EFFECT OF A POLYUNSATURATED FATTY ACID MIMETIC ON THE DEVELOPMENT OF ATHEROSCLEROSIS IN THE APOE DEFICIENT MOUSE

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Summary

Atheroma, heart attacks and strokes continue to be a major cause of morbidity and mortality in our community. Atherosclerosis is a chronic inflammatory vascular disease, characterised by thickening of the vascular wall due to lipid accumulation, infiltration by circulating monocytes and T cells and proliferation of smooth muscle cells. Leukocyte adherence to the blood vessel wall is promoted by the up-regulation of cell adhesion molecules (CAM) by atherogenic substances such as tumour necrosis factor (TNF-α) and oxidised low density lipoprotein (oxidised-LDL). Recently our group has synthesised a novel polyunsaturated fatty acid, β-oxa 23:4*n-6* which inhibits CAM up-regulation in blood vessel walls. It was therefore the objective of this thesis to determine whether this fatty acid protects against atherosclerosis. Advantage was taken of an experimental model of this disease, the apoE deficient mouse (apoE$^{-/-}$) which spontaneously develop atherosclerosis.

To assist our studies on MP3, we established an appropriate classification of different stages of atherosclerotic lesions and defined the kinetics of development of the disease in this model. By examining of the sections at the level of aortic roots the atherosclerotic lesions were classified into six categories. This classification was based on the histological characteristics of the plaque component including the degree of macrophage infiltration and foam cells formation, the presence of cholesterol clefts and confluent lipid cores, calcification and ossification, the composition of the fibrous cap, the media involvement and the incipient/actual aneurysm formation and inflammation, including neutrophils.

Kinetics of plaque development under the influence of a high fat and high cholesterol diet followed an exponential relationship of $y=-e^{-x}$. The asymptotic characteristic of this lesion development was however a function of compensatory aortic enlargement which accompanied the increase in lesion development and size. Thus it is concluded that the level of atherosclerosis needs to be gauged by the size of the lesion *per se*. This may be particularly important for the assessment of anti-atherogenic effects of drugs. Therefore attempts to develop a quantitative system to assess plaques revealed that expression of plaque size as % of occupation of blood vessel had limitations.

Using this model we were able to demonstrate that injections of the novel polyunsaturated fatty acid, MP3 led to a significant reduction/inhibition (70%) of plaque area and a corresponding 60% inhibition of aortic size. As expected this inhibition was not as evident when results were expressed as % of aortic lumen size. The results also suggested that protection by MP3 was dependent on conditions which promoted increased uptake into tissues by, for example, preloading animals with MP3 prior to commencing the high fat high cholesterol diet. The protective effects of MP3 are consistent with a role for the activation of the transcriptional factor, NFκB and up-regulation of cell adhesion molecules in this disease, and the ability of MP3 to inhibit these targets. Thus the objective of this research has been achieved and the hypothesis proven.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I contest to the thesis being made available for photocopying and loan in accepted for the award of the degree.

Fatemeh Moheimani

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ABREVIATIONS

AA	arachidonic acid
AGE	Advanced glycation end products
AHA	American heart association
Akt (PKB)	activating protein kinase B
ALA	α-linoleic acid
ApoE	Apolipoprotein E
$ApoE^{-/-}$	homozygous apoE deficient
BP	blood pressure
CAM	cell adhesion molecule
CHD	Coronary heart disease
COX	cyclooxygenases
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
ES	embryonic stem cell
FA	Fatty acid
HBSS	hannks balanced salt solution

HDL	high density lipoprotein
HUVEC	human umbilical vein endothelial cell
ICAM-1	intercellular adhesion molecule 1
IDL	intermediate density lipoprotein
IEL	internal elastic lamina
IL-1	interleukin 1
IP	intraperitoneal injection
LA	linoleic acid
LDL	low density lipoprotein
Li_2CO_3	Lithium Carbonate
LOX	lipoxygenases
LPS	lipopolysaccharide
MCP-1	monocyte chemotactic protein 1
M-CSF	macrophage colony-stimulating factor
MMP	matrix metalloproteinase
NE	neutrophil elastase
NO	nitric oxide
Oxidised-LDL	oxidised low density lipoprotein

PDK	phosphoinositide-dependent kinase
PI3K	phosphatidylinositol 3 kinase
PIP2	phosphatidylinositol 4, 5 bisphosphate
PMA	phorbol 12-myristate 13-acetate
PPARα	peroxisome proliferator-activated receptor α
PUFA	polyunsaturated fatty acid
SEM	standard error of the sample mean
TBS	Tris buffered saline
TNF-α	tumour necrosis factor α
VCAM-1	vascular-cell adhesion molecule 1
VLDL	very low density lipoprotein