

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E41059

**DIVALENT METAL TRANSPORTER-1 (DMT-1) EXPRESSION IN
CADMIUM ACCUMULATED HUMAN PLACENTA**

KEERAKARN SOMSUAN

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

October 2011

Copyright 2011 by Naresuan University

600255574

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



E41059

**DIVALENT METAL TRANSPORTER-1 (DMT-1) EXPRESSION IN
CADMIUM ACCUMULATED HUMAN PLACENTA**



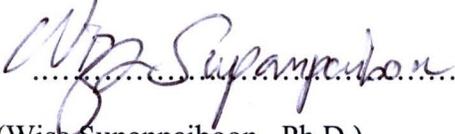
KEERAKARN SOMSUAN

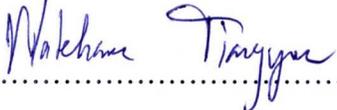
**A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of science Degree in Anatomy
October 2011
Copyright 2011 by Naresuan University**

This thesis entitled "Divalent metal transporter-1 (DMT-1) expression in cadmium accumulated human placenta" submitted by Keerakarn Somsuan in partial fulfillment of the requirements for the Master of Science Degree in Anatomy is hereby approved.


..... Chair
(Assistant Professor Supaporn Chuenchoojit, Ph.D.)


..... Committee
(Natthiya Sakulsak, Ph.D.)


..... Committee
(Wisa Supanpaiboon, Ph.D.)


..... Committee
(Watcharee Tiangyou, Ph.D.)

Approved


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

Dean of the Graduate School

7 October 2011

ACKNOWLEDGMENTS

This thesis was accomplished with the aid and generosity of Dr. Natthiya Sakulsak, my advisor and Dr. Wisa Supanpaiboon, my co-advisor on hand to share valuable advice on the thesis with care and support for research always as well as to alter volume, please review this thesis. I would like to thank to Assistant Dr. Tantip Boonsong, Assistant Professor Dr. Wiphawi Hipkaeo and Assistant Professor Dr. supaporn Chuenchoojit for giving the guidance in reviewing this thesis to best completion.

I thank funding support from the Research Council of Thailand (NRCT) and the Research Fund (TRF) as well as personnel in the Mae Sot Hospital, Mae Sot, Tak province to cooperate very well in between gathering volunteers who joined the research and collected in a mess.

Thank the Department of Anatomy, Faculty of Medical Sciences, Naresuan University for locations in this study, analysis and research throughout the study.

Thank colleagues in Toxic Metal Research Group for cooperation in all aspects as well.

Finally, I respectfully thank my parents and family for their support. The power and inspiration to overcome obstacles I fought on all fronts. And research to be done with great success. Researchers show their gratitude for family, teachers and gratitude upon the grace you have come here.

Keerakarn Somsuan

Title DIVALENT METAL TRANSPORTER-1 (DMT-1)
EXPRESSION IN CADMIUM ACCUMULATED HUMAN
PLACENTA

Author Keerakarn Somsuan

Advisor Natthiya Sakulsak, Ph.D.

Co – Advisor Wisa Supanpaiboon, Ph.D.

Academic Paper Thesis M.S. in Anatomy, Naresuan University, 2011

Keywords Cadmium, Placenta, Divalent metal transporter-1 (DMT-1)

ABSTRACT

E 41059

Cadmium (Cd) is a toxic heavy metal which was reported that it contaminated in soil and rice in local areas of Mae Sot, Tak. Human beings are normally exposed to environmental Cd, leading to gradual Cd accumulation in their bodies, including in the placenta. The placenta is a temporary organ formed during pregnancy which is necessary for the exchange of nutrients from mother to fetus. DMT-1 is a divalent metal transporter which was found in placental tissue. The aims of this study were to localize DMT-1 in term human placenta and to determine DMT-1 protein and mRNA expressions in term human placenta of pregnant women living in Cd contaminated area (n=6) comparing to those in non-Cd contaminated area (n=6) of Mae Sot, Tak. Interestingly, maternal blood Cd and placental Cd of pregnant women living in Cd contaminated area was significantly higher than that in non-Cd contaminated area. In fetal portion of placenta, it showed that DMT-1 was localized in the apical portion of cytoplasm and the basal portion of syncytiotrophoblastic cell, endothelium of fetal capillary which is a part of placental barrier, and also found in cytoplasm of Hofbauer cell. DMT-1 localization in placental maternal portion was detected in most decidual cells. The DMT-1 protein and mRNA expressions in placentas of pregnant women living in Cd contaminated area were significantly higher than that in non-Cd contaminated area ($p = 0.001$ and $p = 0.032$, respectively). According to these results, we suggest that pregnancies exposed in environment of Cd contamination may expose

Cd and it can be accumulated in placenta. Intracellular Cd may induce DMT-1 mRNA transcription and further translates into DMT-1 protein that functions as Cd transport in placental tissue.

LIST OF CONTENTS

Chapter	Page
I INTRODUCTION	1
Rationale for the Study.....	1
Objective of the Study.....	2
The Scope of the Study.....	2
Hypothesis.....	2
The importance of the Study.....	3
II REVIEW LITERATURE	4
Cadmium (Cd).....	4
Major sources of Cd.....	5
Cd adsorptions in the body.....	6
Health effect of Cadmium	7
Cd transporters in enterocyte.....	11
Decidual formation and early placental development.....	17
The structure of mature placenta.....	18
Placental function	24
Divalent metal transporter-1 (DMT-1).....	28
Major function of DMT-1.....	29
Multiple functions of DMT-1.....	35
Structure properties of DMT-1.....	37
Association of DMT-1 in several organs	44
Association of Cd and DMT-1 in placenta	51

LIST OF CONTENTS (CONT.)

Chapter	Page
III RESEARCH METHODOLOGY	53
Study design.....	53
Subjects.....	53
Materials and Instruments.....	54
Methods.....	59
IV RESULTS	63
The body mass index (BMI) of pregnancies and fetal birth	
Weight.....	62
The concentration of serum ferritin, placental Fe, blood Cd,	
urinary Cd and placental Cd.....	64
Localization of DMT-1 protein in placental tissues.....	69
DMT-1 protein expression in placental tissues.....	80
DMT-1 mRNA expression in placental tissues.....	83
V DISCUSSION	89
VI CONCLUSION	95
REFERENCES	97
APPENDIX	118
BIOGRAPHY	134

LIST OF TABLES

Table	Page
1 Multiples name of DMT-1 transporter.....	28
2 Discover of NRAMP family members.....	29
3 DMT-1 interaction with metals.....	36
4 Expression of four DMT-1 isoforms in several species.....	40
5 Localization of DMT-1 protein.....	42
6 Oligonucleotide primers.....	58
7 The maternal weight, height and fetal birth weight of non-Cd and Cd contaminated groups.....	63
8 The concentration of serum ferritin, placental Fe, blood Cd, urinary Cd and placental Cd of non-Cd and Cd contaminated groups.....	64
9 The concentration of blood Cd, placental Cd, urinary Cd, serum ferritin and placental Fe of low-Cd and high-Cd groups.....	68
10 The DMT-1 positive immunoreactivity in human placental cells.....	74
11 The quantitative of DMT-1 localization compared between three areas of placental section of low-Cd and high-Cd groups.....	78
12 The mean value of relative DMT-1/ β -actin protein intensity	82
13 The mean value of relative DMT-1/ β -actin mRNA intensity.....	85
14 The mean value of relative DMT-1/ β -actin protein and mRNA intensity	86
15 Data of pregnant women who live in non-Cd contaminated area.....	132
16 Data of pregnant women who live in Cd contaminated area.....	133

LIST OF FIGURES

Figure	Page
1 Handling of cadmium in human body	7
2 The cadmium mechanism in proximal tubular cells and hepatocyte....	8
3 Possible pathways of Cd transport in enterocyte.....	13
4 Fertilization and cleavage of the embryo	14
5 Implantation of the embryo	15
6 Stages of Chorionic villi	16
7 Decidua formation and early placental development.....	17
8 Placental feature and placental structure.....	18
9 Blood circulation between mother and fetus.....	19
10 A section through the chorionic villus at approximately 10 weeks and the chorionic villus at full term	20
11 Fetal and maternal portions of the placenta.....	21
12 Chorionic villous.....	22
13 Decidual cells.....	23
14 Exchange of substances across the placenta.....	24
15 Function of DMT-1 in enterocytes.....	30
16 DMT-1 in endosome and transferring cycle.....	32
17 Iron distributions in the adult human body.....	33
18 Homeostatic responses to iron by IRE–IRP interactions of TfR, ferritin and DMT-1 transporters.....	35
19 DMT1 involved in the transport of protons and metal-ions.....	37
20 Schematic represent of DMT-1 isoforms including isoform I has iron responsive element (IRE) in 3' UTR, whereas in 3' UTR of isoform I has likely lacking iron responsive element (IRE) and alternative 5'exons designated DMT-1 exon 1A and 1B.....	38

LIST OF FIGURES (CONT.)

Figure	Page	
21	Expression of Human SLC11A2 Transporter mRNAs in Various Tissues.....	41
22	The iron absorption in enterocytes of the upper GI tract.....	44
23	The iron transport in proximal tubule via DMT-1.....	46
24	Iron transport across the Blood Brain Barrier (BBB)	47
25	Iron transportation in human syncytiotrophoblast model.....	49
26	Model of iron transfer across the placenta.....	50
27	The placental tissue collection.....	60
28	Cross plane of placental section for immunohistological collection and metal and molecular collection.....	60
29	The correlation between Cd concentration in maternal blood and placental tissue.....	65
30	The correlation between Cd concentration in maternal blood and urine.....	66
31	The correlation between placental Cd and placental Fe.....	67
32	Localization of DMT-1 in STB	70
33	Localization of DMT-1 in fetal capillary	71
34	Localization of DMT-1 in cytoplasm of hofbuaer cell.....	72
35	Localization of DMT-1 in decidual cells.....	73
36	The drawing diagraeme showing placental membrane (B) DMT-1 localization in placental membrane observed under 100X	75
37	Counting of the number of DMT-1 positive cells in STB per total STB	77
38	The % quantitative of DMT-1 localization in three areas compared between low-Cd (blue bar) and high-Cd (red bar) groups.....	79

LIST OF FIGURES (CONT.)

Figure	Page
39 The photograph of placental protein bands on SDS-PAGE and transferred to membrane.....	80
40 Western blotting showing DMT-1 expressions in term human placental tissues of low-Cd and high-Cd groups and β -Actin protein.....	81
41 Quantitative analysis of DMT-1 protein in placental tissues.....	82
42 The photograph of DMT-1 and β -actin mRNA expressions in term human placental tissues on 1% agarose gel.....	83
43 RT-PCR of DMT-1 mRNA expression in placental tissues of low-Cd and high-Cd groups.....	84
44 Quantitative analysis of DMT-1 mRNA in placental tissues.....	85
45 The correlation of DMT-1 protein and mRNA expression.....	87
46 The correlation between placental Cd and DMT-1 protein expression...	88
47 The correlation between placental Cd and DMT-1 mRNA expression...	88
45 A possible pathway of DMT-1 expression under placental Cd accumulation.....	94
46 Novel mechanism of placental Cd transport mediated by DMT-1 transporter in STB.....	96

ABBREVIATIONS

pH	=	power of hydrogen ion concentration
UV/VIS	=	Ultraviolet and Visible
AAS	=	Atomic Absorption Spectrophotometer
M	=	Molar
mg/l	=	Milligram/liter
ml	=	Milliliter
μ l	=	Microliter
bp	=	base pair
Cd	=	Cadmium
Non-Cd	=	Non-Cadmium
Fe	=	Iron
Zn	=	Zinc
Mn	=	Manganese
Ni	=	Nickle
Co	=	Cobalt
Cu	=	Copper
Pb	=	Lead
Cd-MT	=	Cadmium-Metallothionine complex
DMT-1	=	Divalent Metal Transporter-1
DW	=	Distilled Water
g	=	gram
kg	=	kilogram
μ g	=	microgram
WI	=	Weekly Intake
PTWI	=	Provisional Tolerable Weekly Intake
GI	=	Gastro-Intestinal tract
Nramp2	=	Natural resistance-associated macrophage protein

ABBREVIATIONS (CONT.)

DCT1	=	Divalent Cation Transporter1
SCL11A2	=	Solute carrier family 11, member 2
kg	=	kilogram
µg	=	microgram
WI	=	Weekly Intake
PTWI	=	Provisional Tolerable Weekly Intake
H ⁺	=	Hydrogen ion
IRE	=	iron responsive element
IREG1, FPN1	=	ferroportin 1
Tf-TfR	=	Transferrin bound Transferrin receptor
Tf-Fe	=	Transferrin bound iron
BBB	=	Blood Brain Barrier
FP1-Hp	=	ferroportin-hephaestin
BALT	=	Bronchus-Associated Lymphatic Tissue
GFAAS	=	Graphite Furnace Atomic Absorption Spectroscopy
kD	=	Kilo Dalton