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Case report

A clinical approach to solving discrepancies in therapeutic drug monitoring results for patients on sirolimus or tacrolimus: Towards personalized medicine, immunosuppression and pharmacogenomics

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ABSTRACT

Background: Unexpected clinical laboratory concentrations often need to be investigated before they are acted upon in a clinical setting. Therapeutic drug monitoring (TDM) frequently involves drugs with narrow therapeutic windows and can be harmful to the patient if changes are made based on erroneous serum drug concentrations. Too little of the drug will result in ineffective therapy and too much of the drug can cause life threatening toxicities. There are many factors that can result in unexpected serum drug concentrations including differences in analytical methods being used, diet, timing of blood draw, genotype and compliance. All these factors should all be considered before deciding if changes should be made in a patient's therapeutic course.
Case report: We determined the cause of 2 patient's unexpected TDM concentrations for sirolimus and tacrolimus. Using this approach in 2 patient cases, we describe how co-treatment and uncommon genotypes result in unexpected drug concentrations.
Conclusions: Both cases involved unexpected drug values. In the first case, the cause was revealed to be a drug that was added to the patient's treatment regimen (posaconazole) that inhibits CYP3A4 which is responsible for sirolimus metabolism. In the second case, the patient was revealed to have an uncommon genotype for CYP3A5, causing higher metabolism and lower serum tacrolimus concentrations than the general population.

1. Introduction

Sirolimus and tacrolimus are immunosuppressant drugs of different classes. Sirolimus is an mTOR inihibitor while tacrolimus is a calcineurin inhibitor. Sirolimus is an immunosuppressive drug that has been used since its approval in 1999 to prevent organ rejection in transplantation. Sirolimus inhibits mammalian target of rapamycin (mTOR) which is a kinase required for control of hormone signals and multiple cellular processes including growth. Because the use of mTOR inhibitors is increasing in transplantation [1,2] as well as in cancer therapies [2], there is a need to better predict the correct patient dose and calculated adjustments. Sirolimus is primarily metabolized by cytochrome P450 3A4 (CYP3A4) enzyme and exhibits large pharmacokinetic variability. Efforts have been made to predict correct patient dosing including a physiologically-based pharmacokinetics model (PBPK) [3]. Tacrolimus (FK-506), first described in the 1980s, is a 23-membered macrolide lactone and is isolated from the fermentation of *Streptomyces tsukubaensis* [4]. CYP3A4 and CYP3A5 are both responsible for the metabolism of tacrolimus. Unlike sirolimus, the intrinsic clearance of tacrolimus for CYP3A5 is 2-fold higher than for CYP3A4 [5]. Although the metabolism of sirolimus and tacrolimus exhibits large pharmacokinetic variability, genotyping patients is not commonly performed. When an unexpected drug concentration occurs, a rigorous and systematic approach including genotyping should be considered to reveal the cause and to correct the dose for treatment.

1.1. Unexpected increased sirolimus drug concentration/Patient A

An unexpected sirolimus concentration of 55.2 ng/ml (therapeutic range 5–15 ng/ml) was reported from the laboratory at our hospital using an enzyme multiplied immunoassay (EMIT) methodology performed on a Viva-E analyzer (Siemens Diagnostics). Because this concentration was much greater than the upper end of the therapeutic range, over time the development of toxicities including thrombocytopenia, anemia, leukopenia and infections were of concern. To further investigate this drug concentration, a systematic approach was applied which included 3 phases of assurance and investigation: I) confirmation of the concentration using a different analytical method, II) reviewing the patient's history and III) determining the patient's genotype.

Phase I involved using a different analytical method to confirm the drug concentrations. This provides confidence that the drug concentration obtained is a true representation of the patient's serum drug

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concentration. To ensure that the sirolimus concentration obtained using the immunoassay was an accurate representation of the patient's serum sirolimus concentration, the same samples were confirmed using LC/MS/MS (Waters Xevo TQD).

Once there was confidence that the concentrations obtained were representative of the patient's true serum sirolimus concentrations, we moved onto phase II and reviewed the patient's clinical status, concurrent medications and history. It is absolutely essential to review any changes in treatment that may affect drug concentrations including drugs that are metabolized by the same enzyme or inhibit an enzyme that metabolizes another drug. These drug–drug interactions are extremely important to monitor while a patient is on an mTOR inhibitor.

Lastly, for phase III, we had the patient genotyped for CYP3A4. Sirolimus (an mTOR inhibitor) is predominantly metabolized by hepatic and intestinal cytochrome P450-3A4 (CYP3A4) enzymes and is a substrate and inhibitor of P-glycoprotein [6]. Variability in CYP3A4 can alter the amount of drug that is active and available in the serum. Extensive metabolizers are expected to have less of the parent drug available while poor metabolizers will have a greater amount of the parent drug biologically available [7].

2. Results

2.1. Phase I/Patient A

A sirolimus concentration of 55.2 ng/ml (therapeutic range 5–15 ng/ml) was reported from the laboratory using a Viva-E analyzer (Siemens). To ensure that no error in collection occurred, a recollect was ordered later that day and the sirolimus serum concentrations were measured again. The result was reported as >60 ng/ml. To ensure that there was no bias caused by the analytical method used, the same samples were confirmed using LC/MS/MS. These concentrations are reported in Table 1.

2.2. Phase II/Patient A

The patient's medical history and concurrent therapies were reviewed. A 43-y-old male was admitted to hospital due to fever and hypotension. The patient has a history of chronic myelogenous leukemia (CML) for which he received an allogeneic hematopoietic cell transplant. Immunocompromised patients are at increased risk for fungal infections and the mortality of fungal infections necessitates the use of antifungals. The risk of drug toxicity with co-administration of both sirolimus and triazoles often limits their co-administration. The patient had been treated with sirolimus for over 1.5 y (3 mg qd orally) and concentrations were consistently in the therapeutic range (5–15 mg/dl). Seven days following the initiation of posaconazole treatment, the patient's sirolimus drug concentrations reached a dangerously high concentration of 63.5 mg/dl which was greater than 4 times the upper limit of the therapeutic range. A single study has been previously published reporting the safety and feasibility of co-administration by empirically reducing the sirolimus dosage upon initiation of posaconazole [8]. Currently, clinicians determine dose adjustments empirically because no algorithm is available to provide guidance for dose adjustments of sirolimus for coadministration with posaconazole.

Table 1
Sirolimus levels before, during, and after initiation of posaconazole using both immunoassay and LC/MS/MS.

Date collected	VivaE	Waters Xevo TQD
	EMIT result ng/mL	LC/MS/MS result ng/mL
6-Mar	13.9	Not available
13-Mar	55.2	63.5
13-Mar	>60	34.3
17-Mar	29.1	15.1
19-Mar	11	6.8
24-Mar	3.2	<2.0

2.3. Phase III/Patient A

Variability in CYP3A4 can alter the amount of sirolimus that is active and available in the serum. A 2013 publication indicated that CYP3A4*22 results in 20% lower metabolic rate of sirolimus [9] which would produce higher serum drug concentrations. Although not routinely performed, knowing the CYP3A4 genotype can help predict in vivo behavior and appropriate drug amounts to be administered. We had our patient genotyped (PGXL Laboratories, Louisville, KY) for the major CYP3A4 variants that are known to affect drug metabolism including CYP3A4*2, *3, *17, and *22. Our patient was a *1/*1 extensive metabolizer (wild type for the all variants tested). This would indicate that the patient would not have a higher concentration of parent drug due to his genotype.

2.3.1. Patient resolution/Patient A

Six days following posaconazole initiation, the patient's sirolimus concentrations were increased 4.2 fold over the concentration prior to posaconazole initiation (63.5 ng/ml vs 13.9 ng/ml). These concentrations were obtained using an immunoassay (Viva-E) and were confirmed using LC/MS/MS (Table 1). Following the zenith (63.5 ng/ml) result, both medications were discontinued. Micafungin was initiated immediately to replace posaconazole. The sirolimus was discontinued long enough to allow the concentrations to drop below the therapeutic range. Eleven days were required for the sirolimus concentrations to drop below 2 ng/ml Fig. 1. Once the sirolimus concentrations dropped, a lower dose of sirolimus (1 mg) was reinitiated on that same day to allow posaconazole to be reinitiated.

2.3.2. Unexpected decreased tacrolimus drug concentration/Patient B

An unexpected decreased tacrolimus concentration of 3.1 ng/ml followed by a second concentration of 3.9 ng/ml was reported from the laboratory using the Viva-E analyzer. Because these concentrations are below the therapeutic window for tacrolimus (5–20 ng/ml), the patient was likely not receiving therapeutic benefit of immunosuppression. To further investigate the unexpected drug concentration, the same systematic approach described for Patient A (above) was applied.

Phase I included using a different analytical method to confirm the drug concentrations. This provides confidence that the drug concentration obtained is a true representation of the patient's true serum drug concentration. The tacrolimus concentrations obtained by immunoassay were confirmed using LC/MS/MS, the gold standard for tacrolimus measurement.

Phase II included reviewing the patient's clinical status including concurrent medications and medical history. History of patient

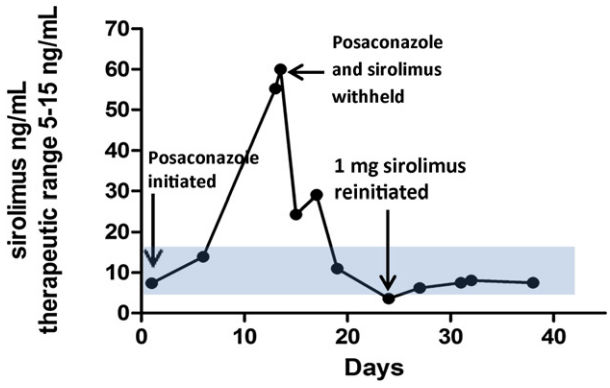

Fig. 1. Sirolimus levels before, during, and after initiation of posaconazole during year 3 of sirolimus treatment.