

Editorial

Oxidised low density lipoproteins and atherogenesis

Research into atherogenesis has been altered radically in the past decade, mainly by the realisation that the macrophage has an important (maybe central) role in this disease and that low density lipoprotein (LDL) itself may not be atherogenic but rather LDL modified by oxidation (and perhaps other mechanisms)

About a decade ago in Steinberg's laboratory in San Diego LDL was first shown to be taken up much faster by macrophages after it had been incubated with arterial endothelial cells in culture.¹ This effect was later shown to be due to the oxidation of the LDL by the endothelial cells.² Oxidised LDL (but not normal LDL) is recognised by a receptor (or possibly a family of receptors) on macrophages known as the scavenger receptor, which has now been cloned.³ Oxidised LDL is then rapidly endocytosed by the macrophages, delivered to the lysosomes, and degraded. The cholesterol accumulates within the macrophage, mainly as cholesteryl esters.

Scavenger receptors are also found on endothelial cells,⁴ but only in low numbers, and on arterial smooth muscle cells, but only after stimulation by phorbol esters or platelet products.⁵

Sequence of events

We can therefore envisage a scenario in which plasma LDL enters the arterial wall from the luminal surface and is locally oxidised within the intima of the artery. All four major cell types within atherosclerotic lesions (endothelial cells, smooth muscle cells, macrophages, and lymphocytes) can oxidise LDL⁶⁻⁹ but the macrophage seems to be the most active. The oxidised LDL may then be internalised rapidly by macrophages, converting them into cholesterol-laden foam cells. Many of the foam cells in human atherosclerotic lesions are now known to be derived from macrophages.¹⁰

LDL oxidation is believed to occur locally within atherosclerotic lesions rather than within the general circulation, because even a few per cent of serum will completely inhibit oxidation of LDL.⁸

LDL oxidation by cells is believed to be caused by free radicals, substances with unpaired electrons which tend to be highly reactive. The nature of these free radicals and their sources within the cells are still highly controversial, because free radical chemistry is difficult to study. We still do not know whether cells oxidise LDL by releasing simple oxidising agents (such as superoxide (O_2^-) or hydrogen peroxide) which then attack LDL or by releasing lipid peroxidation products, formed by the oxidation of the cells' own lipids. These lipid peroxidation products may then become incorporated into the LDL particles and initiate the oxidation of the LDL's own lipids.

Whatever the mechanism, the polyunsaturated fatty acids in LDL become oxidised and converted into lipid hydroperoxides. These fragment into aldehydes, which are believed to combine covalently with lysine and maybe other amino acids in apolipoprotein B-100, the protein moiety of LDL.^{11,12} The modified apolipoprotein B-100 then becomes recognised by the scavenger receptors of

macrophages.

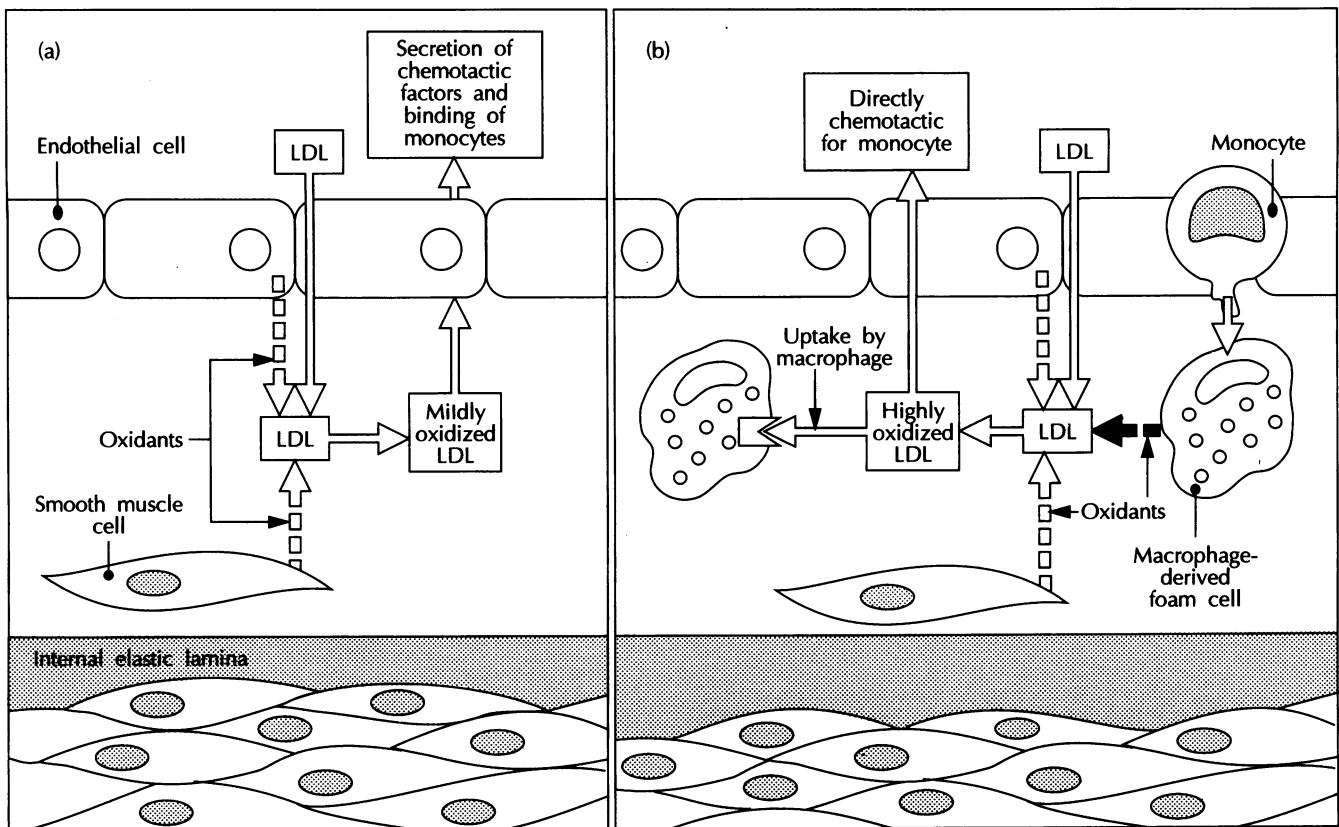
The evidence that this is what happens in atherosclerotic lesions in vivo is accumulating rapidly. LDL gently extracted from human or animal lesions was shown to be oxidised and was taken up faster by macrophages by their scavenger receptors.¹³ Also antibodies against oxidised LDL stained lesions but not the normal arterial wall.¹⁴ In addition, lipid-soluble antioxidants that inhibited LDL oxidation in vitro protected against atherosclerosis in laboratory animals. Probucol protected against lesion development in most^{15,16} but not all studies¹⁷ in rabbits, butylated hydroxytoluene protected in rabbits,¹⁸ and vitamin E protected in primates.¹⁹ Evidence about the effects of vitamin E in rabbits was conflicting and difficult to interpret.) Definitive proof that oxidised LDL is causally related to atherogenesis in humans, however, is still awaited.

As well as converting macrophages into cholesterol-laden foam cells, oxidised LDL may be involved in atherogenesis in several other ways (reviewed by Steinberg *et al*¹). It is directly chemotactic for monocytes and once they have entered the arterial wall and differentiated into macrophages it may inhibit their motility, trapping them within the wall. Oxidised LDL is cytotoxic, because of the lipid peroxidation products it contains, and this may explain some of the damage that occurs to the endothelium and macrophages in the more advanced lesions. It also inhibits the generation or effects or both of endothelium derived relaxing factor (nitric oxide) in arteries and therefore could contribute to vasospasm in diseased vessels.

There has recently been much interest in the effects of mildly oxidised LDL (or minimally modified LDL), which has effects that are very different from those of the highly oxidised LDL studied previously. Thus mildly oxidised LDL (but not highly oxidised LDL) increases the binding of monocytes to cultured endothelial cells²⁰ and activates the gene for monocyte chemotactic factor-1 in human aortic endothelial cells and smooth muscle cells.²¹ This could explain why monocytes enter the arterial wall in the first place. Once present in the wall, monocytes may be largely responsible for converting LDL into highly oxidised LDL which (unlike mildly oxidised LDL) binds to the scavenger receptors of macrophages and leads to their conversion into cholesterol-laden foam cells (reviewed by Leake²²) (figure).

Susceptibility to oxidation

Polyunsaturated fatty acids are readily oxidised by free radicals, whereas monounsaturated fatty acids are much more resistant and saturated fatty acids are not oxidisable. Parthasarathy *et al* made an important discovery when they found that feeding rabbits a diet high in monounsaturated fatty acids rather than one rich in polyunsaturated fatty acids enriched their LDL with monounsaturated fatty acids and made it much less susceptible to oxidation in vivo.²³ This effect has now been shown in several studies in humans.²⁴ It is now known that replacing saturated fatty acids in the diet by either



Mildly oxidised LDL may be involved in the initiation of fatty streaks and highly oxidised LDL may be involved in their progression. (a) LDL may enter the arterial intima, which may be very thin in animals but thicker in humans because of diffuse intimal thickening, and may then be converted into mildly oxidised LDL by free radicals or oxidised lipids released from the endothelial or smooth muscle cells (\rightarrow). This mildly oxidised LDL may cause the endothelial cells to release greater amounts of a chemotactic factor for monocytes and to bind the monocytes to a greater extent. (b) The monocytes may then enter the arterial intima, differentiate into macrophages, release large amounts of free radicals or oxidised lipids (\rightarrow), and convert the LDL into highly oxidised LDL. This may bind to scavenger receptors on the macrophages, be internalised rapidly, and convert them into cholesterol-laden foam cells. The highly oxidised LDL may be directly chemotactic for monocytes (because of its lysophosphatidylcholine content) and may attract more monocytes into the arterial wall, leading to the progression of the lesions. Reproduced with permission from *Current Opinion in Lipidology*.²²

polyunsaturated or monounsaturated fatty acids will lower plasma cholesterol concentrations in humans, although their relative efficacies are still controversial. There is general agreement that reducing the saturated fat content of the diet is advisable but what is much less clear is whether we should aim to replace it by polyunsaturates or by the less oxidisable monounsaturates? This is a complex question that also encompasses effects of various fatty acids on thrombosis and at present is not easy to answer.

Vitamin E seems to be the single most important antioxidant in LDL and when vitamin E is taken by mouth its concentration in LDL increases 2–4 fold. After isolation this vitamin E enriched LDL was less susceptible to oxidation in *in vitro* test systems, including oxidation by macrophages.^{25–27} LDL is golden yellow because it contains the antioxidant β carotene. The β carotene content of LDL was greatly increased when subjects were given β carotene by mouth, but the LDL did not seem to be any less susceptible to oxidation after isolation.²⁷

There is epidemiological evidence that high vitamin E or β carotene intake is associated with a low rate of coronary heart disease. Gey *et al* found that mortality from coronary heart disease was strongly inversely related to dietary vitamin E when they compared several different European populations.²⁸ Though β carotene does not apparently render LDL less susceptible to oxidation, its intake was also inversely correlated with coronary heart disease but less strongly so. In a Scottish study, the risk of angina was inversely and independently related to the plasma concentration of vitamin E.²⁹ In a very large study of nurses in the United States the risk of coronary heart

disease was 30–40% less in those with a high intake of vitamin E or β carotene and a prospective study of elderly people also in the United States, showed independent inverse relation between β carotene intake and cardiovascular mortality.³⁰ The epidemiological evidence for a protective effect of the water soluble antioxidant vitamin C is weaker but Gey *et al* reported an apparent synergism between vitamins E and C.²⁸ It may be worth noting that there is biochemical evidence that vitamin C can react with vitamin E radicals that have already acted as lipid soluble antioxidants and convert them back into intact vitamin E molecules that can act as antioxidants again.³¹

Evidence from trials

Epidemiological studies do not, of course, provide evidence of cause and effect: clinical studies are required for this. There is only one such study at the moment, the Harvard Physicians' Health Study, which has been reported in preliminary form.³² This trial involved 333 men with chronic stable angina or who had had coronary revascularisation, some of whom were given β carotene. This compound reduced the number of cardiovascular events by 50%. Because of this apparently very large effect, the trial is continuing as a primary prevention trial on over 20 000 physicians and the outcome should be known in about three years' time. A primary prevention trial has recently begun on 40 000 women in the United States who will be given vitamin E, β carotene, or aspirin in a factorial design. The results of the Swedish PQRST study on the effects of probucol on the progression of femoral atherosclerosis measured by angiography should

be available shortly. Even if protection against atherosclerosis can be demonstrated, however, this trial may not provide conclusive proof of the possible efficacy of antioxidants in cardiovascular disease because, in addition to inhibiting LDL oxidation, probucol lowers the plasma concentrations of LDL and HDL cholesterol and has several other effects that may be related to atherogenesis.

Great progress has been made in the past few years in our understanding of the pathogenesis of atherosclerosis, especially of the role of oxidation mediated by free radicals and before the start of the next millenium we may know whether antioxidants protect against coronary heart disease in humans. Instead of merely trying to correct the risk factors for atherosclerosis, we may then be able to try to directly influence the course of the disease within the arterial wall.

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