Mildly oxidised LDL induces more macrophage death than moderately oxidised LDL

Mildly oxidised LDL induces more macrophage death than moderately oxidised LDL: roles of peroxidation, lipoprotein-associated phospholipase A_2 and PPARγ

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Abstract Death of macrophages and smooth muscle cells (SMC) can lead to progression of atherosclerosis. Mildly oxidised low-density lipoprotein (mildly-oxLDL) induced more overall death and apoptosis than moderately oxidised LDL, in human monocyte-macrophages (HMM). Mildly-oxLDL also induced more overall death in human SMC than did moderately-oxLDL. Mildly-oxLDL contained more hydroperoxides, but less oxysterol, malondialdehyde and negative charge than moderately-oxLDL. Specific inhibition of lipoprotein-associated phospholipase A_2 (by SB222657) diminished death induction in HMM by both oxLDL types. Peroxisome proliferator-activated receptor γ (PPARγ) antagonist (GW9662) and agonist (ciglitazone) experiments suggested that non-hydrolysed, oxidised phospholipids in oxLDL activate PPARγ as a cellular defence mechanism. These results may be relevant to LDL oxidation within atherosclerotic plaques and may suggest strategies for combating atherosclerosis progression.

Key words: Cell death; Oxidized low-density lipoprotein; Peroxidation; Lipoprotein-associated phospholipase A_2; Peroxisome proliferator-activated receptor γ; Monocyte-macrophage (human)

1. Introduction

Death of macrophages and smooth muscle cells (SMC) occurs in human advanced atherosclerotic lesions (plaques), by apoptosis and necrosis [1–3]. Macrophage foam cells that die and are not phagocytosed can spill lipid into the extracellular environment and so contribute to the lipid core of the plaque [3,4], whilst death of SMC erodes the fibrous cap [2]. Such changes destabilise plaques, increasing the risk of rupture and consequent thrombosis [5–7].

Evidence implicating oxidation of low-density lipoprotein (LDL) in atherosclerosis progression includes that oxidised LDL (oxLDL) and lipid peroxidation products occur in atherosclerotic lesions [8–10], and that oxLDL is toxic and apoptosis-inducing for macrophages and SMC in vitro [11–15].

OxLDL contains a complex, variable, incompletely characterised mixture of toxic oxidation products. Lipid hydroperoxides appear relatively early on in the oxidation and aldehydes arise from their subsequent breakdown [16]. Aldehydes modify the LDL protein (apoB-100), increasing its negative charge [16]. Cholesterol oxidation products (also termed oxysterols) appear consequentially to the peroxidation of polyunsaturated fatty acids (PUFA) within LDL [17,18]. The LDL-borne enzyme lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also termed platelet-activating factor acetylhydrolase, hydrolyses oxidised phosphatidylcholine (oxPC), but not non-oxidised phosphatidylcholine (PC), producing lysophosphatidylcholine (lyso-PC) and oxidised, non-esterified fatty acids (oxNEFA)[19,20].

Lipid oxidation products can activate cell transcription factors known as peroxisome proliferator-activated receptors (PPAR), especially PPARγ, which may induce apoptosis. Various synthetic PPARγ activators induced apoptosis in vitro, in macrophages [21] and in cancer cell lines [22,23]. A natural PPARγ activator, 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2), a cyclo-oxygenase metabolite of arachidonic acid, induced apoptosis in vitro, in macrophages and endothelial cells [21,24]. OxLDL and certain LDL oxidation products, 9- and 13-hydroxyoctadecadienoic acids, activated PPARγ in CV-1 cells [25]. In human atherosclerotic lesions, PPARγ occurs in macrophages in a pattern highly correlated with oxidation-specific epitopes [26–28].

A moderately oxidised form of LDL decreased SMC viability and induced SMC apoptosis in vitro, whereas a more strongly oxidised form of LDL did not, and neither did a very mildly oxidised form of LDL or native LDL (natLDL)[15]. In macrophages, moderately oxidised LDL induced death, including apoptosis, which was diminished by inhibiting Lp-PLA_2 within natLDL prior to oxidation [20]. The present study's purpose was to investigate the effect of degree of

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Abbreviations: AO, acridine orange; 15d-PGJ_2, 15-deoxy-Δ12,14-prostaglandin J_2; ELISA, enzyme-linked immunosorbent assay; FCS, foetal calf serum; FOX, ferrous oxidation of xylenol orange; GC, gas chromatography; HMM, human monocyte-macrophages; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; lyso-PC, lysophosphatidylcholine; MDA, malondialdehyde; NA, no additions; natLDL, native low-density lipoprotein; oxLDL, oxidised low-density lipoprotein; ox(1)LDL, ox(2)LDL and ox(3)LDL, very mildly, mildly and moderately oxidised low-density lipoprotein; oxNEFA, oxidised non-esterified fatty acid; oxPC, oxidised phosphatidylcholine; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PFB, Pefabloc; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; REM, relative electrophoretic mobility; SMC, smooth muscle cells; TBARS, thiobarbituric acid-reactive substances

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LDL oxidation, focusing on mild and moderate degrees, on macrophage death (overall death, as well as apoptosis), and to explore the roles of Lp-PLA$_2$ and PPARγ therein. SMC were compared in selected cases.

2. Materials and methods

Ciglitazone and M199 medium were from Sigma. SB222657 and GW9662 were gifts of Glaxo SmithKline. Other materials were as described previously [20]. Dimethyl sulphoxide (final concentration ≤0.5% v/v) was the solvent vehicle for ciglitazone, SB222657 and GW9662.

Isolation (from buffy coat) and culture conditions for human monocyte-macrophages (HMM) were as described previously [20]. HMM were cultured for 1 day before starting experiments, unless stated otherwise. Mature HMM were produced by culturing monocytes for 4–5 days before starting experiments. All HMM cultures and experiments were in Gibco Macrophage-SFM (serum-free medium), in 24-well plates. SMC derived from human aortic explants were cultured in M199 medium (Sigma) plus 20% foetal calf serum (FCS) in T75 (75-cm^2) tissue culture flasks (Falcon, Becton Dickinson). Medium was renewed every 2–3 days. Cultures were passaged when nearing confluence, and were not used above passage 11. For toxicity experiments, SMC were plated into 24-well plates in M199 (without phenol red) plus 2% FCS and were used at c. 80% confluence.

Isolation of natLDL and oxidation at 1 mg LDL protein/ml by Cu^{2+} ions (10 μM) in phosphate-buffered saline (PBS; pH 7.4) for 15 h at 37°C, producing moderately-oxLDL (ox(3)LDL) were as described previously [20]. We adapted Fe^{2+}-mediated LDL oxidation conditions from the literature [16,29,30], as follows. Fe^{2+} ions (iron-(II) sulphate, 10 μM) were used to oxidise LDL (1 mg LDL protein/ml) for 15 h at 37°C, either in saline (sodium chloride 0.9% w/v in high-purity Milli-Q deionised water) producing mildly-oxLDL (ox(2)LDL) or at pH 5.5 in PBS producing very mildly-oxLDL (ox(1)LDL). Treatment of natLDL with SB222657 (2 μM) or Pefabloc (PFB; 500 μM) prior to oxidation was as described previously [20].

After 15 h oxidation, oxLDLs were Chelex-treated and diluted to 250 μg LDL protein/ml with culture medium for incubation with cells. Triplicate or duplicate culture wells of cells were used for each oxLDL (or control) treatment for the nucleosome enzyme-linked immunosorbent assay (ELISA) and triplicate culture wells for the lactate dehydrogenase (LDH) release assay (see below). Incubations of oxLDLs with cells were for 48 h unless stated otherwise. Negative controls were cells incubated in these media with no additions (NA), or with natLDL. Gliotoxin (3 μM), an established apoptosis-inducing agent for HMM, was used for a positive control.

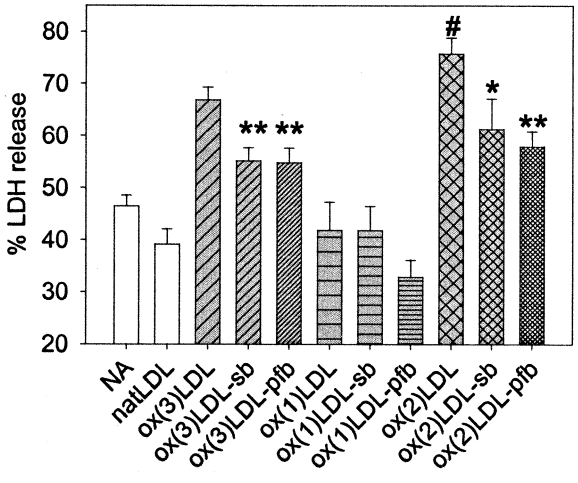

Fig. 1. Cytotoxicity of oxLDLs, measured by the LDH release assay in HMM, after 48 h exposure. Control cells were cultured without LDL or oxLDLs, i.e. NA. The suffixes -sb or -pfb indicate that the oxLDL was prepared with pretreatment (prior to oxidation) of natLDL with SB222657 or with PFB. Each histogram bar represents mean ± S.E.M., $n = 9$–15. ANOVA $P < 0.0001$. $^{**}P < 0.01$, $^{*}P = 0.037$, significantly different from the corresponding oxLDL prepared without SB222657 or PFB pretreatment. $^{\#}P = 0.0323$, ox(2)LDL significantly different from ox(3)LDL. OxLDLs were significantly different from NA ($P < 0.05$), except for ox(1)LDL and ox(1)LDL-sb.

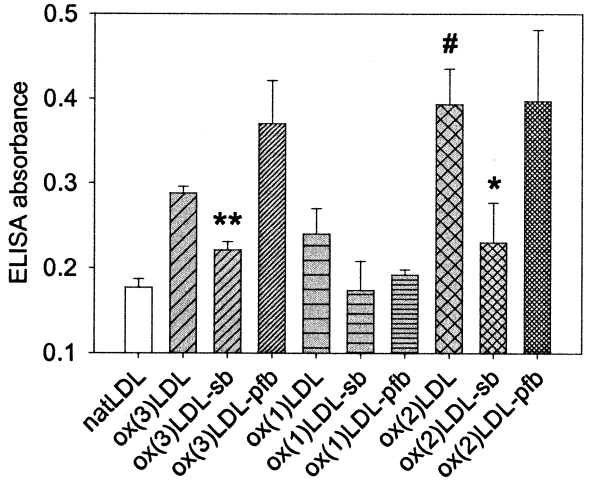

Fig. 2. Apoptosis, measured by nucleosome ELISA, in HMM exposed to oxLDLs for 48 h. Each histogram bar represents mean ± S.E.M., $n = 5$–9. ANOVA $P < 0.0001$. $^{**}P = 0.0002$, $^{*}P = 0.027$, significantly different from the corresponding oxLDL prepared without SB222657 pretreatment. $^{\#}P = 0.0232$, ox(2)LDL significantly different from ox(3)LDL. Gliotoxin (3 μM for 4 h), a positive control, gave absorbance 0.389 ± 0.019 ($n = 6$). OxLDLs were significantly different from NA ($P < 0.05$), except for ox(2)LDL-sb, ox(1)LDL-sb and ox(1)LDL-pfb.

Total hydroperoxides were measured by the ferrous oxidation of xylenol orange (FOX) assay, version 'FOX2' [31], and in selected cases additionally by an iodometric method [32]. Other assays were as described previously [20].

Data are reported as mean ± S.E.M.; n is the number of individual specimens or culture wells. Statistical analysis was by ANOVA and Student's t-test (unpaired), using StatView 5.0.1 software. The level of significance was $P < 0.05$. Experiments were repeated several times and representative data are presented.

3. Results and discussion

Ox(3)LDL, ox(2)LDL and ox(1)LDL were compared for cell death-inducing effects and for chemical composition.

Ox(2)LDL induced significantly more overall death of HMM than ox(3)LDL, measured by the LDH release assay (Fig. 1). Likewise, for SMC, LDH release after 48 h was significantly higher ($P = 0.0009$) for ox(2)LDL (73.2 ± 0.6%, $n = 3$) than for ox(3)LDL (44.2 ± 3.2%, $n = 3$). SMC LDH release was 12.2 ± 1.2% ($n = 3$) for NA and 13.4 ± 1.7% ($n = 3$) for natLDL. Ox(2)LDL induced significantly more HMM apoptosis (measured by nucleosome ELISA) than did ox(3)LDL (Fig. 2). Ox(1)LDL was non-toxic to HMM, measured by LDH release (Fig. 1), although it induced a low (yet statistically above-background) degree of HMM apoptosis (Fig. 2), less than ox(3)LDL.

Time-course measurements (0–24 h; Fig. 3) by the FOX assay and relative electrophoretic mobility (REM) on agarose gels demonstrated that 15 h oxidation appeared optimal for differentiating composition between the three types of oxLDL, and so was adopted to prepare oxLDLs for cell death induction studies, reported above. During moderate LDL oxida-

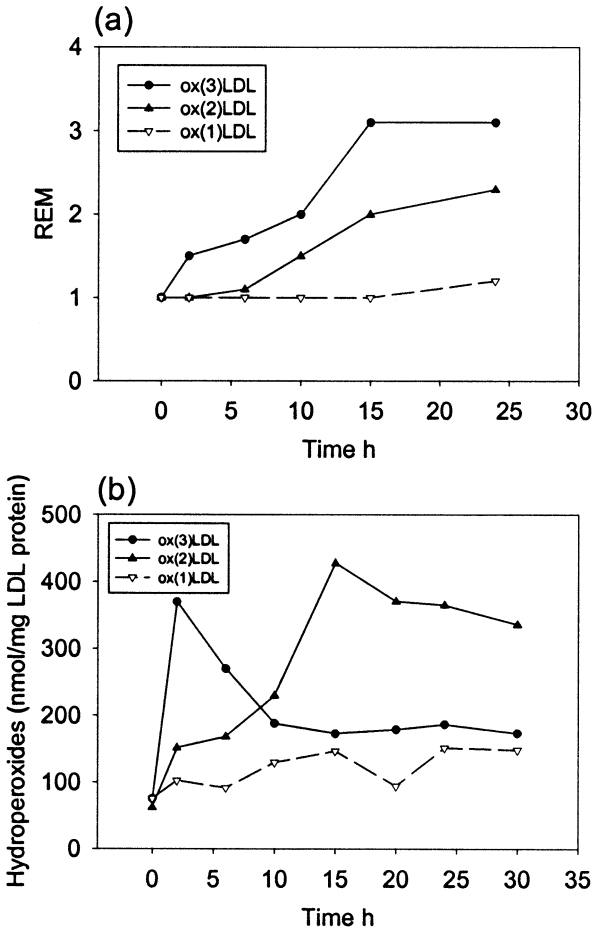

Fig. 3. Time course of LDL oxidation. a: REM, measured on agarose gels. b: Levels of hydroperoxides, assayed in triplicate (data points are means) by the FOX method, for ox(3)LDL, ox(2)LDL and ox(1)LDL.

tion, hydroperoxides peak early on, and then decline rapidly. For mild LDL oxidation, hydroperoxides peak later and remain high, as decline is slow. After 15 h oxidation, ox(2)LDL consistently contained higher levels of hydroperoxides (FOX assay), lower levels of oxysterols measured by gas chromatography (GC), lower levels of malondialdehyde (MDA) equivalents measured by the thiobarbituric acid-reactive substances (TBARS) assay and lower REM (i.e. less negative charge), than did ox(3)LDL (Fig. 4). Hydroperoxide levels in ox(1)LDL were only slightly above natLDL, whilst REM, TBARS and 7β-hydroxycholesterol in ox(1)LDL were similar

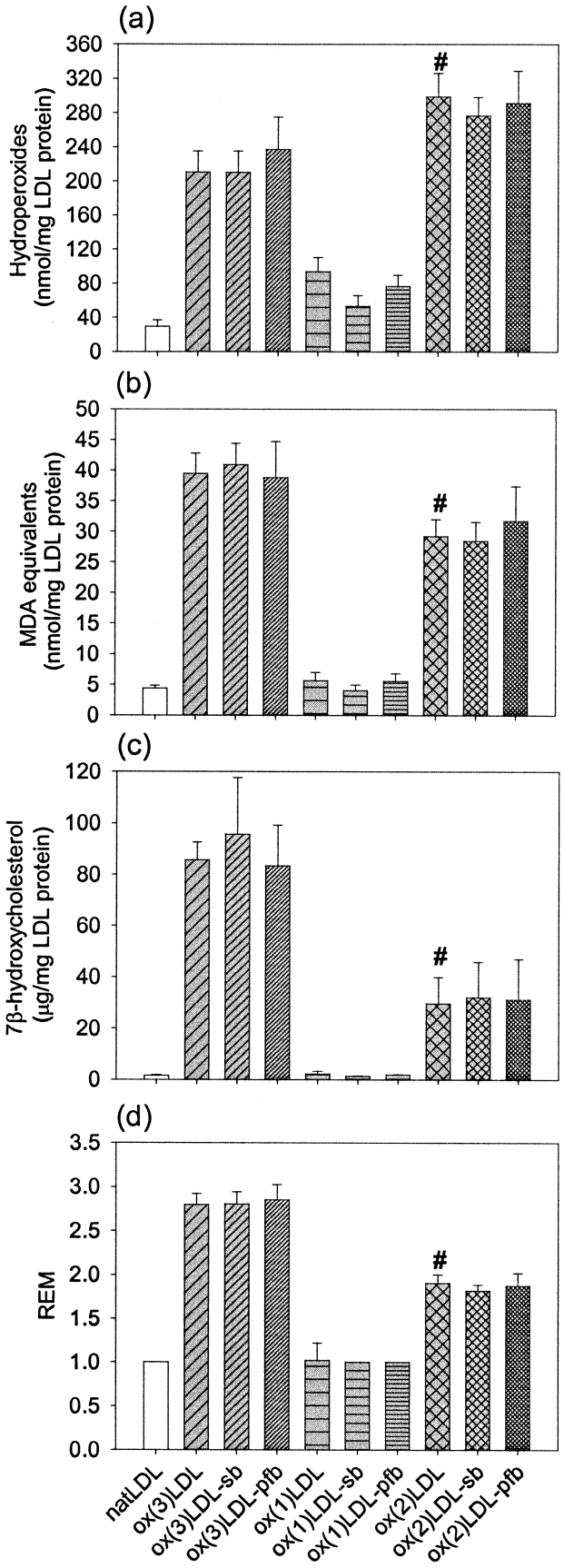

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Fig. 4. Degree of oxidation of oxLDLs used for cell death studies. Oxidation was for 15 h. Levels of (a) hydroperoxides, measured by the FOX assay, (b) MDA equivalents, measured by the TBARS assay, (c) 7β-hydroxycholesterol, measured by GC and (d) REM, measured on agarose gels. Each histogram bar represents mean ± S.E.M., n = (a) 9–18, (b) 7–17, (c) 5–6, except for ox(1)LDL, 2, ox(1)LDL-sb, 2, and ox(1)LDL-pfb, 2, (d) 9–19. ANOVA $P < 0.0001$ for each of a–d. #Ox(2)LDL significantly different from ox(3)LDL, (a) $P = 0.0209$, (b) $P = 0.026$, (c) $P = 0.0012$, (d) $P < 0.0001$. Corresponding oxLDLs prepared with or without SB222657 or PFB pre-treatment were not significantly different from each other. OxLDLs were significantly different from natLDL ($P < 0.05$), except for (a) ox(1)LDL-sb, (b–d) ox(1)LDL, ox(1)LDL-sb and ox(1)LDL-pfb.

to natLDL (Fig. 4). Hydroperoxide results by the iodometric method (data not shown) were similar to the FOX assay.

The nucleosome ELISA measures cytoplasmic histone-associated DNA fragments (mono- and oligo-nucleosomes), apoptosis markers arising from internucleosomal cleavage as a result of activation of endogenous endonucleases and proteases. We also used acridine orange (AO) staining of HMM (fixed in ethanol–PBS 70:30 v/v) to measure apoptosis by fluorescence microscopy as percentage of HMM with morphologically condensed nuclear chromatin, another characteristic of apoptosis [33]. In principle, nuclear condensation can occur before DNA fragmentation. Such cell counts are, however, less quantitatively discriminating than the nucleosome ELISA. Assessed by AO staining, ox(2)LDL and ox(3)LDL appeared both very potent, respectively giving apoptotic cells as 88% and 87% of total cells, whilst natLDL and NA gave 7% and 4% respectively, all after 48 h incubation, and gliotoxin (3 μM; positive control) gave 88% apoptosis after 24 h. Ox(1)LDL was not assessed using AO.

Our results suggest that hydroperoxides, which are early-stage oxidation products, are more potent toxins in oxLDL than are the more advanced oxidation products such as aldehydes and oxysterols. Plotting hydroperoxide levels (FOX assay) in natLDL, ox(1)LDL, ox(2)LDL and ox(3)LDL v. % LDH release measured in HMM (1 day in culture before starting experiments), after 48 h exposure to these oxLDLs or natLDL, revealed a highly significant positive correlation (least-squares linear regression $R^2 = 0.586$, $P < 0.0001$, $n = 51$). Weaker (though still significant) positive correlations with % LDH release were seen for 7β-hydroxycholesterol, MDA equivalents and REM. Hydroperoxide-rich ox(2)LDL might constitute a 'loaded weapon' that can decompose at the cell surface membrane and/or within the cell to give high local concentrations of aldehydes and free radicals. The results for ox(1)LDL suggest that a low, though above-background, level of hydroperoxides (and possibly other uncharacterised entities produced in the earliest stages of LDL oxidation) could trigger a low degree of apoptosis but not yet lead to loss of integrity of the cell surface membrane within the 48 h duration of the experiment. In contrast, for ox(2)LDL and ox(3)LDL, apoptosis and loss of cell surface membrane integrity (LDH release) go hand-in-hand by 48 h.

The hydroperoxides detected are probably derived from PUFAs (esterified and non-esterified) rather than from cholesterol (esterified and non-esterified), as our oxysterol assay protocol, which included a sodium borohydride ($NaBH_4$) reduction step in the sample processing, would encompass cholesterol hydroperoxides by converting them to the corresponding hydroxides. Hydroperoxides are thermally unstable, so would decompose on GC. $NaBH_4$ also converts any 7-ketocholesterol present to 7β-hydroxycholesterol (c. 85%) and to 7α-hydroxycholesterol (c. 15%) [34]. Because it elutes very close to cholesterol, we do not normally quantify 7α-hydroxycholesterol by this analytical protocol [35]. The 7β-hydroxycholesterol levels in Fig. 4 are thus effectively a summation of the oxysterols 7-ketocholesterol, 7β-hydroperoxycholesterol and 7β-hydroxycholesterol present in the original samples. All the above oxysterols are cytotoxic [36–38]. Part of the TBARS detected might arise from decomposition of fatty acid hydroperoxides originally present in the samples, forming MDA during the necessary heating step of the assay [39], thereby possibly underestimating the differential between ox(2)LDL and ox(3)LDL for MDA levels. Hydroperoxides are 'reaction intermediates' chemically, rising then declining as the oxidation progresses [16], whereas 7β-hydroxycholesterol and REM are cumulative markers of oxidation that rise and do not decline even for strong, prolonged oxidation [18].

Toxicity was not due to residual free Fe^{2+} or Cu^{2+} ions remaining in solution after the Chelex treatment of the oxLDLs, because controls consisting of solutions of Fe^{2+} or Cu^{2+} that we put through the whole of the preparation procedure, but omitting the LDL, were non-toxic to HMM. However, oxLDL-associated iron or copper ions, if not removed by Chelex, might play a role in toxicity, possibly in conjunction with lipid hydroperoxides in oxLDL. Human advanced atherosclerotic lesions contain 'catalytic' iron and copper ions [40].

Lp-PLA_2 is a phospholipase that hydrolyses oxPC possessing an oxidised PUFA chain ester-linked in the *sn*-2 position, but does not hydrolyse non-oxidised PC. Lp-PLA_2 is a serine esterase, and is LDL-borne. Also, mature macrophages and to a lesser degree freshly isolated monocytes secrete Lp-PLA_2 identical to LDL-borne Lp-PLA_2 [41–43]. Pre-treatment of natLDL with SB222657, a specific Lp-PLA_2 inhibitor [19], diminished the toxicity and apoptosis induction that ensued when the LDL was mildly or moderately oxidised and then added to HMM (Figs. 1, 2 and 5). PFB, a broad-spectrum serine esterase/protease inhibitor, had a similar effect to SB222657 on toxicity but did not inhibit the corresponding apoptosis induction (Figs. 1 and 2). PFB might inhibit anti-apoptotic serine esterases/proteases, presumably within the cell. SB222657 and PFB did not act as antioxidants, either here (Fig. 4) or in earlier studies [19,20], and have no known chelating ability, consistent with their lack of effect on LDL oxidation per se (as opposed to hydrolysis).

Our results agree with evidence that Lp-PLA_2 can hydrolyse oxPC, whether possessing full-length PUFA-hydroperoxide chains or oxidatively fragmented PUFA chains, liberating lyso-PC and oxNEFA [19]. Lyso-PC was more toxic than an oxPC possessing an oxidatively fragmented PUFA chain, for HMM [20]. PC is the main phospholipid class in LDL. However, Lp-PLA_2 has rather permissive substrate requirements [44,45], so it might also hydrolyse oxidised forms of the less abundant LDL phospholipid classes, and maybe also hydrolyse oxidised tri- and di-acylglycerols.

The role of PPAR in oxLDL-induced cell death was explored using the PPARγ-preferential antagonist GW9662, which also inhibits PPARα and PPARδ less potently, binding irreversibly to PPAR [46]. Cells were pretreated for 20 h with 2 μM GW9662, which was replenished at 2 μM when the oxLDLs were added. GW9662 (2 μM) almost completely inhibits PPARγ activation [46,47]. GW9662 (2 μM) was not toxic to HMM or SMC, and it did not significantly affect the toxicity of ox(2)LDL or ox(3)oxLDL, for HMM (Fig. 5) or for SMC (data not shown; $P > 0.05$). GW9662 (2 μM) treatment of HMM tended to counteract the lowering of the oxLDL toxicity resulting from Lp-PLA_2 inhibition with SB222657, for HMM that had been cultured either for 4 days (Fig. 5) or for 1 day (data not shown) before starting experiments. Possibly, PPARγ activation by intact (i.e. non-hydrolysed) oxPC triggers a cellular defence mechanism. Oxidised alkyl PC (the *O*-alkyl linkage in the *sn*-1 position, and the oxidised chain ester-linked in the *sn*-2 position), a sub-class of oxPC in oxLDL, can specifically bind to and activate PPARγ

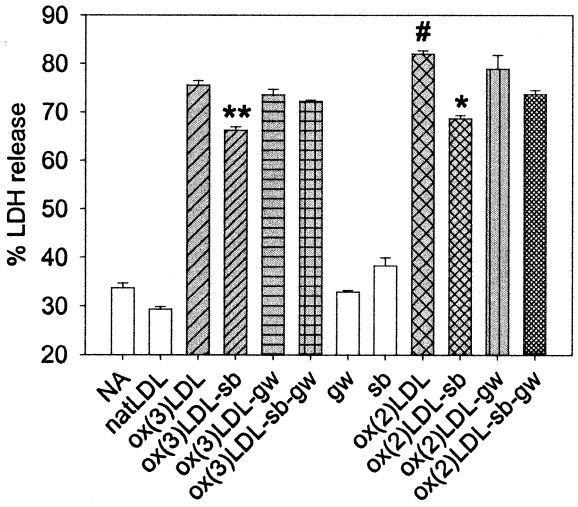

Fig. 5. Effect of treatment of cells with the PPARγ antagonist GW9662 (2 μM) on cytotoxicity of oxLDLs, measured by the LDH release assay, in mature HMM, after 50 h exposure. Abbreviations: gw, GW9662; sb, SB222657. The suffix -gw indicates that cells were pretreated with GW9662 for 20 h before adding the relevant oxLDL prepared with or without SB222657 pretreatment of natLDL. Each histogram bar represents mean ± S.E.M., $n = 3$. ANOVA $P < 0.0001$. **Ox(3)LDL-sb significantly different from ox(3)LDL ($P = 0.0013$) or from ox(3)LDL-sb-gw ($P = 0.0014$); *ox(2)LDL-sb significantly different from ox(2)LDL ($P = 0.0009$) or from ox(2)LDL-sb-gw ($P = 0.0223$); #ox(2)LDL significantly different from ox(3)LDL ($P = 0.005$). OxLDLs were significantly different from NA ($P < 0.0001$); gw and sb were not significantly different from NA.

[48]. The binding appeared a characteristic of the intact (non-hydrolysed) form since oxNEFAs were inefficient competitors [48]. OxPC can activate PPARα [49], but this requires phospholipase A_2 activity [50], suggesting that the oxNEFA thereby liberated were the actual PPARα ligands. PPARγ thus appears more likely to be important than PPARα in the putative defence mechanism triggered by intact oxPC in our study.

The PPARγ agonist ciglitazone at 3 μM, its EC_{50} [51], was innocuous to HMM. The addition of ciglitazone (3 μM) to mature HMM with ox(3)LDL and ox(2)LDL prepared with inhibition of Lp-PLA_2, by pre-treatment of natLDL with SB222657, significantly diminished toxicity, by 27% ($P = 0.0374$) and 24% ($P = 0.0032$) respectively after 24 h, consistent with a protective role for PPARγ activation by oxPC. When the SB222657 pre-treatment was omitted, ciglitazone did not diminish toxicity of the oxLDLs. Ciglitazone and rosiglitazone had biphasic effects on T-lymphocyte death [52]. Another PPARγ activator, 15d-PGJ_2, was toxic to HMM [53]. The highly unsaturated arachidonic and docosahexaenoic acids, incubated with HMM under conditions allowing peroxidation, induced both apoptosis and PPARγ activation, whereas the less highly unsaturated linoleic and oleic acids, which were less readily peroxidisable, were innocuous and did not activate PPARγ [53].

PPARγ activation might thus be a cellular death mechanism or a defence, depending on conditions. Caveats are that inhibitors and activators might have additional non-specific effects, and that co-demonstration of PPARγ activation and apoptosis does not prove causation. The putative dual function of PPARγ – defence or death – would be broadly akin to other cellular damage responses, e.g. involving p53 and mismatch repair, where the cell is either repaired or deleted depending on the degree of damage. PPARγ appears to act defensively under the present study's conditions.

Our induction of cell death by very mildly-, mildly- and moderately-oxLDLs indicates that the presence of strongly oxidised LDL is not necessary to kill cells, a finding relevant to atherosclerotic lesions where the overall degree of oxidation appears less than that of strongly oxidised LDL. Very mildly-oxLDL induced HMM apoptosis above background, whilst mildly- and moderately-oxLDLs produced both apoptosis and overt toxicity. In human advanced atherosclerotic lesions, macrophage foam cells die by apoptosis and necrosis [3]. Classical apoptosis and necrosis represent the ends of a continuum of death modes, with varying contributions of the cellular machinery, and both apoptosis and necrosis can involve controlled cellular events [54,55].

Macrophage death in lesions may be anti-inflammatory and anti-atherogenic if the dying or dead macrophages are efficiently scavenged by viable neighbouring macrophages. If not scavenged, dead macrophages contribute to the lesion's acellular lipid core, exacerbating the lesion. Inhibition of Lp-PLA_2 might be ameliorative, as the fewer ensuing dead cells would be more easily scavenged by the remaining viable macrophages. Since oxPC are epitopes, on oxLDL and on apoptotic cells, recognised by CD36 (scavenger receptor type B) [56–58], preservation of oxPC by inhibiting their hydrolysis, by means of inhibiting Lp-PLA_2, might improve the efficiency of scavenging of oxLDL and of apoptotic macrophages. Whilst theoretically this might increase foam cell formation, this might be counterbalanced if the oxPC also activate PPARγ, since activation of PPARγ in macrophages upregulates both CD36 [59] and the ABCA1 cholesterol efflux pathway, and the latter counteracts the effect of the former [60].

In conclusion, we have shown that mildly-oxLDL, rich in lipid hydroperoxides, is a potent death inducer for macrophages and SMC. Moderately-oxLDL was a less potent, though clearly significant, inducer of death in these cell types. Induction of macrophage death appears partly as a consequence of the hydrolytic action of Lp-PLA_2 on oxPC. Elevated plasma levels of Lp-PLA_2 were a strong independent predictor of coronary artery disease in men [61]. Non-hydrolysed, oxidised phospholipids in oxLDL may activate PPARγ in macrophages, as a cellular defensive response. These results may be relevant to the progression of atherosclerotic lesions to an unstable, rupture-prone state with thinned fibrous caps and enlarged lipid cores, and may suggest dietary and pharmacological strategies for combating the disease.

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