

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47352

MODULATION OF ERYPTOSIS IN THALASSEMIC RED BLOOD CELLS

NANGNOI JERMNIM

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of science Degree in Biomedical sciences

April 2012

Copyright 2011 by Naresuan University

600254891

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



E47352

MODULATION OF ERYPTOSIS IN THALASSEMIC RED BLOOD CELLS



NANGNOI JERMNIM


**A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of science Degree in Biomedical sciences**


April 2012

Copyright 2011 by Naresuan University

This thesis entitled “Modulation of eryptosis in thalassemic red blood cells” submitted by Nangnoi Jermnim in partial fulfillment of the requirements for the Master of Science Degree in Biomedical Sciences is hereby approved.



..... Chair
(Assistant Professor Surapon Tangvarasittichai, Ph.D.)


..... Committee
(Associate Professor Orathai Tangvarasittichai, Ph.D.)


..... Committee
(Daniel Sagan, Ph. D.)


..... Committee
(Assistant Professor Tanett Pongtheerat, Ph.D.)

Approved


.....
(Assistant Professor Kanungnit Phupatwibol, Ph.D.)

Dean of the Graduate School

25 April 2012

ACKNOWLEDGEMENT

I would like to express the extensive support and assistance from my major advisor, Associate Professor Dr.Orathai Tangvarasittichai. I wish to express my deep appreciation and sincere gratitude for her kindness, valuable suggestion, and constant encouragement throughout this research.

I would like to thank my co-advisor, Dr.Daniel Sagan, and Assistant Professor Dr.Surapon Tangvarasittichai for their valuable supervision, guidance and proofreading of the thesis. Without their continuous encouragements this thesis would not be possible. I also would like to thank them for giving me the opportunity to learn new things and the best experience in life.

I would like to thank all staffs at the Thalassemia Research Unit, Naresuan University Hospital who donated their time and samples to incorporate in this study and whom I have had the good fortune to interact. Thanks for their kindness, valuable teaching and encouragement.

I would like to acknowledge the Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University for their support to this study.

Finally, sincere appreciation is expressed to my family for their infinite love, understanding, encouragement throughout the entire period of my study and support in all achievement. Especially,my mother who is the wonderful mom and the most important person in my life.

Nangnoi Jermnim

Title	MODULATION OF ERYPTOSIS IN THALASSEMIC RED BLOOD CELLS
Author	Nangnoi Jermnim
Advisor	Associate Professor Orathai Tangvarasittichai, Ph.D.
Co - Advisor	Daniel Sagan, Ph.D.
Academic Paper	Thesis M.S. in Biomedical Sciences, Naresuan University, 2011
Keywords	CD95, apoptosis, eryptosis

ABSTRACT

E47352

Eryptosis, a form of programmed cell death in red blood cells, is triggered by oxidative stress or energy depletion. Apotent inducer of apoptosis in nucleated cells is the CD95 pathway and expressed in red blood cells. The function of this death receptor in red blood cells is not well documented. Red blood cell is an anucleated cells can undergo cell death via eryptosis, which similarites to apoptosis in nucleated cells. Modulation of mechanisms regulating energy supply or oxidative stress impacts eryptotic behavior of red blood cells. In thalassemic red blood cells enhanced eryptosis causes anemia. Insulin enhances the flux of glucose by stimulating glycolysis to produce ATP and NADH in human erythrocytes. Thalassemic red blood cells are prone to undergo eryptosis leading to anemia. This study has two objectives for modulation of eryptosis in thalassemic red blood cells. First objective is activations of the death receptor CD95 on red blood cell by CH11 antibody for induction of phosphatidylserine (PS) exposure. Second objective is activation of insulin receptor that the stimulatory effect of insulin leads to a protection of oxidative-stress-induced eryptosis, especially in red blood cells of thalassemic donors. Oxidative stress in beta-thalassemic red blood cells was induced by (*tert*-butylhydroperoxide, tBOOH) with or without supplementing the media with insulin. Phosphatidylserine exposure, measured as annexin V-binding, was used as a marker for eryptosis in beta- thalassemia blood cells. The death receptor CD95 on red blood cells treated with CH11 antibody that no significantly increased PS-positive cells. Insulin significantly decreased (P -value<0.05) the percentage of phosphatidylserine exposing red blood cells in both, normal and thalassemic red blood cells. In conclusion, the death receptor CD95 on red blood cells is not functional. Insulin can decrease PS-positive cells of both normal and thalassemic red blood cells in oxidative stress lead to exposure of phosphatidylserine.

LIST OF CONTENTS

Chapter	Page
I INTRODUCTION.....	1
Objective of the study	3
Scope of the study.....	3
Benefit of the study.....	3
II REVIEW OF RELATED LITERATURE AND RESEARCH.....	4
Apoptosis.....	4
Apoptosis in mature erythrocytes called “eryptosis”.....	8
Thalassemias.....	10
CD95-mediated role for eryptosis.....	12
Influence of insulin on eryptosis.....	13
III RESEARCH METHODOLOGY.....	14
Materials.....	14
Methods.....	16
IV RESULTS AND DISCUSSION.....	19
A functional impact of CD95 on the induction of eryptosis.....	19
Influence of insulin to reduce eryptosis.....	21
Effects of the red blood cells with tBOOH and H ₂ O ₂ to determine a suitable concentration range for induction of red blood cells eryptosis.....	23
Investigation of eryptotic cells on oxidative stress of normal and thalassemic red blood cells.....	24

LIST OF CONTENTS (CONT.)

Chapter	Page
V CONCLUSION.....	31
Conclusion.....	31
REFERENCES.....	32
APPENDIX.....	39
BIOGRAPHY.....	41

LIST OF TABLES

Table	Page
1 List of chemicals equipments and plastic wares.....	14
2 List of instrument used in the studies.....	15
3 The effect of CH11 on the function of death receptor CD95 red blood cells	19
4 Lactate concentration was determined using the automated chemistry analyzer by Hitachi 912, Roche.....	22
5 Lactate concentration with 10 mM insulin in red blood cells.....	22
6 Percentage of eryptotic cells various concentration of tBOOH The data demonstrated the percentage of eryptotic cells from three experiment of Normal, Beta thalassemia/HbE and Hb H disease red blood cells.....	25
7 Mean ± S.E. of percentage of normal eryptotic cells of normal and thalassemic group in oxidative stress.....	26

LIST OF FIGURES

Figure	Page
1 Receptor-mediated caspase activation at the DISC.....	5
2 Apoptosis signaling pathway.....	6
3 Intrinsic apoptotic pathway.....	8
4 CD 95 ligand-induced apoptosis is independent of CD95 expression.....	9
5 Effects of insulin on erythrocytes.....	13
6 BD FACSCalibur Flow Cytometer; BDBiosciences, Heidel berg, Germany.....	18
7 Histogram of the effect of CH11 on the function of death receptor CD95 red blood cells. Red blood cells were treated with increasing concentrations of CH11 to stimulation of eryptotic cells..	20
8 Positive control of activity CH11for stimulation of apoptosis in Jurkat Cells and red blood cells with ionomycin	21
9 Histogram of effects of the red blood cells with tBOOH and H ₂ O ₂ to determine a suitable concentration range for induction of red blood cells eryptosis.....	23
10 Eryptotic cells of normal and thalassemic group in oxidative stress absence and presence insulin.....	27
11 Histogram of the percentage at each conditions of PS positive normal red blood cells in oxidative stress.....	28
12 Histogram of the percentage at each condition of PS positive Betathal/HbE red blood cells in oxidative stress.....	29
13 Histogram of the percentage at each conditions of PS positive Hb H red blood cells in oxidative stress.....	30

ABBREVIATIONS

AIF	=	Apoptosis-inducing factor
ATP	=	Adenosine triphosphate
BAK	=	Bcl-2 homologous antagonist killer
BAX	=	Bcl-2-associated X protein
Bcl-2	=	B-cell leukemia/lymphoma 2
Bcl-X _L	=	B-cell lymphoma-extra large
BID	=	BH3 interacting domain death agonist
c-FLIC	=	Caspase FLICE-like inhibitory protein
CH11	=	CD95/FAS agonistic antibody
DISC	=	Death-inducing signaling complex
FACS	=	Fluorescence activated cell sorter
FADD	=	Fas-Associated protein with Death Domain
FCS	=	Fetal Calf Serum
FITC	=	Fluorescein-5-isothiocyanate
H ₂ O ₂	=	Hydrogen peroxide
IAP	=	Inhibit the inhibitor of apoptosis
NADH	=	Nicotinamide adenine dinucleotide
NADPH	=	Nicotinamide adenine dinucleotide phosphate-oxidase
PFK	=	Phosphofructokinase
PKC	=	Protein kinase C
PS	=	Phosphatidylserine
RBCs	=	Red blood cells
ROS	=	Reactive oxygen species
t-BOOH	=	<i>tert</i> -butylhydroperoxide
XLAP	=	X-linked inhibitor of apoptosis protein