Biogenesis of Plasma CD36+ Microparticles in Human Diabetes and the Metabolic Syndrome

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BSc in Pharmacy

&

MSc in Clinical Pharmacy

A thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy (Experimental Pharmacology)

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University of Newcastle
Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution 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.

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Mohammad Alkhatatbeh

Date
Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper (Chapter 4) of which I am the first and major contributing author of a joint publication. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution (see next page).

15th August, 2012

Mohammad Alkhatatbeh

Date
Endorsement of Authorship by the Supervisor

I attest that Research Higher Degree candidate Mohammad Alkhatatbeh contributed to
1) the conception and design of the research, 2) collection, analysis and interpretation of
research data and 3) drafting and revision of the major part of the work to contribute to
the interpretation of the publication entitled:

*The putative diabetic plasma marker, soluble CD36, is non-cleaved, non-soluble and
entirely associated with microparticles.*

Alkhatatbeh MJ, Mhaidat NM, Enjeti AK, Lincz LF, Thorne RF. J Thromb Haemost,

On behalf of all co-authors and co-supervisors,

-----------------------------------------------
Dr. Rick Thorne (primary supervisor) 15th August, 2012
-----------------------------------------------
Date
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Abstract

CD36 is a widely expressed cell surface receptor that binds lipoproteins and its function has been implicated in many complications of the metabolic syndrome. A cell-free form of CD36, soluble CD36 (sCD36), has been reported in human plasma and found to be elevated in obesity and diabetes and claimed to be a marker of insulin resistance. The nature and origin of the sCD36 has not been determined previously, although it has been postulated to be either a product of proteolytic cleavage or as an intact glycoprotein in the form of circulating microparticles (MPs) which are defined as small (0.1 - 1 µm in diameter) membranous microvesicles that can be specifically and selectively released from the cell membrane and retain many features of their cell of origin. MPs are present in the peripheral blood of normal healthy individuals but their numbers can increase dramatically in various pathological states, including type 2 diabetes and various vascular diseases. Given that MPs are enriched with bioactive proteins and nucleic acids, MPs bearing CD36 may not just be a marker of insulin resistance but may in fact contribute to disease pathogenesis. Thus the overarching hypothesis for this thesis is that plasma derived CD36 (sCD36) identifies a specific subset of MPs which contributes to the pathogenesis of type 2 diabetes and/or its complications.

The first objective of this thesis was to determine the nature of sCD36; in particular whether sCD36 is truly soluble or, as hypothesized, found as a component of circulating MPs. Biochemical experiments done on plasma of normal subjects revealed that the cell-free plasma CD36 was not associated with its lipoprotein ligands and was not a proteolytic fragment; rather it was associated with the plasma MP fraction suggesting that sCD36 is a product of circulating MPs. Flow cytometric and immunoblotting analyses of plasma from normal donors showed these MPs were derived mainly from platelets. Analysis of in vitro activated platelets also showed that CD36 was secreted in the form of MPs.

The second objective of this thesis was to further understand the potential role of CD36 in obesity and the pathogenesis of diabetes. The aim was to determine the levels and cellular sources of the CD36+MPs in patients with type 2 diabetes compared to normal lean and obese controls. Levels of CD36+MPs were found to correspond to approximately 50% of those of platelet derived MPs, were significantly higher in obese patients with type 2 diabetes compared to the obese controls (p<0.00001), and were primarily derived from mature erythrocytes (35.8 ± 14.6%). Plasma CD36 protein concentration measured by ELISA was positively correlated with CD36+MPs measured by flow cytometry but only weakly associated with the distribution of controls and patients with diabetes. Multivariate analysis confirmed that CD36+MP levels were a much better marker of diabetes than CD36 protein concentration.

The third objective of this thesis was determine if there was any pattern of elevated MP levels related to diabetic complications and medications. Analysis by self-reported
diabetic complications did not show significant differences in most of the MP subsets between patient subgroups except for the significant increase in % of CD36+/PS+MPs ([PS] phosphatidylserine; activation/apoptosis marker) in patients with microangiopathy and peripheral ulcer and the significant decrease in CD14+MPs (monocyte marker) and CD36+/CD41+MPs (platelet marker) in patients with reported nephropathy. Analysis by self-reported medications showed a significant increase in absolute numbers of CD36+/CD105+MPs (endothelial marker) and CD36+/CD45+MPs (leukocyte marker) in patients with diabetes taking sulfonylurea and a significant increase in % of CD36+/CD235a+MPs (erythrocyte marker) in patients taking metformin compared to those who were not. Total CD36+MP levels were not significantly associated with any of the self-reported diabetic complications and medications except for patients who were treated with calcium blockers.

The last objective of this thesis was to determine if CD36+MPs could be released from cells of organs exposed to diabetic conditions. To this end, in vitro models were developed to represent complications of type 2 diabetes including diabetic nephropathy and fatty liver disease. Treatment of cell lines using advanced glycosylation end products (AGEs) and palmitic acid (PA) induced cellular death and CD36+MP production from HK-2 cells (nephropathy model) and HepG2 cells (fatty liver model) under circumstances resembling those that occur in diabetic plasma. If similar processes occur in human liver and kidney, it will be expected that CD36+MPs could be produced from CD36 expressing tissues especially those which are involved in diabetes and its complications.

Collectively, this thesis establishes that the reported diabetic marker sCD36 in human plasma is entirely associated with circulating MPs (CD36+MPs). Interestingly, CD36+MP levels were found to be a better marker of diabetes than sCD36 protein concentration. The origin of circulating MPs could be easily determined as they also express antigens of their cellular source. CD36+MPs in patients with type 2 diabetes were mainly derived from mature erythrocytes but the underlying pathophysiology behind involvement of erythrocytes in diabetes requires further investigations. In addition, further investigations are needed to determine whether CD36+MPs could contribute as mediators of diabetes or if they are purely just biomarkers.
Acknowledgments

I would like to thank my Supervisor, Dr. Rick Thorne and all the staff and students at the Cancer Research Unit, School of Biomedical Science and Pharmacy. Thank you Dr. Rick for your instruction and guidance throughout all aspects of this project. Thank you also for being friend more than being supervisor and for your social support during the last three years when I was away from my home. I will never forget your positive effect on my life and I am very proud of your supervision throughout my candidature.

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<tr>
<td>%CV</td>
<td>coefficient of variation</td>
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<tr>
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<td>messenger ribonucleic acid</td>
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Mr  molecular weight
nm  nanometer
O   oxygen
N   nitrogen
NaCl sodium chloride
NaHCO₃ sodium hydrogencarbonate
NaH₂PO₄ sodium dihydrogen phosphate
PAGE polyacrylamide gel electrophoresis
pAb polyclonal antibody
PBS phosphate buffered saline
pH  potential hydrogen
PMA phorbol 12-myristate 13-acetate
PTA1 platelet and T cell antigen 1
RNA ribonucleic acid
PNGase-F peptide N-glycosidase F
r  correlation coefficient
RT room temperature
SDS sodium dodecyl sulphate
T  thyonine
TBST tris buffered saline with tween 20
Tris (2-amino-2-hydroxy-(hydroxymethyl)-propane-1, diol, (tris))
U  unit
U.V. ultra violet
V  volt
vs.  versus
v/v, w/v volume per volume, weight per volume
List of Publications

Journal articles


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