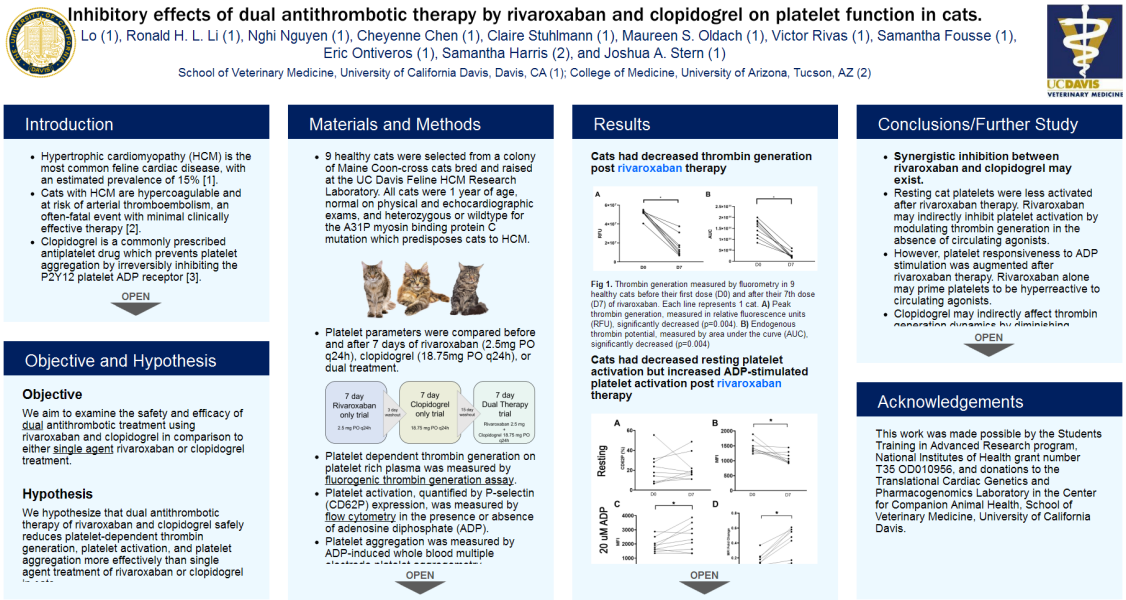
Inhibitory effects of dual antithrombotic therapy by rivaroxaban and clopidogrel on platelet function in cats.



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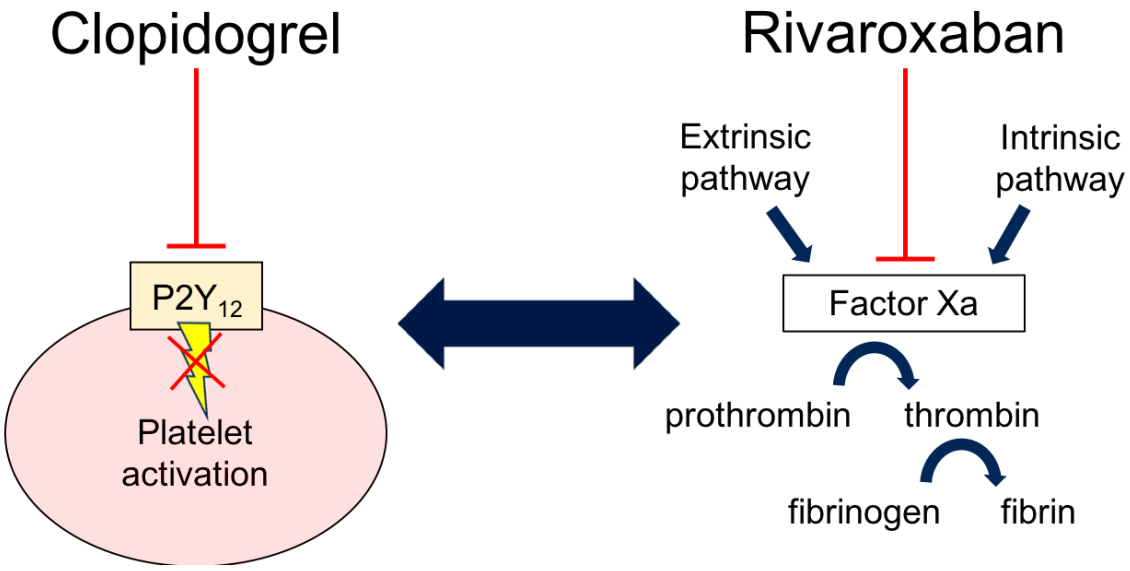
INTRODUCTION

- Hypertrophic cardiomyopathy (HCM) is the most common feline cardiac disease, with an estimated prevalence of 15% [1].
- Cats with HCM are hypercoagulable and at risk of arterial thromboembolism, an often-fatal event with minimal clinically effective therapy [2].
- Clopidogrel is a commonly prescribed antiplatelet drug which prevents platelet aggregation by irreversibly inhibiting the P2Y12 platelet ADP receptor [3].
- Rivaroxaban is a newer anticoagulant drug that inhibits activated factor X, preventing thrombin generation [4].



- Dual antithrombotic therapy consisting of rivaroxaban and clopidogrel has been shown to be superior to single-agent therapy in humans with acute cardiovascular events [5].
- Little is known about the synergistic inhibitory effects of rivaroxaban and clopidogrel on platelet function in cats.

OBJECTIVE AND HYPOTHESIS

Objective

We aim to examine the safety and efficacy of dual antithrombotic treatment using rivaroxaban and clopidogrel in comparison to either single agent rivaroxaban or clopidogrel treatment.

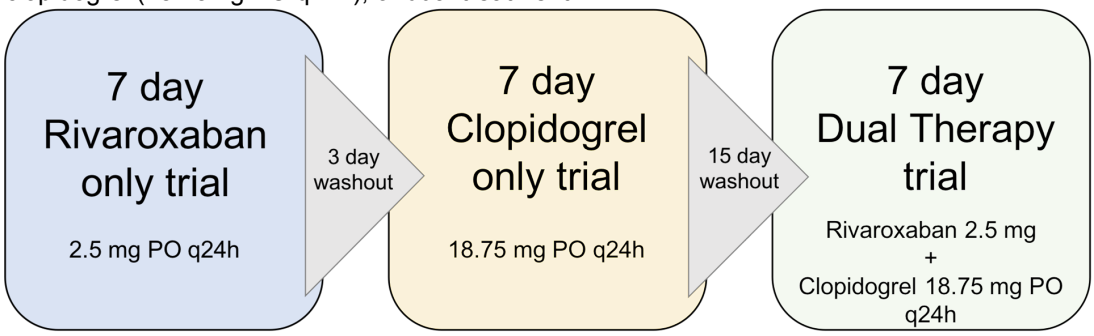
Hypothesis

We hypothesize that dual antithrombotic therapy of rivaroxaban and clopidogrel safely reduces platelet-dependent thrombin generation, platelet activation, and platelet aggregation more effectively than single agent treatment of rivaroxaban or clopidogrel in cats.

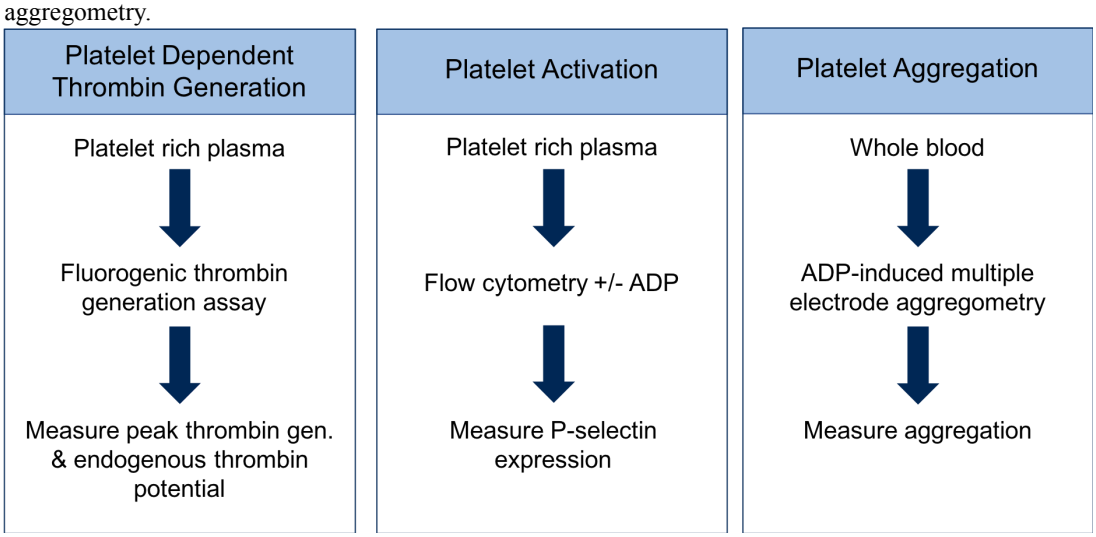
MATERIALS AND METHODS

- 9 healthy cats were selected from a colony of Maine Coon-cross cats bred and raised at the UC Davis Feline HCM Research Laboratory. All cats were 1 year of age, normal on physical and echocardiographic exams, and heterozygous or wildtype for the A31P myosin binding protein C mutation which predisposes cats to HCM.

- Platelet parameters were compared before and after 7 days of rivaroxaban (2.5mg PO q24h), clopidogrel (18.75mg PO q24h), or dual treatment.



- Platelet dependent thrombin generation on platelet rich plasma was measured by fluorogenic thrombin generation assay.
- Platelet activation, quantified by P-selectin (CD62P) expression, was measured by flow cytometry in the presence or absence of adenosine diphosphate (ADP).
- Platelet aggregation was measured by ADP-induced whole blood multiple electrode platelet aggregometry.



RESULTS

Cats had decreased thrombin generation post rivaroxaban therapy

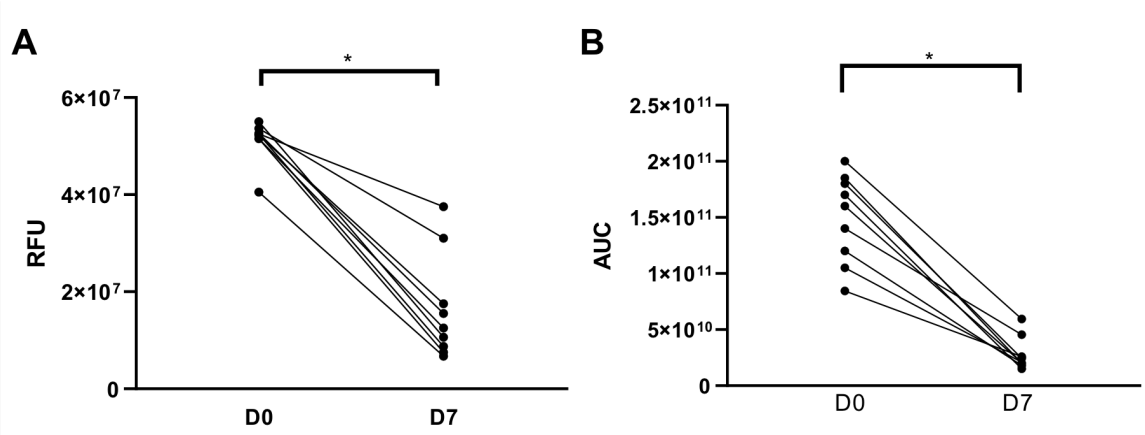


Fig 1. Thrombin generation measured by fluorometry in 9 healthy cats before their first dose (D0) and after their 7th dose (D7) of rivaroxaban. Each line represents 1 cat. **A)** Peak thrombin generation, measured in relative fluorescence units (RFU), significantly decreased (p=0.004). **B)** Endogenous thrombin potential, measured by area under the curve (AUC), significantly decreased (p=0.004)

Cats had decreased resting platelet activation but increased ADP-stimulated platelet activation post rivaroxaban therapy

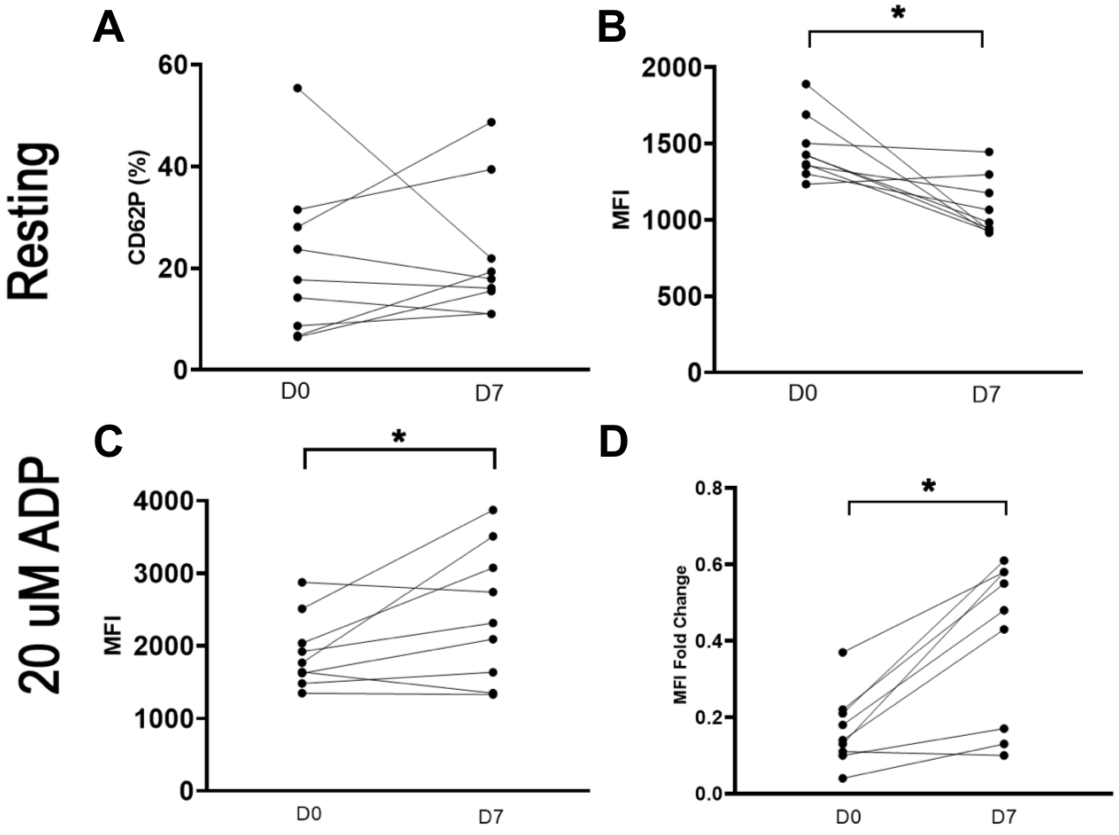


Fig 2. Platelet activation, expressed as P-selectin expression, measured by flow cytometry in the absence (resting) or presence of 20 uM ADP. Each line represents 1 of 9 healthy cats before their first dose (D0) and after their 7th dose (D7) of rivaroxaban. **A)** Percentage of platelets expressing CD62P (P-selectin) was not affected. **B)** P-selectin expression in resting platelets, measured in mean fluorescence units (MFI), significantly decreased (p=0.008). **C)** P-selectin expression in ADP-stimulated platelets, measured in MFI, significantly increased (p=0.05). **D)** Platelet responsiveness to ADP, measured by MFI fold change, significantly increased (p=0.002)

Cats had decreased platelet activation post clopidogrel therapy

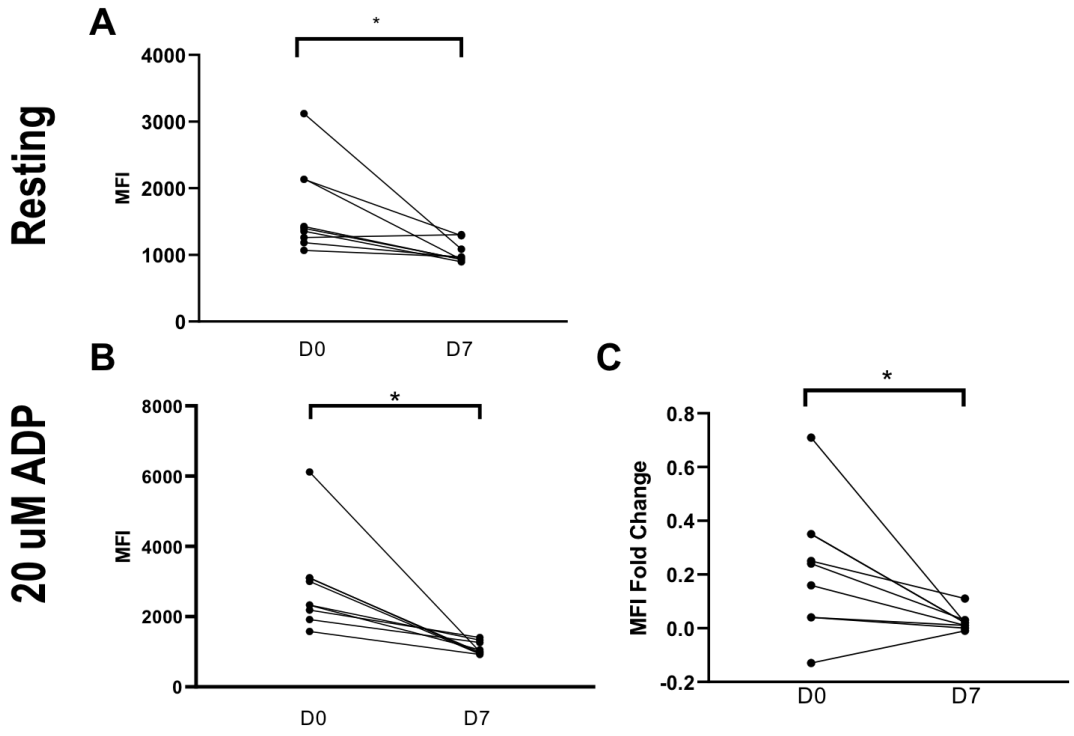


Fig 3. Platelet activation, expressed as P-selectin expression, measured by flow cytometry in the absence (resting) or presence of 20 uM ADP. Each line represents 1 of 9 healthy cats before their first dose (D0) and after their 7th dose (D7) of clopidogrel. **A)** P-selectin expression in resting platelets, measured in MFI, significantly decreased (p=0.017). **B)** P-selectin expression in ADP-stimulated platelets, measured in MFI, significantly decreased (p=0.004). **C)** Platelet responsiveness to ADP, measured by MFI fold change, significantly decreased (p=0.002)

Thrombin generation dynamics in cats were affected by clopidogrel

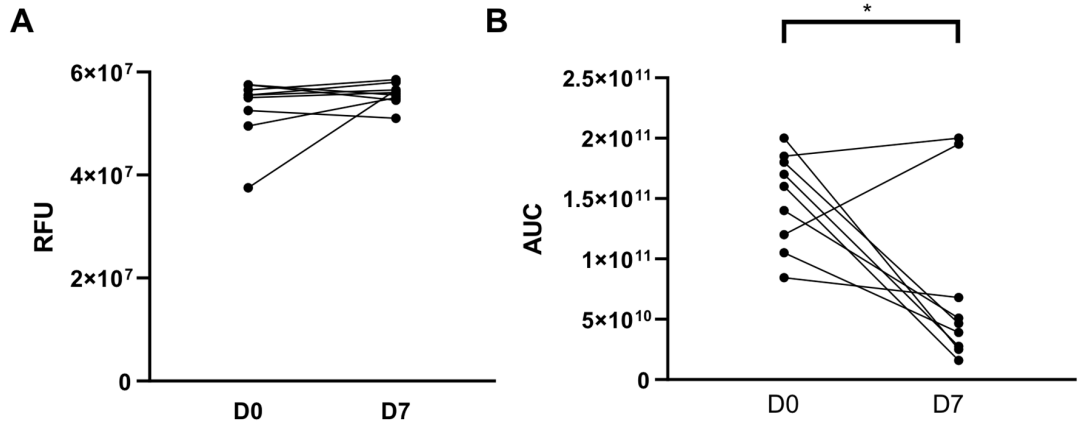


Fig 4. Thrombin generation measured by fluorometry in 9 healthy cats before their first dose (D0) and after their 7th dose (D7) of clopidogrel. Each line represents 1 cat. **A)** Peak thrombin generation, measured in relative fluorescence units (RFU), was not affected. **B)** Endogenous thrombin potential, measured by area under the curve (AUC), significantly decreased (p=0.03)

CONCLUSIONS/FURTHER STUDY

- **Synergistic inhibition between rivaroxaban and clopidogrel may exist.**
- Resting cat platelets were less activated after rivaroxaban therapy. Rivaroxaban may indirectly inhibit platelet activation by modulating thrombin generation in the absence of circulating agonists.
- However, platelet responsiveness to ADP stimulation was augmented after rivaroxaban therapy. Rivaroxaban alone may prime platelets to be hyperreactive to circulating agonists.
- Clopidogrel may indirectly affect thrombin generation dynamics by diminishing endogenous thrombin potential.
- Future directions include ongoing dual therapy trial data generation and a retrospective study of client-owned cats receiving rivaroxaban and clopidogrel at the UC Davis Veterinary Medical Teaching Hospital.

ACKNOWLEDGEMENTS

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is the most common feline cardiac disease. Cats with HCM are at risk of arterial thromboembolism, an often-fatal event with poor prognosis. Despite this outcome, little is known about the synergistic inhibitory effects of rivaroxaban and clopidogrel on platelet function. We aim to examine the safety and efficacy of dual antithrombotic treatment using rivaroxaban and clopidogrel in comparison to either rivaroxaban or clopidogrel treatment. We hypothesize that dual treatment safely reduces platelet-dependent thrombin generation, platelet activation, and platelet aggregation more effectively than single agent treatment of rivaroxaban or clopidogrel in cats. Platelet parameters were compared before and after 7 days of rivaroxaban (2.5mg PO q24h), clopidogrel (18.75mg PO q24h), or dual treatment. Thrombin generation on platelet rich plasma was measured by fluorogenic thrombin generation assay. Platelet activation, quantified by P-selectin expression, was measured by flow cytometry in the presence or absence of adenosine diphosphate (ADP). Platelet aggregation was measured by ADP-induced whole blood platelet aggregometry. Preliminary data showed no adverse events in cats receiving single agent rivaroxaban therapy. Rivaroxaban treatment lowered P-selectin expression in unstimulated platelets (Mean Fluorescence: 1157 ±197 vs 821 ±126, p=0.004) but augmented platelet responsiveness to ADP (Fold Change: 0.17 ± 0.095 vs. 0.40 ±0.21, p=0.002). Rivaroxaban prolonged (1683 s ±811 vs 3588 s ±812, p=0.01) and diminished (RFU: 5.13x107 ±4.20 vs 1.64 x107 ±1.09, p=0.004) thrombin generation. Rivaroxaban did not alter ADP-induced platelet aggregation. These findings suggest rivaroxcaban may indirectly inhibit platelet activation by modulating thrombin generation. Dual therapy data generation is ongoing.

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