

BRIEF REPORT

Medication contaminants as a potential cause of anaphylaxis to vincristine

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Abstract

Vincristine (VCR) is a vinca alkaloid and common chemotherapeutic that is used to treat multiple pediatric and adult malignancies. Despite its common use, cases of anaphylaxis to VCR are rare and typically isolated to a single individual. We report a series of eight patients with adverse reactions to VCR over the course of 11 months at a single institution, four of which progressed to anaphylaxis and one of which resulted in cardiac arrest. Mass spectrometry analysis of medication lots was performed to test for possible contaminant(s). Our findings highlight the risk of anaphylaxis during therapy with VCR.

KEYWORDS

allergy, anaphylaxis, contaminant, vincristine, mass spectrometry

1 | INTRODUCTION

Vincristine (VCR) is a vinca alkaloid that was historically purified from the Madagascar periwinkle *Catharanthus roseus*, but is now synthesized.^{1,2} VCR is a common chemotherapeutic that is used to treat multiple pediatric and adult malignancies. Cases of anaphylaxis to VCR have been reported, but are rare and typically isolated to a single individual.^{3,4}

Between July 2015 and May 2016, eight patients had symptoms of adverse reactions immediately after receiving VCR at The Children's Hospital of Philadelphia (CHOP), and similar reactions were colloquially reported at other institutions around the country. A subset of the patients treated at CHOP (four of eight) progressed to anaphylaxis. We performed a manual chart review of the electronic medical records for these patients, as well as mass spectrometry (MS) analysis of medication lots, to determine the cause of the reactions. This study was

reviewed by the CHOP Institutional Review Board and determined to be exempt from requiring approval.

2 | RESULTS

The demographic and clinical characteristics of the patients are shown in Table 1. There were five male and three female patients, with an average age of 5 years. Patients were treated with three lots of VCR from two different manufacturers. Underlying oncologic diagnoses were acute lymphoblastic leukemia, vaginal sarcoma, oligodendroglioma, medulloblastoma, lymphoblastic lymphoma, and Wilms' tumor. On review, we found no obvious change in the medication administration protocol, equipment associated with the medication infusion, or other similarity among the patients in question (other medications, underlying diagnosis, etc.) that would explain the reactions. Attention was given to other chemotherapeutic agents administered in proximity to VCR. In all cases, the reaction occurred during or immediately following VCR administration. In seven of eight cases,

Abbreviations: CHOP, The Children's Hospital of Philadelphia; MS, mass spectrometry; VCR, vincristine

TABLE 1 Demographic and clinical characteristics of patients and reactions

Patient			Sex	Diagnosis	Age at reaction (years)	Symptoms										Therapy																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
						First exposure (Y/N)	Tolerated subsequently (Y/N or N/A)	Respiratory	Vomiting	Flushing	Hypotension	Mouth Itch	Headache	Cardiac arrest	Hives	Antihistamine	Intravenous fluids	Steroids	Epinephrine	Oxygen	Albuterol																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
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N, no; Y, yes; N/A, not applicable.

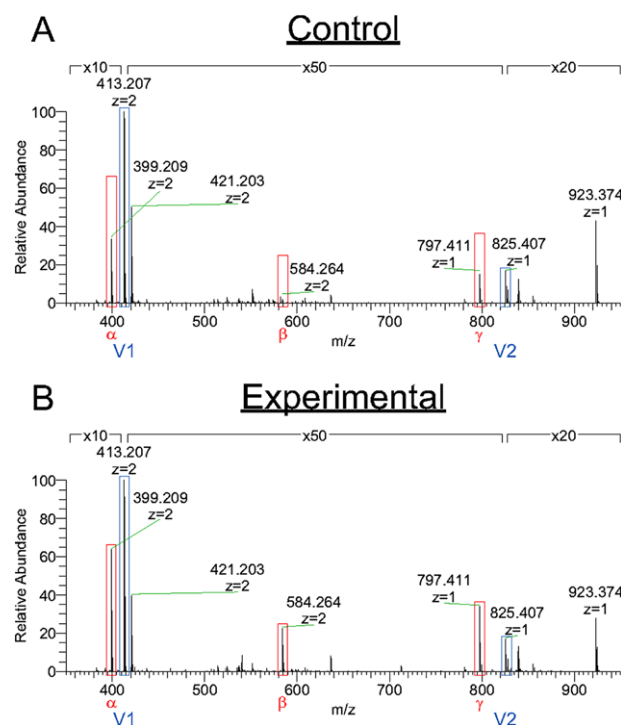


FIGURE 1 Mass spectra of vincristine samples. (A) Control sample to which no patients reacted. (B) Experimental sample to which patients reacted

VCR was the first or only chemotherapeutic administered, excluding other chemotherapeutic agents as the etiology of the reactions. In the remaining case (patient number 8), pegaspargase was administered 6 hours prior to VCR without complication. As most medication reactions occur within 1 hour of administration, it is unlikely that pegaspargase was the cause of the reaction observed in this patient. VCR is administered in a saline mini-bag of 0.9% NaCl at our institution, offering an additional potential etiology of the reactions. There was no consistency of mini-bag lot numbers across patients that reacted, and no reactions were reported with other medications prepared with the same mini-bags, making it unlikely that the mini-bags were related to the reactions in question.

Symptoms experienced were immediate and included respiratory (100%), vomiting (50%), flushing (25%), hypotension (25%), mouth itch (13%), headache (13%), and cardiac arrest (13%) (Table 1). While two patients experienced flushing, no patients experienced hives. Patients required therapy with antihistamines (100%), intravenous fluids (75%), steroids (50%), epinephrine (50%), supplemental oxygen (25%), and albuterol (13%) (Table 1). Reactions occurred on first exposure to VCR in two of the eight patients. Seven of the patients were treated with VCR subsequently without reaction, and four of these received premedications (anti-histamines $\pm$ steroids) prior to subsequent infusions. One patient required no further VCR due to completion of therapy (patient number 4). There were no long-term, medically related negative sequelae of these reactions.

Given the number of cases over a short period of time, and that most patients were tolerating VCR prior to their initial reaction or could tolerate VCR subsequently, we suspected a contaminant as the etiology of the reactions. To identify potential contaminants, we

performed MS analysis. The experimental VCR sample was taken from a lot of medication to which patients clinically reacted. A control sample of VCR was taken from a lot from the same manufacturer to which patients did not react. For each sample, 20 μ l of 1 mg/ml VCR was prepared for MS analysis by C18 reversed solid phase extraction (Zip Tip, Millipore Corp.). Briefly, columns were activated with 20 μ l of 70% acetonitrile and VCR was bound via three aspiration/dispense cycles. Columns were washed seven times with 0.1 % formic acid to remove mannitol and residual salts. After eluting VCR in 20 μ l 0.1% formic acid and 50% acetonitrile, the solution was analyzed on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) by nanoelectrospray ionization in positive ion mode, infused at a rate of 250–500 nL/min, and analyzed with a normalized collision energy of 30%, spray voltage of 1.8 kV, and ion transfer tube temperature of 275°C. Full scan MS and tandem MS spectra were recorded for both samples at a resolution of 240,000.

The spectra and analysis of the control and experimental VCR samples are presented in Figure 1 and Supplementary Table S1. Most of the molecules in both the control and experimental preparations were VCR in either mono- or diprotonated forms (Fig. 1 and Supplementary Table S1, peaks V1 and V2). Tandem MS analysis confirmed the identity of these molecules as VCR (Supplementary Fig. S1). We also identified three molecules present at up to 7% relative abundance in the experimental sample, and reduced or near absent in the control sample (Fig. 1 and Supplementary Table S1, peaks α , β , and γ). Tandem MS analysis revealed common product ions between these peaks and VCR, indicating that these molecules were likely related to the vinca alkaloids (Supplementary Fig. S1). Peaks α and γ , with m/z values of 399.209 and 797.411, respectively, represent di- or monoprotonated forms of a molecule with a combined relative abundance of 6.71% in the experimental sample compared with the predominant form of VCR.^{5,6} This molecule is related to VCR, but predicted to be missing one carbon and one oxygen atom; therefore likely representing N-dimethyl-vinblastine, a known decomposition product and proposed intermediate in the synthetic preparation of VCR.^{7,8} The third molecule had a m/z value of 584.264 and was present at 0.35% relative abundance compared with the predominant form of VCR (Fig. 1 and Supplementary Table S1, peak β). The composition of peak β relative to VCR was not able to be predicted.

3 | DISCUSSION

VCR is a chemotherapeutic that is used to treat multiple pediatric and adult malignancies. Because of its common use, it is essential to understand the risks of adverse reactions with this medication. Although cases of anaphylaxis to VCR have been reported, they are rare and typically isolated to a single individual.^{3,4} We report a series of eight adverse reactions to VCR, four of which progressed to anaphylaxis.

It is thought that most drugs are too small to directly illicit an IgE-dependent immune response. Rather, they act as haptens or prohaptens by binding endogenous proteins and causing an IgE-mediated reaction. Alternatively, small molecules can lead to direct,

IgE-independent mast cell activation, complement activation, and/or kallikrein activation.⁹ It is therefore possible that seemingly subtle differences in molecular composition can result in dramatically different immune reactivity.

We cannot be certain that the identified molecules in our study were the etiology of the observed reactions, and we do not know the mechanism of the reactions in our population. While MS is a very sensitive technique for identification of compounds, it is not without limitations and can have difficulty identifying nonvolatile and very rare substances, as well as compounds not currently available in spectra databases.^{10,11} Furthermore, very small amounts of allergen can elicit clinically significant reactions making identification of the causal compound difficult.

Nevertheless, the frequency and characteristics of the reactions reviewed here are mostly consistent with a reaction to a contaminant. It is possible that the contaminants identified represent degradation products. However, VCR concentrate is stable for 24 months when stored appropriately, and 7 days once diluted. All doses administered in this case series strictly adhered to these recommended time periods. As such, we favor a contaminant resulting from the manufacturing process as the etiology of the reactions. There is precedent for this hypothesis, as a similar series of anaphylactic reactions occurred in the United States and Germany in 2007 resulting from heparin contaminated with oversulfated chondroitin.¹² Our findings highlight the importance of recognizing the potential for complications related to anaphylaxis when treating patients with VCR specifically, as well as the importance of recognizing the potential for allergic complications as a result of impure medication preparations in general.

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AUTHORS' CONTRIBUTION

D.A.H., J.M.S., and T.B.W. made contributions to conception and design of the work; D.A.H., S.H.S., and T.B.W. made contributions to the acquisition, analysis, and/or interpretation of the data; All authors made intellectual contributions during drafting and revision of the work, approved the final version to be published, and agree to be accountable for all aspects of the work including accuracy and integrity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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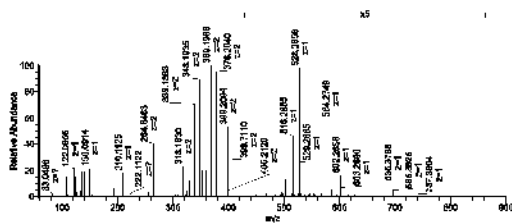
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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SUPPLEMENTAL TABLE S1 Analysis of molecules in control and experimental vincristine samples

Peek	m/z	Intensity	Relative Abundance (Control)	Relative Abundance (Experimental)	Charge	Measured Mass	Delta Composition	Predicted Mass
	383.196	10125691	0.14	0.17	2	-60.0228	-CH4N2O	-60.0324
	392.201	9049138	0.13	0.15	2	-42.0122	-C2H2O	-42.0106
α	399.209	366678336	3.35	6.19	2	-27.9962	-CO	-27.9949
	412.200	11956217	0.37	0.20	2	-2.0148	-H2	-2.0157
V1	413.207	5926512640	100.00	100.00	2	-0.0008	C46H56N4O10+2H+	413.2071
	421.203	49138952	1.03	0.83	2	15.9926	+O	15.9949
	540.282	11245577	-	0.19	2	254.1502		
β	584.264	20964042	0.04	0.35	2	342.1142		
	636.377	9780648	0.08	0.17	2	446.3394		
γ	797.411	31082270	0.30	0.52	1	-27.9955	-CO	-27.9949
	823.391	2680218	0.08	0.05	1	-2.0155	-H2	-2.0157
V2	825.407	902158080	16.87	15.22	1	-0.0002	C46H56N4O10+H+	825.4068
	839.385	36494420	0.64	0.62	1	13.9785	+O -H2	13.9793
	855.380	8905911	0.19	0.15	1	29.9732	+O2 -H2	29.9742
	923.374	76840904	2.17	1.30	1	97.967	+H2SO4	97.967