Received: 19 April 2023 | Revised: 26 April 2023 | Accepted: 28 April 2023

DOI: 10.1002/pbc.30430

Pediatric Blood & Cancer | SIOP International Society of Paediatric Oncology | aspho The American Society of Pediatric Hematology/Oncology | WILEY

LETTER TO THE EDITOR

Spontaneous fractures during 13-cis retinoic acid therapy for neuroblastoma

To the Editor:

Neuroblastoma accounts for approximately 10% of childhood malignancies and is the most common extracranial paediatric solid malignancy.[1] Current management of high-risk neuroblastoma includes a final treatment phase that alternates differentiation therapy with isotretinoin (13-cis-retinoic acid [13-cis-RA]) and anti-GD2 antibody immunotherapy.[2] 13-cis-RA is a vitamin A derivative, which demonstrated differentiation and growth arrest of neuroblastoma cell lines preclinically,[3,4] and was subsequently developed successfully in clinical trials.[5,6] 13-cis-RA drug exposure has been shown to exhibit marked inter-patient variability and the potential benefits of therapeutic drug monitoring (TDM) have been highlighted.[7] We here report spontaneous fractures in a 3-year old receiving 13-cis-RA treatment and investigate a potential causal relationship with drug exposure.

The patient presented at 21 months with left temporal swelling, exophthalmos and periorbital bruising. He was diagnosed with a primary adrenal MYCN-amplified neuroblastoma disseminated to skull base and sphenoidal bones only. Induction chemotherapy was completed as per guidelines,[8] followed by high-dose chemotherapy with autologous stem cell return and complete resection of the small primary adrenal lesion. Histopathological review revealed complete necrosis, and radiotherapy was omitted. Organ function, including kidney, liver and bone profile analysis, remained normal. The patient proceeded with six cycles of oral 13-cis RA according to the HR-NBL/SIOPEN protocol.[9]

Following the first cycle of 13-cis-RA, the patient was noted to not weight-bear on the right leg; there was no swelling, redness or local irritation on palpation and passive movement including the hip joint was normal. Plain x-rays performed 3 weeks later revealed a right proximal tibial shaft healing fracture. There were no concerning features typical for metastasis or non-accidental injury (Figure 1A). No specific orthopaedic treatment was necessary as the fracture was aged and healing. Biochemistry investigations were normal for adjusted calcium, phosphate and alkaline phosphatase, but revealed vitamin D deficiency (39.7 nmol/L; normal: 50–125 nmol/L); necessitating a 3-month treatment course of daily oral cholecalciferol supplements. The patient continued treatment and started walking again soon after. Three months later, during the fifth course of 13-cis-RA, the child presented again with a 3-day history of non-weight-bearing, this time of the left lower limb. Immediate radiographs revealed a left proximal tibial healing fracture, estimated to be over 10 days old, thus preceding the clinical manifestations (Figure 1B). No vertebral fractures were identified by lateral spinal x-ray.

To investigate 13-cis-RA exposure in this patient, pharmacokinetic studies were performed during the sixth cycle of therapy. Plasma was collected over 6 hours following drug administration and drug levels quantified as previously described.[10] Maximal plasma concentrations of 10.3 and 19.6 μM were observed for 13-cis-RA and 4-oxo-13-cis-RA, respectively. These concentrations were markedly higher than a 13-cis-RA C_{max} value of 2 μM targeted in a previously published TDM study, with observed C_{max} ranges of 0.4–11.2 and 0.5–14.3 μM for the parent drug and major metabolite, respectively, observed across 103 patients.[7] Indeed, drug exposures in excess of those observed in this patient have rarely been reported across multiple studies (Table 1), with levels in this range associated with increased incidence of skin lesions, liver impairment, and hypercalcemia.[5,11] However, no spontaneous fractures following 13-cis-RA treatment have previously been reported.

Retrospective review of the patient's previous imaging did not suggest obvious signs of bone demineralisation antedating treatment with 13-cis-RA. Apart from the fractures, the treatment was overall well tolerated, with mild skin changes the only other adverse event of note. End-of-treatment imaging including a whole-body magnetic resonance imaging (MRI) confirmed remission and did not show any other focal bony changes (Figure 1C). The patient remains well and in remission, with no further long bone fractures, now 2.5 years out of therapy.

Genetic variation influencing 13-cis-RA pharmacokinetics has previously been questioned,[7] and a pharmacogenomic defect seems unlikely in our patient as drug metabolite levels were also elevated. To discount other explanations for the fractures, we excluded obvious signs for osteoporosis pre-dating treatment and considered biochemical abnormalities of bone metabolism, with no laboratory findings sufficient to explain the fractures. Indeed, the effect of the bone-remodeling properties of vitamin A derivatives have been highlighted in studies on rodents and spontaneous long bone fractures observed in rats treated with all-trans retinoic acid.[13,14]

Importantly, clinical studies in adults have documented the effects of retinoids on human bones. Postmenopausal women with long-term intake of diets high in retinol are at increased risk of hip fractures,[15] with middle-aged men at increased risk of fracture if serum retinol levels are above 103 μg/dL (3.6 μM).[16] Two further studies have identified an increase in osteoporosis and fracture risk when high retinol levels were observed alongside vitamin D deficiency.[17,18] A recent

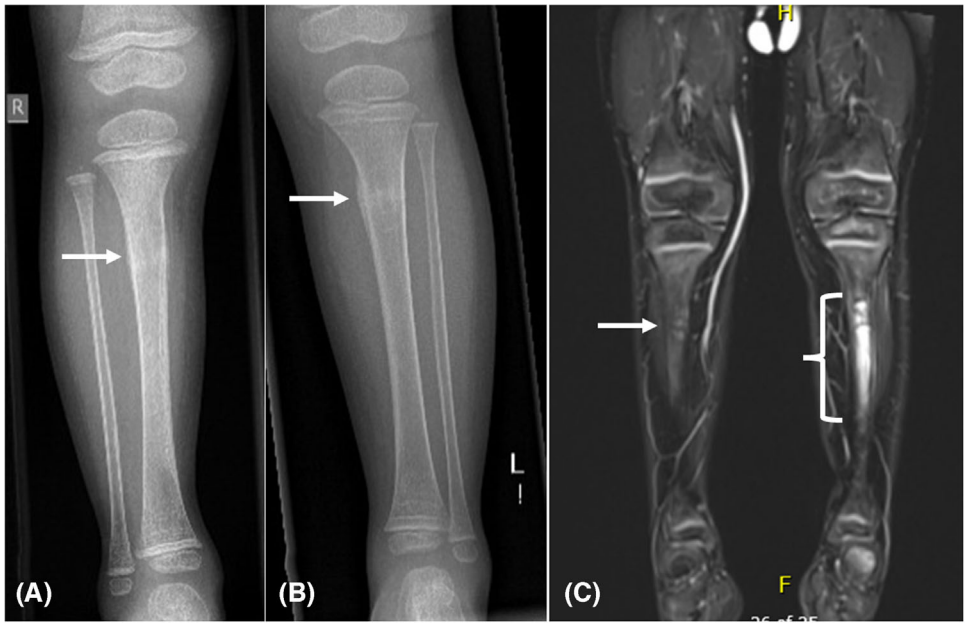

FIGURE 1 (A) Plain x-ray of the right tibia after two cycles of anti-GD2, 3 weeks after the child was unable to bear weight, showing a healing fracture through the proximal diaphysis of the right tibia with periosteal reaction around the area of fracture (indicated by an arrow). (B) Plain radiograph of the left tibia performed during the fifth cycle of 13-cis-RA, following a 72-hour history of limping, and revealing a similar tibial shaft fracture (indicated by an arrow). (C) Whole-body short tau inversion recovery (STIR) magnetic resonance imaging (MRI) at completion of treatment showing extensive high signal in the left tibia (bracket) and a small focus of higher signal on the right tibia (arrow), related to the fractures. Another focus of high signal is seen in the left talus, likely due to altered weight-bearing constraints. There is no other focal bone lesion noted on imaging.

TABLE 1 Summary of exposures to 13-cis-RA and 4-oxo-13-cis-RA in high-risk neuroblastoma patients from published data.

Author	Number of patients	Age range (years)	13-cis-RA dose (mg/m^2)	13-cis-RA C_{max} (μM)	4-oxo-13-cis-RA C_{max} (μM)	Reference
Khan (1996)	31	2–12	100–200	4.9 ± 3.6–8.9 ± 10	ND	11
Veal (2007)	28	1.1–18.7	160	0.2–5.3	0.7–12.1	12
Veal (2013)	103	0.8–20.5	160–290	0.4–11.2	0.5–14.3	7
Veal (2021)	20	1.0–11.6	160–200	0.7–9.6	1.5–16.4	10

Abbreviations: 13-cis-RA, 13-cis-retinoic acid; C_{max}, maximum plasma concentration.

retrospective study in neuroblastoma patients revealed that six of 15 patients (all receiving 13-cis-RA) had multifocal physeal changes on whole-body MRI following multi-modal therapy, with three of six developing progressive changes in lesions eventually affecting bone growth.[19] An end-of-treatment whole-body MRI scan in our patient showed no evidence of physeal changes, but remnants of the healing fractures. Regardless, this recent study provides further support that bone changes during retinoid treatment could be more common than previously thought. A high index of suspicion and low threshold for imaging is therefore recommended. In our patient, a combinatorial effect of low vitamin D levels and high retinoid levels should be considered as the most likely cause of the fractures observed. Assessment of vitamin D levels and appropriate supplementation if required, prior to the start of 13-cis-RA treatment in neuroblastoma patients, could potentially be beneficial.

ACKNOWLEDGEMENTS

This work was supported in part by Cancer Research UK, the Experimental Cancer Medicine Centre Network, the National institute for Health Research and the Little Princess Trust.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

Claire Geurten[1,2]
Julie Errington[3]
Shelby Barnett[3]
Hiten Patel[4]
Saqib Noor[5]
Vrinda Saraff[6]

Gareth J. Veal[3]
Susanne A. Gatz[1,7]

[1]*Department of Paediatric Haematology/Oncology, Birmingham Women and Children's NHS Foundation Trust, Birmingham, UK*
[2]*Department of Paediatric Haematology/Oncology, Centre Hospitalier Universitaire de Liège, Liège, Belgium*
[3]*Newcastle University Centre for Cancer, Translational and Clinical Research Institute, Newcastle upon Tyne, UK*
[4]*Department of Paediatric Radiology, Birmingham Women and Children's NHS Foundation Trust, Birmingham, UK*
[5]*Department of Paediatric Orthopaedic Surgery, Birmingham Women and Children's NHS Foundation Trust, Birmingham, UK*
[6]*Department of Paediatric Endocrinology, Birmingham Women and Children's NHS Foundation Trust, Birmingham, UK*
[7]*Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK*

Correspondence
Susanne A. Gatz, Institute of Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.
Email: s.gatz@bham.ac.uk

REFERENCES

1. Steliarova-Foucher E, Colombet M, Ries L, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. *Lancet North Am Ed*. 2017;18:719-731.
2. Smith V, Foster J. High-risk neuroblastoma treatment review. *Children*. 2018;5:114.
3. Sidell N. Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells in vitro. *J Natl Cancer Inst*. 1982;68(4):589-596.
4. Reynolds CP, Kane DJ, Einhorn PA, et al. Response of neuroblastoma to retinoic acid in vitro and in vivo. *Prog Clin Biol Res*. 1991;366:203-211.
5. Villablanca JG, Khan AA, Avramis VI, et al. Phase I trial of 13-cis-retinoic acid in children with neuroblastoma following bone marrow transplantation. *J Clin Oncol*. 1995;13:894-901.
6. Matthay KK, Villablanca JG, Seeger RC, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *N Engl J Med*. 1999;341(16):1165-1173.
7. Veal GJ, Errington J, Rowbotham SE, et al. Adaptive dosing approaches to the individualization of 13-cis-retinoic acid (isotretinoin) treatment for children with high-risk neuroblastoma. *Clin Cancer Res*. 2013;19(2):469-479.
8. Simon T, Hero B, Schulte J, et al. 2017 GPOH guidelines for diagnosis and treatment of patients with neuroblastic tumors. *Klin Padiatr*. 2017;229:147-167.
9. Ladenstein R. High-risk neuroblastoma study 1.5 of SIOP-Europe (SIOPEN). International Society of Pediatric Oncology European Neuroblastoma High Risk; 2014:132-134.
10. Veal GJ, Tweddle DA, Visser J, et al. Pharmacokinetics and safety of a novel oral liquid formulation of 13-cis retinoic acid in children with neuroblastoma: a randomized crossover clinical trial. *Cancers*. 2021;13(8):1868.
11. Khan AA, Villablanca JG, Reynolds CP, et al. Pharmacokinetic studies of 13-cis-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation. *Cancer Chemother Pharmacol*. 1996;39:34-41.
12. Veal GJ, Cole M, Errington J, et al. Pharmacokinetics and metabolism of 13-cis-retinoic acid (isotretinoin) in children with high-risk neuroblastoma - study of the United Kingdom Children's Cancer Study Group. *Br J Cancer*. 2007;96(3):424-431.
13. Miziołek B, Bergler-Czop B, Stańkowska A, Brzezińska-Wcisło L. The safety of isotretinoin treatment in patients with bone fractures. *Adv Dermatol Allergol*. 2019;36:18-24.
14. Hotchkiss CE, Latendresse J, Ferguson SA. Oral treatment with retinoic acid de-creases bone mass in rats. *Comp Med*. 2006;56:502-511.
15. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. *JAMA*. 2002;287:47-54.
16. Michaëlsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med*. 2003;348:287-294.
17. Mata-Granados JM, Cuenca-Acevedo JR, Luque de Castro MD, et al. Vitamin D insufficiency together with high serum levels of vitamin A increases the risk for osteoporosis in postmenopausal women. *Arch Osteoporos*. 2013;8:124.
18. Caire-Juvera G, Ritenbaugh C, Wactawski-Wende J, et al. Vitamin A and retinol intakes and the risk of fractures among participants of the Women's Health Initiative Observational Study. *Am J Clin Nutr*. 2009;89:323-330.
19. Koh K-N, Jeon JY, Park S-S, et al. Physeal abnormalities in children with high-risk neuroblastoma intensively treated with/without 13-cis-retinoic acid. *J Pediatr Orthop*. 2021;41(9):e841-e848.