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Evaluating Anticoagulant and Antiplatelet Therapies in Rhesus and Cynomolgus Macaques for Predictive Modeling in Humans

Sydney N. Phu [1,2], David J. Leishman [1], Sierra D. Palmer [1,2], Scott H. Oppler [1,2], Melanie N. Niewinski [1], Lucas A. Mutch [1], Jill S. Faustich [3], Andrew B. Adams [1], Robert T. Tranquillo [4] and Melanie L. Graham [1,2,*]

[1] Department of Surgery, University of Minnesota, Minneapolis, MN 55455, USA; phu00004@umn.edu (S.N.P.); leish008@umn.edu (D.J.L.); opple001@umn.edu (S.H.O.)
[2] Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN 55108, USA
[3] Experimental Surgical Services, Department of Surgery, University of Minnesota, Minneapolis, MN 55455, USA
[4] Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA; tranquillo@umn.edu
* Correspondence: graha066@umn.edu

Abstract: Anticoagulant and antiplatelet therapies are used to prevent life-threatening complications associated with thrombosis. While there are numerous clinical guidelines for antithrombotic medications, there is an incomplete understanding of whether these interventions yield similar effects in preclinical models, potentially impacting their predictive value for translational studies on the development of medical devices, therapies, and surgical techniques. Due to their close physiologic similarities to humans, we employed nonhuman primates (NHPs) using a reverse translational approach to analyze the response to clinical regimens of unfractionated heparin, low-molecular-weight heparin (LMWH) and aspirin to assess concordance with typical human responses and evaluate the predictive validity of this model. We evaluate activated clotting time (ACT) in nine rhesus and six cynomolgus macaques following the intraoperative administration of intravenous unfractionated heparin (100–300 U/kg) reflecting the clinical dose range. We observed a significant dose-dependent effect of heparin on ACT (low-dose average = 114.1 s; high-dose average = 148.3 s; $p = 0.0011$). LMWH and aspirin, common clinical antithrombotic prophylactics, were evaluated in three rhesus macaques. NHPs achieved therapeutic Anti-Xa levels (mean = 0.64 U/mL) and ARU (mean = 459) via VerifyNow, adhering to clinical guidance using 1.0 mg/kg enoxaparin and 81 mg aspirin. Clinical dosing strategies for unfractionated heparin, LMWH, and aspirin were safe and effective in NHPs, with no development of thrombosis or bleeding complications intraoperatively, postoperatively, or for prophylaxis. Our findings suggest that coagulation studies, performed as an integrative part of studies on biologics, bioengineered devices, or transplantation in NHPs, can be extrapolated to the clinical situation with high predictive validity.

Keywords: anticoagulation; nonhuman primates; surgery; unfractionated heparin; low-molecular-weight heparin; aspirin; antiplatelet

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1. Introduction

Nonhuman primates (NHP) serve as essential models for the development of medical devices, therapies, and surgical techniques [1–4]. They play a crucial role in assessing their safety and efficacy before translation into the clinical setting. Devices, bioengineered materials, and surgical manipulations can influence hemostasis, and concurrent antithrombotic therapy is often needed for optimal outcomes. Unfractionated heparin has been used for decades for the prevention and treatment of thrombosis, with numerous applications, including pulmonary embolism, cardiovascular disease, stroke, and surgical vascular interventions [5–11]. Low-molecular-weight heparin (LMWH) and aspirin are used similarly, often for thromboprophylaxis, to reduce the risk of venous and arterial thromboembolism

in clinical patients [10,12–16]. There are numerous guidelines for the dosing and monitoring of unfractionated heparin, LMWH, and aspirin in the clinical setting, with well-known safety and efficacy profiles [17–22]. However, there is a limited foundation for practice concerning the dosing, monitoring, and adjustment of common anticoagulant and antiplatelet therapies in NHPs, which could potentially impact the translation and predictive validity of NHP models [23,24]. Given the close approximation of NHP coagulation and fibrinolysis physiology to the human condition [25], NHPs are frequently employed as the preferred model for investigating these parameters.

The utility of the NHP model is particularly evident in the field of solid organ transplantation, where NHPs are physiologically and immunologically similar to humans [4,26]. With advancements in immunogenetic analysis enabling complete major histocompatibility complex (MHC) characterization [27]—a critical determinant in transplant graft rejection—NHPs have been instrumental in modeling the transplantation of various solid organs, such as the kidney, liver, lung, and heart [28]. NHPs closely mirror the clinical transplantation scenario, beginning with crossmatching practices and extending through immunosuppressive drug administration, immune monitoring, and especially the transplantation surgery itself. These procedures involve the manipulation and anastomosing of blood vessels, necessitating intraoperative anticoagulation to prevent thrombosis and subsequent graft failure. This practice may extend to the immediate post-operative period, where continued anticoagulation or antiplatelet therapy is administered to mitigate the risk of vascular thrombosis. Therefore, understanding the appropriate dosing strategies for these drugs in experimental transplant models is paramount.

In this study, we evaluated the anticoagulant and antiplatelet responses in NHPs exposed to unfractionated heparin, LMWH, and aspirin, aiming to model various clinical scenarios. We functionally assessed the safety and efficacy of unfractionated heparin use during kidney transplant surgery in NHPs, employing different dosing strategies representative of those conventionally utilized in surgeries involving vascular manipulation.

A subset of NHPs underwent a period of the administration of LMWH and aspirin, modeling antithrombotic prophylaxis, with the option to adjust dosages based on measured LMWH and aspirin levels. This approach aimed to replicate the clinical practices for dose adjustment to achieve the therapeutic range.

2. Materials and Methods

The protocol and procedures related to the use of animals described in this study were approved by the University of Minnesota's Institutional Animal Care and Use Committee (IACUC). The use of animals was in adherence with the United States Department of Agriculture's (USDA) Animal Welfare Act and Animal Welfare Regulations, and the standards outlined in both the Guide for the Care and Use of Laboratory Animals, and USDA Animal Care Blue Book [29]. The response to unfractionated heparin was evaluated retrospectively by analyzing 15 NHPs enrolled in our solid organ transplant program. The response to LMWH and aspirin as antithrombotic prophylaxis was prospectively assessed in a comparable group of three NHPs with similar demographics. All animals were purpose-bred and acquired through institutionally approved commercial vendors. Unless social incompatibilities were indicated, all animals were housed in same-sex pairs. Enrolled animals' general appearance and behavior were observed a minimum of twice daily as part of routine health monitoring. Weights were collected at least once a month and veterinary rounds were performed weekly for routine evaluation. Individual animals had semi-annual physical veterinary examinations, as well as complete blood counts and chemistry panels.

The screening process for anticoagulation disorders in animals involved a comprehensive approach, incorporating clinical assessment, laboratory tests, and a medical history review. The clinical assessment encompassed a thorough physical examination to detect signs indicative of bleeding or clotting disorders, including bruising, petechiae, swelling, and an examination of mucous membranes. Laboratory tests comprised a complete hema-

tology and chemistry panel, including platelet studies, with baseline evaluations of relevant coagulation parameters. Additionally, the medical history was reviewed, specifically focusing on previous surgeries involving vascular access port placement, ensuring no abnormal bleeding was observed.

All animals had access to water ad libitum and were fed twice daily (2055c or 7195 Envigo Harlan Teklad Nonhuman Primate Diet or 5048 LabDiet Certified Primate Diet), with the type and quantity determined by individual preference, study goals, and percentage of body weight. An environmental enrichment program included supplemental food enrichment offered on a daily basis and provided animals with opportunities that included music, novel items designed to promote foraging, grooming, problem solving, and social play, fostering species-typical behavior. This program included routine access to large play areas, allowing for additional exercise and swimming opportunities.

The housing areas were maintained at temperatures between 20 and 26.7 °C, with 30–70% humidity. Lights were programmed to a 12 h on and 12 h off circadian light cycle, with 30 min dawn and dusk fading intervals. All animals had a subcutaneous vascular access port placed into the femoral vein [30] for routine blood collection and were trained for cooperation [31,32] with study tasks that include physical examination, drug administration, and blood collection.

2.1. Unfractionated Heparin

To assess intraoperative heparin response, we conducted a retrospective analysis of the activated clotting time (ACT) following heparinization in both rhesus (male = 8, female = 1) and cynomolgus macaques (male = 6) for a total of 15 NHPs aged 4.6–7.4 years (median = 5.4) and weighing 6.3–12.1 kg (median = 8.14) during kidney transplantation (Figure 1), which was performed at our center aiming to advance novel treatment approaches in transplantation medicine. Intravenous heparinization was performed as a part of renal transplant surgery prior to vascular clamping. NHPs dosed with 100 U/kg heparin (Meitheal Pharmaceuticals, Chicago, IL, USA) were categorized as 'low-dose'. NHPs dosed with 300 U/kg heparin were categorized as 'high-dose'. NHPs dosed with 100 U/kg initially, followed by 50 U/kg approximately 30 min later to reflect the scenario of the administration of additional doses, were categorized as 're-dose' (Figure 1). The average clamp times were 17 ± 4.3 min for the inferior vena cava and 17 ± 6.1 min for the aorta. ACTs were collected and evaluated within a 60–150 min window post-heparin administration using an iSTAT ACT point-of-care analyzer (Abbott Laboratories, Green Oaks, IL, USA).

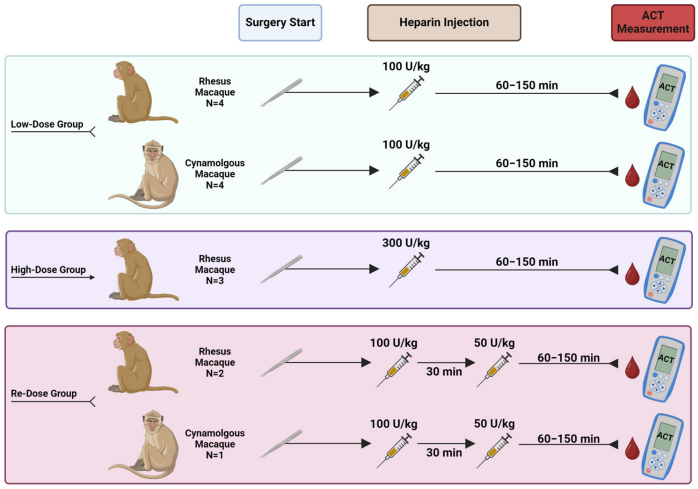
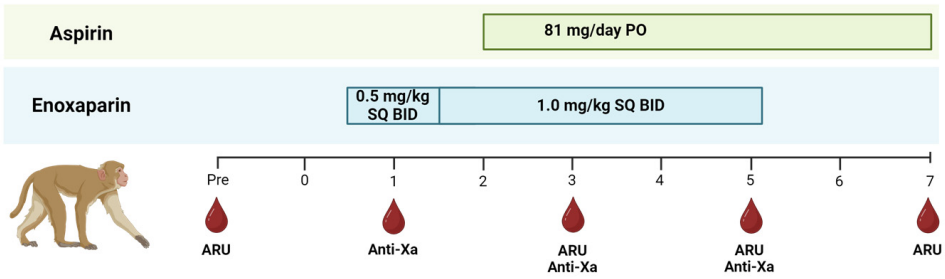


Figure 1. Overview of different intraoperative heparin dosing strategy groups. NHPs were divided into low-dose (100 U/kg), high-dose (300 U/kg), and re-dose (100 U/kg followed by 50 U/kg) groups. In preparation for anastomosis creation and prior to vascular clamping, intravenous unfractionated heparin was administered, and ACT was measured 60–150 min afterward. In the re-dose group, an additional 50 U/kg was administered 30 min after initial heparin injection.

2.2. Low-Molecular-Weight Heparin and Aspirin

To assess the hemostatic response to LMWH (Amphastar Pharmaceuticals, Rancho Cucamonga, CA, USA) and aspirin (Bayer Pharmaceuticals, Leverkusen, North Rhine-Westphalia, Germany), we adapted a clinical regimen of enoxaparin and aspirin to attain target Anti-Xa and ARU levels in three healthy male rhesus macaques, aged 9–14 years old, and weighing 11.4–15.8 kg. Enoxaparin was administered subcutaneously, starting at 0.5 mg/kg q12h and increasing to 1.0 mg/kg q12h on day one. Aspirin was administered orally at a dose of 81 mg daily, starting on day two. Blood samples were collected approximately 4 h after enoxaparin administration on days 1, 3, and 5 (Figure 2A). For the determination of the plasma levels of LMWH, the anti-Xa activity on antithrombin was measured in a competitive assay using a synthetic chromogenic substrate at M Health Fairview's clinical laboratory within 4 h of sample collection. Blood was collected on days 3, 5, and 7 to measure Aspirin Reaction Units (ARU), a measure of thromboxane A2-mediated glycoprotein IIb/IIIa receptor activation during platelet aggregation. ARU was calculated as a function of the rate and extent of platelet aggregation using the VerifyNow point-of-care testing platform (Accumetrics, Latham, NY, USA). Dosing adjustments adhered to the clinical guidance [33–35] provided in Figure 2B,C.

A



B

Enoxaparin Dose: 0.5–1.0 mg/kg/day SC Target Anti-Factor Xa: 0.5–1.0 U/mL
If Xa level is <0.35 U/mL, increase dose by 25%.
If Xa level is 0.35–0.49 U/mL, increase dose by 10%.
If Xa level is 0.5–1.0 U/mL, keep same dose.
If Xa level is 1.1–1.5 U/mL, decrease dose by 20%.
If Xa level is 1.6–2.0 U/mL, decrease dose by 30%.
If Xa level is >2.0 U/mL, decrease dose by 40%.
If Xa level is unstable, apixaban may be substituted at 0.05–0.2 mg/kg PO BID.

C

Aspirin Dose: 3–10mg/kg/day PO QD Target ARU: <550
If ARU is <550, keep same dose.
If ARU is >550, increase dose by 100%.
If ARU does not reach target, substitute clopidogrel 1–6 mg/kg/day with target PRU <194.

Figure 2. Overview of LMWH and aspirin administration and dose adjustment. (**A**) Administration and sampling timeline for the dose optimization of LMWH and aspirin. (**B**) Protocol for adjusting LMWH dosage based on measured Anti-Xa level. (**C**) Protocol for adjusting aspirin dosage based on measured ARU.

2.3. Statistical Analysis

Nonparametric tests were employed for the statistical analysis of results. The relationship between ACT and unfractionated heparin dose was assessed using one-way analysis of variance (ANOVA) with Kruskal–Wallis correction. The Mann–Whitney test was utilized to evaluate species-specific differences in ACT response. To assess the effects of enoxaparin and aspirin regimens, Mann–Whitney tests were performed to compare baseline and combined treatment Anti-Xa and ARU levels. Secondary outcomes were reported through descriptive statistics. Data were analyzed using the statistical software GraphPad Prism version 9.5.1 (Boston, MA, USA).

3. Results

3.1. Unfractionated Heparin

The response to intraoperative unfractionated heparin is illustrated in Figure 3. To compare responses to heparin between species, a *t*-test analysis was performed on rhesus (n = 4) and cynomolgus (n = 5) macaques who received low-dose heparin (Figure 3A). Although the mean ACT for rhesus macaques was noted to be slightly higher, at 117 ± 13.3 s, than in cynomolgus macaques, at 111 ± 8.4 s, there was no significant difference in mean ACT between rhesus and cynomolgus macaques in the low-dose group (p = 0.6111). Therefore, data for rhesus and cynomolgus macaques were combined.

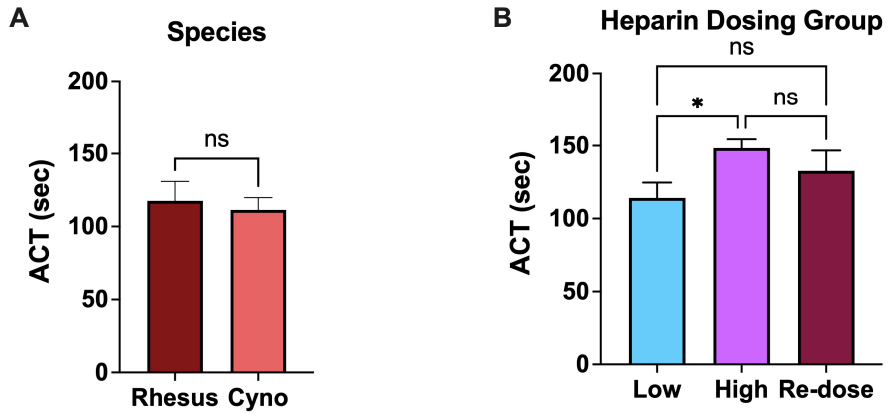


Figure 3. Mean activated clotting time (ACT) following intraoperative unfractionated heparin administration in rhesus and cynomolgus macaques. (**A**) Rhesus (n = 4) and cynomolgus (n = 5) ACT response to low-dose unfractionated heparin. Rhesus macaques had a mean ACT of 117.5 s, and cynomolgus macaques had a mean ACT of 111.4 s. There was no significant difference in ACT between species in the low-dose group (p = 0.6111). (**B**) ACT response by dosing group for both rhesus and cynomolgus macaques combined. The low-dose group had a mean ACT of 114.1 s. The high-dose group had a mean ACT of 148.3 s. The re-dose group had a mean ACT of 132.7 s. There was a significant difference between low-dose (n = 9) and high-dose (n = 3) groups (p = 0.0190), but no significant difference between low-dose and re-dose (n = 3) groups (p = 0.2881) or between high-dose and re-dose groups ($p \geq 0.9999$). Data are presented as mean ± SD. * $p < 0.05$, ns = not significant.

Mean ACT for NHPs increased as heparin dose increased, with the mean ACT for NHPs receiving low-dose heparin at 114 ± 10.59 s (n = 9), the mean ACT for NHPs who received high-dose heparin at 148 ± 6.11 s (n = 3), and the mean ACT for NHPs who were re-dosed at 133 ± 14.01 s (n = 3) (Figure 3B. The Kruskal–Wallis ANOVA test found there was a significant difference found between animals who received low-dose heparin compared to animals who received high-dose heparin (p = 0.0190). With a difference in means of −8.111 s, animals who received high-dose heparin could achieve ACT values up to 8 s greater than low-dose animals, on average, and are likely to take longer to return to normal range. However, this difference is unlikely to be clinically significant.

3.2. Low-Molecular-Weight Heparin and Aspirin

Figure 4A shows the average Anti-Xa levels at baseline and at the end of low-molecular-weight heparin and aspirin treatment. NHPs had a mean anti-Xa level of 0.29 ± 0.95 IU/mL at baseline and 0.82 ± 0.20 IU/mL with treatment. *T*-test analysis shows there was significant increase in Anti-Xa levels with treatment ($p = 0.0238$). Figure 4B shows individual Anti-Xa levels by treatment day, with a maximum Anti-Xa of 1.04 IU/mL and a minimum Anti-Xa of 0.64 IU/mL at the end of treatment.

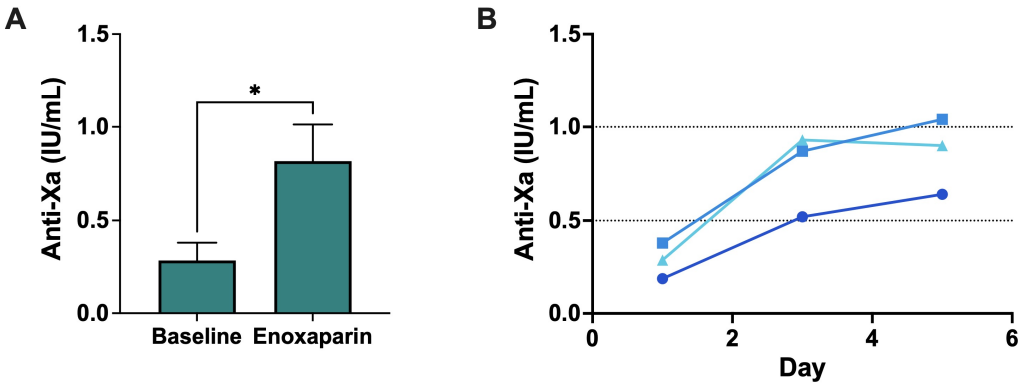


Figure 4. Anti-Xa levels following the subcutaneous administration of low-molecular-weight heparin (LMWH) in rhesus macaques ($n = 3$). (**A**) Mean anti-Xa levels with standard deviation at baseline and post low-molecular-weight heparin and aspirin treatment. Animals had a mean Anti-Xa level of 0.29 IU/mL at baseline and mean Anti-Xa level of 0.82 IU/mL with treatment. (**B**) Individual Anti-Xa levels across treatment days with target levels of 0.5–1.0 IU/mL, as shown by the dotted lines. There was a significant increase in Anti-Xa levels with treatment ($p = 0.0238$). Data are presented as mean ± SD. * $p < 0.05$, ns = not significant

Figure 5A shows the average ARU with standard deviation at baseline and at the end of low-molecular-weight heparin and aspirin treatment. NHPs had a mean of 636 ± 7.21 ARU at baseline and 459 ± 38.9 ARU with treatment. The *t*-test analysis shows there was a significant decrease in ARU levels with treatment ($p = 0.0091$). Figure 5B shows individual ARU values according to treatment day, with a minimum of 404 ARU and a maximum of 440 ARU at the end of treatment. No adverse events were observed.

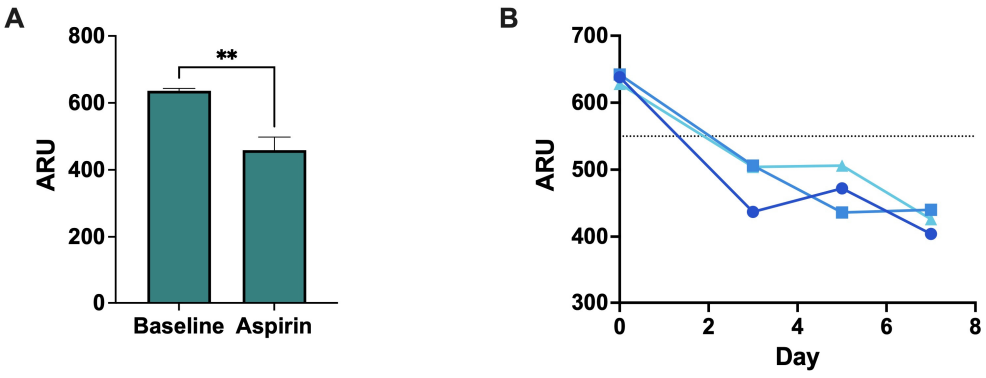


Figure 5. Aspirin reaction units (ARU) following oral aspirin administration in rhesus macaques. (**A**) Mean ARU with standard deviation at baseline and after low-molecular-weight heparin and aspirin treatment. Animals had a mean of 636 ARU at baseline and a mean of 459 ARU with treatment. (**B**) Individual ARU values over the course of treatment with target ARU levels of <550, as shown by the dotted line. There was a significant reduction in ARU levels with treatment ($p = 0.0091$). Data are presented as mean ± SD. ** $p < 0.01$, ns = not significant.

4. Discussion

The purpose of this study was to assess the utilization of unfractionated heparin, LMWH, and aspirin in NHPs to determine concordance with common clinical practices. Additionally, the study aimed to offer guidance on optimal dosing and monitoring protocols to ensure safety when administering these agents to NHPs. Given their close phylogenetic relationship, and thus, their close anatomic and physiologic similarity [4,26,36,37], NHPs are a crucial translational model for the understanding of disease states and the development of new surgical techniques and medicines. Modeling various conditions often necessitates anticoagulation or platelet inhibition. However, the existing literature on their application in NHPs is limited. Our findings suggest that replicating clinical dosing strategies for unfractionated heparin, LMWH, and aspirin effectively achieves therapeutic levels with a similar safety profile to that observed in the clinical setting. This underscores the predictive validity and relevance of the NHP model in accurately assessing the safety and efficacy of novel approaches requiring concurrent antithrombotic therapy.

We evaluated three different intraoperative heparin dosing strategies in NHPs undergoing kidney transplantation at our center to advance novel treatment approaches in transplantation medicine. In general, heparin is commonly utilized during vascular surgery in order to mitigate thrombotic complications [38]. In kidney transplantation, both arterial and venous anastomoses are performed, and thrombosis of either vessel during the immediate postoperative period is a devastating complication, often leading to graft loss [39,40]. Despite this, few guidelines exist for best practices related to vascular surgery in NHPs. Accordingly, our study aimed to assess whether the ACT profile in NHPs mirrors that observed in humans. To achieve this, we evaluated the effects of low-dose heparin (100 U/kg), high-dose heparin (300 U/kg), and the intraoperative redosing of heparin (100 U/kg followed by 50 U/kg) in NHPs. These administration strategies align with the clinical dosing protocols observed in a range of cardiac and vascular procedures [6,38,40–42].

In all three dosing groups, successful anticoagulation was achieved throughout the approximately 30 min cross-clamp time, as evidenced by the surgical success achieved for all animals. ACT levels returned to normal or near-normal 60–150 min after heparin administration or re-dose. While the literature reports variable upper limits of normal (up to 180 s [43–46]), 70–120 s is typically used in clinical practice [47,48]. No significant differences in ACT were detected between rhesus and cynomolgus macaques with dosing intervention held constant. Similarly, no sex-based differences in ACT were observed in either species with fixed dosing, consistent with the findings of other coagulation studies in rhesus and cynomolgus macaques [49–52]. A dose-dependent effect was observed with heparin, as the average measured ACT was higher in the high-dose group, followed by the re-dose group and the low-dose group. Clinically, no NHPs in any group experienced bleeding complications intra- or post-op. Furthermore, heparin has a relatively short half-life, reported to be between 60 and 150 min depending on dose [53,54]. Given this, it would be expected that those with near-normal ACTs would soon reach the normal range, mitigating further risk due to anticoagulation.

In this study, we also explored antithrombotic prophylaxis with LMWH and aspirin by performing a dose optimization study aiming to achieve therapeutic levels. Clinically, LMWH is used in a variety of applications, including the treatment and prevention of arterial and venous thromboembolism [10,12,14,15]. Aspirin is often used in the management of coronary, cerebral, and peripheral artery disease [13,16,18]; when combined with unfractionated heparin or LMWH, aspirin is also commonly used for thrombosis prophylaxis after coronary artery surgery/intervention [55–57] and after major non-cardiac vascular surgery [38]. To mimic the clinical need for therapeutic anticoagulation and platelet inhibition over a period of time, we administered LMWH and aspirin to NHPs over 7 days while monitoring anti-Xa and ARU levels. Our goal was to maintain therapeutic levels and, if necessary, adjust subsequent doses based on an algorithm derived from pediatric experience [33–35]. Therapeutic anti-Xa and ARU levels were achieved and remained within the target range throughout the 7-day period in all subjects, requiring no adjustments. No NHP

experienced complications or adverse events. Based on our results, combination therapy with LMWH and aspirin could be a viable option for maintenance therapy in NHPs when anticoagulation and platelet inhibition is required by the study.

While our findings contribute new data to the field, this study is not without its limitations. In our heparin studies, we focused solely on safety following dosing and did not capture measurements immediately after injection. Consequently, we were unable to present a complete profile of heparin following injection, which would indicate the level of anticoagulation while clamped in addition to assessing the risk of bleeding complications following anticoagulation. Although no anastomotic thrombosis occurred in any of our subjects, considering the half-life of heparin, it is likely the ACT reached levels of 250 or greater during cross-clamp. However, obtaining ACT measurements just prior to surgical manipulation that carries a risk of thrombosis remains ideal to ensure that the appropriate target levels for the procedure are achieved. The sample size in the retrospective analysis was confined to 18 animals to ensure comparability in terms of age and weight within the defined dose groups. Although there was not an equal distribution regarding species or sex in the groups, antithrombotic regimens have not shown sex-specific effects [49–52]. The absence of a species-specific effect was apparent when comparing across species in our low-dose condition. The inclusion of only one female precludes us from drawing conclusions about sex-specific effects.

The clinical dosing strategy for LMWH and aspirin yielded therapeutic anti-Xa and ARU levels without the need for adjustment. Consequently, the effectiveness of our dose adjustment algorithm remains uncertain, as no adjustments were necessary. Future studies could explore extending the treatment or sampling timeline to further refine the precision of anticoagulant use in NHP models.

5. Conclusions

Unfractionated heparin, LMWH, and aspirin have demonstrated safety and efficacy in NHPs when administered using clinically applicable dosing strategies for anticoagulation and platelet inhibition, supporting their use in modeling requiring intraoperative vascular manipulation or chemical thromboembolic prophylaxis. The use of these antithrombotic medications in applicable preclinical NHP models further enhances their predictive translational value in the development of novel procedures, techniques, and devices.

Author Contributions: Conceptualization, S.N.P., D.J.L., S.D.P., S.H.O., A.B.A., R.T.T. and M.L.G.; methodology, S.N.P., D.J.L., S.D.P., S.H.O., A.B.A., R.T.T. and M.L.G.; validation, S.N.P., D.J.L., S.D.P., S.H.O., M.N.N., L.A.M., J.S.F., A.B.A., R.T.T. and M.L.G.; formal analysis, S.N.P., S.D.P. and S.H.O.; investigation, S.N.P., D.J.L., S.D.P., S.H.O., M.N.N., L.A.M., J.S.F., A.B.A., R.T.T. and M.L.G.; resources, M.L.G.; writing—original draft preparation, S.N.P., D.J.L., S.D.P., S.H.O. and M.L.G.; writing—review and editing, S.N.P., D.J.L., S.D.P., S.H.O., M.N.N., L.A.M., J.S.F., A.B.A., R.T.T. and M.L.G.; visualization, S.N.P., D.J.L., S.D.P. and S.H.O.; supervision, A.B.A., R.T.T. and M.L.G.; project administration, M.N.N., L.A.M. and M.L.G.; funding acquisition, A.B.A., R.T.T. and M.L.G. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lifson, J.D.; Haigwood, N.L. Lessons in nonhuman primate models for AIDS vaccine research: From minefields to milestones. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a007310. [CrossRef]
2. Shen, S.; Pyo, C.-W.; Vu, Q.; Wang, R.; Geraghty, D.E. The essential detail: The genetics and genomics of the primate immune response. *ILAR J.* **2013**, *54*, 181–195. [CrossRef]
3. Sibal, L.R.; Samson, K.J. Nonhuman Primates: A Critical Role in Current Disease Research. *ILAR J.* **2001**, *42*, 74–84. [CrossRef]
4. Phillips, K.A.; Bales, K.L.; Capitanio, J.P.; Conley, A.; Czoty, P.W.; 't Hart, B.A.; Hopkins, W.D.; Hu, S.L.; Miller, L.A.; Nader, M.A. Why primate models matter. *Am. J. Primatol.* **2014**, *76*, 801–827. [CrossRef]
5. Hirsh, J.; Anand, S.S.; Halperin, J.L.; Fuster, V. Guide to anticoagulant therapy: Heparin: A statement for healthcare professionals from the American Heart Association. *Circulation* **2001**, *103*, 2994–3018. [CrossRef]
6. Lax, M.; Pesonen, E.; Hiippala, S.; Schramko, A.; Lassila, R.; Raivio, P. Heparin dose and point-of-care measurements of hemostasis in cardiac surgery—Results of a randomized controlled trial. *J. Cardiothorac. Vasc. Anesth.* **2020**, *34*, 2362–2368. [CrossRef]
7. O'Gara, P.T.; Kushner, F.G.; Ascheim, D.D.; Casey, D.E.; Chung, M.K.; De Lemos, J.A.; Ettinger, S.M.; Fang, J.C.; Fesmire, F.M.; Franklin, B.A. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.* **2013**, *61*, e78–e140. [CrossRef]
8. Ramelli, E.; Durry, A.; Ruffenach, L.; Bruant-Rodier, C.; Bodin, F. Decrease of thrombosis in microsurgical anastomoses: The use of intraoperative pure intraluminal unfractionated heparin. *J. Reconstr. Microsurg.* **2020**, *36*, 16–20. [CrossRef]
9. Ruff, I.M.; Jindal, J.A. Use of heparin in acute ischemic stroke: Is there still a role? *Curr. Atheroscler. Rep.* **2015**, *17*, 51. [CrossRef]
10. Simonneau, G.; Sors, H.; Charbonnier, B.; Page, Y.; Laaban, J.-P.; Azarian, R.; Laurent, M.; Hirsch, J.-L.; Ferrari, E.; Bosson, J.-L. A comparison of low-molecular-weight heparin with unfractionated heparin for acute pulmonary embolism. *N. Engl. J. Med.* **1997**, *337*, 663–669. [CrossRef]
11. You, J.J.; Singer, D.E.; Howard, P.A.; Lane, D.A.; Eckman, M.H.; Fang, M.C.; Hylek, E.M.; Schulman, S.; Go, A.S.; Hughes, M. Antithrombotic therapy for atrial fibrillation: Antithrombotic therapy and prevention of thrombosis: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* **2012**, *141*, e531S–e575S. [CrossRef]
12. Guyatt, G.H.; Eikelboom, J.W.; Gould, M.K.; Garcia, D.A.; Crowther, M.; Murad, M.H.; Kahn, S.R.; Falck-Ytter, Y.; Francis, C.W.; Lansberg, M.G. Approach to outcome measurement in the prevention of thrombosis in surgical and medical patients: Antithrombotic Therapy and Prevention of Thrombosis: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* **2012**, *141*, e185S–e194S. [CrossRef]
13. Johnston, S.C.; Easton, J.D.; Farrant, M.; Barsan, W.; Conwit, R.A.; Elm, J.J.; Kim, A.S.; Lindblad, A.S.; Palesch, Y.Y. Clopidogrel and aspirin in acute ischemic stroke and high-risk TIA. *N. Engl. J. Med.* **2018**, *379*, 215–225. [CrossRef]
14. Kay, R.; Wong, K.S.; Yu, Y.L.; Chan, Y.W.; Tsoi, T.H.; Ahuja, A.T.; Chan, F.L.; Fong, K.Y.; Law, C.B.; Wong, A. Low-molecular-weight heparin for the treatment of acute ischemic stroke. *N. Engl. J. Med.* **1995**, *333*, 1588–1594. [CrossRef]
15. Segon, Y.S.; Summey, R.D.; Slawski, B.; Kaatz, S. Surgical venous thromboembolism prophylaxis: Clinical practice update. *Hosp. Pract.* **2020**, *48*, 248–257. [CrossRef]
16. Zheng, S.L.; Roddick, A.J. Association of aspirin use for primary prevention with cardiovascular events and bleeding events: A systematic review and meta-analysis. *JAMA* **2019**, *321*, 277–287. [CrossRef]
17. Kleindorfer, D.O.; Towfighi, A.; Chaturvedi, S.; Cockroft, K.M.; Gutierrez, J.; Lombardi-Hill, D.; Kamel, H.; Kernan, W.N.; Kittner, S.J.; Leira, E.C. 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline from the American Heart Association/American Stroke Association. *Stroke* **2021**, *52*, e364–e467. [CrossRef]
18. Jneid, H.; Addison, D.; Bhatt, D.L.; Fonarow, G.C.; Gokak, S.; Grady, K.L.; Green, L.A.; Heidenreich, P.A.; Ho, P.M.; Jurgens, C.Y. 2017 AHA/ACC clinical performance and quality measures for adults with ST-elevation and non–ST-elevation myocardial infarction: A report of the American College of Cardiology/American Heart Association Task Force on Performance Measures. *Circ. Cardiovasc. Qual. Outcomes* **2017**, *10*, e000032. [CrossRef]
19. Ortel, T.L.; Neumann, I.; Ageno, W.; Beyth, R.; Clark, N.P.; Cuker, A.; Hutten, B.A.; Jaff, M.R.; Manja, V.; Schulman, S. American Society of Hematology 2020 guidelines for management of venous thromboembolism: Treatment of deep vein thrombosis and pulmonary embolism. *Blood Adv.* **2020**, *4*, 4693–4738. [CrossRef]
20. Calderone, D.; Greco, A.; Ingala, S.; Agnello, F.; Franchina, G.; Scalia, L.; Buccheri, S.; Capodanno, D. Efficacy and safety of aspirin for primary cardiovascular risk prevention in younger and older age: An updated systematic review and meta-analysis of 173,810 subjects from 21 randomized studies. *Thromb. Haemost.* **2022**, *122*, 445–455. [CrossRef]

21. Kakkar, V. Effectiveness and safety of low molecular weight heparins (LMWH) in the prevention of venous thromboembolism (VTE). *Thromb. Haemost.* **1995**, *74*, 364–368. [CrossRef]
22. Hirsh, J.; Warkentin, T.E.; Dalen, J.E.; Deykin, D.; Poller, L. Heparin: Mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest* **1995**, *108*, 258S–275S. [CrossRef]
23. Sheppard, F.R.; Macko, A.R.; Glaser, J.J.; Vernon, P.J.; Burdette, A.J.; Paredes, R.M.; Koeller, C.A.; Pusateri, A.E.; Tadaki, D.K.; Cardin, S. Nonhuman primate (rhesus macaque) models of severe pressure-targeted hemorrhagic and polytraumatic hemorrhagic shock. *Shock* **2018**, *49*, 174–186. [CrossRef]
24. Arndt, T.; Meindel, M.; Clarke, J.; Shaw, A.; Gregori, M. Comparison of Routine Hematology, Coagulation, and Clinical Chemistry Parameters of Cynomolgus Macaques of Mauritius Origin With Cynomolgus Macaques of Cambodia, China, and Vietnam Origin. *Toxicol. Pathol.* **2022**, *50*, 591–606. [CrossRef]
25. Myers Jr, D.D. Nonhuman primate models of thrombosis. *Thromb. Res.* **2012**, *129*, S65–S69. [CrossRef]
26. Aulbach, A.D.; Patrick, D.J. Nonhuman primates in preclinical research. In *Biomarkers in Toxicology*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 91–101.
27. Kean, L.S.; Singh, K.; Blazar, B.; Larsen, C. Nonhuman primate transplant models finally evolve: Detailed immunogenetic analysis creates new models and strengthens the old. *Am. J. Transplant.* **2012**, *12*, 812–819. [CrossRef]
28. Fitch, Z.; Schmitz, R.; Kwun, J.; Hering, B.; Madsen, J.; Knechtle, S.J. Transplant research in nonhuman primates to evaluate clinically relevant immune strategies in organ transplantation. *Transplant. Rev.* **2019**, *33*, 115–129. [CrossRef]
29. Doganer, O.; Wiersema, A.M.; Pierie, M.; Blankensteijn, J.D.; Yeung, K.K.; Jongkind, V. More effective anticoagulation during non-cardiac arterial procedures using activated clotting time guided heparin administration. *Ann. Vasc. Surg.* **2021**, *76*, 378–388. [CrossRef]
30. Council, N.R. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*; The National Academies Press: Washington, DC, USA, 2011; p. 246.
31. Graham, M.L.; Rieke, E.F.; Dunning, M.; Mutch, L.A.; Craig, A.M.; Zolondek, E.K.; Hering, B.J.; Schuurman, H.J.; Bianco, R.W. A novel alternative placement site and technique for totally implantable vascular access ports in non-human primates. *J. Med. Primatol.* **2009**, *38*, 204–212. [CrossRef]
32. Graham, M.L.; Rieke, E.F.; Mutch, L.A.; Zolondek, E.K.; Faig, A.W.; DuFour, T.A.; Munson, J.W.; Kittredge, J.A.; Schuurman, H.-J. Successful implementation of cooperative handling eliminates the need for restraint in a complex non-human primate disease model: Cooperative handling in a macaque disease model. *J. Med. Primatol.* **2012**, *41*, 89–106. [CrossRef]
33. Palmer, S.; Oppler, S.H.; Graham, M.L. Behavioral Management as a Coping Strategy for Managing Stressors in Primates: The Influence of Temperament and Species. *Biology* **2022**, *11*, 423. [CrossRef] [PubMed]
34. Emani, S.; Zurakowski, D.; Mulone, M.; DiNardo, J.A.; Trenor, C.C., III; Emani, S.M. Platelet testing to guide aspirin dose adjustment in pediatric patients after cardiac surgery. *J. Thorac. Cardiovasc. Surg.* **2017**, *154*, 1723–1730. [CrossRef] [PubMed]
35. Fung, L.S.; Klockau, C. Effects of age and weight-based dosing of enoxaparin on anti-factor xa levels in pediatric patients. *J. Pediatr. Pharmacol. Ther.* **2010**, *15*, 119–125. [CrossRef] [PubMed]
36. Monagle, P.; Michelson, A.D.; Bovill, E.; Andrew, M. Antithrombotic therapy in children. *Chest* **2001**, *119*, 344S–370S. [CrossRef] [PubMed]
37. Olson, H.; Betton, G.; Robinson, D.; Thomas, K.; Monro, A.; Kolaja, G.; Lilly, P.; Sanders, J.; Sipes, G.; Bracken, W. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.* **2000**, *32*, 56–67. [CrossRef] [PubMed]
38. Bogers, W.M.; Haanstra, K.G.; Verreck, F.A.; Kocken, C.H. The translational value of non-human primates in preclinical research on infection and immunopathology. *Eur. J. Pharmacol.* **2015**, *759*, 69–83.
39. Assadian, A.; Senekowitsch, C.; Assadian, O.; Eidher, U.; Hagmüller, G.; Knöbl, P. Antithrombotic strategies in vascular surgery: Evidence and practice. *Eur. J. Vasc. Endovasc. Surg.* **2005**, *29*, 516–521. [CrossRef]
40. Bakir, N.; Sluiter, W.; Ploeg, R.; Van Son, W.; Tegzess, A. Primary renal graft thrombosis. *Nephrol. Dial. Transplant.* **1996**, *11*, 140–147. [CrossRef] [PubMed]
41. Denize, J.; Defortescu, G.; Guerrot, D.; Jeannot, P.; Bertrand, D.; Cornu, J.-N.; Pfister, C.; Nouhaud, F.-X. Is intraoperative heparin during renal transplantation useful to reduce graft vascular thrombosis? *Progrès En Urol.* **2021**, *31*, 531–538. [CrossRef]
42. Gravlee, G.P.; Haddon, W.S.; Rothberger, H.K.; Mills, S.A.; Rogers, A.T.; Bean, V.E.; Buss, D.H.; Prough, D.S.; Cordell, A.R. Heparin dosing and monitoring for cardiopulmonary bypass: A comparison of techniques with measurement of subclinical plasma coagulation. *J. Thorac. Cardiovasc. Surg.* **1990**, *99*, 518–527. [CrossRef]
43. Philip, B.M.; Brock-Utne, J.G.; Lemmens, H.J.; Jaffe, R.A.; Shuttleworth, P.E. Does a delay in performing an activated clotting (ACT) test really matter? A study in nonheparinized blood and a single ACT machine. *J. ExtraCorporeal Technol.* **2008**, *40*, 193–195. [CrossRef]
44. Hattersley, P.G. Activated coagulation time of whole blood. *JAMA* **1966**, *196*, 436–440. [CrossRef] [PubMed]
45. Kratz, A.; Van Cott, E.M. Activated clotting time: Methods and clinical applications. *Point Care* **2005**, *4*, 90–94. [CrossRef]
46. Lewandrowski, E.L.; Van Cott, E.M.; Gregory, K.; Jang, I.-K.; Lewandrowski, K.B. Clinical evaluation of the i-STAT kaolin activated clotting time (ACT) test in different clinical settings in a large academic urban medical center: Comparison with the Medtronic ACT Plus. *Am. J. Clin. Pathol.* **2011**, *135*, 741–748. [CrossRef]
47. Pagana, K.; Pagana, T.; Pagana, T. *Mosby's Diagnostic & Laboratory Test Reference*, 14th ed.; Elsevier: St. Louis, MO, USA, 2019.

48. Shore-Lesserson, L. Coagulation monitoring. In *Essentials of Cardiac Anesthesia E-Book: A Volume in Essentials of Anesthesia and Critical Care*; Elsevier: Philidelphia, PA, USA, 2018; pp. 299–319.
49. Chen, Y.; Qin, S.; Ding, Y.; Wei, L.; Zhang, J.; Li, H.; Bu, H.; Lu, Y.; Cheng, J. Reference values of clinical chemistry and hematology parameters in rhesus monkeys (*Macaca mulatta*). *Xenotransplantation* **2009**, *16*, 496–501. [CrossRef] [PubMed]
50. Lee, J.I.; Shin, J.S.; Lee, J.E.; Jung, W.Y.; Lee, G.; Kim, M.S.; Park, C.G.; Kim, S.J. Reference values of hematology, chemistry, electrolytes, blood gas, coagulation time, and urinalysis in the Chinese rhesus macaques (*Macaca mulatta*). *Xenotransplantation* **2012**, *19*, 244–248. [CrossRef]
51. Li, X.; Li, D.; Biddle, K.E.; Portugal, S.S.; Li, M.R.; Santos, R.; Burkhardt, J.E.; Khan, N.K. Age-and sex-related changes in body weights and clinical pathology analytes in cynomolgus monkeys (*Macaca Fascicularis*) of Mauritius origin. *Vet. Clin. Pathol.* **2022**, *51*, 356–375. [CrossRef] [PubMed]
52. Park, H.-K.; Cho, J.-W.; Lee, B.-S.; Park, H.; Han, J.-S.; Yang, M.-J.; Im, W.-J.; Park, D.-Y.; Kim, W.-J.; Han, S.-C. Reference values of clinical pathology parameters in cynomolgus monkeys (*Macaca fascicularis*) used in preclinical studies. *Lab. Anim. Res.* **2016**, *32*, 79–86. [CrossRef]
53. Bussey, H.; Francis, J.L.; Heparin Consensus Group. Heparin overview and issues. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* **2004**, *24*, 103S–107S. [CrossRef]
54. Hirsh, J.; Warkentin, T.E.; Shaughnessy, S.G.; Anand, S.S.; Halperin, J.L.; Raschke, R.; Granger, C.; Ohman, E.M.; Dalen, J.E. Heparin and low-molecular-weight heparin mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest* **2001**, *119*, 64S–94S.
55. The CREATE Trial Group Investigators. Effects of reviparin, a low-molecular-weight heparin, on mortality, reinfarction, and strokes in patients with acute myocardial infarction presenting with ST-segment elevation. *JAMA* **2005**, *293*, 427–436. [CrossRef] [PubMed]
56. Eikelboom, J.; Hirsh, J. Combined antiplatelet and anticoagulant therapy: Clinical benefits and risks. *J. Thromb. Haemost.* **2007**, *5*, 255–263. [CrossRef] [PubMed]
57. Wallentin, L.; Ohlsson, J. Low-molecular-weight heparin during instability in coronary artery disease. *Lancet* **1996**, *347*, 561–568.