Chylomicron remnants: mediators of endothelial dysfunction?

C.P.D. Wheeler-Jones1
Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, U.K.

Abstract
Vascular disease is initiated by activation of the endothelium characterized by the predominance of pro-inflammatory and pro-coagulant changes in endothelial cells (ECs) referred to collectively as ‘endothelial dysfunction’. There is increasing evidence that lipoproteins of dietary origin modulate EC function and the use of artificial chylomicron remnant-like particles (CRLPs) in vitro is now beginning to shed light on the molecular mechanisms through which these particles influence cell behaviour. CRLPs enriched in n−6 PUFAs (polyunsaturated fatty acids) influence the production of vasoactive mediators by ECs in a pro-inflammatory manner. Thus CRLPs reduce the synthesis and release of nitric oxide and alter the balance of release of vasodilator versus vasoconstrictor eicosanoids. These changes are accompanied by induction of cyclo-oxygenase-2 expression and activity as well as increased expression of adhesion molecules and the antioxidant defence enzyme haem oxygenase-1. CRLPs also activate a number of intracellular signalling pathways, including NF-κB (nuclear factor κB) and MAPKs (mitogen-activated protein kinases), which may be involved in mediating their effects on gene expression. The effects of CRLPs on EC behaviour can also be modulated by the type of fat/oxygenation status of the particles. These findings support the hypothesis that lipoproteins of dietary origin directly regulate molecular events in the vascular wall.

CMRs (chylomicron remnants) and the vessel wall
EC (endothelial cell) dysfunction is characterized by impairment of endothelial-dependent vasodilatation and by the prevalence of both pro-inflammatory and pro-coagulant events in the endothelium [1]. This ‘smouldering’ endothelial activation can predispose to disorders of the cardiovascular system, including hypertension and atherosclerosis, and is additionally associated with atherothrombotic conditions such as ischaemic stroke and acute coronary syndromes. Endothelial dysfunction is also an underlying feature of several other disorders including metabolic syndrome, diabetes, rheumatoid arthritis and cancer. A reduction in inflammatory processes in the vascular wall should therefore have cardiovascular benefits. Thus an understanding of the mechanisms that generate endothelial dysfunction is an important prerequisite for designing therapeutic strategies to limit aberrant EC behaviour.

There is now substantial evidence that dietary components strongly influence the inflammatory status of the vessel wall. In particular, it is well established that the type of fat consumed modifies the risk of developing cardiovascular disease with increased risk associated with ingestion of SFAs (saturated fatty acids), compared with PUFAs (polyunsaturated fatty acids) or MUFAs (mono-unsaturated fatty acids) [2]. Often referred to as ‘exogenous’ lipoproteins, CMs (chylomicrons) are heterogeneous TAG (triaclyglycerol)-rich particles synthesized by intestinal cells to transport dietary TAG and fat soluble vitamins to peripheral tissues. CMs enter the systemic circulation via the thoracic duct and are then converted into CMRs by lipoprotein lipase-mediated removal of 70–90% of the TAG [3]. While the pro-atherogenic role of endogenous lipids is well established (e.g. [4]) the effects of lipoproteins of dietary origin on the vasculature have received little attention, despite the fact that these particles carry fat from the diet to the tissues through the vasculature. Since conditions associated with delayed CMR clearance from the blood result in premature atherosclerosis [5], these particles may accelerate disease progression through heightened and/or prolonged interaction with ECs followed by modification of vessel wall function. The molecular mechanisms through which these changes occur are currently undefined, but since the type of fat consumed in the diet directly affects the fatty acid composition of CMRs, such effects provide a mechanism by which fat ingestion impacts directly on EC behaviour.

Owing to the difficulties associated with isolating homogeneous preparations of CMRs without contamination with other TAG-rich lipoproteins, the Botham Laboratory developed methodology for the preparation of artificial CMRs (CRLPs (CMR-like particles)) which are similar in size, density and lipid composition to physiological CMRs [5].
Molecular mechanisms of EC activation by n−6 PUFAs

CRLPs interact with the plasma membrane of ECs and elicit rapid changes in the activation status of MAPK signalling pathways, including ERK1/2, p38MAPK and the JNKS (c-Jun N-terminal kinases). Activation of these signalling elements may be dependent upon the CRLP-induced generation of ROS, which in turn depends upon the redox status of the cells and the oxidation state of the CRLPs. Increased NF-κB activity can participate in the regulation of COX-2 and HO-1 expression, as well as controlling the expression of adhesion molecules which drive interaction of inflammatory cells with the vascular wall. Early activation of COX-1, together with induced COX-2, results in the generation of cytoprotective PGI₂ as well as the pro-atherogenic molecule TxA₂. Increased expression of HO-1 protects the cells from apoptosis and generates carbon monoxide, thus enhancing cell survival and improving dilator/constrictor balance.

Several pharmacological studies in rat and porcine vessels have now shown that CRLPs inhibit relaxation and potentiate vasoconstriction in an endothelium-dependent manner which is mediated, at least partly, by impaired generation of the vasodilator molecule NO (nitric oxide) [6,7]. Evidence that these particles also reduce basal NO production by cultured porcine ECs was subsequently provided [8].

Effects of CRLPs on cultured endothelial and VSMCs (vascular smooth muscle cells)

More recently, the effects of exposure of human ECs to CRLPs has been examined. We have now established that HUVECs (human umbilical vein ECs) respond both acutely and chronically to CRLP treatment (M. Evans, Y. Berhane, J. Elliott, K.M. Botham and C.P.D. Wheeler-Jones, unpublished work) [9,10]. Our findings so far are consistent with the hypothesis that ECs are key targets for CMR action and that CRLPs increase inflammatory activation of ECs (Figure 1). Accordingly, n−6 PUFAs CRLP treatment abrogated basal NO synthesis, as assessed by measurements of cGMP formation, and also significantly decreased agonist-stimulated cGMP formation [9]. This effect is clearly not due to changes in eNOS (endothelial nitric oxide synthase) expression since this was not modified by exposure to CRLPs for periods up to 24 h. CRLPs strongly up-regulate COX (cyclo-oxygenase)-2 protein expression in HUVECs, the Eahy.926 EC line and human coronary artery ECs, without modifying constitutive COX-1 expression. COX-2 induction was accompanied by biphasic release of PGI₂ (prostacyclin), a potent vasodilator, with the later peak abolished by the COX-2 selective inhibitor, NS-398. CRLPs also increased release of the vasoconstrictor molecule TxA (thromboxane) A₂ providing further evidence that these particles may alter the balance of vasodilator/vasoconstrictor release from the vascular wall and hence contribute to the development of endothelial dysfunction. Furthermore, CRLPs promote EC release of cytokines (e.g. interleukin-6) strongly implicated in the progression of vascular disease, thus reinforcing their pro-inflammatory actions. CRLP-mediated activation of ECs is also associated with enhanced expression of the antioxidant molecule HO-1 (haem oxygenase-1) which is induced in ECs with similar kinetics to COX-2.

Importantly, our data are beginning to reveal how the type of fatty acids in the triacylglycerols contained within the particles alters these functional effects. Modification from trilinolein n−6 PUFAs to triolein/tripalmitin (1:1) MUFA/SFA significantly influences the effects observed. Thus MUFA/SFA CRLPs do not promote either a decrease in cGMP formation or increased COX-2 protein expression, but retain ability to marginally increase TxA₂ production and enhance HO-1 expression [10]. Since the techniques utilized to make these particles were identical, their size and composition, including the presence of apoE, is comparable, and thus the differing effects of the two types of CRLPs can be attributed to the difference in their fatty acid composition, with particles containing PUFAs being more effective than those containing more SFAs. Therefore, overall, our in vitro studies provide evidence to support the hypothesis that CMRs could differentially influence the initiation of atherosclerotic lesion formation in vivo by altering EC functions that regulate production of vasoactive mediators. They additionally suggest that the fat composition of the diet may also be an important determinant of the ability of CRLPs to promote EC dysfunction. Current work is interrogating how the oxidative state of the particles influences their effects on ECs.

Our recent studies have also shown that in addition to their effects on ECs, CRLPs modify intracellular signalling events and enzyme expression in VSMCs. Thus CRLPs containing n−6 PUFAs induce expression of both COX-2 and HO-1 proteins, without significantly modifying the levels of COX-1 or eNOS. In contrast, CRLPs enriched in MUFA/SFAs did not influence the expression of these proteins [10]. There was
also a clear trend for an increase in release of both PGI2 and TxA2 in response to n-6 PUFA CMR-LPs, but only TxA2 in response to MUFA/SFA particles. These data demonstrate that the effects of CRLPs on VSMCs, in common with ECs, are modulated by the fatty acid composition of the particles, with PUFAs being more active than SFAs. Together, these data suggest that CMRs could potentially affect the function of multiple cell types in the vessel wall, both through direct interaction of the particles with individual cell types as well as through CMR-induced release of paracrine mediators (e.g. eicosanoids).

CRLP-mediated activation is likely to be accompanied by changes in cellular expression of a large number of genes. Identification of additional genes altered in cells exposed to CRLPs of different composition will provide important insights into the molecular control of EC and VSMC function by lipoproteins derived from the diet. Frequent ingestion of food places many individuals in a more or less constant post-prandial state for much of the day. Our in vitro data strongly imply that repeated exposure of the vascular wall to post-prandial lipoprotein particles in normal and compromised individuals is likely to have highly significant outcomes in terms of vessel homeostasis. Further work is now needed to establish the exact molecular mechanisms through which CRLPs bring about these effects.

Activation of signalling pathways by CRLPs

Our studies have provided direct evidence that CRLPs trigger EC signalling to enhance inflammatory activation, and the pathways activated have similarities to those evoked by classic pro-inflammatory agonists such as interleukin-1. For example, key players in the molecular regulation of pro-inflammatory gene expression in the vascular wall are the NF-κB (nuclear factor-κB) and MAPK (mitogen-activated protein kinase) signalling pathways and hence both are attractive targets for anti-inflammatory intervention. We have shown that MAPKs, in particular the ERK1/2 (extracellular-signal-regulated kinase 1/2) and p38MAPK families, control both acute and chronic prostanoid production by ECs through their regulation, respectively, of cytosolic phospholipase A2α activation and COX-2 induction [11–13]. NF-κB also directs inflammatory events in ECs [13]. We have recently shown that n-6 PUFA CRLPs rapidly activate ERK1/2 and p38MAPK in HUVECs, whereas MAPK activation is not observed in cells exposed to CRLPs enriched in MUFA/SFAs. Transfection studies using an adenoviral reporter construct suggest that CRLPs also activate NF-κB and that changes in gene expression may be regulated, at least in part, through activation of this transcription factor. The p38MAPK pathway, but not ERK1/2, may also be involved in CRLP-induced gene expression (M. Evans, Y. Berhane, J. Elliott, K.M. Botham and C.P.D. Wheeler-Jones, unpublished work).

One mechanism that could provide a link between the early signalling events and functional effects observed in CRLP-stimulated ECs is the generation of ROS (reactive oxygen species). ROS, including superoxide and hydrogen peroxide, are recognized as important signalling molecules in cardiovascular tissues and participate in normal physiological signalling processes, as well as contributing to the detrimental events associated with oxidative stress. There is increasing evidence that oxidative stress is a critical factor in the pathogenesis of cardiovascular disease, and that oxidized lipids play a central role in the initiation and progression of atherosclerotic lesions [14]. The influence of CRLPs on ROS production in ECs, however, is currently unknown, but our preliminary data suggest that n-6 PUFA CRLPs increase ROS generation in HUVECs, as assessed by DCF (2′,7′-dichlorofluorescin) fluorescence, and are able to potentiate ROS production by pro-inflammatory stimuli (J. Dalla-Riva, T. Carter, J. Elliott, K. Botham and C.P.D. Wheeler-Jones, unpublished work). Studies using heterogeneous RLP (remnant-like particle) isolates also indicate that oxidant-dependent pathways may mediate RLP effects on ECs, although the contribution of CMRs was not explored and remains to be defined [15–17]. The likelihood that redox-sensitive pathways are involved in CRLP-induced signalling and gene expression is supported by our recent observation that oxidized n-6 PUFA particles are more effective inducers of both COX-2 and HO-1 expression in ECs than their non-oxidized counterparts [18]. The effects of CRLPs on isolated rodent vessels to reduce endothelium-dependent relaxation and potentiate vasoconstriction are also enhanced when the particles are oxidized [7].

There is therefore growing support for the hypothesis that CMRs of distinct fatty acid composition (and therefore reflecting the type of fat in the diet) differentially influence signalling events, gene expression and the release of vasoactive mediators by both ECs and VSMCs. Conceivably, these effects may predispose to or indeed protect the vascular wall from developing the early dysfunction that signals the onset of vascular disease.

CRLPs and vascular protection?

The physiological versus pathological roles of COX-2 in ECs and in the vessel wall in general are currently the subject of much debate. Thus COX-2, which is strongly induced by pro-inflammatory cytokines, is overexpressed in atherosclerotic lesions but apparently not in the normal vasculature, suggesting that COX-2 is involved in disease pathogenesis. However, prostanoid release depends not only upon the level of COX-2 expression and activity but also on cell-specific expression of the downstream terminal synthases that ultimately dictate the profile of cellular prostanoid production. PGI2, generated through the sequential activities of COX-2 and PGIS (prostacyclin synthase), is a strongly vasculoprotective molecule and ECs exhibit high expression of PGIS. The importance of PGI2 as a cardiovascular protector is highlighted by its ability to promote vasodilatation, inhibit platelet and lymphocyte adhesion to endothelium, reduce VSMC proliferation, counteract the production of profibrotic growth factors and prevent free radical production (e.g. [19–21]). Moreover, there is evidence that circulating PGI2 in healthy humans is COX-2-derived [22] raising the
possibility that the COX-2–PGIS–PGI2 axis is operative in vivo and thus physiologically relevant. The ability of CRLPs enriched in n–6 PUFA to enhance COX-2 expression and promote concomitant PGI2 synthesis in ECs is consistent with the hypothesis that as well as contributing to inflammation these lipoproteins may trigger protective responses in the vasculature. This is also supported by the observation that exposure to n–6 PUFA particles co-induces the antioxidant enzyme HO-1 [23] which protects ECs from apoptosis and generates carbon monoxide, which has vasodilator activity. Such protective responses, particularly if they occur in the absence of existing endothelial dysfunction, may help to limit the potentially detrimental effects of inflammatory mediators. On the other hand, CRLPs can trigger TxA2 release from ECs, a potent vasoconstrictor and pro-inflammatory mediator. On the other hand, CRLPs can trigger TxA2 release from ECs, a potent vasoconstrictor which promotes platelet activation and VSMC proliferation [24], and has pro-apoptotic effects on ECs [25]. A full understanding of the pro-inflammatory versus cytoprotective actions of CRLPs of different fatty acid composition awaits the results of our current studies which aim to define the vasoactive mediator profile of vascular cells exposed to CRLPs of varying composition and oxidative state. However, our data, and those of others [26,27], support the working hypothesis that dietary fats play a central role in differentially regulating molecular events in the vascular wall.

Studying the vascular effects of postprandial lipoprotein particles continues to be a challenging area of basic research and has increasing relevance in the light of the increased incidence of obesity, diabetes, and the metabolic syndrome and recognition of their cardiovascular consequences. Our data, and those of others using lipoprotein isolates, strongly suggest that lipoproteins derived from the diet modify signalling events and gene expression in the vascular wall through direct actions on both endothelial and smooth muscle cells. Work in this area is in its infancy and many questions remain unanswered. For example, how exactly do CRLPs interact with the endothelium? What are the molecular mechanisms underlying the varied actions of CRLPs on ECs? How does exposure to CRLPs influence the ability of the endothelium to respond to subsequent pro-inflammatory challenge? Are the functional effects of CRLPs modified by shear stress? And crucially, how are these events modified by the type of fat ingested in the diet? Further study of the effects of CRLPs on the vasculature should go some way towards answering these and other questions in the near future and will facilitate our understanding of the mechanisms underlying diet-induced vascular wall dysfunction. Such information will allow the development of more focused therapeutic strategies that combine dietary modifications with pharmacological intervention to limit endothelial dysfunction and cardiovascular disease.

I thank Dr Kathleen Botham, Professor Jonathan Elliott, Dr Michelle Evans, Dr Yoel Berhane and Mr Jonathan Dalla-Riva for their valued contributions to the work described, and the British Heart Foundation for financial support.

References


Received 4 December 2006