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ARTICLE

Pharmacokinetics of apixaban and tacrolimus or cyclosporine in kidney and lung transplant recipients

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Abstract

Apixaban is frequently used off-label in transplant recipients. However, a potential drug interaction exists with the calcineurin inhibitors. We conducted an open-label drug–drug interaction study to determine the pharmacokinetics of apixaban in lung and kidney transplant recipients who were taking a calcineurin inhibitor. A single dose of apixaban 10 mg was administered orally to kidney and lung transplant recipients maintained on either tacrolimus or cyclosporine, and pharmacokinetic parameters were compared to a reference cohort of 12 healthy subjects who used the same apixaban dose and pharmacokinetic blood sampling. Fourteen participants were enrolled ($n = 6$ kidney, $n = 8$ lung), with 10 maintained on tacrolimus and four on cyclosporine. Data from 13 participants was usable. Participants were taking triple therapy immunosuppression and had a mean (SD) of 12 (3) medications. Participants receiving tacrolimus and cyclosporine had area under the plasma concentration–time curve from time zero to infinity ($AUC_{0\text{-inf}}$) geometric least square means (90% confidence interval [CI]) of 4312 (95% CI 3682, 5049) and 5388 (95% CI 3277, 8858), respectively. Compared to healthy subjects, the associated geometric mean ratios (GMRs) for apixaban maximum plasma concentration (C_{max}), AUC from time zero to the last quantifiable concentration ($AUC_{0\text{-tlast}}$) and $AUC_{0\text{-inf}}$ were 197% (95% CI 153, 295), 244% (95% CI 184, 323), and 224% (95% CI 170, 295) for transplant recipients on tacrolimus. The GMR (90% CI) C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ of apixaban for patients on cyclosporine were 256% (95% CI 184, 358), 287% (95% CI 198, 415), and 280% (95% CI 195, 401). Kidney and lung transplant recipients receiving tacrolimus had higher apixaban exposure. A similar trend was noted for patients receiving cyclosporine, but additional patients are needed to confirm this interaction. Future studies are needed before apixaban can be safely recommended in this population, and the impact of dose staggering should be investigated. This

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study highlights the importance of pharmacokinetic studies in actual patient populations.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Apixaban is frequently used off label in transplant recipients. However, a potential drug interaction exists with the calcineurin inhibitors. A randomized crossover drug–drug interaction study in healthy subjects suggested that the combination of apixaban and calcineurin inhibitors is safe, but this has not been evaluated in actual patients.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study explored the pharmacokinetics of apixaban in lung and kidney transplant recipients in a real-world setting to determine the impact of calcineurin use on apixaban systemic exposure.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Kidney and lung transplant recipients receiving tacrolimus experienced significantly higher apixaban exposure compared with healthy subjects receiving apixaban alone, and there was a similar trend for patients receiving cyclosporine.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Significantly higher apixaban exposure suggests that future studies are needed before this drug can be safely recommended in this population, and highlight the importance of pharmacokinetic studies in actual patient populations.

INTRODUCTION

New medications, such as the direct-acting oral anticoagulants (DOACs), offer novel solutions for improving patient outcomes. Solid organ transplant recipients (SOTRs), however, are neglected in the drug evaluation process. The use of off-label medications carries a significant risk for drug–drug interactions (DDIs), adverse medication-related events, and can be a potential liability for prescribers.

In the general population, the four DOACS demonstrate comparable/superior efficacy for treatment of venous thromboembolism (VTE) and stroke prevention in non-valvular atrial fibrillation (NVAF) with significantly lower bleeding compared to vitamin k antagonists (VKAs).[1–8] As such, the DOACs have been rapidly incorporated into standard practice. For SOTRs, a population with a significantly higher risk for VTE, NVAF, stroke, and associated negative outcomes,[9–13] studies on the safe use of DOACs are lacking. Current evidence is limited to single arm descriptive reports, single-center retrospective cohort studies, and one single-center prospective study ($n = 19$).[14–20] Some of these studies have indicated that the incidence of bleeding with DOACs was similar, if not lower, to that of VKAs, but no pharmacokinetic studies in this population have been performed.[15–18]

Several appealing characteristics make DOACs advantageous for SOTRs. Compared to warfarin, DOACs have fewer drug interactions, a wider therapeutic window, and the ability to use fixed-dosing without routine international normalized ratio monitoring.[21] Of the currently available DOACs, apixaban is postulated to be the safest for this population.[14,20] Only 27% of an apixaban dose is removed by the kidneys[22] and dosage adjustment is not necessary for compromised renal function alone. Apixaban's safety has been noted in other high-risk patient populations, including elderly, patients with moderate renal insufficiency, and patients with prior stroke.[23,24] However, a major concern is the potential for a DDI between apixaban and the calcineurin inhibitors (CNIs), tacrolimus and cyclosporine. These CNIs and apixaban are known substrates of CYP3A4 and P-glycoprotein (P-gp). Cyclosporine also has the potential to inhibit systemic and intestinal CYP3A4/P-gp, and tacrolimus likely only has the potential to inhibit intestinal CYP3A4/P-gp due to its low systemic maximum plasma concentration (C_{max}; at therapeutic dose) and fraction unbound in the plasma. Therefore, co-administration with CNIs may result in increases in exposure to apixaban.[14,25]

A randomized crossover DDI study evaluated the pharmacokinetics of apixaban when co-administered with cyclosporine and tacrolimus in healthy volunteers

at Thomas Jefferson University.[26] Twelve subjects received apixaban 10 mg alone; cyclosporine 100 mg daily for 3 days, followed by apixaban; or tacrolimus 5 mg daily for 3 days, followed by apixaban. All subjects received all three treatments. Following multiple doses of the CNI, and compared to apixaban alone, the apixaban area under the plasma concentration–time curve from time zero to the last quantifiable concentration ($AUC_{0\text{-tlast}}$) and C_{max} increased by 20% and 43% with cyclosporine, but decreased by 22% and 13% with tacrolimus, respectively. These results, which were clinically insignificant, suggested that the combination of apixaban and CNIs in healthy subjects is safe.[26]

Pharmacokinetic studies in healthy volunteers are a first step for characterizing DDIs, but follow-up studies in the actual patient population are essential for ensuring safety. The goal of this single dose DDI study was to explore the pharmacokinetics of apixaban in lung and kidney transplant recipients in a real-world setting to determine the impact of CNI use on apixaban systemic exposure. Because stopping the CNI was not feasible for providing a control condition, the pharmacokinetic data from the previous study in healthy subjects[26] was used as the comparator.

MATERIALS AND METHODS

A prospective, open-label, DDI clinical trial was undertaken in kidney and lung transplant recipients on cyclosporine or tacrolimus. Stable adult SOTRs (≥18 years old) who received their transplant more than 6 months ago were eligible to participate. All patients provided informed consent before their inclusion in the study. Participants were required to have a creatinine clearance above 15 ml/min (Cockcroft–Gault formula as per the Canadian product monograph for Eliquis[27]), a hemoglobin of at least 80 g/L, and no clinically significant bleeding risk or hepatic disease defined by the Child-Pugh score B or C. Patients who were taking an antiplatelet or anticoagulant (except acetylsalicylic acid 81 mg) or strong inhibitors of both CYP3A4 and P-gp (e.g., ketoconazole and voriconazole), as described in the Canadian product monograph,[27] were excluded from participation, as well as those who had CNI dosage changes within the previous 2 weeks. Participants had to refrain from smoking for at least 6 months prior, avoid drinking grapefruit juice, or consuming natural health products (for 2 weeks) and cannabis (for 2 days) prior to and during the study period. The study was approved by the Biomedical Ethics Board at the University of Saskatchewan (Beh-235; Clinicaltrials.gov #NCT04023760) and was performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

Subjects were recruited from the Saskatchewan Transplant Program between October 2019 and March 2020. All signed written informed consent and a patient-centered approach was undertaken, whereby the researchers collaborated to determine mutually convenient logistics. On the day of the study, subjects reported to St. Paul's Hospital in Saskatoon, Saskatchewan. A single dose of apixaban 10 mg was orally administered (two 5 mg tablets -Eliquis; Bristol-Myers Squibb Canada Co., and Pfizer Canada ULC). Participants fasted for at least 8 h overnight to 2 h after administration of apixaban. Blood samples for apixaban plasma concentrations were collected prior to apixaban administration at 0 h, and then at ~1, 2, 3, 4, 6, 12, 24, 48, and 72 h. Because this was a real-world study conducted in a medical setting versus in a clinical trials unit, the blood samples were drawn as close as possible to the specified time, and the actual time in hours and minutes was recorded. Tacrolimus or cyclosporine trough levels were collected at 0, 12, 24, and 72 h. Participants remained at the site until the 6-h blood draw was performed and then met the study nurse for the 12-h blood draw and safety assessment (either at the study location or participant's residence or hotel). The 24, 48, and 72-h blood draws were performed either at: (a) St. Paul's Hospital, (b) the participant's local laboratory, or (c) the participant's home, depending on patient preference and convenience. Safety and tolerability of apixaban when co-administered with tacrolimus or cyclosporine was assessed by adverse events, vital sign measurements, physical examinations, and clinical laboratory tests (a summary of the safety assessments is shown in Figure 1). The study conditions (e.g., fasting and sampling times) were consistent with the Thomas Jefferson historical control.[26]

Sample analysis

The CNI plasma levels and laboratory safety tests were processed immediately by the clinical diagnostic laboratory at the Saskatchewan Health Authority. The CNI levels were determined using electrochemiluminescence immunoassay. Whole blood for apixaban samples was collected into 7 ml plain red top tubes, and centrifuged once clotted. The serum was stored at −20°C until the end of the study, when the tubes were sent on dry ice to the St. Paul's Hospital Department of Pathology and Laboratory Medicine. Plasma apixaban concentrations were determined using a validated liquid chromatography tandem mass spectrometry (LC–MS/MS) assay (SCIEX 5500QTrap).[28] Briefly, 50 µl of calibrators, quality controls, and patient samples were mixed with 25 µl

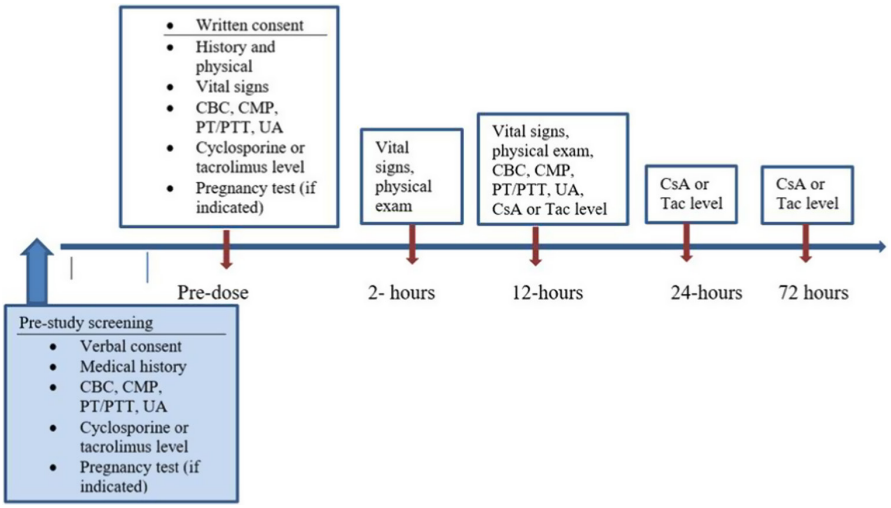

FIGURE 1 Safety assessments

CBC= complete blood cell count, CMP= complete metabolic profile, PT= prothrombin time, PTT= partial prothrombin time, CsA= cyclosporine, Tac = tacrolimus; UA = uric acid

of internal standard solution, and then precipitated with 400 µl of 10 g/L zinc sulfate w/v in 70:30 MeOH:water in a 2 ml deep 96-well plate. The 96 deep well plant was mixed, centrifuged, and 10 µl of supernatant injected into the LC–MS/MS system. Calibrators were prepared in-house by spiking apixaban (Toronto Research Chemicals) into blank plasma. Apixaban-d3 (Santa Cruz Biotechnology, Inc.) was used as the internal standard. The assay was linear over a range of 2.5–500 ng/ml. Based on a comparison of quadratic curve to linear calibration curve results, levels above 500 ng/ml were expected to be underestimated by ~2%–4%. Two comparison studies were performed for the coefficient of determination; one with another LC–MS/MS assay (r^2 value = 0.96; n = 24), and another with the house hematology ACL TOP700 AntiCoag (r^2 value = 0.95; n = 23). The lower limit of quantification was 2.86 ng/ml. The between-run imprecision was between 5.3% and 6.6% coefficient of variation (CV) at levels from 2.54 to 426.4 ng/ml. Apixaban within run (intraday) imprecision (CV) was between 1.9% and 4.0% at levels from 2.54 to 426.4 ng/ml. Recovery was between 98.2% and 103% at low and high levels. Analytical interferences and ion suppression effects were not observed in the assays.

Samples from the cohort at Thomas Jefferson University[26] were analyzed based on existing literature for direct oral anticoagulant measurement by LC–MS/MS,[29] using commercial calibrators (Hyphen Biomed) with d_4-rivaroxaban as an internal standard (Santa Cruz Biotechnology). The calibration curve in plasma is linear over the range of 2.0–600 ng/ml. The between-run precision for all levels of quality control samples was below 10% CV; accuracy/recovery centered on 100%. No analytical interferences or ion suppression effects was observed in precedent LC–MS/MS assays. Calibration took place on each day of actual testing.

Pharmacokinetic analysis

Plasma concentrations over time were used to determine the single dose pharmacokinetic parameters of apixaban administered to transplant recipients. Individual pharmacokinetic parameters were determined by noncompartmental methods using GraphPad Prism software (version 9.0.1). The C_{max} and the time required to achieve the C_{max} (T_{max}) were determined by visual inspection of the plasma concentration–time curves. The $AUC_{0\text{-tlast}}$ and extrapolated to infinity ($AUC_{0\text{-inf}}$) were calculated using the log-linear trapezoidal method. The slope of the terminal phase of the plasma concentration–time curve (λ_z) was estimated by the least squares method (natural log-linear regression of at least three data points) with a weighting factor of one. The half-life was estimated as $0.693/\lambda_z$, while the equation dose/(λ_z*$AUC_{0\text{-inf}}$) was used to calculate the apparent volume of distribution (V_z/F), and oral clearance (CL/F) was estimated by dividing the oral dose by $AUC_{0\text{-inf}}$. Geometric least square means and 90% confidence intervals (CIs) were calculated from the fitted models for the cyclosporine and tacrolimus groups. The raw data was obtained from the Thomas Jefferson study,[26] and pharmacokinetic parameters were analyzed using the linear up log down trapezoidal method for AUC estimation. Because AUC estimation depends upon the method used, the resulting pharmacokinetic parameters may show slight differences. Hence, we opted to reanalyze the Thomas Jefferson data so that both datasets could be compared using the same approach.

The pharmacokinetic data from the SOTR and healthy subjects[26] were compared using recommended methods for parallel design drug–drug studies and accepted thresholds for DDIs (no-effect boundary of 80%–125%).[30,31] C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ were log-transformed and analysis of variance tests were performed in SAS (version 9.4) to obtain point estimates and 90% CIs. Results were back-transformed to the original scale to present adjusted geometric mean ratios (GMRs) and corresponding 90% CIs for the difference in means between the test (transplant recipients) and reference (apixaban with and without tacrolimus or cyclosporine in healthy subjects). Unpaired parametric *t*-tests were used to test for significant differences between the groups in terminal half-life, V_z/F and CL/F, and within each CNI cohort of transplant recipients to explore potential differences among demographic variables (organ type, sex, body weight, and serum creatinine) and pharmacokinetic parameters. The significance level was $p < 0.05$.

RESULTS

To determine the presence of a clinically significant DDI between the CNIs and apixaban, 14 stable transplant participants were enrolled in the study, including six kidney and eight lung recipients. Ten of the participants were maintained on tacrolimus, whereas four participants received cyclosporine. All participants were receiving triple therapy immunosuppression, consisting of their respective CNI, an antiproliferative (either a mycophenolic acid derivative or azathioprine) and prednisone at a dose of 5 or 10 mg of day. Participants were taking a mean of 12.1 (2.9) medications (Table S1). Concurrent medications were taken at approximately the same time in all patients, with the exception of one participant (tacrolimus, kidney 2), who took their other medications ~35 min prior to apixaban dosing. One participant (who also had cystic fibrosis) was concurrently taking an additional moderate inhibitor of CYP3A4 and/or P-gp (diltiazem; cyclosporine, lung 1) and one participant (cyclosporine, lung 2) was taking divalproex, a potential CYP3A4 inhibitor. Table 1 describes the participant demographics. No serious adverse events were noted and no statistically significant differences in the tacrolimus and cyclosporine plasma concentrations were observed before and after the administration of apixaban.

Although a larger sample size was originally planned, enrollment was stopped in March 2020, due

TABLE 1 Participant demographics

		All transplant participants ($n = 14$)	Transplant participants taking tacrolimus ($n = 10$)	Transplant participants taking cyclosporine($n = 4$)	Healthy subjects from Thomas Jefferson study[26]($n = 12$)
Age, years	Mean (SD)	59 (7)	61 (6)	53 (3)	41 (11)
	Range	50, 68	50, 68	50, 56	25, 54
Organ type *n* (%)	Kidney	6 (43)	4 (40)	2 (50)	Not applicable
	Lung	8 (57)	6 (60)	2 (50)	
Sex *n* (%)	Male	7 (50)	4 (40)	3 (75)	12 (100)
	Female	7 (50)	6 (60)	1 (25)	00
Weight, kg	Mean (SD)	77 (14)	74 (15)	84 (9)	89 (13)
	Range	54, 98	54, 98	75, 97	73, 106
BMI, kg/M^2	Mean (SD)	28 (5)	27 (5)	29 (7)	29 (3)
	Range	22, 37	22, 36	22, 37	24, 33
Creatinine, µmol/L	Mean (SD)	149 (57)	136 (47)	182 (73)	81 (9)
	Range	76, 241	76, 224	89, 241	71, 97
(mg/dl)	Mean (SD)	2 (1)	2 (1)	2 (1)	1 (0)
	Range	1, 3	1, 3	1, 3	1, 1
Estimated creatinine clearance[a] (ml/min)	Mean (SD)	54 (23)	53 (22)	56 (29)	141 (27)
	Range	30, 105	30, 105	34, 96	82, 188
Hemoglobin, g/L	Mean (SD)	124 (20)	122 (23)	129 (9)	unavailable
	Range	93, 167	93, 167	120, 142	

Abbreviation: BMI, body mass index.

[a]Estimated creatine clearance calculated by the Cockcroft-Gault equation (using actual body weight).

to study restrictions with the coronavirus disease 2019 (COVID-19) pandemic. One of the participants from the tacrolimus group (lung 1) was removed from the analysis because the study nurse was only able to collect three blood samples. Apixaban samples were stored in a −20°C freezer until they were batched and sent to the processing laboratory. During the COVID-19 pandemic lockdown, samples were moved between freezers and 13 of the 130 samples could not be retrieved (total missing data = 10%). Missing timepoints are shown in Table S2.

Tacrolimus drug-interaction study

The effect of tacrolimus on apixaban oral exposure metrics was determined in SOTRs stabilized on tacrolimus and administered a single-dose of apixaban. The plasma concentration–time profiles and summary pharmacokinetic parameters of apixaban are shown in Figure 2 and Table 2. The geometric least square mean (90% CI) was 353 (90% CI 306, 407) for C_{max} and 4312 (90% CI 3682, 5049) for $AUC_{0\text{-inf}}$. Using the data from healthy subjects on apixaban + tacrolimus as a reference, the GMR (90%

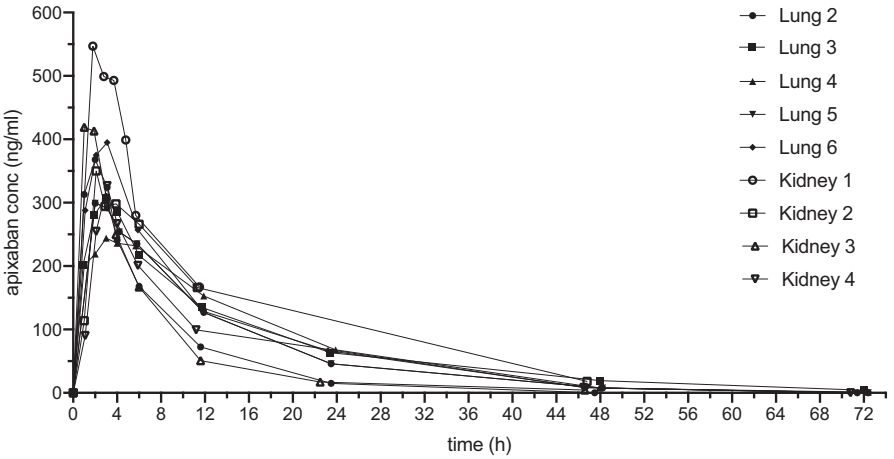

FIGURE 2 Plasma apixaban concentration–time profiles in transplant recipients ($n = 9$) stabilized on tacrolimus following a single oral dose of apixaban (10 mg)

TABLE 2 Summary of apixaban pharmacokinetic parameters following a single oral dose (10 mg) administration to transplant recipients ($N = 9$) on tacrolimus compared with healthy subjects ($n = 12$)

	Transplant recipients ($n = 9$)	Healthy subjects ($n = 12$)		Transplant recipients vs. healthy subjects on apixaban + tacrolimus[26]	Transplant recipients versus healthy subjects on apixaban[26]
Pharmacokinetic parameter	**Apixaban + tacrolimus**	**Apixaban + tacrolimus**	**Apixaban alone**	**Point estimate of GMR (90% CI)**	**Point estimate of GMR (90% CI)**
C_{max} (ng/ml)	353 [306, 407]	157 [125, 196]	179 [145, 221]	2.25 [1.72, 2.55]	1.97 [1.53, 2.95]
$AUC_{0\text{-tlast}}$ (h ng/ml)	4243 [3606, 4992]	1374 [1151, 1641]	1741 [1394, 2172]	3.09 [2.43, 3.92]	2.44 [1.84, 3.23]
$AUC_{0\text{-inf}}$ (h ng/ml)	4312 [3682, 5049]	1511 [1221, 1870]	1926 [1484 2500]	2.85 [2.30, 3.54]	2.24 [1.70, 2.95]
T_{max} (h)	2.3 (0.7) [1, 3.1]	2.6 (0.9) [1, 4]	2.7 (0.8) [1, 4]		
$t_{1/2}$ (h)	8.6 (2.2) [4.9, 12.2]	7.0 (2.1) [2.1, 9.8]	10.5 (5.9) [4.5, 21.7]	n.s. ($p = 0.11$)	n.s. ($p = 0.37$)
CL/F (L/h)	2.4 (0.6) [1.6, 3.5]	7.0 (2.3) [4.3, 11.8]	5.6 (2.6) [3.1, 10.9]	$p < 0.0001$	$p < 0.002$
V_z/F (L)	29 (7) [17, 41]	71 (29) [13, 124]	80 (46) [26, 174]	$p = 0.0002$	$p = 0.004$

Note: Geometric least square mean [90% confidence intervals] for C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$. Arithmetic mean (standard deviation) [minimum, maximum] for T_{max}, $t_{1/2}$, CL/F, and V_z/F.

Abbreviations: $AUC_{0\text{-tlast}}$, area under the plasma concentration–time curve from time zero to the last quantifiable concentration; $AUC_{0\text{-inf}}$, AUC and extrapolated to infinity; CI, confidence interval; CL/F, oral clearance (F is oral bioavailability) based on dose divided by $AUC_{0\text{-inf}}$; C_{max}, observed peak plasma concentration; GMR, geometric least square mean ratio; n.s., not significant; $t_{1/2}$, elimination half-life; T_{max}, time taken to reach C_{max}; V_z/F, apparent volume of distribution based on dose divided by the product of terminal elimination rate constant and $AUC_{0\text{-inf}}$.

CI) for apixaban C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ were 225% (90% CI 172, 255), 309% (90% CI 243, 392), and 285% (90% CI 230, 354), respectively. Compared to the healthy subjects on apixaban alone, the GMR (90% CI) for apixaban C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ were 197% (90% CI 153, 295), 244% (90% CI 184, 323), and 224% (90% CI 170, 295), respectively. The mean CL/F and V_z/F of apixaban were significantly lower in SOTRs compared to the healthy subjects ($p < 0.005$). One participant (kidney 1) achieved a peak level higher that 500 ng/ml. No significant difference was found in the mean tacrolimus trough levels before and after apixaban administration (7.4 [1.5] ng/ml vs. 8.0 [2.7] ng/ml, respectively).

Within the tacrolimus transplant cohort, no significant differences between the pharmacokinetic parameters were found between organ type or sex. Patients with creatinine levels of greater than 133 μmol/L had significantly higher mean AUCs ($AUC_{0\text{-inf}}$ [h ng/ml] 5213 vs. 3806, $p = 0.035$) and lower apixaban CL/F values (1.9 vs. 2.8 [L/h], $p = 0.044$). Patients that weighed <60 kg had significantly higher mean C_{max} (483 vs. 327 [ng/ml], $p = 0.012$) and lower T_{max} (1.4 vs. 2.6 [h], $p = 0.028$).

Cyclosporine drug-interaction study

The effect of cyclosporine on apixaban oral exposure metrics was determined in SOTRs stabilized on cyclosporine and administered a single-dose of apixaban. The plasma concentration–time profiles and summary pharmacokinetic parameters are shown in Figure 3 and Table 3. The geometric least square means (90% CI) were 458 (90% CI 330, 637) for C_{max} and 5388 (90% CI 3277, 8858) for $AUC_{0\text{-inf}}$. Using the data from healthy subjects on apixaban + cyclosporine as a reference, the GMR (90% CI) for apixaban C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ were 179% (90% CI 126, 254), 235% (90% CI 172, 321), and 229% (90% CI 172, 303), respectively. Compared to the healthy subjects on apixaban alone, the GMR (90% CI) for apixaban C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$ were 256% (90% CI 184, 358), 287% (90% CI 198, 415), and 280% (90% CI 195, 401). The mean CL/F and V_z/F of apixaban in transplant recipients was significantly lower than healthy subjects on apixaban only ($p < 0.03$). No differences were noted in the pharmacokinetic parameters between demographics. No significant difference was found in the mean cyclosporine trough levels before and after apixaban administration (146.8 [62.8] ng/ml vs. 147.5 [68.4] ng/ml, respectively). Two participants (lung 1 and kidney 2) achieved levels higher that 500 ng/ml, including participant lung 1, who was on concurrent therapy with diltiazem.

DISCUSSION

Apixaban has been used off-label in SOTRs without pharmacokinetic studies within this patient population to guide dosing, therefore, we undertook a real-world DDI study to investigate the impact CNI use on apixaban systemic exposure. Although small single-center studies have indicated that apixaban may be safe, results from the present study give us pause from recommending apixaban at standard doses.

Patients in the current study received a 10 mg oral dose of apixaban to simulate a typical initial treatment regimen of VTE posing the highest risk for anticoagulation associated bleeding. This dose also allowed a comparison with previous data (i.e., Thomas Jefferson study) obtained from healthy subjects.[26] In the Thomas Jefferson study, compared to apixaban alone, the apixaban AUC in healthy subjects increased by 20% for cyclosporine, but decreased by 22% for tacrolimus.[26] In the present study, SOTRs experienced significantly higher plasma concentrations and total body exposures

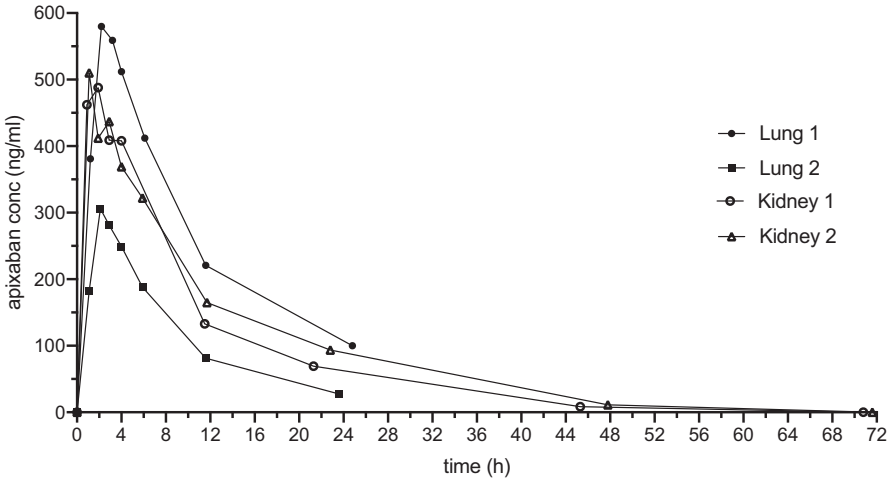

FIGURE 3 Plasma apixaban concentration–time profiles in transplant recipients ($n = 4$) stabilized on cyclosporine following a single oral dose of apixaban (10 mg)

TABLE 3 Summary of apixaban pharmacokinetic parameters following a single oral dose (10 mg) administration to transplant recipients ($N = 4$) on cyclosporine compared with healthy subjects ($n = 12$)

	Transplant recipients ($n = 4$)	Healthy subjects ($n = 12$)		Transplant recipients versus healthy subjects on apixaban + cyclosporine[26]	Transplant recipients versus healthy subjects on apixaban alone[26]
Pharmacokinetic parameter	**Apixaban + cyclosporine**	**Apixaban + cyclosporine**	**Apixaban alone**	**Point estimate of GMR (90% CI)**	**Point estimate of GMR (90% CI)**
C_{max} (ng/ml)	458 [330, 637]	257 [211, 312]	179 [145, 221]	1.79 [1.26, 2.54]	2.56 [1.84, 3.58]
$AUC_{0\text{-tlast}}$ (h ng/ml)	4990 [3065, 8122]	2121 [1813, 2482]	1741 [1394, 2172]	2.35 [1.72, 3.21]	2.87 [1.98, 4.15]
$AUC_{0\text{-inf}}$ (h ng/ml)	5388 [3277, 8858]	2356 [2065, 2689]	1926 [1484 2500]	2.29 [1.72, 3.03]	2.80 [1.95, 4.01]
T_{max} (h)	1.8 (0.5) [1.1, 2.2]	2.2 (1.1) [1, 4]	2.7 (0.8) [1, 4]		
$t_{1/2}$ (h)	7.9 (1.3) [6.2, 9.0]	7.0 (4.1) [2.7, 18.9]	10.5 (5.9) [4.5, 21.7]	n.s. ($p = 0.68$)	n.s. ($p = 0.41$)
CL/F (L/h)	2 (0.9) [1.3, 3.4]	4.3 (0.9) [3.0, 5.5]	5.6 (2.6) [3.1, 10.9]	$p = 0.0006$	$p < 0.02$
V_z/F (L)	22 (6) [17,30]	45 (35) [14, 151]	80 (46) [26, 174]	n.s. ($p = 0.22$)	$p < 0.03$

Note: Geometric least square mean [90% confidence intervals] for C_{max}, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-inf}}$. Arithmetic mean (standard deviation) [minimum, maximum] for T_{max}, $t_{1/2}$, CL/F, and V_z/F.

Abbreviations: $AUC_{0\text{-tlast}}$, area under the plasma concentration–time curve from time zero to the last quantifiable concentration; $AUC_{0\text{-inf}}$, AUC extrapolated to infinity; CI, confidence interval; CL/F, oral clearance (F is oral bioavailability) based on dose divided by $AUC_{0\text{-inf}}$; C_{max}, observed peak plasma concentration; GMR, geometric least square mean ratio; $t_{1/2,}$ elimination half-life; T_{max}, time taken to reach C_{max}; V_z/F, apparent volume of distribution based on dose divided by the product of terminal elimination rate constant and $AUC_{0\text{-inf}}$.

(2 to 3-fold increase) compared to healthy subjects on apixaban alone. The transplant recipients had multiple comorbidities, an average of 12 medications, and steady-state CNI levels of 7.4 (1.5) ng/ml and 146.8 (62.8) ng/ml for tacrolimus and cyclosporine, respectively. The reference group of healthy subjects had an abbreviated dosing schedule and lower systemic concentrations of CNIs (tacrolimus trough level 3.8 (2.3) ng/ml and cyclosporine 22.4 (3.9) ng/ml). They were all male subjects, younger, and were not taking other medications. These results identify the limitations of healthy volunteer DDI studies and support the need to undertake pharmacokinetic studies in actual patient populations to understand the true extent of DDIs.

In this real-world study, we included all patients with an estimated creatinine clearance (eCrCl) >15 ml/min. According to the Canadian product monograph, dose adjustments are not necessary for patients with eCrCl 25–30 ml/min, unless two of the following are present: age ≥80 years, body weight ≤60 kg, or serum creatinine greater than or equal to 133 μmol/L (1.5 mg/dl).[27] None of our participants required dosage adjustment according to the criteria. In the tacrolimus group, patients with creatinine levels of >133 μmol/L had significantly higher mean AUCs and lower apixaban oral clearance values ($p < 0.05$), and patients that weighed <60 kg had significantly higher mean C_{max} ($p < 0.03$). Nevertheless, the mean AUCs in the patients with optimal renal function were 1.8-fold higher than the healthy participants. The higher exposure in all transplant recipients (regardless of renal function), indicates that these changes are likely a result of the drug interaction.

This study has clinical significance. Whereas clinician confidence with prescribing apixaban has increased, our data suggest caution is warranted in transplant recipients. In healthy subjects, co-administration of a single dose of apixaban and ketoconazole (a strong inhibitor of both CYP3A4 and P-gp) resulted in a twofold and 1.6-fold increase in apixaban AUC and C_{max}, respectively.[32] Diltiazem, a moderate inhibitor of CYP3A4 and P-gp, contributed to a 1.4- and 1.3-fold increase in apixaban AUC and C_{max}, respectively.[32] As such, the Canadian product monograph states that concomitant treatment with strong inhibitors of both CYP3A4 and P-gp (e.g., ketoconazole, itraconazole, voriconazole, or posaconazole, and HIV protease inhibitors) are contraindicated,[27] whereas the US monograph states that dosage reduction by 50% is recommended in such instances.[33] The higher exposures of apixaban in our study are comparable to those presented in the ketoconazole study. Hence, further investigation is recommended to explore the pharmacokinetics of using apixaban at a reduced dose.

Notably, two patients in the cyclosporine group were also taking drugs which could potentially interact with apixaban. Consistent with the literature,[32] participant lung 1 who was taking diltiazem experienced the highest AUC and C_{max} values in the entire cohort. Participant lung 2 who was taking divalproex had the lowest AUC and C_{max} values in the cyclosporine cohort. Although divalproex is typically considered an inhibitor, it may diminish the therapeutic effect of apixaban. In a recent nested case–control study (n = 89,284), patients treated with DOACs (54.8% apixaban) in combination with valproic acid were at significantly higher risk of stroke or systemic embolism compared with those without valproic acid (odds ratio 2.58, 95% CI 1.5 to 4.45).[34]

The mechanism for a putative DDI between apixaban and the calcineurin inhibitors based upon concomitant administration is not clear. Although, apixaban is a substrate for P-gp and BCRP efflux transporters,[35] it is classified as a high permeability and high solubility drug and therefore might suggest that apixaban is less likely to experience clinically relevant DDIs at intestinal efflux transporters at clinical doses. However, clinically relevant DDIs at presystemic intestinal and hepatic CYP3A4 enzymes are possible. The literature postulates that clinically relevant drug interactions with apixaban are related to intestinal CYP3A4,[36,37] but hepatic presystemic drug interactions at CYP3A4 can also contribute to the observed increase to AUC that result in enhanced bioavailability.

In this real-world study, we asked participants to continue their regular medications at the usual dosing intervals. This resulted in the co-administration of the CNIs with apixaban, or in one case, the administration of tacrolimus 35 min prior to apixaban. If the nature of the apixaban-CNI is primarily intestinal as the literature suggests,[36,37] it is possible that spacing the doses of these medications may reduce the interaction. In one study, which used midazolam as a probe to examine the inhibitor effect of CNIs in CYP3A4- and CYP3A5-expressing microsomes, in vitro-in vivo extrapolations estimated that separating the dosing times by only a few hours would eliminate this interaction.[25]

Several limitations of this study deserve consideration. Our study consisted of a combination of both lung and kidney transplant recipients and several physiologic differences exist between these populations. These cohorts were specifically chosen because their immunosuppressive regimens are similar, and generally consist of triple therapy. Our data was compared with data obtained from healthy subjects. Apixaban levels from both studies were analyzed using a validated LC–MS/MS method, and because the samples were processed from different facilities, some laboratory variations may be expected. However, these variations are expected to be negligible. Three participants achieved levels higher than standard curve range for the LC–MS/MS method (500 ng/ml), and the AUCs on these participants may be underestimated. In a typical drug interaction study, participants are exposed to at least two conditions (in a crossover fashion) and pharmacokinetic parameters are compared: (a) the drug is administered alone (control condition) and (b) the drug is administered simultaneously with the potential offender. DDI studies in transplant recipients cannot be performed in this manner because the immunosuppressants cannot be stopped. A larger sample size would have been desirable for further exploring differences between the transplant types and patient demographics, but our study was halted early due to the COVID-19 pandemic. Firm conclusions cannot be drawn in the cyclosporine group due to the small sample size and because two participants were taking CYP3A4 interacting medications. Finally, this study examined drug exposure and was not designed to measure clinical end points.

CONCLUSION

Significantly higher apixaban exposure in transplant recipients maintained on CNIs compared with healthy subjects suggests that additional studies are needed before apixaban can be safely recommended. Future studies are necessary for determining the optimal dosage of apixaban or possible need for dose staggering when concomitantly administering apixaban with CNIs in transplant recipients.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

H.M., A.S., J.A., M.F., J.T., W.S., B.B., W.K., S.Y., and J.D. wrote the manuscript. H.M., A.S., J.A., M.F., W.S., B.B., W.K., and J.D. designed the research. H.M., A.S., M.F., and J.T. performed the research. H.M., J.A., and S.Y. analyzed the data.

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REFERENCES

1. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation [published correction appears in *N Engl J Med*. 2010 Nov 4;363(19):1877]. *N Engl J Med.* 2009;361(12):1139-1151.
2. Schulman S, Kearon C, Kakkar AK, et al. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. *N Engl J Med.* 2009;361(24):2342-2352.
3. Bauersachs R, Berkowitz SD, Brenner B, et al. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med.* 2010;363(26):2499-2510.
4. Granger CB, Alexander JH, McMurray JJ, et al. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2011;365(11):981-992.
5. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med.* 2011;365(10):883-891.
6. Agnelli G, Buller HR, Cohen A, et al. Oral apixaban for the treatment of acute venous thromboembolism. *N Engl J Med.* 2013;369(9):799-808.
7. Buller HR, Decousus H, Grosso MA, et al. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med.* 2013;369(15):1406-1415.
8. Giugliano RP, Ruff CT, Braunwald E, et al. Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2013;369(22):2093-2104.
9. Sáez-Giménez B, Berastegui C, Loor K, et al. Deep vein thrombosis and pulmonary embolism after solid organ transplantation: an unresolved problem. *Transplant Rev (Orlando).* 2015;29(2):85-92.
10. Oliveras A, Roquer J, Puig JM, et al. Stroke in renal transplant recipients: epidemiology, predictive risk factors and outcome. *Clin Transplant.* 2003;17(1):1-8.
11. Morgan CT, Manlhiot C, McCrindle BW, et al. Outcome, incidence and risk factors for stroke after pediatric heart transplantation: An analysis of the International Society for Heart and Lung Transplantation Registry. *J Heart Lung Transplant.* 2016;35(5):597-602.
12. Yegen HA, Lederer DJ, Barr RG, et al. Risk factors for venous thromboembolism after lung transplantation. *Chest.* 2007;132(2):547-553.
13. Lenihan CR, Montez-Rath ME, Scandling JD, et al. Outcomes after kidney transplantation of patients previously diagnosed with atrial fibrillation. *Am J Transplant.* 2013;13:1566-1575.
14. Bixby AL, Lichvar AB, Salerno D, Park JM. Use of direct-acting oral anticoagulants in solid organ transplantation: A systematic review. *Pharmacotherapy.* 2021;41(1):28-43.
15. Bixby AL, Shaikh SA, Naik AS, et al. Safety and efficacy of direct-acting oral anticoagulants versus warfarin in kidney transplant recipients: a retrospective single-center cohort study. *Transplant Int*. 2020;33(7):740-751.
16. Henricksen EJ, Tremblay-Gravel M, Moayedi Y, et al. Use of direct oral anticoagulants after heart transplantation. *J Heart Lung Transplant.* 2020;39(4):399-401.
17. Leon J, Sabbah L, Aubert O, et al. Efficacy and safety of direct oral anticoagulants in kidney transplantation: a single-center pilot experience. *Transplantation.* 2020;104(12):2625-2631.
18. Santeusanio AD, Weinberg AD, Florman SS, et al. Safety of direct-acting oral anticoagulants relative to warfarin in a matched cohort of liver transplant recipients. *Clin Transplant.* 2020;34(1):e13756.
19. Reininger KA, Sam T, Patel RS, et al. A retrospective analysis of the safety and efficacy of apixaban use after lung transplant. *Clin Transplant.* 2021;35(7):e14327.
20. Green H, Rahamimov R, Spectre G, et al. Apixaban level and its influence on immunosuppression and graft outcome in kidney transplant recipients with atrial fibrillation. *Ther Drug Monit.* 2021;43(5):637-644.
21. Baumann Kreuziger LM, Morton CT, Dries DJ. New anticoagulants: a concise review. *J Trauma Acute Care Surg.* 2012;73(4):983-992.
22. Hanna MS, Mohan P, Knabb R, et al. Development of apixaban: a novel anticoagulant for prevention of stroke in patients with atrial fibrillation. *Ann N Y Acad Sci.* 2014;1329:93-106.
23. Hohnloser SH, Hijazi Z, Thomas L, et al. Efficacy of apixaban when compared with warfarin in relation to renal function in patients with atrial fibrillation: insights from the ARISTOTLE trial. *Eur Heart J.* 2012;33:2821-2830.
24. Peterson BE, Al-Khatib SM, Granger CB. Apixaban to prevent stroke in patients with atrial fibrillation: a review. *Ther Adv Cardiovasc Dis*. 2017;11(3):91-104.
25. Amundsen R, Åsberg A, Ohm IK, Christensen H. Cyclosporine A- and tacrolimus-mediated inhibition of CYP3A4 and CYP3A5 in vitro. *Drug Metab Dispos.* 2012;40(4):655-661.
26. Bashir B, Stickle DF, Chervoneva I, et al. Drug–drug interaction study of apixaban with cyclosporine and tacrolimus in healthy volunteers. *Clin Transl Sci*. 2018;11(6):590-596.
27. Eliquis® [product monograph] Quebec, Canada. Pfizer Canada ULC and Bristol-Myers Squibb Canada Co. https://www.pfizer.ca/sites/default/files/201910/ELIQUIS_PM_229267_07Oct2019_Marketed_E.pdfwebsite
28. Schmitz EMH, Boonen K, van den Heuvel DJA, et al. Determination of Dabigatran, Rivaroxaban and Apixaban by ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. *J Thromb and Haemost*. 2014;12:1636-1646.
29. Korn WR, Paziuk TM, Stickle DF. Measurement of plasma Apixaban by LC-MS/MS using the AB Sciex API 3200MD. *Clin Chem*. 2016;62:S214.
30. Huang SM, Temple R, Throckmorton DC, et al. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. *Clin Pharmacol Ther.* 2007;81(2):298-304.
31. U.S. Department of Health and Human Services Food and Drug Administration. Center for Drug Evaluation and Research (CDER). 2020. Transporter-mediated drug interactions guidance for industry. https://www.fda.gov/media/134581/download.

32. Frost CE, Byon W, Song Y, et al. Effect of ketoconazole and diltiazem on the pharmacokinetics of apixaban, an oral direct factor Xa inhibitor. *Br J Clin Pharmacol*. 2015;79(5):838-846.
33. Eliquis® [package insert] New York, USA. Bristol-Myers Squibb Company Pfizer Inc. https://packageinserts.bms.com/pi/pi_eliquis.pdf.
34. Gronich N, Stein N, Muszkat M. Association between use of pharmacokinetic interacting drugs and effectiveness and safety of direct acting oral anticoagulants: nested case–control study. *Clin Pharmacol Ther*. 2021;110(6):1526-1536.
35. Zhang D, He K, Herbst JJ, et al. Characterization of efflux transporters involved in distribution and disposition of apixaban. *Drug Metab Dispos*. 2013;41(4):827-835.
36. Sodhi JK, Liu S, Benet LZ. Intestinal efflux transporters P-gp and BCRP are not clinically relevant in apixaban disposition. *Pharm Res*. 2020;37(10):208.
37. Yamada M, Inoue SI, Sugiyama D, et al. Critical impact of drug–drug interactions via intestinal CYP3A in the risk assessment of weak perpetrators using physiologically based pharmacokinetic models. *Drug Metab Dispos*. 2020;48(4):288-296.

SUPPORTING INFORMATION

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