MECHANISMS OF THROMBOGENESIS
IN ATRIAL FIBRILLATION

Han Sung Lim
MBBS, FRACP

Department of Cardiology
Royal Adelaide Hospital
and
Discipline of Physiology
The University of Adelaide

A thesis submitted to The University of Adelaide in
fulfillment of the requirements of the degree of

Doctor of Philosophy

December 2011
To my wife Lufee

and our daughter Gloria
TABLE OF CONTENTS

ABSTRACT ........................................................................................................................................... XII

DECLARATION ..................................................................................................................................... XV

ACKNOWLEDGEMENTS ................................................................................................................... XVI

PUBLICATIONS AND COMMUNICATIONS TO LEARNED SOCIETIES ........................................ XVIII

PRIZES AND AWARDS DURING CANDIDATURE ......................................................................... XXIV

CHAPTER ONE

LITERATURE REVIEW

1.1 INTRODUCTION ............................................................................................................................. 1

1.1.1 Epidemiology of Atrial Fibrillation ........................................................................................... 1

1.1.1.1 Incidence and Prevalence ...................................................................................................... 1

1.1.1.2 Predisposing Conditions ..................................................................................................... 2

1.1.2 Epidemiology of Stroke ........................................................................................................... 4

1.1.3 Atrial Fibrillation and Stroke .................................................................................................. 5

1.1.4 Emerging Epidemic and Health Burden .................................................................................. 7

1.2 MECHANISMS OF THROMBOGENESIS IN ATRIAL FIBRILLATION ..................................... 8

1.2.1 Abnormal Blood Flow .............................................................................................................. 9

1.2.1.1 Blood Stasis ......................................................................................................................... 9

1.2.1.2 Atrial Mechanical Remodeling ............................................................................................ 11

1.2.2 Abnormal Blood Constituents .................................................................................................. 14

1.2.2.1 Platelets ............................................................................................................................... 14
1.2.2.2 Coagulation Cascade.................................................................18
1.2.3 Abnormal Vessel Wall...............................................................20
1.2.3.1 Endothelial Dysfunction.........................................................20
1.2.3.2 Structural Remodeling.........................................................23
1.2.3.3 Inflammation.................................................................25
1.3 INTRACARDIAC VERSUS PERIPHERAL SAMPLING............................28
1.4 RATE VERSUS RHYTHM CONTROL IN RELATION TO STROKE AND CLINICAL OUTCOMES.................................................................29
1.4.1 Evidence from Clinical Trials..................................................30
1.4.2 Limitations of Existing Clinical Trials......................................32
1.5 CATHETER ABLATION FOR ATRIAL FIBRILLATION..........................34
1.5.1 Techniques for Ablation and Clinical Outcome........................34
1.5.2 Complications Related to Atrial Fibrillation Ablation.................36
1.5.3 Catheter Ablation and Stroke Outcome....................................36
1.5.3.1 Peri-procedural Stroke.....................................................36
1.5.3.2 Long Term Stroke Outcome.............................................37
1.6 EXISTING THERAPEUTIC OPTIONS FOR STROKE PREVENTION IN ATRIAL FIBRILLATION.................................................................39
1.6.1 Antiplatelet Therapy............................................................39
1.6.1.1 Aspirin.................................................................39
1.6.1.2 Aspirin and Clopidogrel..................................................40
1.6.2 Anticoagulation Therapy..........................................................40
1.6.2.1 Vitamin K Antagonist Therapy (Warfarin)..........................40
1.6.2.2 Limitations of Vitamin K Antagonist Therapy.................................42

1.7 NEWER ANTITHROMBOTIC THERAPIES FOR STROKE PREVENTION.........43

1.7.1 Pharmacological Antithrombotic Therapies........................................43

1.7.1.1 Direct Thrombin Inhibitors............................................................43

1.7.1.2 Factor Xa Inhibitors.....................................................................46

1.7.2 Novel Non-Pharmacological Therapy...............................................48

CHAPTER TWO

PLATELET ACTIVATION AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH ATRIAL FIBRILLATION: IMPORTANCE OF CO-MORBID CONDITIONS

2.1 OVERVIEW...............................................................................................50

2.2 INTRODUCTION........................................................................................51

2.3 METHODS................................................................................................52

2.3.1 Study population................................................................................52

2.3.2 Definitions............................................................................................53

2.3.3 Electrophysiology Study and Ablation..................................................54

2.3.4 Study Protocol.......................................................................................54

2.3.5 Statistical analysis................................................................................56

2.4 RESULTS..................................................................................................57

2.4.1 Patient Characteristics..........................................................................57

2.4.2 Platelet Activation................................................................................58

2.4.3 Endothelial Dysfunction........................................................................58

2.5 DISCUSSION..............................................................................................59
CHAPTER THREE

THROMBOGENESIS IN THE HUMAN LEFT ATRIUM IN PATIENTS WITH ATRIAL FIBRILLATION: IMPACT OF ATRIAL RATES AND ATRIO-VENTRICULAR DYSSYNCHRONONY

3.1 OVERVIEW .................................................................71
3.2 INTRODUCTION ..........................................................72
3.3 METHODS .................................................................74
  3.3.1 Study Population .....................................................74
  3.3.2 Electrophysiology Study and Ablation ................................75
  3.3.3 Study Protocol .......................................................75
  3.3.4 Blood Sampling and Analysis .......................................76
  3.3.5 Statistical Analysis ..................................................77

3.4 RESULTS ......................................................................78
  3.4.1 Patient Characteristics ............................................78
  3.4.2 Thrombin Generation ...............................................79
  3.4.3 Platelet Activation ..................................................80
  3.4.4 Endothelial Dysfunction ..........................................80
CHAPTER FOUR
EFFECT OF ATRIAL FIBRILLATION ON ATRIAL THROMBOGENESIS IN HUMANS:
IMPACT OF RATE AND RHYTHM

4.1 OVERVIEW .................................................................................................................. 102

4.2 INTRODUCTION ............................................................................................................. 103

4.3 METHODS ..................................................................................................................... 104

4.3.1 Study Population .................................................................................................... 104

4.3.2 Electrophysiology Study and Ablation .................................................................. 105

4.3.3 Study Protocol ....................................................................................................... 106

4.3.4 Blood Analysis ....................................................................................................... 107
4.3.5 Statistical Analysis

4.4 RESULTS

4.4.1 Patient Characteristics

4.4.2 Platelet Activation

4.4.3 Thrombin Generation

4.4.4 Endothelial Dysfunction

4.4.5 Platelet-derived Inflammation

4.4.6 AF versus Pacing

4.5 DISCUSSION

4.5.1 Left Atrial Platelet Activation with AF and Pacing

4.5.2 Left Atrial Thrombin Generation with AF and Pacing

4.5.3 Endothelial Dysfunction with AF Induction

4.5.4 Inflammation with AF Induction

4.5.5 Mechanisms of Left Atrial Thrombogenesis in AF: the Fulfilment of Virchow’s Triad

4.5.6 Mechanisms of Thrombogenesis in Atrial Flutter

4.5.7 Clinical Implications

4.6 STUDY LIMITATIONS

4.7 CONCLUSIONS

Table

Figures
CHAPTER FIVE

TIME COURSE OF INFLAMMATION, MYOCARDIAL INJURY AND PROTHROMBOTIC RESPONSE FOLLOWING RADIOFREQUENCY CATHETER ABLATION FOR ATRIAL FIBRILLATION

5.1 OVERVIEW.......................................................................................................................133

5.2 INTRODUCTION...............................................................................................................134

5.3 METHODS........................................................................................................................136

5.3.1 Patient Selection..........................................................................................................136

5.3.2 Peri-procedural Care....................................................................................................136

5.3.3 Ablation Procedure......................................................................................................137

5.3.4 Blood Collection.........................................................................................................138

5.3.5 Markers of Inflammation, Myocardial Injury and Thrombosis.................................138

5.3.6 Statistical Analysis......................................................................................................139

5.4 RESULTS.........................................................................................................................140

5.4.1 Patient and Procedural Characteristics........................................................................140

5.4.2 Time Course of Inflammation, Myocardial Injury and Prothrombotic Markers........140

5.4.3 Correlation between Inflammatory, Myocardial Injury and Prothrombotic Markers .................................................................................................................................................. 141

5.4.4 Predictors of Rise for Inflammatory, Myocardial Injury and Prothrombotic Markers .................................................................................................................................................. 141

5.4.5 AF Recurrence............................................................................................................ 142

5.5 DISCUSSION..................................................................................................................... 143
5.5.1 Main Findings.................................................................143
5.5.2 Inflammation Post RF ablation for AF.................................143
5.5.3 Myocardial injury post RF ablation for AF..............................144
5.5.4 Inflammation and AF recurrence..........................................145
5.5.5 Thrombotic Risk Post Ablation..............................................146
5.5.6 Clinical Implications.........................................................147
5.6 STUDY LIMITATIONS..........................................................148
5.7 CONCLUSIONS.................................................................148

Tables..........................................................................................150
Figures..........................................................................................155

CHAPTER SIX
SUCCESSFUL CATHETER ABLATION AND MAINTENANCE OF SINUS RHYTHM DECREASES PLATELET ACTIVATION AND IMPROVES ENDOTHELIAL FUNCTION IN PATIENTS WITH ATRIAL FIBRILLATION
6.1 OVERVIEW..............................................................................166
6.2 INTRODUCTION......................................................................167
6.3 METHODS...............................................................................168
6.3.1 Study Population.................................................................168
6.3.2 Catheter Ablation Procedure.................................................169
6.3.3 Post Ablation Follow-up.......................................................170
6.3.4 Blood Sampling and Analysis...............................................171
6.3.5 Statistical Analysis.............................................................172
6.4 RESULTS.......................................................................................................................173

6.4.1 Baseline Patient and Procedural Characteristics.................................................173

6.4.2 Platelet Activation Following Catheter Ablation................................................174

6.4.3 Endothelial Function Following Catheter Ablation.............................................174

6.5 DISCUSSION.............................................................................................................175

6.5.1 Platelet Activation after Successful Reversion to Sinus Rhythm.......................175

6.5.2 Endothelial Function after Successful Reversion to Sinus Rhythm.....................177

6.6 STUDY LIMITATIONS..............................................................................................179

6.7 CONCLUSIONS......................................................................................................179

Table.............................................................................................................................180

Figures..........................................................................................................................183

CHAPTER SEVEN

SUMMARY.......................................................................................................................186

CHAPTER EIGHT

FUTURE DIRECTIONS......................................................................................................191

CHAPTER NINE

REFERENCES...................................................................................................................194
ABSTRACT

Atrial fibrillation (AF) is the commonest sustained heart rhythm disorder in clinical practice. Non-valvular AF confers a 5-fold increased risk of stroke. Stroke in AF is mainly due to thromboembolic phenomenon from the left atrium (LA). It is well known that atrial mechanical dysfunction contributes to thrombus formation. However, patients with AF are also known to exhibit a prothrombotic state and endothelial dysfunction, further contributing to this thromboembolic risk.

There is debate as to whether the prothrombotic state and endothelial dysfunction seen in patients with AF are due to AF per se or the patients’ concurrent comorbidities. Chapter 2 examined the LA milieu in patients with lone non-valvular AF compared to patients with AF and comorbidities and controls. The study demonstrated increased platelet activation in the LA compared to the periphery in patients with lone AF. There was a step-wise increase in endothelial dysfunction in the lone AF cohort and AF with comorbidities compared to controls, indicating that both AF per se and its concurrent comorbidities contribute to endothelial dysfunction and thrombotic risk.

Chapter 3 investigated the effect of rapid atrial rates in patients with AF compared to patients with supraventricular tachycardia. The study demonstrated rapid atrial rates increased LA platelet activation and thrombin generation in patients with AF. Left atrial thrombogenesis was markedly accentuated with atrio-ventricular dyssynchrony.
In contrast, rapid atrial rates did not result in abnormal changes in patients with supraventricular tachycardia. These findings suggest rapid atrial rates, atrio-ventricular dyssynchrony and the abnormal substrate in patients with AF contribute to LA thrombogenesis in these patients.

The relative contribution of the atrial rate or rhythm to LA thrombogenesis is unknown. Chapter 4 examined the effects of atrial rate and abnormal rhythm on LA thrombogenesis and demonstrated both rapid atrial rates and AF result in increased platelet activation and thrombin generation in the LA. However, AF also induced endothelial dysfunction and inflammation, not seen with rapid atrial rates alone. These findings suggest that while rapid atrial rates increase the thrombogenic risk, abnormal rhythm may further potentiate this risk.

Catheter ablation therapy has emerged as an effective strategy for rhythm control in patients with AF. However, radiofrequency ablation is known to cause an increase in various markers of inflammation and patients are at risk of peri-procedural thromboembolic events. Chapter 5 examined inflammatory, myocardial injury and prothrombotic markers in AF patients undergoing catheter ablation during the peri-procedural period. The study demonstrated that patients exhibit an inflammatory response within the first few days post-ablation, and that this response predicted immediate AF recurrence. Prothrombotic markers were elevated one week post-ablation and may contribute to the increased peri-procedural thrombotic risk.
Whether catheter ablation for AF confers a benefit on prevention of future thromboembolic stroke is a vital question. Chapter 6 demonstrated that successful catheter ablation and maintenance of sinus rhythm leads to a decrease in platelet activation and improvement in endothelial function. These findings suggest that the prothrombotic state in patients with AF can be reduced with successful maintenance of sinus rhythm following catheter ablation.
DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Han Lim and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

Han Sung Lim

December 2011


ACKNOWLEDGEMENTS

I would like to thank Professor Prashanthan Sanders, my primary supervisor, for his mentorship and support during these doctoral years. His passion and pursuit of excellence in both clinical care and academia is a personal inspiration. I am also thankful to my co-supervisors, Dr Scott Willoughby, for his guidance and encouragement throughout this entire period, and Dr Matthew Worthley for his support and advice. I am grateful for the support received from the Medical Postgraduate Scholarship from the National Health and Medical Research Council of Australia, which was a timely encouragement, and the Divisional Postgraduate Scholarship and Earl Bakken Scholarship from the University of Adelaide. I am also indebted to the patients who volunteered their additional time for the purpose of research. Seeing their quality of life improve through the course of follow up was an added inspiration to the research work.

The clinical electrophysiology training at the Royal Adelaide Hospital with Drs Glenn Young, Kurt Roberts-Thomson and Richard Hillock was exceedingly rewarding and I admire their dedication to teaching and knowledge of their chosen field. I enjoyed working and sharing the journey with co-fellows Drs Dennis Lau, Muayad Alasady, Darryl Leong, Gautam Sharma, Narayanan Namboodiri, Hany Abed, Rajiv Mahajan, Sachin Nayyar and Anand Ganesan. Their diverse backgrounds have tremendously enriched the fellowship experience. Special thanks go to Ms Carlee Schultz for her ready assistance with the many concurrent projects and Ms Cheryl Gan for her support.
invaluable contribution in the area of enzyme linked immunosorbent assays. Additional thanks go to Mr Jerry Dang and Dr Adhiraj Chakrabarty for their assistance in the research experiments. My sincere appreciation goes to Mr Tom Sullivan and Dr Anthony Brooks for statistical support, and Professor Karlheinz Peter for his help in the initial setting up of flow cytometry testing. I am also grateful to the staff of the Cardiovascular Investigation Unit, Royal Adelaide Hospital and Centre for Heart Rhythm Disorders, University of Adelaide, in particular Ms Judith Hunt, Ms Lauren Wilson, Ms Melissa Middeldorp and Ms Aimie Paukner, whose professional yet friendly attitudes have made the research all the more enjoyable.

A special thanks to my parents Ban Beng and Chang Mee Lim, whose unyielding love and support have been a constant encouragement. Mum’s pursuit of academia and intellectual interests has always been an inspiration to me. I would also like to thank my parents-in-law Kin Siong and Lee Hung Wong who offered enormous support during Lufee’s pregnancy. I treasure the special bond with my brother Chris, Ee Lyn and James, Annie, Trevor, Noah and Jacob throughout this period.

Most importantly, I would like to thank my beloved wife Lufee whom this thesis is dedicated. This PhD journey encompasses our miraculous meeting in Adelaide, courtship, marriage and beginning of a new family, and would not be possible without her constant support and loving understanding. I am sure we will remember fondly our time in Adelaide and the friends we made here. Above all, thanks to God, who has proven Himself time and time again to be faithful and sovereign in all circumstances.
Chapter One


Chapter Two


Chapter Three

in the Human Left Atrium in Patients with Atrial Fibrillation: Impact of Atrial Rates and Atrio-Ventricular Dyssynchrony. Submitted for publication.


4. Presentation: Presented at the 3rd Asia Pacific Heart Rhythm Society Scientific Sessions, Jeju Island, Korea, October 2010 and published in abstract form (Journal of Arrhythmia 2010; 26:1)

**Chapter Four**


XX
2. Presentation: Presented at the American Heart Association Scientific Sessions 2010 in Chicago, United States of America and published in abstract form (Circulation 2010)


7. Presentation: Presented at the Medical Grand Round, Royal Adelaide Hospital, 2010. Finalist for the 2010 Nimmo Prize for Full-Time Research
Chapter Five


Chapter Six


PRIZES AND AWARDS DURING CANDIDATURE

1. National Health and Medical Research Council of Australia Medical Postgraduate Scholarship, 2009-2011

2. Cardiac Society of Australia and New Zealand Travel Award, 2010

3. Nimmo Prize Finalist for Full-time Research, Royal Adelaide Hospital, 2010

4. National Heart Foundation of Australia (South Australia) EO Myers Trust Fund Travel Award, 2010

5. Postgraduate Travel Fellowship Award, Faculty of Health Sciences, The University of Adelaide, 2010

6. National Heart Foundation of Australia (South Australia) EO Myers Trust Fund Travel Award, 2009

7. Faculty of Sciences Divisional Scholarship, Postgraduate Scholarship, The University of Adelaide, 2008

8. Biotronik Educational Grant for Asia Pacific Heart Rhythm Society Conference Registration, 2011

9. National Heart Foundation of Australia Travel Award, 2011