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Pharmacokinetics and Pharmacodynamics of Apixaban in Nephrotic Syndrome: Findings From a Phase 1a Trial

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To the Editor:

Nephrotic syndrome is associated with an elevated risk of venous thromboembolism (VTE), occurring in up to 25% of NS patients.[1–3] Guidelines advocate for primary thromboprophylaxis in selected NS patients.[4] Warfarin, the most studied oral anticoagulant in NS, has been supplanted by direct oral anticoagulants (DOACs) in many other indications. Apixaban, a direct factor Xa (FXa) inhibitor, has particular appeal due to limited renal clearance and observational data suggesting it may be safe and effective when glomerular filtration is reduced.[5,6] Because apixaban is highly protein bound (87-93% with ~66% bound to albumin), the hypoalbuminemia and proteinuria commonly encountered in NS could significantly influence apixaban pharmacokinetics and pharmacodynamics (PK/PD).[7,8]

This single-institution, parallel-arm Phase 1a study evaluated safety and PK/PD of a single apixaban dose in NS subjects (ClinicalTrials.gov: NCT02599532). On Day 1, subjects presented fasting and were administered 10 mg of oral apixaban to mirror initial FDA dosing guidance for VTE treatment. Blood was collected prior to apixaban administration, and 0.5, 1, 3, 4, 6, 8, and 24h after administration for PK analyses and anti-FXa levels. Additional blood samples were collected at baseline and 3, 6 and 24h post-dose to evaluate thrombin generation, and at baseline and 24h for D-dimer. Urine protein:creatinine ratio (UCPR), baseline PT/INR, APTT and platelet count were evaluated prior to apixaban administration. Safety assessments were performed at baseline, prior to 24h time point on Day 2, and for the entire AE-reporting period (0-48h) (Figure S1). We measured total and free fraction apixaban concentrations by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Laboratory Corporation of America [Labcorp], Burlington, NC). Functional apixaban concentrations were determined by a chromogenic anti-FXa

assay (Colorado Coagulation, Labcorp, Englewood, CO). Additional PD assay and biostatistical analysis details are noted in Item S1.

Eleven subjects with NS (6 membranous nephropathy [MN]; 4 focal segmental glomerulosclerosis [FSGS]; 1 minimal change [MCD]) and 11 healthy controls were enrolled. Nineteen subjects completed the study. Three MN subjects were ineligible at the study visit (Figure S2). Baseline demographic characteristics were similar in both groups (Table S1). Apixaban PK parameters are summarized in Table 1. We examined both total apixaban (i.e., the sum of the protein-bound apixaban fraction plus the protein-unbound "free" apixaban fraction) and free apixaban (the protein-unbound apixaban fraction only). Percent of total circulating apixaban versus free apixaban fraction differences for PK parameters among NS subjects versus healthy controls were utilized for comparisons. The T_{max} of both total and free apixaban was 3h, indicating similar gastrointestinal absorption rates among all subjects. There was no statistically significant difference observed for peak concentration (C_{max}) or overall exposure (AUC_{0-24}) of total or free apixaban between and NS subjects and healthy controls. However, one interesting observed trend was the C_{max} for percent of the circulating free apixaban fraction was lowest among healthy controls (6.5%, range 3.6-13.0%) and but rose among NS subjects (9.1%, 6.3-13.7%). This was highest among the four NS subjects with the most severe disease who presented with albumin <3.0 g/dL or UPCR > 7 (10.3%, range 6.3-13.7%). Similarly, the percent of the circulating free apixaban fraction for AUC_{0-24} was also lowest healthy among healthy controls (6.4%, range 5.0-9.5%),and increased among NS subjects (8.8%, range7.0-12.2%) and the subjects with most severe nephrosis (9.7%, range 7.6-12.2%). While the observed ranges of free circulating apixaban are consistent with published apixaban plasma protein binding estimates (87-93%),[7,8] they suggest a trend

towards greater free fraction exposure in NS subjects, possibly due to decreased circulating albumin levels.

When evaluating the severe NS subjects, we also noted 23% lower exposure (AUC_{0-24}), and 28% higher apparent clearance (CL/F) of total apixaban when compared to healthy controls (Table 1). In these severe NS subjects, the higher clearance of total apixaban was observed concurrently with 14% lower free apixaban clearance. Together these differences would suggest the more rapid clearance of total apixaban in severe NS subjects was driven by a disproportionately greater clearance of the protein-bound apixaban fraction, possibly as a function of increased proteinuria.

Among NS subjects overall, maximum free apixaban concentration and AUC_{0-24} were higher (25% and 28%, respectively), while CL/F was approximately 18% slower than in healthy controls. Collectively, these data could suggest that greater exposure to pharmacologically active free apixaban fraction could possibly translate to higher bleeding risk. At baseline, D-dimer and TG trended higher in NS subjects versus healthy controls, but did not reach statistical significance. However, D-dimer at 24h post-dose was significantly higher in NS subjects (Figure S3). These and additional PK/PD results are available in Item S2 and Figures S3-S9.

From our data, a single 10 mg dose of apixaban was safe, and non-steady-state PK/PD was similar between NS subjects and healthy controls, with some variance dependent upon disease severity. Severity of hypoalbuminemia in NS is the most well-established risk factor for VTE,[9] particularly for MN; NS subjects with the highest VTE risk and greatest need for pharmacologic thromboprophylaxis may be most likely to have altered PK/PD for highly protein-bound drugs, such as apixaban.[7,10] While we observed relatively similar PK/PD characteristics in NS compared to healthy controls (Figure 1; Figures S3-S8), notable PK/PD differences could have potential clinical importance if validated, particularly among subjects with the most severe NS (e.g.,

differences in total apixaban AUC_{0-24} and CL/F (Table 1). However, the clinical implications of reduced total apixaban exposure and increased total apixaban clearance are currently unknown in a disease whose cardinal features could potentially affect the free fraction PK because all of the above PK/PD measures in this study were assessed only after a single 10 mg apixaban dose.

Clinically, we have observed apixaban anticoagulation failure in an MN patient who experienced recurrent VTE with lower than expected peak apixaban Xa activity, suggesting potentially reduced therapeutic efficacy.[11] Among NS subjects with severe nephrosis, we noted increased exposure of free fraction apixaban with slower CL/F compared to control subjects, which was in contrast to the findings of total apixaban (Table 1). These latter findings could suggest that NS patients may have increased bleeding risk from free drug due to prolonged exposure. However, the exact concentration at which apixaban treatment leads to increased bleeding risk remains unknown, and these observed exposure differences from our study may not meet that threshold. Moreover, our toxicity data and present PD data do not confirm such an increased bleeding risk, but only capture changes from a single dose and not steady-state PD.

While PK parameter differences for the pharmacologically free apixaban are descriptively different between NS subjects and healthy controls, these data need both confirmation and assessment of their clinical relevance in a larger cohort of NS subjects, and with steady-state apixaban PK measurements. PD assessments at the steady-state may also reveal more apparent differences between NS subjects and controls. Our observations remain hypothesis generating given the single dose nature of the study and small sample size. Future studies incorporating steady-state apixaban PK and PD will be needed to reveal the clinical significance of any alterations to free and total apixaban PK in NS subjects, including those patients that present with more severe hypoalbuminemia or proteinuria.

Our study utilized traditional approaches to explore apixaban PK/PD (e.g., quantification of total circulating apixaban and anti-FXa), in combination with innovative approaches to quantify circulating free apixaban and apixaban PD (e.g., D-dimer and thrombin generation quantitation), to explore pharmacological differences for both bound and unbound apixaban *vis-a-vis* proteinuria and hypoalbuminemia in NS subjects. We did note PK/PD differences, as described, above whose clinical consequences warrant further evaluation. Future multi-dose studies with steady-state measures of apixaban exposure, elimination and pharmacodynamics (ClinicalTrials.gov NCT04278729) can advise potential clinical studies of NS and VTE and inform clinically appropriate use of this agent for this population.

Article Information

Authors' Contributions: Research idea and study design: VKD, TP, KAM, NSK, DJC; data acquisition: VKD, MLC, JRG, TP, AF, MRS, AHS, SMP, CDT, DMA, ST, RPG, NSK, and DJC; data analyses and interpretations: VKD, JZ, MLC, JRG, SS, SMP, CDT, DMA, ST, RPG, NSK, DJC; statistical analyses: JZ, DJC; supervision or mentorship: VKD, DMA, ST, RPG, NSK, DJC. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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Data Sharing: Deidentified individual data that supports the results will be shared beginning 9 to 36 months following publication provided the investigator who proposes to use the data has

approval from an IRB, Independent Ethics Committee (IEC), or Research Ethics Board (REB), as applicable, and executes a data use/sharing agreement with UNC.

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Table 1. Summary statistics for apixaban and anti-FXa parameters.

Parameter Estimate	Healthy Control Subjects (n=11)				All Nephrotic Syndrome Subjects (n=8)				Severe Nephrotic Syndrome Subjects (n=4)			
	Total Apixaban	Free Apixaban	Free % of Total Apixaban	Anti-FXa	Total Apixaban	Free Apixaban	Free % of Total Apixaban	Anti-FXa	Total Apixaban	Free Apixaban	Free % of Total Apixaban	Anti-FXa
C_{max} (ng/mL), geometric mean (%CV)	162.3 (38.2)	10.6 (38.5)	6.5% (3.6-13.0%	166.3 (34.9)	146.8 (32.8)	13.3 (33.1)	9.1% (6.3-13.7%)	155.3 (33.1)	132.0 (21.9)	13.6 (35.6)	10.3% (6.3-13.7%)	68.0 (67.4)
T_{max} (hours), median (range)	3.0 (1.0-3.0)	3.0 (1.0-4.0)	-	2.2 (1.0-3.0)	3.0 (1.0-4.0)	3.0 (1.0-4.0)	-	2.8 (1.0-4.0)	3.0 (0)	3.0 (0)	-	3.0 (0)
AUC_{0-24} (ng*h/mL), geometric mean (%CV)	1654.9 (31.6)	106.2 (41.9)	6.4% (5.0-9.5%)	1576.9 (26.4)	1539.4 (50.9)	135.8 (46.5)	8.8% (7.0-12.2%	1651.0 (43.6)	1274.9 (45.4)	124.1 (59.0)	9.7% (7.6-12.2%)	1478.6 (54.1)
Vd/F (L), geometric mean (%CV)	55.9 (39.4)	1162.8 (47.6)	-	65.2 (32.1)	65.3 (35.9)	919.6 (44.6)	-	74.6 (42.4)	69.8 (25.1)	1037.9 (13.9)	-	91.5 (23.6)
$t_{1/2}$ (hours), mean SD)	7.3 (1.7)	13.3 (4.2)	-	12.6 (7.5)	9.2 (4.6)	12.6 (2.6)	-	8.2 (1.5)	7.4 (3.4)	13.3 (1.8)	-	14.6 (10.2)
CL/F (mL/h), geometric mean (%CV)	5389.0 (29.8)	62929.3 (21.9)	-	5592.6 (22.1)	5386.4 (68.9)	51608.7 (43.1)	-	4614.9 (58.6)	6923.0 (40.9)	54512.7 (40.3)	-	5016.7 (46.9)

Parameter estimates for total and free apixaban PK, and anti-FXa activity are based on non-compartmental analyses. Significant differences were not observed for any of the parameters between the NS and healthy control subjects, or between the severe NS and healthy control subjects, for total apixaban concentrations, free apixaban concentrations, or anti-FXa activity (P>0.05). All Nephrotic Syndrome subjects includes those with severe NS. Severe NS was defined as UPC ratio >7 and/or albumin < 3.0 g/dL. Abbreviations, AUC_{0-24}, area under the plasma concentration–time curve from time zero to 24 hours; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero extrapolated to infinity; CL/F, apparent clearance; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; FXa, factor Xa; NS, nephrotic syndrome; PK, pharmacokinetic; SD, standard deviation; $t_{1/2}$, plasma terminal half-life; T_{max}, time of observed maximum plasma concentration; UPC, urine protein to creatinine; Vd/F, apparent volume of distribution.

Figure Legend

Figure 1. Plasma concentration versus time profiles for total apixaban, free apixaban, free to total apixaban ratio, and anti-FXa. Panel A is the plasma concentration profile for total apixaban among healthy control subjects (n=11) and NS subjects (n=8) from 0-24 hours after a single orally administered 10 mg dose of apixaban. **Panel B** is the plasma concentration profile for free apixaban over the same time interval. **Panel C** shows the ratio of free apixaban to total apixaban over the same interval. **Panel D** shows the plasma concentration profile for anti-FXa over the same time interval. Error bars represent standard deviation of the mean. Abbreviations: FXa, factor Xa; NS, nephrotic syndrome.