mg of nitisinone daily. This is expected to influence the monitoring of patients on nitisinone. Incidence of tyrosine keratopathy in AKU patients receiving 2 mg daily dose of nitisinone is estimated at 5% (Introne et al. 2011). In our centre we have over 50 patients on low dose nitisinone and we have reported a case of symptomatic tyrosine keratopathy (Stewart et al. 2014).

Table 1 Summary of the metabolic data for case subject

	Weight (kg)	BMI (kg/m ²)	Serum measurements (μmol/L)		24-h urine measurements			Recommended dietary protein intake (g/kg) ^a	Estimated dietary protein intake (g/kg) ^b
			HGA	Tyrosine	HGA (μmol/24 h)	Tyrosine (μmol/24 h)	Urine nitrogen ^c (g/kg)		
Baseline (visit 1)	78.2	25.8	16.3	38	17,337	163	–	1.0	1.8
12 months (visit 2)	69.9	22.8	4.6	815	1,487	1,797	0.95	0.8	0.77
24 months (visit 3)	74.3	24.5	10.2	113 ^d	1,785	1,357	0.74	0.8	0.78
36 months (visit 4)	74.3	24.5	<3.1	964	1,107	1,912	0.80	0.75	0.98
40 months	–	–	–	745^e	–	–	–	–	–
41 months	–	–	–	578^e	–	–	–	–	–
43 months	–	–	–	518^e	–	–	–	–	–
48 months (visit 5)	79.4	26.2	2.5	841	920	1,354	0.63	0.83	1.1

^a Represents recommended dietary protein intake^b Protein intake was estimated from the patient food diary^c Estimated from urinary urea excretion^d Nitisinone was not detected in this sample^e These were done using blood spot samples

In the literature, there are only two cases describing symptomatic tyrosine keratopathy in AKU patients receiving nitisinone (Table 2). The first case was from a 3-year randomised trial that assessed the safety and efficacy of nitisinone in AKU patients. The affected subject was a 48-year-old male who had symptomatic tyrosine keratopathy after 6 weeks of daily 2 mg nitisinone treatment (Introne et al. 2011). The second case was described by Stewart et al. (2014). The patient was 25-year-old male who experienced typical ocular symptoms of blurred vision, ocular pain, red eyes and epiphora as well as a concomitant urticarial skin rash. He was taking 2 mg nitisinone on alternate days and was not compliant with a low protein diet.

In both published cases of tyrosine corneal keratopathy in AKU, eye symptoms resolved on discontinuation of nitisinone. In the first case, eye symptoms recurred and led to permanent withdrawal of nitisinone; while in the second case, the patient was able to tolerate a once weekly dose of 2 mg. In addition to the two published AKU corneal keratopathy reports, another patient who was commenced on nitisinone 2 mg daily at the NAC, subsequently reported severe symptoms of photophobia, red eye, ocular pain, tearing and visual impairment consistent with tyrosine keratopathy post nitisinone but was then no longer able to be followed up in the NAC (Table 2 summarises the demographics and the clinical features of the four patients).

The lack of pain despite clear corneal involvement in the patient described here is difficult to explain. The cornea is very well innervated and has a rich supply of sensory and autonomic fibres (Muller et al. 2003). Corneal hypoesthesia

is well documented in many circumstances including post herpetic infection, corneal surgery, damage to the trigeminal cranial nerve, and systemic conditions such as diabetes (Sacchetti and Lambiase 2014). However, this patient had no such history. The lack of symptoms could be explained by the fact that while the corneal epithelium is well supplied with nerve endings, the underlying stroma is not (Shaheen et al. 2014), and it is tempting to speculate that in this case, if tyrosine deposits were mostly confined to the sub-epithelial stroma, pain would not be a feature. This is supported by descriptions of other stromal lesions of the cornea without the presence of pain (Sacchetti et al. 2016). However, confocal corneal microscopy in a hereditary tyrosinaemia type 2 (HT-2) case has demonstrated the presence of tyrosine crystal in the corneal epithelium even after the resolution of eye symptoms (Kocabeyoglu et al. 2014). Similarly, Schauwvlieghe et al. (2013) described asymptomatic tyrosine corneal deposits in a nitisinone treated HT-1 patient. In both cases the stroma was spared. However, there was no mechanism offered in either report to explain the lack of pain.

Experiments in rats support our speculation to some extent. It is important to remember that the tyrosine concentration in the aqueous humor of the anterior chamber of the eye is much higher than in circulating plasma (Lock et al. 1996). Aqueous humor diffuses into the avascular cornea providing crucial nourishment to the endothelium and stroma. Moreover, due to the unique needs of the eye, concentration of tyrosine is much higher than in plasma. The endothelium on the posterior surface of the cornea is

Fig. 1 No evidence of keratopathy in on the first (not shown here), second (**a, b**) and third visit (**c, d**). On the fourth visit, tyrosine keratopathy lesions are seen in both eyes (red arrows in **e, f**).

Fifth visit: Minimal corneal epithelial disturbance with minimal fluorescein uptake in the left eye (red arrow, **h**) and normal right eye (**g**)

leaky compared to the tight epithelium of the anterior corneal surface, allowing the aqueous humor containing the tyrosine to permeate through the cornea stroma. Tyrosine is normally soluble in aqueous humor and precipitates out of solution when its concentrations exceed its solubility in water (Lock et al. 2006). Precipitation of tyrosine in the cornea leads to tyrosine keratopathy. It is likely that the posterior stromal parts of the cornea are exposed to higher tyrosine first and/or affected to a greater extent with a relative sparing the corneal epithelium. This might explain why the richly innervated corneal epithelium is minimally involved, if any, and why symptoms may be lacking.

Serum tyrosine monitoring is important in the context of nitisinone therapy although it may not be very helpful in identifying keratopathy per se. The apparent lack of correlation between serum tyrosine concentrations and eye symptoms has been noted before (Holme and Lindstedt 1998). In a small cohort of nitisinone treated HT-1 children,

there were no ocular symptoms despite non-compliance with low tyrosine diet in four patients and serum tyrosine concentrations were as high as 1,240 and 1,410 $\mu\text{mol/L}$ (Gissen et al. 2003). It can be conjectured that the aqueous humor tyrosine is more meaningful than the circulating tyrosine concentrations.

In this present case, the serum tyrosine concentrations were the highest on the fourth (964 $\mu\text{mol/L}$) and the fifth visit (841 $\mu\text{mol/L}$). His dietary protein intake was estimated to be 0.98 g/kg on his fourth visit and 1.1 g/kg on his fifth visit; both consistent with lapses in dietary protein restriction. One could speculate that there may be a correlation between circulating and aqueous humor tyrosine concentrations, in the present case, even though the aqueous humor concentrations were not measured for obvious impractical reasons.

Tyrosine keratopathy can be potentially sight threatening. While there are no reliable predictors of tyrosine keratopathy in AKU patients receiving nitisinone, there are measures that

Table 2 Summary of nitisinone induced tyrosine keratopathy cases in AKU

Case	Age	Gender	Serum tyrosine ($\mu\text{mol/L}$)	Nitisinone dose	Onset of eye symptoms in relation to starting nitisinone	Outcome	Symptoms
Stewart et al. (2014)	21	Male	941	2 mg alternate days	7 weeks post nitisinone	Restarted nitisinone 2 mg once weekly	Epiphora on alternate evenings while watching television
Introne et al. (2011)	48	Male	600	2 mg daily	6 weeks post nitisinone	Restarting nitisinone attempted twice but eye symptoms recurred	Corneal irritation
Present case	25	Male	964 ^a 841 ^b	2 mg daily	36 months post nitisinone	Nitisinone 2 mg daily restarted after resolution of keratopathy	No symptoms on visit 4 Dry eyes on visit 5
NAC case (unpublished)	55	Male	1,214	2 mg alternate days	3 months post nitisinone (normal slit lamp examination)	Nitisinone stopped	Experienced right eye discomfort towards the end of the day every other day

^a Annual visit 4 (V4)^b Annual visit 5 (V5)

can be taken to ensure the safe use of nitisinone. Locally, we have a robust protocol for initiating nitisinone and monitoring serum tyrosine concentrations during treatment. Patients are commenced on 2 mg alternate day for 3 months which are then increased to 2 mg daily. Serum tyrosine concentrations are monitored 3 and 6 months after nitisinone initiation and at all annual visits. Patients are counselled regarding low diet tyrosine as well as the potential eye symptoms resulting from hypertyrosinaemia. They are given an alert card and also advised to report eye symptoms promptly and to stop nitisinone. Additionally, ophthalmological assessments are done before starting nitisinone and annually thereafter using slit lamp examination. The present case does not clarify a rationale for frequency of slit lamp examinations required post-nitisinone. It may be necessary to have a higher index of suspicion for potential keratopathy by identifying any atypical or mild ocular symptoms and screening by slit lamp examination. It may also be better to carry out a biannual slit lamp examination. These findings highlight the necessity for further research on managing serum tyrosine concentrations in AKU by dietary or other therapeutic interventions. Further work is also required to elucidate the clinical and prognostic implications of asymptomatic corneal depositions in nitisinone treated patients.

In summary, this is the first case of asymptomatic tyrosine keratopathy in an AKU patient receiving 2 mg daily dose of nitisinone. Elective and symptom-based slit lamp examination may be needed to detect corneal tyrosine keratopathy. Discontinuation of nitisinone, low tyrosine diet and frequent serum tyrosine monitoring remain a key in managing tyrosine keratopathy. The oversight of a meta-

bolic physician and specialised dietary support are paramount.

Take Home Message

Asymptomatic tyrosine keratopathy may occur in AKU patients taking low dose nitisinone. It can be detected using elective and symptom-based slit lamp examination and it should be managed by a metabolic physician and a specialised dietitian.

Compliance with Ethics Guidelines

Conflict of Interest

M. Khedr, S. Judd, M. C. Briggs, A. T. Hughes, A. M. Milan, R. M. K. Stewart, E. A. Lock, J. A. Gallagher and L. R. Ranganath declare that they have no conflict of interest.

Informed Consent

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 2000 (5). Informed consent was obtained from all patients for being included in the study.

Animal Rights

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Details of the Contributions of Individual Authors

M. Khedr wrote the first draft.

S. Judd carried out dietary assessments.

A. T. Hughes, A. M. Milan, M. C. Briggs, R. M. K. Stewart, E. A. Lock, J. A. Gallagher and L. R. Ranganath: Intellectual input and support, editing the manuscript.

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None.

Details of Ethics Approval

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 2000. In addition, the institutional review body (Royal Liverpool University Hospital) explicitly approved the National Alkaptonuria Service from which this data was generated.

A Patient Consent Statement

Informed consent was obtained from all patients for being included in the study. This is being published as a clinical practice article and standard research ethics process is not therefore appropriate. The data from this patient have been completely anonymised to ensure he is not recognised from the publication of this manuscript. The data obtained were following standard clinical assessments upon referral to the National Alkaptonuria Service in Liverpool. Patients are informed verbally and through being handwritten materials about the activities of the National AKU Service. They are

explicitly informed in the Patient information booklet of the National AKU Service that:

We could publish results from the study but if we do, we will make sure you cannot be identified in any way. All data used for publicity or for other research purposes will ensure total anonymity. Please let us know when you are visiting Ward 9 B (where the National AKU Service is located) that you understand this and have no objection to it.

All the ocular photos were acquired during the standard assessments during the patient visit.

References

- Gissen P, Preece M, Willshaw H, McKiernan PJ (2003) Ophthalmic follow-up of patients with tyrosinaemia type I on NTBC. *J Inher Metab Dis* 26:13–16
- Holme E, Lindstedt S (1998) Tyrosinaemia type I and NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione). *J Inher Metab Dis* 21(5):507–517
- Introne WJ, Perry MB, Troendle J et al (2011) A 3-year randomized therapeutic trial of nitisinone in alkaptonuria. *Mol Genet Metab* 103(4):307–314
- Keenan CM, Preston AJ, Sutherland H et al (2015) Nitisinone arrests but does not reverse Ochronosis in Alkaptonuric mice. *JIMD Rep* 24:45–50
- Kocabeyoglu S, Mocan MC, Irkeç M (2014) In vivo confocal microscopic features of corneal pseudodendritic lesions in tyrosinemia type II. *Cornea* 33(10):1106–1108
- Lock EA, Gaskin P, Ellis MK et al (1996) Tissue distribution of 2-(2-Nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC): effect on enzymes involved in tyrosine catabolism and relevance to ocular toxicity in the rat. *Toxicol Appl Pharmacol* 141:439–447
- Lock EA, Ellis MK, Gaskin P et al (1998) From toxicological problem to therapeutic use: the discovery of the mode of action of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), its toxicology and development as a drug. *J Inher Metab Dis* 21:498–506
- Lock EA, Gaskin P, Ellis M, Provan WM, Smith LL (2006) Tyrosinaemia produced by 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC) in experimental animals and its relationship to corneal injury. *Toxicol Appl Pharmacol* 215:9–16
- Meissner T, Betz RC, Pasternak SM et al (2008) Richner-Hanhart syndrome detected by expanded newborn screening. *Paediatr Dermatol* 25:378–380
- Milan AM, Hughes AT, Davison AS et al (2017) The effect of nitisinone on homogentisic acid and tyrosine: a two-year survey of patients attending the National Alkaptonuria Centre, Liverpool. *Ann Clin Biochem* 54:323–330
- Muller LJ, Marfurt CF, Krusec F, Tervod TM (2003) Corneal nerves: structure, contents and function. *Exp Eye Res* 76:521–542
- O'Brien WM, BNL D, Bunim JJ (1963) Biochemical, pathologic and clinical aspects of alcaptonuria, ochronosis and ochronotic arthropathy. *Am J Med* 34:813–838
- Phornphutkul C, Introne WJ, Perry MB et al (2002) Natural history of alkaptonuria. *N Engl J Med* 347:2111–2121
- Preston AJ, Keenan CM, Sutherland H et al (2014) Ochronotic osteoarthropathy in a mouse model of alkaptonuria, and its inhibition by nitisinone. *Ann Rheum Dis* 73(1):284–289

- Ranganath LR, Jarvis JC, Gallagher JA (2013) Recent advances in management of alkaptonuria (invited review; best practice article). *J Clin Pathol* 66:367–373
- Ranganath LR, Timmis OG, Gallagher JA (2015) Progress in alkaptonuria – are we near to an effective therapy? *J Inher Metab Dis* 38:787–789
- Ranganath LR, Milan AM, Hughes AT et al (2016) Suitability of nitisinone in alkaptonuria 1 (SONIA 1): an international, multi-centre, 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. *Ann Rheum Dis* 75(2):362–367
- Sacchetti M, Lambiase A (2014) Diagnosis and management of neurotrophic keratitis. *Clin Ophthalmol* 8:571–579
- Sacchetti M, Macchi I, Tiezzi A, La Cava M, Massaro-Giordano G, Lambiase A (2016) Pathophysiology of corneal dystrophies: from cellular genetic alteration to clinical findings. *J Cell Physiol* 231:261–269
- Schauwvlieghe P-P, Jaeken J, Kestelyn P, Claerhout I (2013) Confocal microscopy of corneal crystals in a patient with hereditary tyrosinemia type I, treated with NTBC. *Cornea* 32(1):91–94
- Shaheen B, Bakir M, Jain S (2014) Corneal nerves in health and disease. *Surv Ophthalmol* 59(3):263–285
- Stewart R, Briggs MC, Jarvis JC et al (2014) Reversible keratopathy due to hypertyrosinaemia following intermittent low-dose nitisinone in alkaptonuria: a case report. *JIMD Rep* 17:1–6
- Zannoni VG, Lomtevas N, Goldfinger S (1969) Oxidation of homogentisic acid to ochronotic pigment in connective tissue. *Biochim Biophys Acta* 177:94–105