70% Ethanol for Decontamination of Central Venous Lines Exposed to Calcineurin Inhibitors

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Abbreviation	Key
CVL	Central venous line
CNI	Calcineurin inhibitor
HSCT	Hematopoietic stem cell transplantation
GvHD	Graft versus host disease
MDM	Medical decision making
SD	Standard deviation
CV	Coefficient of variance
SCL	Significant change limit

Abstract

Introduction: Tacrolimus, commonly used for GvHD prophylaxis is usually administered via a dedicated CVL and trough levels drawn from the unexposed lumen. Being an oil-based medication, it may be adsorbed to the inside lumen of the CVL and result in falsely high levels drawn from an inadvertently exposed lumen. There is no treatment for decontamination of such CVLs and natural decay occurs over months before the CVL can be used to draw reliable trough levels. Methods: We studied the efficacy of 70% ethanol lock in decontaminating CVLs exposed to tacrolimus in children during transplant. Trough tacrolimus levels were drawn from the exposed and unexposed (control) lumens at 8:00AM, followed by a 2 ml 70% ethanol lock instilled for a 2hour dwell into the exposed (intervention) lumen. Trough tacrolimus levels were again drawn from both lumens at 8:00PM and levels compared for efficacy. Results: All 20 sets showed a high 8am trough level in the exposed intervention arm (median = 30 ng/ml) to be significantly greater ($p < .0001$) than the control arm (median = 9.05 ng/ml) and were contaminated. After the 2h ethanol lock, 65% of the lumens were decontaminated. The difference between the control and intervention arms were no longer found to be statistically significant ($p = 0.0826$). Conclusion: A 2-hour 70% ethanol lock is effective for decontamination of CVLs exposed to tacrolimus. These results will help to easily fix a tenacious problem encountered in the allogeneic HSCT field.

Introduction

Graft versus host disease (GvHD) is a common complication after an allogeneic hematopoietic stem cell transplant (HSCT) and calcineurin inhibitors (CNI) along with methotrexate are the standard of care for GVHD prophylaxis (1). Patients undergoing HSCT usually have a double lumen central venous line (CVL), with one lumen dedicated to administer the CNI and the other (unexposed to CNI) to measure trough CNI levels, besides other uses. Tacrolimus and cyclosporine A are oil-based medications and can be adsorbed to the inside lumen of the CVL catheter. Many studies have demonstrated that trough CNI levels drawn through a lumen exposed to CNI can be falsely elevated, complicating the medical decision making (MDM) process (2, 3). As the levels could be up to 8 times higher than those from the unexposed lumen; it is standard practice that trough CNI levels are not measured from blood drawn from a lumen used to infuse the CNI (3, 4).

A retrospective analysis at Nationwide Children's Hospital's (NCH) BMT unit over a two-year period (2010-12), showed that 17% of the unexposed CVL lumens were contaminated with a CNI during the life of a CVL (personal communication, unpublished data). Once a CVL lumen is contaminated, the only alternative to ensure accurate trough CNI levels is to obtain peripheral blood sample via a needle stick. Continued use of such contaminated CVL lumens leads to natural decay of the adsorbed CNI over many weeks to months, however until then, peripheral blood samples have to be collected. This can cause additional physical and/or mental trauma to the patient and the family. Moreover, the need to perform additional trough CNI levels results in added financial burden to the patient and increased resource utilization for medical

centers. There is no known published method or process reported for decontamination of CNI-exposed CVLs.

Miscibility is the ability of two or more liquids to mix and form an even solution, thus substances with bipolar molecules will dissolve with other substances with bipolar molecules. Intravenous cyclosporine is formulated in polyoxyethylated castor oil and alcohol and i.v tacrolimus is formulated in polyoxyl-hydrogenated castor oil and alcohol (5, 6). As alcohol (70% ethanol), a bipolar solvent is easily miscible with these solvents, we hypothesized that a 70% ethanol lock instilled into the CVL lumen will elute out the oil-based CNI adsorbed to the inside lumen of the CVL thus decontaminating it and making it reusable for drawing trough CNI levels.

Safety of using 70% ethanol in CVLs has already been proven in multiple studies using ethanol locks to prevent or treat CVL related bacterial infections in pediatrics (7-10). Ethanol and hydrochloric acid locks have also been used for clearing CVLs occluded with lipid and/or drug precipitates (11). Crnich et al. exposed polyurethane and silicone catheters to 70% ethanol for as long as 10 weeks and did not find any reductions in mechanical integrity of the CVLs(12). Studies have also shown that small volumes of ethanol are tolerated by pediatric patients without any untoward effects (9). However, there are no previous studies on the use of 70% ethanol for decontamination of CVLs exposed to CNI, which is a commonly encountered problem in BMT patients. The primary objective of this study was to estimate the efficacy of 70% ethanol locks for

decontaminating CVLs exposed to tacrolimus. The secondary objective was to estimate the incidence of side effects of ethanol locks in the study population.

Methods and Statistics

This prospective study was approved by the Institutional Review Board (IRB14-00028). Twelve patients (contributing 20 sample sets) between the ages of 3 months and 30 years, undergoing allogeneic HSCT were included in the study after informed consent. Patients, who were hemodynamically compromised or in the intensive care unit were excluded from the study. Standard practice at our institution is to administer intravenous tacrolimus over a period of 120 mins starting from day -3 of HSCT via the white lumen of the CVL (labelled as "exposed to CNI") and to obtain trough levels via the red lumen (never exposed to CNI). For this study the red lumen was labelled as the *control arm* of the study and the white lumen (the exposed and hence contaminated lumen) was marked as the *intervention arm*. As per standard practice we measure trough tacrolimus levels twice a week (every Monday and Thursday). For this study, trough tacrolimus levels were drawn from both lumens at 8:00AM on the first Monday or Thursday after day -3, followed by infusion of tacrolimus via the white lumen as per standard practice. A 2-ml 70% ethanol was then instilled for two hours in the white lumen only (intervention arm) and allowed to dwell. At the end of two hours, ethanol was removed; the lumen flushed with 0.9% sodium chloride and normal use of the CVL was resumed. To measure the efficacy of the ethanol intervention, trough tacrolimus levels were again drawn from the control and the intervention arms at 8:00PM, before infusing the evening dose of tacrolimus. The two levels drawn at 8am and at 8pm constituted one data set and were labelled as A1/B1 and A2/B2 respectively as shown in Figure 1. The first data set was collected on the first Monday or

Thursday after the start of tacrolimus and additional data set(s) were collected subsequently. The procedural methods for the ethanol lock and the flow diagram to illustrate the schema of the study are shown in Figure 1.

Tacrolimus was measured in the clinical laboratory at NCH on the Abbott Architect by a one-step chemiluminescent micro-particle immunoassay in whole blood following a protein-precipitation pretreatment with methanol and zinc sulfate, according to the manufacturer's recommendations. The 120-day running assay precision is defined by three levels of quality control materials as follows: level 1 mean: 4.6 ng/mL, SD: 0.22, coefficient of variance (CV): 4.7%, level 2 mean: 8.6 ng/mL, SD: 0.38, CV: 4.4%, level 3 mean: 17.4 ng/mL, SD: 0.79, CV: 4.5%. The analytical measurement range of the tacrolimus assay is 2-30 ng/mL, with sample dilution permitting reporting up to 60ng/mL; we follow a clinical range of 8-14ng/ml for therapeutic dose adjustments. An analytically significant change is defined as exceeding the significant change limit (SCL) which is the initial/control value +/- 2.8 x the assay analytical CV (13). Since all three levels of tacrolimus quality control (mentioned above) had nearly the same variance (% CVs ranging from 4.4-4.7%) with an average of 4.5%, the average % CV was used to represent assay analytical variance. A significant change limit (SCL) for this study was defined by the following equation:

SCL = 4.5% x 2.8 = +/- 12.6%

Thus, any change from the control +/- 12.6% was considered analytically significant. Trough tacrolimus levels from both lumens drawn at 8:00AM (A1 and B1) were compared with levels drawn at 8:00PM (A2 and B2). The study was set up to

analyze data after 20 data sets were collected. Data were summarized with descriptive statistics. Data was analyzed to be compared based on analytical reference ranges with SCL and additional analysis was done based on the clinical reference range used for MDM. Comparisons between control and intervention arms were assessed using nonparametric methods. Analysis was not performed at the patient-level, but rather observation-level; because the overall aim of the study was to see if existing contamination could be removed with an ethanol lock, and since every observation had its own comparative control, each patient could provide multiple sets of data. The aspect of how tacrolimus level varies due to biological differences was not of interest, thus the lack of truly independent sets of observations was not a large concern. All statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC).

A lumen of a CVL was considered to be potentially contaminated with CNI, if CNI had been infused through that lumen or if a high trough level obtained from that lumen could not be clinically justified. The ethanol intervention was considered effective in decontaminating a CVL lumen, if the 8pm tacrolimus level from the intervention lumen (B2) was within the SCL of the control arm (A2) at 8 pm or if the level fell within or below the clinical reference range. The ethanol intervention was considered partially effective if the 8pm tacrolimus level (B2) decreased by a value equal to or greater than the SCL of the intervention arm at 8am (B1). Patients were monitored for symptoms and signs of ethanol intoxication, including drowsiness, ataxia, slurred speech, loss of consciousness, or memory loss. In addition, monitoring for signs of hypoglycemia, blood pressure and blood alcohol levels (if needed) was also done.

Results

Between day "0" and day +7 post-transplant, most patients are on relatively fewer medications, thus allowing for a 2 hour CVL free time (lock time) and to be part of the study. Twelve patients participated in the study contributing a total of 20 sets of data. Median age of study subjects was 7 years and no patient experienced any adverse event necessitating removal from study. There were only two instances when the ethanol lock could not be fully withdrawn from the CVL; in each of these cases, the line was flushed with normal saline and returned to normal use with no notable side effects. Table 1 shows the raw data of all 20 sets.

All 20 sets showed that the 8am trough tacrolimus level (B1) in the exposed intervention arm (median = 30 ng/ml, range: 11.8 – 60 ng/ml) to be significantly greater ($p < .0001$) than the control arm (A1) (median = 9.05 ng/ml, range: 2.7 – 18.9 ng/ml) and were contaminated as defined by the SCL above. The level of contamination in the exposed lumen as measured by percentage difference from the control arm, ranged from 33% to 1011% (median 238% (Table 1 and 2). Seventeen of the 20 (85%) trough tacrolimus levels at 8am from the intervention lumen were greater than the clinical reference range of 8-14ng/ml. The 3 lumens which were within the clinical reference range were still significantly higher as compared to the levels from the control lumens (Table 1, IDs 18-20).

Effect of ethanol lock therapy: Figure 2 and 4 show a comparison of tacrolimus levels in the intervention arm and the control arm for each observation. After a 2-hour ethanol lock, the 8pm trough tacrolimus levels (B2) decreased from the 8am levels in all 20 (100%) observations, with

measurements ranging from 3.3ng/ml to 24.2ng/ml and a median of 12.14 ng/ml. The median percent decrease from 8am to 8pm in the intervention lumen was 66% (range16% to 80%). Based on the laboratory analytical reference range and SCL as defined above, 8/20 (40%) lumens were considered decontaminated (Table1, Column L). However, based on the clinical reference range of tacrolimus (8-14ng/ml), after the ethanol lock 13/20 (65%) of the lumens were decontaminated and only 7 were still above the clinical reference range. The difference between the control and intervention results were no longer found to be statistically significant ($p = 0.0826$).

Twelve out of 20 (60%) sets had extremely high trough tacrolimus levels (≥30ng/ml) at 8:00AM in the intervention arm (B1) (Table 1 and Figure 2, ID: 1-12). After the 2h ethanol lock, median trough tacrolimus levels (B2) were 15.25 ng/ml (range 7.7 to 24.2 ng/ml); representing a median percent decrease of 71% (range 40-81% decrease) compared to its 8am measurement. In 6/12 (50%) of these lumens, the levels decreased below the clinical reference range (8-14ng/ml), in 4/12 lumens with levels still >14ng/ml after the ethanol lock, the corresponding 8pm control lumen level was also >14ng/ml.

There was no correlation of the duration of CVL placement (the life of CVL) and degree of contamination of the white lumen ($p = 0.8998$) as shown in Figure 3A and there was no correlation of the degree of CVL contamination with the amount/dose of tacrolimus infused through the white lumen ($p = 0.6949$) as shown in Figure 3B.

Discussion

CVL contamination with a CNI is a common problem encountered in patients undergoing allogeneic HSCT and institutions using intermittent tacrolimus infusions. The current solution is to check trough CNI levels via peripheral venipuncture for MDM and wait for the contamination to resolve itself with natural decay, which can take weeks or months. Replacing the contaminated CVL with a new CVL will expose the patient to additional risks of anesthesia and surgery is not practically feasible. Hence it is important to prevent contaminations with diligent nursing care. While such a contaminated CVL by itself has no detrimental effects on the patient, it does affect the MDM process, causing indirect harm.

This study showed high tacrolimus levels in the intervention arm and confirmed that once a lumen is exposed to tacrolimus, it cannot be used to draw blood for reliable trough tacrolimus levels. Extremely high levels of contamination were seen in 60% of the lumens exposed to tacrolimus, though there was no correlation of the degree of contamination to the duration of CVL days, or to the amount/dose of tacrolimus infused via that lumen. This is the first study of its kind to find a solution to the problem of CVL contamination with CNIs. We show that in 100% of the contaminated lumens, a 2-hour ethanol lock was effective in decreasing the degree of contamination with 65% of the lumens being fully decontaminated. Even in lumens with extremely high levels of contamination ($\geq$ 30ng/ml), a 2-hour lock was able to fully decontaminated 50% of such lumens and the remaining 50% showed a significant decrease. We did not use a second ethanol lock to further decontaminate such lumens; however, based on our encouraging

results a second ethanol lock maybe safely used. Crnich et al (12) showed that prolonged exposure of CVLs to 70% ethanol for 10 weeks did not cause any damage to the mechanical integrity of the CVL material, hence a second 2 hour ethanol lock, if needed would be reasonable for lumens with extremely high degree of contaminations.

In case of suspicion that a CVL lumen may be contaminated, trough tacrolimus levels from both lumens should be compared with a peripheral blood sample. If the trough level from the peripheral blood and the CVL lumen (s) are within the SCL (as defined above); then that lumen should be considered safe and not contaminated. If the level from the lumen is more than 12.6% higher than the peripheral or the level from the unexposed lumen then it should be considered contaminated, provided other clinical causes of a high level are ruled out. To help decontaminate a CVL we propose the guideline as detailed in Table 2. Study limitations were mainly time-related, as the ethanol locked CVL lumen was not available for routine medication administration for 2 hours. Another limitation was the biological variation in the serum tacrolimus levels, which may have played a role by affecting the levels in the control and intervention arms simultaneously.

In conclusion, a single 2hour 70% ethanol lock was effective in decontaminating majority of the CVLs exposed to tacrolimus. The 2hour ethanol lock had no adverse effects on patients, and its administration did not affect patient care. This new intervention would allow us to treat contaminated CVLs, change the current practice, improve therapeutic efficacy and ultimately decrease the financial burden on the patient and medical center.

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Legends to Figures

Figure 1: flowsheet showing the procedureal methods and schema fo the study

Figure 2: bar diagram showing the tacrolimus levels before and after the ethanol lock in the intervention arm and the control arm for each set

Figure 3 A: showing correlation of the CVL duration (in days) and contamination, Figure 3B showin g correlation of dose of tacrolimus used and the degree of contamination

Figure 4: Side by side boxplots comparing morning (AM) and evening (PM) tacrolimus levels in Intervention arm (A) and Control arm (B). Paired morning and evening tacrolimus levels are connected by lines