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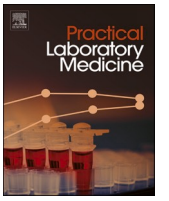

Treatment of graft versus host disease with photopheresis interferes in voriconazole therapeutic drug monitoring: A case study

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ABSTRACT

Extracorporeal photopheresis is an established procedure for refractory graft-versus-host disease, a major complication associated with notable morbidity and mortality in patients with allogeneic hematopoietic stem cell transplant. Despite being implemented over a decade ago, there is scant information about potential interactions or analytical interferences with concomitant drugs in this polymedicated population. Here we report the case of a pediatric patient diagnosed with cutaneous steroid-refractory acute graft-versus-host disease after unrelated allogeneic hematopoietic stem cell transplant that was treated with photopheresis. Analytical quantification of voriconazole by HPLC-PDA the day following photopheresis treatment did not permit therapeutic drug monitoring (TDM) due to the presence of interference at the voriconazole retention time. Following investigations, it was demonstrated that the interference is likely attributable to a psoralen-based compound. The interference was not present when samples were obtained prior to photopheresis, enabling TDM. This case underscores the relevance of communication among the members of the treating team to perform reliable TDM, especially in routine clinical practice of pediatric patients with complex diseases undergoing innovative treatments. This finding is relevant to voriconazole quantification by HPLC-PDA, frequently used in laboratories based in middle-income countries.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for a variety of hematologic and non-hematologic disorders. Despite being widely used, graft-versus-host disease (GvHD) is one of the most frequent and serious complications in patients undergoing HSCT with an incidence that varies between 30 and 70%, depending on the type of transplant and several risk factors [1]. The clinical management of patients with GvHD is challenging due to the wide variability of disease manifestations, clinical course, infectious complications, and treatment-related toxicity [1,2].

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The first-line therapy for GvHD relies on the administration of corticosteroids with a calcineurin inhibitor. However, its lack of efficacy in steroid-resistant patients, the intense immunosuppression, and significant adverse events stresses the need for new alternative therapies. In these cases, Extracorporeal Photopheresis (ECP) is a preferred second-line treatment [1].

ECP is a leukapheresis-based immunomodulatory treatment that involves the reinfusion of autologous mononuclear cells after exposure to 8-methoxypsoralen (8-MOP) and ultraviolet A (UVA) light irradiation; this process results in the apoptosis of lymphocytes and differentiation of dendritic cells which explains part of the mechanism of action [2,3].

Patients undergoing HSCT require antifungal prophylaxis. Voriconazole is the most frequently recommended agent for antifungal prophylaxis for children. Due to its narrow therapeutic index (1000–5000/6000 ng/ml) and wide inter- and intra-individual variability in the pharmacokinetics, the azole is subjected to therapeutic drug monitoring (TDM) for dosage adjustment according to individual requirement [4–6]. It is uncertain whether 8-MOP or the compounds generated after light irradiation may interact with concomitant medications or interfere with the bioanalytical assay of those drugs subjected to TDM.

Here we report a case that demonstrates interference for voriconazole quantification performed by HPLC-PDA, when samples are obtained during, or soon after, ECP. We perform investigations that demonstrate that the interference is attributable to psoralen-based compound, similar in structure to 8-MOP. Analyses were performed in the setting of bioanalytical laboratory in a middle-income country using instrumentation, thus our finding is relevant to other centres operating in a similar setting that also provide ECP.

2. Materials and methods

For ECP, peripheral blood mononuclear cells were collected by leukapheresis with a SPECTRA OPTIA separator, and the buffy coat was transferred to a UVA light permeable bag and exposed to 2 mL of 8-MOP (PIT Medical Systems GmbH) 20 μg/ml as a photoactive and photosensitizing agent. Irradiation was performed using UVA PIT system set at 2J/cm3 and then the cell suspension was re-infused to the patient.

Voriconazole (Sigma-Aldrich, #1718008) stock solution was prepared at a concentration of 1mg/ml in methanol and stored at −20 °C. Working solutions were obtained after dilution in methanol and were used to spike drug-free pool serum to obtain the calibration standards (final concentrations: 100, 200, 400, 500, 750, 1000, 2000, 3000, 4000, and 5000 ng/ml) and quality controls (1500 and 3000 ng/ml).

To carry out voriconazole TDM, blood samples (2 ml) were collected from a peripheral access in serum gel and activator clot tubes (DVS, Buenos Aires, Argentina), centrifuged at 3000 rpm during 10 min and serum was separated. To 200 μl of serum sample, calibrator, or quality control, 50 μl of the internal standard lormetazepam (Lipomed, #LOR-141)100 μg/ml in methanol were added, and vortexed for 30 seconds. Then, specimens were subjected to liquid-liquid extraction using 1 ml of hexane (Merck; Darmstadt, Germany): dichloromethane (Sintorgan; Buenos Aires, Argentina) (70:30). The organic phase was separated and evaporated under nitrogen, the residue was reconstituted in 200 μlof acetonitrile (Sintorgan; Buenos Aires, Argentina) and 5μl was injected in the chromatographic system. Liquid chromatographic analysis was performed on a Shimadzu 20A system coupled with a diode array detector set at 256 nm and data was acquired and processed using the LC solution software. The chromatographic separation was performed on a Shim-pack VP-ODS C18 column (150mm x 4.6mm, 4.6 μm) maintained at 45 °C. The mobile phase consisted of acetonitrile:water (90:10) delivered at 0.9 ml/min. Deionized water was produced using a MilliQ Water purification system (Merck, Darmstadt, Germany).. The assay was linear in the range of 200–5000/6000 ng/mL and the limit of quantification (LOQ) was 200 ng/mL. The extraction recovery was >80%, and within and inter-day precision was <10%.

3. Case description

The case, a 5-year-old male, was diagnosed with T-type Acute Lymphoblastic Leukemia (ALL), with poor response to prednisone and Minimal Residual Disease (MRD) 19.6 on day 15 following treatment according to ALLIC-2019 protocol. On December 20th, 2021, he received an unrelated allogeneic peripheral blood hematopoietic stem cell transplant. Prior to the transplant, he underwent a conditioning regimen with Total Body Irradiation (TBI) of 1200 cGy, etoposide 60 mg/kg and thymoglobulin 7.5 mg/Kg. As prophylaxis for GvHD, he received tacrolimus (initial dose of 0.03 mg/kg) and methotrexate (10 mg/m^2 on days +1, +3, +6, and +11). Antifungal prophylaxis with voriconazole (tablets) 200 mg twice daily was started on day +1 post-transplant. A blood sample was collected for voriconazole therapeutic drug monitoring (TDM) on day +8 after transplant. The patient sample obtained on day +8 post-transplant (7 days after initiating prophylaxis) showed a concentration of 5106 ng/ml of voriconazole (the sample was processed again after 1/2 dilution with saline solution and the concentration was corrected by the dilution factor). Thus, a 50% dose reduction was recommended corresponding to 100 mg twice daily to attain the prophylaxis range of 1000–2000 ng/mL [5,6].

On day +13 post-HSCT, the patient presented with febrile neutropenia relapse and thus treatment was switched from voriconazole to amphotericin lipid complex 5 mg/kg. Grade II acute cutaneous graft *versus* host disease was diagnosed on day +25 post-HSCT and thus methylprednisolone 2 mg/kg IV was started. After five days of no response to steroids, refractory GvHD was assumed and extracorporeal photopheresis (ECP) consisting of two sessions per week was indicated.

After resolution of febrile neutropenia on day +28, with normal thorax CT scan, voriconazole 100 mg twice daily was restarted. TDM of the antifungal was requested on day +37, the day after the third ECP session.

The blood sample was processed as usual, but the chromatogram showed an interference peak at a retention timeof 5.72 min while the voriconazole standard in serum eluted at 5.81minutes (Fig. 1A and B, respectively). The spectral analysis indicated that the observed peak at 5.72 minutes could not be attributed to voriconazole (Fig. 1B). To our suspicion that the interference peak was a psoralen compound, we tested retention times for standard solutions of psoralen and voriconazole. The 8-MOP solution (10 μg/mL) a

retention time of 5.35 minutes (Fig. 1C) while an external standard of the voriconazole solution (1250ng/mL in methanol) had a retention time of 5.79minutes (Fig. 1D). A solution of 10μg/mL 8-MOP and voriconazole 1250 ng/mL prepared in water showed superimposed peaks as depicted in (Fig. 1E).

Possible drug-drug interactions that might have caused a high systemic voriconazole concentration were discounted and the absence of signs and symptoms of acute voriconazole intoxication (mainly neurological and/or hepatic alterations) suggested the peak was unlikely to reflect extremely high voriconazole concentrations.

A new sample for voriconazole monitoring was requested on day +39 less than 24 hours after the fourth ECP session. Again, the chromatogram showed the interference peak at the retention time of the azole, hindering voriconazole quantification. We attempted modifications to the mobile phase and column with the aim to resolve the chromatographic interference, however these were unsuccessful. Due to clinical context and analytical constraints, it was decided to cease voriconazole and start with anidulafungin 1.5 mg/kg/day.

Following our investigations that suggested the interference was likely related to psoralen-based compound, the clinical decision was to restart voriconazole 100 mg twice daily on day +49 while maintaining anidulafungin, and attempt sampling for voriconazole quantification prior to the next ECP session. Thus, 1 h before starting photopheresis on day +57, a blood sample for voriconazole TDM was obtained.

No interference peak was observed in the chromatogram and a concentration of 200 ng/mL of voriconazole was reported. As this was subtherapeutic, the recommended dose was increased to 150 mg twice daily.

Due to progression of skin lesions, ECP was provided one every other day (Monday, Wednesday, Friday) from day +63. Blood samples for voriconazole quantification were always obtained before each ECP session (about 48 h of the last session). Voriconazole doses were gradually increased to 300 mg twice daily resulting in a blood concentration of 1594 ng/mL on day +86 and anidulafungin was stopped.

4. Discussion

Extracorporeal photopheresis is an efficient and established therapy for cutaneous T-cell lymphomas, GvHD, solid organ transplant rejection, and various autoimmune diseases. The procedure is based on the photochemical inactivation of patient leucocytes collected by apheresis after external incubation with 8-MOP and UVA exposure [7,8]. A fraction of 8-MOP remains in the bag with the cell suspension after UV radiation and is infused to the patient along with treated leukocytes, blood concentrations of 8-MOP in patients have been reported as undetectable as a consequence of rapid renal elimination from the body [9,10]. To our knowledge, there are no previous reports about the bioanalytical interference of 8-MOP or its metabolites influencing quantification of drugs subjected to TDM.

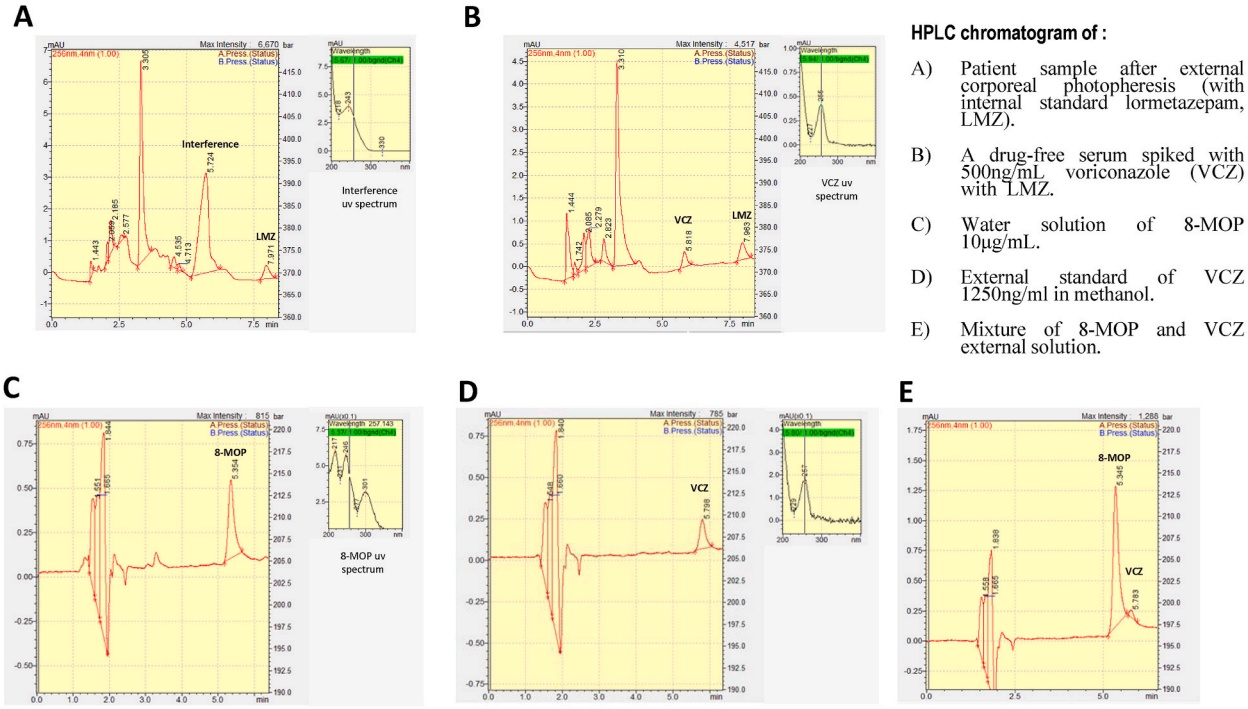

Fig. 1. HPLC chromatogram of A) patient sample after external corporeal photopheresis (with internal standard lormetazepam, LMZ); B) a drug-free serum spiked with 500 ng/mL voriconazole (VCZ) with LMZ; C) Water solution of 8-MOP 10 μg/mL; D) External standard of VCZ 1250 ng/ml in methanol; E) Mixture of 8-MOP and VCZ external solution.

The present case is a patient with an unrelated allogeneic peripheral blood hematopoietic stem cell transplant that, due to refractory cutaneous GvHD, received ECP therapy.

The patient also received voriconazole as antifungal prophylaxis, with dose adjustment performed using TDM, which at our center, involves a validated analytical method based on HPLC-PDA. We observeda chromatographic interference at the retention time of voriconazole when TDM was performed concomitantly with bi-weekly photopheresis procedures. After discounting clinical conditions of the patient or drug-drug interactions, we hypothesized a possible analytical interference by a psoralen-based compound.

Previous studies have shown that 8-MOP is metabolized in the liver through cytochrome P450 and is subjected to renal elimination with a high inter-individual variability in the elimination half-life. Several sources of elimination variability include impairment of hepatic or renal function, drug-drug interactions due to multiple concurrent medications that patients receive during ECP therapy (via induction or competitive inhibition of P450 enzymes) and polymorphisms in CYP450 enzymes. Moreover, previous reports on 8-MOP disposition have shown its binding capacity to blood proteins such as albumin affecting the distribution and elimination of the drug [9–11]. Thus, concomitant drugs that are frequently used in children with complex diseases and that also bind to albumin (such as methylprednisolone, furosemide, enalapril), may displace 8-MOP from albumin and increase the unbound fraction that becomes available for liver metabolism and renal filtration [9].

As expected for cases with allogeneic stem cell transplant, our patient received concomitant drugs that are subjected to cytochrome P450-mediated metabolism such as furosemide, methylprednisolone, enalapril, paracetamol, hydroxyzine, and voriconazole. Hypothetically, some of these drugs may have triggered drug-drug interactions resulting in a longer than expected 8-MOP half-life with quantifiable concentrations observed during voriconazole TDM.

In high-income countries, voriconazole monitoring along with other drugs from the azole pharmacological family is commonly performed using liquid chromatography-tandem mass spectrometry [12–14]. Nonetheless, in middle-income countries we face a limitation in the availability of LC-MS/MS units and the most frequent instruments for analytical quantification are HPLC coupled with PDA or fluorescence detectors. Therefore, in our setting of limited analytical resources but in a clinical center that provides high complex treatments such as ECP, we perform TDM using HPLC-PDA and work in close collaboration with pharmacists and clinicians to optimize the pharmacotherapeutic treatment.

According to our case and the analytical capabilities of our center, we recommend performing voriconazole TDM obtaining blood samples before ECP therapy or apart, at least 48 h, from the last ECP session so as to ensure the complete removal of 8-MOP from the plasma and avoid an analytical interference.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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