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
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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.

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ABSTRACT

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 phosphotidylserine (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.

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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_2O_2	=	Hydrogen peroxide
IAP	=	Inhibit the inhibitor of apoptosis
NADH	=	Nicotinamide adenine dinucleotide
NADPH	=	Nicotinamide adenine dinucleotide phosphate-oxidase
PFK	=	Phosphofructokinas
PKC	=	Protein kinase C
PS	=	Phosphoatidylserine
RBCs	=	Red blood cells
ROS	=	Reactive oxygen species
t-BOOH	=	*tert*-butylhydroperoxide
XLAP	=	X-linked inhibitor of apoptosis protein