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Falsely elevated cyclosporin and tacrolimus concentrations over prolonged periods of time due to reversible adsorption to central venous catheters

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ABSTRACT

Falsely elevated concentrations of immunosuppressants can be caused by reversible adsorption to central venous catheter (CVC) systems. If undetected, this may lead to dose reduction resulting in underdosage which may even entail graft-versus-host disease or organ rejection. We analyzed the adsorption and release for cyclosporine A (CsA) and tacrolimus (Tac) in vitro and in vivo. Four types of CVCs were examined in vitro: two made from polyurethane (PU), one from silicone and one from PU with an incorporated silver ion-based antimicrobial agent. All 26 CVCs analyzed in vitro showed significant reversible adsorption of CsA ($n = 13$; $p = 0.001$) and Tac ($n = 13$; $p = 0.001$, Wilcoxon signed rank test). Immediately after infusing the drugs, the mean concentrations of 6420 ng/mL of CsA and 250 ng/mL of Tac were measured. Flushing with NaCl lowered the drug release. Besides, blood samples of fifteen patients were taken simultaneously from all lumina of the CVC and via venipuncture. The samples from contaminated lumina showed the mean elevations by a factor of 11 for CsA ($n = 12$) and 89 for Tac ($n = 3$). Blood sampling for immunosuppressant monitoring should thus never be performed from lumina previously used for infusing the drug even after prolonged periods of time and extensive rinsing.

1. Introduction

For over 30 years, cyclosporine A (CsA) and tacrolimus (Tac; FK506) have been cornerstones in immunosuppressive therapy after solid organ as well as stem cell transplantation [1,2]. Both CsA, a lipophilic cyclic peptide, and Tac, a macrolide, inhibit calcineurin resulting in a reduction of lymphocytes [3]. For patients receiving these drugs, it is crucial to maintain the correct drug concentration within the narrow therapeutic range. Inappropriate levels can result in renal toxicity, hypertension, severe infections, transplant rejection or graft-versus-host disease [4–6].

Erroneously high immunosuppressant concentrations have been reported repeatedly to be caused by reversible adsorption of the drugs to different intravenous catheter systems [7–20]. This can be especially dangerous since falsely elevated levels may fall within the therapeutic range whereas in reality, the patient is in risky underdosage. Graft-versus-host disease or organ rejection could be a life-threatening consequence [4,5].

A case in our house (cf. 2.2.2, patient A) turned our special attention to this pitfall: two days after being switched to oral Tac administration, a blood sample, accidentally taken from the lumen of the central venous catheter (CVC) previously used for infusing Tac, yielded a toxic concentration of 86 μg/L of Tac. A control sample obtained by venipuncture, though, showed a value of 2.9 μg/L of Tac. This remarkable discrepancy prompted us to perform a thorough in vitro and in vivo study on this effect.

To systematically examine this adsorption and release effect for CsA and Tac in vitro, we studied some of the CVC systems most widely used in Europe and the USA. The 26 analyzed CVCs consisted of different materials, namely polyurethane (PU), silicone and PU with an incorporated silver ion-based antimicrobial agent. To substantiate the findings from our extensive in vitro experiments, a prospective in vivo study was conducted with fifteen patients receiving CsA or Tac via a CVC after stem cell transplantation.

Abbreviations: CsA, cyclosporine A; CVC, central venous catheter; FFP, Fresh Frozen Plasma; P0, first 2 mL portion of the mimicked in vitro blood sampling (discarded); P1–P5, consecutive 2 mL portions of the mimicked in vitro blood sampling; PU, polyurethane; PU-AH-CVC, PU-In-CVC, silver-Vy-CVC, silicone-Vy-CVC, cf. Table 1; Tac, tacrolimus; TTP/HUS, thrombotic thrombocytopenic purpura/hemolytic uremic syndrome.

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Table 1
Central venous catheters (CVCs) used in in vitro and in vivo experiments.

Abbreviation	Name	Material	Brand	Number of lumina	Inner diameter of contaminated lumen (mm)	Total length of contaminated umen (cm)
PU-AH-CVC	Quad-Lumen Central Venous Catheterization Set	Polyurethane	Arrow-Howes™, (Kernen, Germany)	4	1.52	46.6
PU-In-CVC	Trilucath	Polyurethane	Intra, (Rehlingen-Siersburg, Germany)	3	0.80	36.5
Silver-Vy-CVC	Multicath 4 Expert	Polyurethane incorporated with silver ion-based antimicrobial agent	Vygon, (Aachen, Germany)	4	1.25	35.8
Silicone-Vy-CVC	Lifecath Apheresis Plus	Silicone	Vygon (Aachen, Germany)	3	1.9	50.0

2. Material and methods

2.1. In vitro experiments

2.1.1. Basic study

Four types of catheters, made of three different materials, were examined in vitro: Arrow-Howes™ "Quad-Lumen Central Venous Catheterization Set" (n = 6, PU-AH-CVC), Intra "Trilucath" (n = 2, PU-IN-CVC), Vygon "Lifecath Apheresis Plus" (n = 2, silicone-Vy-CVC) and Vygon "Multicath 4 Expert" (n = 2, PU with an incorporated silver ion-based antimicrobial agent, silver-Vy-CVC; Table 1). One of the following doses was infused into one lumen of a CVC to mimic a therapeutic in vivo application of the drug: 250 mg of CsA (n = 10; Sandimmun, Novartis Pharma) in 100 mL NaCl solution (0.9%) (2.5 g/L of CsA) over 6 h or 2 mg of Tac (n = 10; Prograf, Astellas) in 50 mL NaCl (40 mg/L of Tac) over 22 h. Right after infusing the drug, blood sampling was mimicked in all four lumina with 4 mL of Fresh Frozen Plasma (FFP) each, always discarding the first 2 mL. When blood sampling was simulated, special attention was given to avoid cross contamination between the different lumina, particularly at the exits of the individual ports at the distal end of the catheter. Therefore, FFP was infused retrogradely with a syringe and collected at the distal end without getting in contact with the exits of the other lumina. The direction of sampling FFP had no significant effect on the released drug concentrations. The CVC then was recurrently rinsed with NaCl (0.9%), the first volume always being 10 mL, followed by multiple flushes of 1 L of NaCl. Each rinsing was followed by mimicking a blood withdrawal with 12 mL of FFP. To thoroughly analyze the withdrawal, the 12 mL was collected in six separate 2 mL doses, always discarding the first 2 mL. The lumina of the CVCs were not blocked with any fluid at any time.

2.1.2. Water bath

In eight additional cases, infusing the drug was performed in a water bath at 37 °C to create a more realistic setting. All four types of catheters were once infused with CsA (n = 4) and once with Tac (n = 4), each time using a new catheter.

2.1.3. EDTA blood

In addition to the 20 experiments conducted with FFP only, six PU-AH-CVCs were used for further investigations. This time, some mimicked blood sampling was performed with uncontaminated whole blood taken into EDTA containing tubes in order to study whether the whole blood and FFP behave similarly: Four CVCs were investigated in the usual manner, except for mimicking the blood collection alternating with FFP and EDTA blood. These experiments were performed with both drugs once at room temperature and once with the drug being infused in a water bath at 37 °C.

To study the possible adsorption effects with the drugs being in the therapeutic range, two PU-AH-CVCs were put into EDTA blood for 24 h with a concentration of 125 μg/L of CsA (n = 1) and 10 μg/L of Tac (n = 1) respectively. Blood sampling was mimicked with EDTA blood after rinsing the CVCs with 10 mL of NaCl.

2.1.4. Fat emulsion

In order to examine the effect of oily fluid on the release of the drugs, two PU-AH-CVCs were infused with CsA and Tac respectively. After flushing them with 1.01 L of NaCl, 100 mL of fat emulsion (Clinoleic 20%, Baxter) was infused. Blood sampling was then mimicked with 3 × 2 mL of EDTA blood, discarding the first 2 mL. Another 1.01 L of NaCl and 50 mL of fat emulsion were infused into the CVC previously contaminated with Tac. Afterwards, blood sampling was performed in the same manner as just described.

2.2. Patients

We conducted a monocentric, prospective pilot study, approved by the institution's responsible committee and in accordance with the current revision of the Helsinki Declaration. Fifteen in-patients after allogenic stem cell transplantation aged 22 to 68 were included after giving informed consent (patients 1–15; Table 2). Twelve of them were receiving CsA and three were treated with Tac.

In all cases, blood withdrawal was performed at all lumina of the CVC, each time discarding the first 5 mL of blood as it is a clinical practice in our house. Additionally, a blood sample was taken simultaneously via peripheral venipuncture each time. All samples were collected into EDTA containing tubes of 2.7 mL.

2.2.1. Main study

In thirteen cases (patients 1–13, Table 2), the drug had been administered via a PU-AH-CVC (cf. 2.1.1, in vitro experiments). Doses of 75 mg to 750 mg of CsA and 1.0 mg to 2.5 mg of Tac had been administered over 7 to 22 days. The drug was received each day for 22 h, followed by flushing with 50 mL of NaCl for 2 h. Trough blood samples for our study were taken after 2 h of rinsing.

2.2.2. Special cases

The only distinction in patient 14 was the fact that drug administration had been switched to oral medication three days prior to our blood sampling.

Patient 15 had received only a single dose of Tac via a silver-Vy-CVC (cf. 2.1.1, in vitro experiments). Due to high Tac concentrations in the blood, the infusion with a concentration of 60 mg/L of Tac was stopped after 40 mL had been administered. For the following three days, Tac administration was stopped completely. On the third day after withdrawing drug administration, blood samples were taken for our study.

Patient A had received Tac for 27 days via a three-lumen CVC made from polyurethane by Arrow-Howes™. Blood samples were taken on days 7, 8 and 18 after stopping Tac administration through the CVC.

2.3. Measuring

All in vivo and in vitro samples were measured in duplicate by a LC/MS/MS immunosuppressant method modified from Koal et al. [21] and Ceglarek et al. [22] using a two dimensional HPLC system coupled to an ABI 3000 tandem mass spectrometer. Mobile phase chemicals