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Lipid Oxidation Products and Vascular Function

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The endothelium and blood platelets are intimately involved in both the maintenance of vascular tone and in haemostasis. They are also exposed to high concentrations of lipoproteins, either in the plasma or in the sub-endothelial region of the artery wall, and the biological activity of these cells has been shown to be modulated. Oxidative modification of these lipoproteins results in further variations in the properties of these particles in relation to the activities of the endothelium and platelets. These effects and how the work of Hermann Esterbauer on the details of lipoprotein oxidation permitted rapid progress in understanding these phenomena are discussed in this review.

Keywords: Lipoprotein oxidation, endothelium, nitric oxide, platelets

INTRODUCTION

It is now widely accepted that damage to the endothelium plays a pivotal role in the development of early atherosclerosis.[1] The endothelium has so many key functions which are relevant to the both systemic and local functions of the arterial wall. In atherosclerosis, there are key changes which determine many of the processes leading to the multifarious events in atherosclerotic plaque. The recognition of the key role of endothelium led to an explosion of interest in the early 1980s, a process which was contemporary with another major advance in which the potential role of oxidation of lipoproteins in atherogenesis was uncovered. These events predated briefly the recognition that nitric oxide was endothelium-dependent relaxing factor.[2] The conjunction of all these streams of thought, a decade ago, opened a new era of interest in free radical biology and its application to arterial disease. The contribution of Hermann Esterbauer to this synthesis of ideas was very considerable.[3]

The detailed knowledge of the processes and products arising from the oxidation of lipoproteins, particularly those of the low-density lipoproteins (LDLs), has paved the way for a plethora of studies in which the influence of lipoprotein oxidation could be studied on the physiological properties of many cell types, particularly in relation to cells of the arterial wall. The concept of the cellular induction of lipoprotein oxidation originating from the laboratories of Fogelman,[4] Steinberg[5] was a key driving force in this field. Hermann Esterbauer's main contribution was the elaboration of the precise chemistry of lipoprotein oxidation and the sequence of events therein, as well as the

methods he developed for the determination of extent of oxidation. This provided a very significant framework for all to follow and allowed a much greater definition for experimentation, especially for those working *in vitro*. In our case and in that of others, it stimulated a study of the effects of lipoprotein oxidation on the endothelium, smooth muscle and on platelets as part of an axis of interactions which relate to the biology and pathology of nitric oxide.

The Endothelium, Lipoproteins and Oxidation

The endothelium is now recognised to be a layer of cells with a pivotal role in normal regulation of arterial function. This has been brought to the fore by the demonstration that the endothelium controls the relaxation of smooth muscle cells in the artery wall and therefore vascular tone, blood flow and blood pressure and that this is due to the release of nitric oxide synthesised from L-arginine.[2] Lipoproteins are present on both the luminal and abluminal sides of the endothelium at similar concentrations and, since they are considered to have a significant role in the atherogenesis, it was inevitable that the effects of these particles on endothelial function should be investigated (Figure 1).

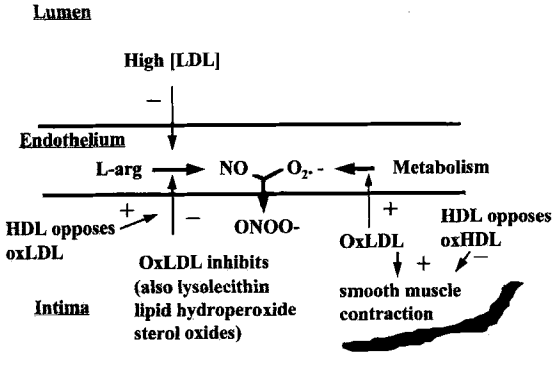

FIGURE 1 The influence of lipoproteins on the release of nitric oxide and superoxide from the endothelium. − represents an inhibition and + a stimulation. OxLDL is oxidised LDL.

Early work had shown,[6] in animal models of atherosclerosis, that the relaxation induced by agonists such as acetylcholine was impaired. In this laboratory, it was then shown in organ bath studies on rings of rabbit aortae that LDLs, at high concentrations, were able to inhibit these relaxations even at relatively short periods of exposure.[7,8] However, these effects were reversible: high-density lipoproteins (HDLs) did not induce these effects. These observations have now been extended into the clinical sphere and there have now been numerous observations which show that patients with hypercholesterolaemia have impaired forearm blood flow as measured by plethysmography or ultrasound methods.[9,10] The impairment of endothelial activity may even be a better indicator of arterial dysfunction than the physical deformations observed by angiography. Furthermore, lowering of plasma cholesterol by cholesterol biosynthesis inhibitors could reverse this impairment in blood vessels.[11] Therefore, hypercholesterolaemia alone, due to elevated concentrations of LDLs could be responsible for the major impairment of endothelial function. It is not clear why native LDLs should have these effects, but they are not free of all oxidation products. Traces of lipid peroxides are present and, indeed, significant quantities of lysophosphatidylcholines, which themselves may influence endothelium-dependent relaxation. Interestingly, this effect of native LDLs could be reversed *in vitro* by the antioxidants such as vitamin C or probucol, although the mechanism by which this is achieved is not clear. Probucol and ascorbate also seem to enhance the improvements in endothelial function produced of lipid-lowering treatments *in vivo*,[12,13] at least when administered at high concentrations. Probucol seemed effective over a relatively short period of time, when regression of atherosclerosis would be unlikely. This was confirmed by the decreased impairment in the relaxation of the aortic arch in WHHL rabbits fed probucol after formation of lesion had already developed.[14] The continuity

between the early studies *in vitro* and the outcome of the clinical studies is an impressive validation of laboratory based science.

Whereas most of the above relates to the consequences of hyperlipidaemia, the effects of LDLs may not explain the observations of impairment of atherosclerosis. It is here that the studies on oxidised lipoproteins have been concentrated. Following the procedures developed by Hermann Esterbauer for the oxidation of LDLs, most observers have shown that copper-oxidised LDLs show a distinct impairment of endothelium-dependent relaxation, although the extent to which this occurs may vary.[15] We have found consistent inhibition by oxidised LDLs from the same donor which related to the extent to which this oxidation occurs as determined by the conjugated diene/lag time assay developed by Hermann Esterbauer. The impairment was observed in vessels from different species,[16] and in different locations e.g. both aortae and in coronary vessels[17] including resistance vessels. Some donors yielded LDLs which produced irreversible impairment of relaxation, whereas others produced a reversible impairment. The variation between laboratories also depends on the concentration of LDLs during the process of oxidation with copper ions. At dilute concentrations, an LDL is oxidised very heavily and produces impairment at low concentrations, whereas at high concentrations of LDL, even with the same ratio of copper ions to protein, a less oxidised form of LDL is produced which requires higher concentrations to cause an inhibition. It can be argued that the LDLs at higher concentrations may be more representative, both in terms of concentration and the degree of oxidation, of LDLs found in the sub-endothelial intimal region of the artery wall where concentrations of LDL may be equal to or greater than that of the plasma.

The mode of action of oxidised lipoproteins on relaxation has been under investigation for some time. It can readily be demonstrated that products of lipid oxidation such as lysophosphatidylcholines[18] inhibit the acetylcholine-induced relaxations of blood vessels, but paradoxically has the ability to induce relaxations in the absence of acetylcholine. Similarly, LDL which has been treated with phospholipase A_2 also impairs receptor-mediated relaxation and this may be neutralised by the presence of albumin and, to a greater extent, of HDL which may sequester the free lysophosphatidylcholine. Hydroperoxides and hydroxy-derivatives of linoleic acid and arachidonic acid[19,20] also impair relaxations. There may be a separate role for sterol oxides,[21] some of which have inhibitory effects on relaxation.

The mechanism by which these effects occur is more controversial. The apparent lower release of NO suggests a reduction in the synthesis of NO by the endothelium, although this is not the only explanation. This is a composite of several processes which lead to changes in the net release of NO. Oxidised LDL has been shown to decrease the release of NO when measured by these techniques[22,23] and the expression of endothelial NO synthase (eNOS) is also diminished,[24] although some reports contradict this. Indeed, other groups have shown that NO synthesis may be increased in atherosclerotic tissue[25] and that there is increased expression of eNOS.[26] Using the human umbilical cord endothelial cells, cultured in parallel with rat RFL-6 fibroblasts, we see a modest reduction of bradykinin-stimulated release of NO following exposure to fully oxidised LDL for 1 h and to a lesser extent with native LDL, but not with mildly oxidised LDL.[27] After 24 h exposure, the differences between the types of LDL diminished.

An important factor in determining the net release of NO from the endothelium is the presence of superoxide anion, since this is capable of reacting with NO to form peroxynitrite[28] measurable amounts of which are reported to be formed by normal endothelium.[29] Increases in the release of superoxide anion have been reported in atherosclerotic tissue.[30] This may also occur in smaller blood vessels in response to hypercholesterolaemia, for example in coronary

resistance vessels of WHHL rabbits.[27] There is also an increase in the activity of superoxide dismutase in human endothelial cells treated with oxidised LDL or with low concentrations of lysophosphatidylcholine.[27]

An alternative outcome may be a direct interaction of NO with oxidation products. Such mechanisms have been proposed for peroxyl radicals interacting with NO to form nitrolipids.[31] However, the inhibition of relaxation induced by authentic NO in the presence of native LDL and of oxidised LDL was modest and may be a minor consequence of the general lipophilicity of NO. There is evidence that NO can, in some circumstances, act as a chain breaking antioxidant[32] but in others, such as the oxidation of LDL by metmyoglobin in the presence of hydrogen peroxide, NO could exhibit pro-oxidant activity.[33] It is now well established that peroxy-nitrite has potent oxidising properties both for lipid and proteins [34] and therefore lipoproteins.[35]

There remains the possibility of a direct action of LDL or mildly oxidised LDL on the target cells which impairs their responsiveness to NO e.g. platelets, smooth muscle cells or even fibroblasts which may depend on the concentrations of small amounts of lipid oxidation products.

Lipoprotein Oxidation and Smooth Muscle Contractility

A number of studies have shown an enhancement in sensitivity to contractile agents during the life history of the atherosclerotic plaque.[36] Angiographic studies of human coronary arteries in patients with CHD show contractions with acetylcholine rather than vasodilatation.[37] However, the fact that there was little impairment of relaxation induced by nitrovasodilators suggests that the effects are primarily endothelium dependent and not due to a defect in guanylyl-cyclase activity in smooth muscle. A selective enhancement of responses to 5-HT and hypo-responsiveness to noradrenaline was observed in WHHL rabbits.[38]

Native LDL has no effect on the contractions of de-endothelialised arterial rings at lower concentrations, but there were effects of the oxidised LDL depending on the contracting agent. With norepinephrine, oxidised LDL impaired the contractions, but enhanced those evoked by 5-HT in rabbit aortae. These effects appear to be influenced by the increase in calcium influx[39] and the activation of phospholipase C–phosphatidylinositol pathway.[40] Oxidised LDL has been shown to inhibit sodium-nitroprusside induced relaxations in arterial rings to a limited extent and to decrease cyclic GMP in smooth muscle cells, but other workers have not confirmed this. However, in pig coronary vessels oxidised LDL was found only to enhance vasoconstriction in an endothelium-dependent manner.[41]

Lysophosphatidylcholine appeared to be less potent in causing this increased contractility than its known effects on the endothelium.[42] However, HDL could reverse the effects of oxidised LDL and lysophosphatidylcholine on guanylyl-cyclase activity. Exposure of rat RFL-6 fibroblasts to fully oxidised LDL, but not minimally oxidised LDL, reduced the response of these cells to authentic NO in terms of cyclic GMP synthesis.[43] Oxidised LDL does have other effects on smooth muscle cells separate from contractility, including proliferation[44] and apoptosis[45] in which lysophosphatidylcholine and sterol oxides may also be involved. Various mechanisms have been proposed which include the sphingomyelin–ceramide pathway[46] and release of fibroblast growth factor-2.[47]

It appears that enhanced responsiveness of the vascular smooth muscle contributes to the impaired vasodilatation of human atherosclerotic vessels and that oxidised LDL can contribute to this while HDL attenuates their action.

Lipoproteins and Platelet Function

Platelets are multifunctional cells which release vasoactive compounds which influence endothelial and smooth muscle function. They also

release growth factors which enhance the repair mechanisms of the endothelium and smooth muscle. There is evidence of a direct interaction of platelets with LDLs (Figure 2). Early data suggested that LDL may enhance the activation of the platelets,[48] but this has not always been confirmed.[49] The diverse results in the literature again depend a great deal on the different ways in which platelet activity can be measured. This ranges from the standard technique *in vitro* of platelet aggregometry to the measurement of thromboxane derivatives in urine to use a whole body approach. Earlier studies[50] showed that patients with hypercholesterolaemia had platelets which were more sensitive to certain agonists and that this could, in part, be explained by the enrichment of platelet plasma membranes by cholesterol.[51]

In vitro, LDLs slightly inhibit platelets, by standard aggregometry and by measurement of platelet activation using a more sensitive flow cytometric technique.[52] Furthermore, native LDLs inhibited the activation of platelets by oxidised LDLs (see below).[53] In contrast, others have shown an increased aggregability, when LDL is added *in vitro*, employing the sensitive filtragometric assay[54] in which all the other blood cell types are present, but not the inhibition by endothelial factors. However, in Types IIa and particularly in IIb hyperlipidaemia there was a decrease in activity of the platelets as measured by the filtragometric technique.[55] Furthermore, lipid lowering with drugs did not decrease platelet activation e.g. had no effect with lovastatin or following LDL apheresis treatment.[55]

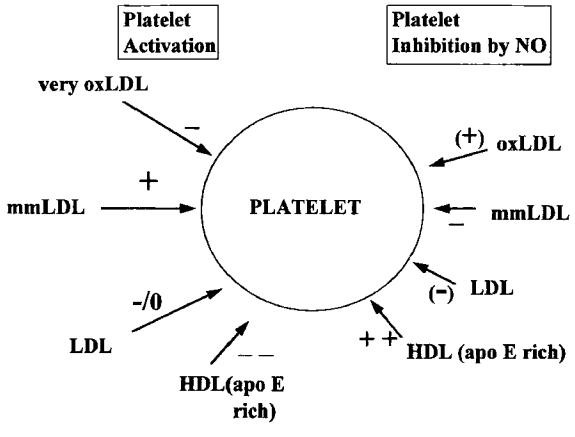

FIGURE 2 The influence of lipoproteins on activation of blood platelets and their inhibition by nitric oxide. – represents inhibition and + an augmentation the agonist-induced activation or inhibition by NO. Symbols in brackets represent a minor effect and where double, a major effect.

Important advances have been made to show that platelets do not have the conventional high affinity receptors for LDL, but that there are binding sites which may be on the key glycoprotein IIb/IIIa complex which is associated with fibrinogen binding,[56] although this has been disputed by others.[57] There may be a domain in the IIIa glycoprotein which has a significant similarity with a domain in the putative LDL binding region of the LDL-receptor.[27] Other lipoproteins are also active on platelets: apo E-rich HDLs inhibit platelets,[58] possibly by increasing the synthesis of NO in these cells.

Oxidised LDL and Platelet Function

Oxidised LDL has been shown to activate platelets more strongly than native LDL. One of the earliest papers on modification of lipoproteins concerned the effects of platelet activation on LDL, which led to their modification by malondialdehyde.[4] The upsurge in interest in the role of oxidised lipoproteins in atherosclerosis prompted the finding that LDL, which had been oxidised by Cu^{2+}, were more effective than native LDL in sensitising platelets,[59] increasing the release of arachidonic acid from platelet phospholipids.[60] Recent work shows that oxidised LDL, but not native LDL, may inhibit the Ca^{2+}–ATPase, thereby increasing the intracellular calcium concentrations.[61] Aviram actually found a reduction in activation of platelets by oxidised LDLs, unless the lipoproteins were subsequently dialysed,[62] after which they stimulated aggregation, presumably

because of residual effects of copper ions and some soluble oxidation products. The addition of malondialdehyde reduced the enhancement of platelet activation by dialysed oxidised LDL. Oxidised total HDL also had a stimulatory effect on platelets,[59] although less than LDL. These findings appear to be anomalous, because there are longstanding reports that oxidation products of arachidonic acid, such as 4-hydroxynonenal, is an inhibitor of platelet aggregation[62] as is lysophosphatidylcholine.[63] 15-hydroxyeicosatetraenoic acid (15-HETE) potentiates the action of thrombin.[64] However, there are also reports that lipid peroxides, at least at low concentrations,[65] enhance the responsiveness of platelets to agonists or promote direct activation.

Meraji *et al.*[66] suggested that mildly oxidised LDL may be more potent than those strongly oxidised in the presence of Cu^{2+} in terms of their effects on platelets, although the active agent was not identified. The minimally modified-LDL was characterised by a lower concentration of lipid peroxides than in the highly oxidised forms and with no significant modification of apolipoprotein B. These observations raised the question whether in the earlier work, where considerable variation in the effects of LDL on platelets was noted in different laboratories, this variation could be due to the presence of variable low concentrations of oxidation products. Some of these, when in excess, may bring about an inhibition of platelet activity. There is also considerable individual variation in the observed platelet responses to LDL obtained from different donors. Salonen *et al.* associated an increase in platelet aggregation and β-thromboglobulin in plasma with plasma lipid peroxide concentrations in individuals with a low plasma antioxidant profile.[67] Several investigators have reported the inhibition of platelet activation by α-tocopherol.[68] We have shown that mildly oxidised LDL, but not fully oxidised LDL, activates platelets as shown by conventional and FACS methodology and that this can be reproduced by enrichment of LDL with 15-hydroperoxyeicosatetraenoic acid (15-HPETE), a product of lipid oxidation in LDL.[52] These findings support the earlier observations of Weidtmann *et al.* showing that mmLDL enhanced platelet activation[69] and influences the tyrosine kinase mechanism. Since fully oxidised LDL is not considered to occur in the plasma, but mildly oxidised LDL may be present, these findings may have a greater physiological reality. The only likely contact that platelets may have with fully oxidised LDL may be in the event of fissuring of the atherosclerotic plaque.

Platelets, Lipoprotein Oxidation and NO

Platelets are important target cells for NO released from the endothelium and will themselves synthesise NO when activated.[70] Furthermore, NO is also present in the blood in the form of nitrosothiols.[71]

LDL or its oxidation products may, therefore, influence the inhibition of platelets by NO (Figure 2). Native LDL appeared to have little effect on inhibition of platelet activation by thrombin using authentic NO, yet enhanced the inhibition of platelets by S-nitrosoglutathione (GSNO), which releases its NO at the platelet membrane and may, therefore, not be sequestered by the lipids of LDL due to the known lipophilicity of NO.[27] In contrast, minimally modified LDL reduced the inhibition of platelets by NO. Lipid peroxides (15-HPETE) also decrease the sensitivity of platelets to NO to a limited extent, but the effect was stronger in the case of cumene hydroperoxide which is water soluble.[27] In contrast to other platelet agonists, oxidised LDL decreased the activation of platelet NO synthase.[72] This effect was reversed by the presence of HDL.[73]

Therefore, mildly oxidised LDL may augment the sensitivity of platelets to their agonists and diminish the influence of an important physiological antagonist.

CONCLUSION

Oxidation of LDL may, therefore, modulate the physiological functions of platelets, smooth muscle and the endothelium, either indirectly by their presence in the subendothelial space or directly because of low concentrations of mildly oxidised LDL in the circulation. At normal concentrations, native LDL may have some positive effects on arterial function and platelet activity. At pathophysiological levels, native LDL have detrimental actions, at least on endothelium-dependent relaxation. However, the oxidation of LDL changes the properties of the lipoproteins in a manner which is clearly disadvantageous and creates significant imbalances in the normal mechanisms of the vasculature.

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References

[1] R. Ross (1986) The pathogenesis of atherosclerosis – an update. *New England Journal of Medicine*, **314**, 488–500.
[2] H. Esterbauer, M. Dieber-Rotheneder, G. Waeg, G. Striegl and G. Jurgens (1990) Biochemical, structural and functional properties of oxidised low-density lipoproteins. *Chemical Research in Toxicology*, **3**, 77–91.
[3] S. Moncada (1990) The first Robert Furchgott lecture: from endothelium-dependent relaxation to the L-arginine: NO pathway. *Blood Vessels*, **27**, 208–217.
[4] A. M. Fogelman, J. S. Schechter, M. Hokom, J. S. Child and P. A. Edwards (1980) Malondialdehyde alteration of low-density lipoprotein leads to cholesterol accummulation in human monocyte-macrophages. *Proceedings of the National Academy of Sciences USA*, **77**, 2214–2218.
[5] D. Steinberg (1993) Modified forms of low-density lipoprotein and atherosclerosis. *Journal of Internal Medicine*, **233**, 227–232.
[6] T. J. Verbeuren, F. H. Jordaens, C. E. Van-Hove, A. E. Van-Hoydonck and A. G. Herman (1990) Release and vascular activity of endothelium-derived relaxing factor in atherosclerotic rabbit aorta. *European Journal of Pharmacology*, **191**, 173–184.
[7] H. E. Andrews, K. R. Bruckdorfer, R. C. Dunn and M. Jacobs (1987) Low-density lipoproteins I inhibit endothelium-dependent relaxation in rabbit aorta. *Nature*, **327**, 237–239.
[8] M. Jacobs, F. Plane and K. R. Bruckdorfer (1990) Native and oxidised low-density lipoproteins have different I inhibitory effects on endothelium-derived relaxing factor in the rabbit aorta. *British Journal of Pharmacology*, **100**, 21–26.
[9] P. J. Chowienczyk, G. F. Watts, J. R. Cockcroft and J. M. Ritter (1992) Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *Lancet*, **340**, 1430–1432.
[10] K.E. Sorensen, D. S. Celermajer, D. Georgokopoulos, G. Hatcher, J. D. Berridge and J. E. Deanfield (1994) Impairment of endothelium-dependent dilation is an early event in children with hypercholesterolaemia and is related to the lipoprotein (a) level. *Journal of Clinical investigation*, **93**, 50–55.
[11] C. B. Treasure, J. L. Klein, W. S. Weintraub *et al.* (1995) Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *New England Journal of Medicine*, **332**, 481–487.
[12] T. J. Anderson, I. T. Meredith, A. C. Yeung, B. Frei, A. P. Selwyn and P. Ganz (1995) The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *New England Journal of Medicine*, **332**, 488–493.
[13] T. J. Anderson, I. T. Meredith, F. Charbonneau, A. C. Yeung, B. Frei, A. P. Selwyn and P. Ganz (1996) Endothelium-dependent coronary vasomotion relates to the susceptibility of LDL to oxidation in humans. *Circulation*, **93**, 1647–1650.
[14] K. R. Bruckdorfer, A. M. Gleeson, C. Allot and M. Jacobs (1995) Regression of atherosclerotic lesions and the associated impairment of vasodilation by probucol. *Endothelium*, **2**, S10.
[15] F. Plane, K. R. Bruckdorfer, P. Kerr, A. Steuer and M. Jacobs (1992) Oxidative modification of low-density lipoproteins and the inhibition of relaxations mediated by endothelium-derived nitric oxide in rabbit aorta. *British Journal of Pharmacology*, **105**, 216–222.
[16] B. C. Simon, L. D. Cunningham and R. A. Cohen (1990) Oxidised low density lipoproteins cause contraction and inhibit endothelium-dependent relaxation in the pig coronary artery. *Journal of Clinical Investigation*, **86**, 75–79.
[17] C. Buckley, K. R. Bruckdorfer and M. Jacobs (1992) 15-lipoxygenase-modified low-density lipoproteins alter vascular tone in isolated rabbit aorta. *Journal of Vascular Research*, **29**, 91.
[18] K. Kugiyama, S. A. Kerns, J. D. Morrisett, R. Roberts and P. D. Henry (1990) Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. *Nature*, **344**, 160–162.
[19] C. Buckley, D. McManus, K. R. Bruckdorfer and M. Jacobs (1994) 9-hydroxy and hydroperoxylinoleic acids inhibit endothelium-dependent relaxations of rabbit aorta. *Br. J. Pharmacol.*, **112**, 222.
[20] C. Buckley, D. McManus, K.R. Bruckdorfer and M. Jacobs (1994) Hydroperoxy- and hydroxy-derivatives of

arachidonic acid inhibit endothelium-dependent relaxation. *British Journal of Pharmacology*, **112**, 222.
[21] A. M. Gleeson, K. R. Bruckdorfer and M. Jacobs (1995) Oxysterols and endothelium-dependent relaxations. *Endothelium*, **2**, S8.
[22] J. H. Chin, S. Azhar and B. B. Hoffmann (1992) Inactivation of endothelium-derived relaxing factor by oxidised lipoproteins. *Journal of clinical Investigation*, **89**, 10–18.
[23] J. A. Mitchell, T. D. Warner, Z-J Huang, U. Forstermann and F. Murad (1992) Native LDL inhibits the release of derived relaxing factor by reducing the activity of endothelial nitric oxide synthase. *Journal of Vascular Research*, **29**, 169.
[24] L. K. Liao, W. S. Shin, W. Y. Lee and S. L. Clark (1995) Oxidised low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. *Journal of Biological Chemistry*, **270**, 319–324.
[25] R. L. Minor, P. R. Myers, R. Guerra, J. N. Bates and D. G. Harrison (1990) Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *Journal of Clinical Investigation*, **86**, 2109–2116.
[26] K. Hirata, N. Miki, Y. Kuroda, T. Sakosda, S. Kawashima and M. Yokoyama (1995) Low concentrations of oxidised low-density lipoproteins and lysophosphatidylcholine upregulate constitutive nitric oxide synthase mRNA expression in bovine aortic endothelial cells. *Circulation Research*, **76**, 958–962.
[27] K. R. Bruckdorfer, K. M. Naseem, B. Mohammadi, S. Chirico, C. Ettelaie, A. Gleeson, T. Young, J. A. Adam, B. Wilbourn, V. O. Skinner, A.H. Goodall, J. Khan, S. C. Gates and M. Jacobs (1997) The influence of oxidised lipoproteins, oxidation products and antioxidants on the release of nitric oxide from the endothelium and the response of platelets to nitric oxide. *Biofactors*, **9**, 1–9.
[28] J. S. Beckman and J. P. Crow (1993) Pathological implications of nitric oxide, superoxide and peroxynitrite formation. *Biochemical Society Transactions*, **21**, 330–334.
[29] N. W. Kooy and J. A. Royall (1994) Agonist-induced peroxynitrite production of peroxynitrite by endothelial cells. *Archives of Biochemistry and Biophysics*, **310**, 352–359.
[30] A. Mugge, R. P. Brandes, R. H. Boger, A. Dwenger, S. Boder-Boger, S. Kienke and J. C. Frolich (1994) Vascular release of superoxide radicals is enhanced in hypercholesterolaemic rabbits. *J. Cardiovasc. Pharmacol.*, **24**, 994–998.
[31] H. Rubbo, S. Parthasarathy, S. Barnes, B. Kalyaranaram, M. Kirk and B. A. Freeman. (1995) Nitric oxide inhibition of lipoxygenase-dependent liposome and low-density lipoprotein oxidation: termination of radical chain propagation reactions and formation of nitrogen-containing oxidised lipid derivatives. *Archives of Biochemistry and Biophysics*, **324**, 25–25.
[32] W. Jessup and R. Dean (1993) Autoinhibition of murine macrophage-mediated oxidation of low-density lipoprotein by nitric oxide synthesis. *Atherosclerosis*, **101**, 145–155.
[33] G. Dee, C. Rice-Evans, S. Obeyesekera, S. Meraji, M. Jacobs and K. R. Bruckdorfer (1991) The modulation of ferryl myoglobin formation by nitric oxide and its oxidative effects on low-density lipoproteins. *FEBS Letters*, **294**, 38–42.
[34] W. A. Pryor and G. L. Squadrito (1995) The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *American Journal of Physiology*, **268**, L699–L722.
[35] R. P. Patel and V. M. Darley-Usmar (1996) Using peroxynitrite as oxidant with low-density lipoprotein. *Methods in Enzymology*, **269**, 375–384.
[36] A. H. Henderson (1991) Endothelium in Control. *British Heart Journal*, **65**, 116–125.
[37] J. A. Vita, C. B. Treasure, A. C. Yeung, V. I. Vekshtein, G. M. Fantasia, R. D. Fish, P. Ganz and A. P. Selwyn (1992) Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. *Circulation*, **85**, 1390–1397.
[38] P. A. Wines, J. M. Schmitz, S. L. Pfister, F. J. Clubb, L. M. Buja, J. T. Willerson and W. B. Campbell (1989) Augmented vasocontrictor responses to serotonin precede development of atherosclerosis in aortae of WHHL rabbits. *Arteriosclerosis*, **9**, 195–202.
[39] J. Galle, A. Luckhoff, R. Busse and E. Bassenge (1990) Low-density lipoproteins stimulate internal free calcium and prostacyclin release in endothelial cells. *Eicosanoids*, **3**, 81–86.
[40] T. J. Resink, V. A. Tkachuk, J. Bernhardt and F. R. Buhler (1992) Oxidised low-density lipoproteins stimulate phosphoinositide turnover in cultured vascular smooth muscle cells. *Atherosclerosis and Thrombosis*, **12**, 278–285.
[41] D. A. Cox and M. L. Cohen (1996) Selective enhancement of 5-hydroxytryptamine-induced contraction of porcine coronary artery by oxidised low-density lipoprotein. *Journal of Pharmacology and Experimental Therapeutics*, **276**, 1095–1103.
[42] K. Schmidt, P. Klatt, W. F. Grailer, G. M. Kostner and W. R. Kukovetz (1992) High density lipoprotein antagonizes the inhibitory effects of oxidised low-density lipoprotein and lysolecithin on soluble guanylyl cyclase. *Biochemical an Biophysical Research Communications*, **182**, 302–308.
[43] M. T. Jay, S. Chirico, R. C. M. Siow, K. R. Bruckdorfer, M. Jacobs, D. S. Leake, J. D. Pearson and G. E. Mann (1997) Effect of low-density lipoproteins on L-arginine transport and nitric oxide and prostacyclin synthesis in human vascular endothelial cells. *Experimental Physiology*, **82**, 349–360.
[44] S. Chatterjee and N. Ghosh (1996) Oxidised low-density lipoprotein stimulates smooth muscle cell proliferation. *Glycobiology*, **6**, 303–311.
[45] E. Nishio, S. Arimura and Y. Watanabe (1996) Oxidised LDL induces apoptosis in cultured smooth muscle cells: a possible role for 7-ketocholesterol. *Biochemical and Biophysical Research Communications*, **223**, 413–418.
[46] N. Auge, N. Andrieu, A. Negre-Salvayre, J. C. Thiers, T. Levase and R. Salvayre (1996) The sphingomyleinceramide signalling pathway is involved in oxidised low-density lipoproteins-induced cell proliferation. *Journal of Biological Chemistry*, **271**, 19251–19255.
[47] Y. C. Chai, P. H. Howe, P. E. Di Corletto and G. M. Chisholm (1996) Oxidised low-density lipoproteins and lysophosphatidylcholine stimulate cell cycle entry in vascular smooth muscle cells – evidence for release of fibroblast growth factor-2. *Journal of Biological Chemistry*, **271**, 17791–17797.
[48] E. Koller, T. Vukovich, W. Doleschel and W. Auerswald (1979) Uber den Einfluss von lipoproteinen auf die ADP-induzierte thrombozytenaggregation. *Atherogenese*, **4** (suppl. IV), 53–68.
[49] J. Zahavi, J. D. Betteridge, N. A. G. Jones, D. J. Galton and V. V. Kakker (1981) Enhanced *in vivo* platelet release

reaction and malondialdehyde formation in patients with hyperlipidaemia. *American Journal of Medicine*, **70**, 59–64.
[50] A. C. Carvalho, R. W. Colman and R. S. Lees (1974) Platelet function in hypercholesterolaemia. *New England Journal of Medicine*, **290**, 430–437.
[51] S. J. Shattil and R. A. Cooper (1977) Membrane microviscosity and human platelet function. *Biochemistry*, **15**, 4832–4838.
[52] K. M. Naseem, A. H. Goodall and K. R. Bruckdorfer (1997) Differentiated effects of native and oxidatively modified lipoproteins on platelet function. *Platelets*, **8**, 163–173.
[53] Y. Takahashi, H. Chiba, K. Matsuno *et al.* (1996) Native lipoproteins inhibit platelet activation induced by oxidised lipoproteins. *Biochemical and Biophysical Research Communications*, **222**, 453–459.
[54] A. Broijersen, N. H. Wallen, P. T. Vitols, P. Larsson and P. Hjemdahl (1993) Autologous low density lipoprotein enhances platelet aggregation in whole blood as measured *in vitro* filtragometry. *Platelets*, **4**, 11–95.
[55] A. Broijerson, M. Eriksson, P. T. Larsson, O. Beck, B. Berglund, B. Angelin and P. Hjemdahl (1994) Effects of selective LDL-apheresis and pravastatin therapy on platelet function in familial hypercholesterolaemia. *European Journal of Clinical Investigation*, **24**, 488–498;
[56] E. Koller, F. Koller and F. R. Binder (1989) Purification and identification of the lipoprotein-binding proteins form human blood platelet membrane. *Journal of Biological Chemistry*, **264**, 12412–12418.
[57] J. Pedreno, J. Fernandez, C. Cullare, A. Barcelo and M. A. Elorza. (1997) Platelet integrin alpha (IIb) beta 3 is not implicated in hte binding of LDL to intact resting platelets. *Arteriosclerosis, Thrombosis and Vascular Biology*, **17**, 156–163.
[58] D. R. Ridell and J. S. Owen (1997) Apolipoproteins E inhibits platelet responsiveness through the L-arginine: nitric oxide pathway – implications for vascular disease. *Journal of Biological Chemistry*, **272**, 69–95.
[59] N. G. Ardlie, M. L. Selley and L. A. Simons (1989) Platelet activation by oxidatively modified low-density lipoproteins. *Atherosclerosis*, **76**, 117–122.
[60] M. Aviram (1989) Modified forms of low-density lipoproteins affect at aggregation *in vitro*. *Thrombosis Res.*, **53**, 561–567.
[61] B. Zhao, R. Dierichs, F. N. Miller and W. L. Dean (1996) Oxidised low density lipoprotein inhibits platelet plasma membrane Ca^{2+}-ATPase. *Cell Calcium*, **19**, 453–458.
[62] M. Aviram (1990) Malondialdehyde affects the physicochemical and biological characteristics of oxidised low density lipoprotein. *Atherosclerosis*, **84**, 141–143.
[63] T. F. Slater, C. Bendetto, K. H. Cheeseman, M. Collins, M. J. Davies, S. Flatman, M. Hayashi, J. S. Hurst, R. G. McDonald-Gibson, A. Morgan, S. Nigam and K. Proudfoot (1986) Free radical and prostaglandin disturbances in cancer. *Free radicals, cell damage and disease* (ed. by C. Rice-Evans), p. 57. Richelieu Press, London.
[64] E. E. M. Besterman and M. P. T. Gillett (1971) Inhibition of platelet aggregation. *Atherosclerosis*, **14**, 323–330.
[65] B. N. Y. Setty, M. H. Werner, Y. A. Hannun and M. J. Stuart (1992) 15-hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositide-derived 2nd messengers – sn-1,2-diacylglycerol and inositol-1,4,5-trisphosphate. *Blood*, **80**, 2765–2773.
[66] Y. Fujimoto, S. Takai, K. Matsuno, T. Sumiya, H. Nishida, S. Sakuma and T. Fujita (1992) Effect of tert-butyl hydroperoxide on cyclooxygenase and lipoxygenase metabolism of arachidonic acid in rabbit platelets. *Prostaglandins Leukotrienes and Essential Fatty Acids*, **47**, 259–264.
[67] S. Meraji, C. E. Moore, V.O. Skinner and K. R. Bruckdorfer (1992) The importance of oxidation or glycosylation of low-density lipoproteins in relation to platelet activation. *Platelets*, **3**, 155–162.
[68] J. T. Salonen, R. Salonen, K. Seppanen, S. Rinta-Kiikka, M. Kuukka, H. Korpela, G. Alfthan, M. Kantola and W. Schalch (1991) Effects of antioxidant supplementation on platelet function: a randomized pair-matched, placebo-controlled, double-blind trial in men with low antioxidant status. *American Journal of Clinical Nutrition*, **53**, 1222–1229.
[69] C. Calzada, C. A. Rice-Evans and K. R. Bruckdorfer (1997) The influence of antioxidant nutrients on platelet function in healthy volunteers. *Atherosclerosis*, **128**, 97–105.
[70] A. Weidtmann, F. Schriethe, N. Hrboticky, A. Pietsch, R. Lorenz and W. Siess (1995) Mildly oxidised LDL enhances platelet aggregation through activation of phospholipase A_2. *Ateriosclerosis Thombosis and Vascular Biology*, **15**, 1131–1138.
[71] S. Moncada, M. Radomski and R. M. J. (1988) Endothelium-derived relaxing factor: identification as nitric oxide and role in the control of vascular tone and platelet function. *Biochemical Pharmacology*, **37**, 2495–2501.
[72] J. S. Stamler, D. I. Simon, J. A. Osbourne, M. E. Mullins, O. Jakari, T. Michel, D. Singel and J. Loscalzo (1992) S-nitrosylation of proteins with nitric oxide: synthesis and characterisation of novel biologically active compounds. *Proc. Natl. Acad. Sci. USA*, **89**, 444–448.
[73] L. Y. Chen, P. Mehta and J. L. Mehta (1996) Oxidised LDL decreases L-arginine uptake and nitric oxide synthase protein expression in human platelets: relevance of the effect of oxidised LDL on platelet function. *Circulation*, **93**, 1740–1746.
[74] L. J. Mehta and L. Y. Chen (1996) Reversal by high density lipoproteins of the effects of oxidised LDL on nitric oxide synthase protein expression in human platelets. *Journal of Laboratory and Clinical Medicine*, **127**, 287–295.