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PPARγ Ligand-Induced Unfolded Protein Responses in Monocytes and Macrophages

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ABSTRACT: Obesity and associated disorders such as Type-2 Diabetes (T2D) and atherosclerosis are associated with elevated levels of circulating oxidized low-density lipoprotein (oxLDL). High levels of oxLDL lead to cell dysfunction and apoptosis, a phenomenon known as lipotoxicity. Disturbing endoplasmic reticulum (ER) function results in ER stress and unfolded protein response (UPR), which tends to restore ER homeostasis but switches to apoptosis when ER stress is prolonged. In the present study the lipotoxic effect of oxLDL was investigated on a monocyte/macrophage cell lines. The results demonstrate that oxLDL could induce ER stress and activation of the UPR pathway in mnocyte/macrophage cell lines as evident of the activation/up-regulation of ER stress/UPR genes. Cholesterol does not seem to exert effects in intact cells in our experiments; in contrast oxLDL did induce ER stress and UPR. In microsomal fractions, cholesterol but not oxLDL inhibit the ER Ca^{2+}-ATPase activity. Gene expression analysis showed that macrophages express high levels of the oxLDL scavenger receptor CD36, than monocytes and oxLDL induced macrophage apoptosis via caspase-3/7 activation. The observations that oxLDL can induce UPRs in macrophages, and that cholesterol inhibit ER Ca^{2+}-ATPase activity, suggest that cholesterol may be the oxLDL component responsible for macrophage lipotoxic ER stress effects as seen in obesity. As disrupted cellular Ca^{2+} homeostasis/ER stress may be linked to macrophage lipotoxicity this data may enhance our understanding of the diverse effects of oxLDL, particularly in the context of obesity, type 2 diabetes and metabolic syndrome.

Keywords: oxLDL; ER stress; UPR; monocyte/macrophage

INTRODUCTION

The endoplasmic reticulum (ER) performs several important functions including post-translational modification, folding and assembly of newly synthesized secretory proteins and calcium homeostasis. However, various conditions can disturb any of the ER functions leading to imbalance between protein-folding load and the capacity of the ER, causing unfolded or misfolded proteins to accumulate in the ER lumen, a condition referred to as ER stress (Araki *et al.*, 2003; Zhang and Kaufman, 2008). To combat the deleterious effects of ER stress, cells have evolved a protective response called the unfolded protein response (UPR) (Patil and Walter, 2001; Ron and Walter, 2007). The main function of the UPR is to reduce the accumulation of the unfolded proteins and restoration of normal ER function. However, prolonged ER stress may lead to apoptotic cell death (Szegezdi *et al.,* 2006). Thus, ER stress and UPR has been linked to several diseases (Marciniak and Ron, 2006)

One of the major complications associated with diabetes is atherosclerosis, and has been regarded as number one cause of mortality in diabetics (Beckman *et al.,* 2002). Monocytes/macrophages play an important role in the pathogenesis of atherosclerosis and several other diseases (Lebovitz and Banerji, 2001). In atherosclerotic lesions for example, macrophages are the most prominent cell type and are associated with lipid deposition and inflammation (Tiwari *et al.,* 2008). One prominent feature of atherosclerotic lesions is macrophage death which affects lesion progression and complications (Yao and Tabas, 2001). Among the likely cause of macrophage death is accumulation of free cholesterol in the ER leading to the activation of the unfolded protein response (Devries-Seimon *et al.,* 2005). Macrophages can acquire cholesterol both from endogenous synthesis and uptake of cholesterol-containing

extracellular materials. However in atherosclerotic lesions the major cholesterol source is plasma low density lipoprotein (LDL) that has been chemically or physically modified forming oxidized LDL (oxLDL) (van Reyk and Jessup, 1999).

A large body of evidence indicates that oxLDL are cytotoxic for several cell types including macrophages (Muller *et al.,* 2001; Heinloth *et al.,* 2002). However, the mechanisms by which oxLDL induce cytotoxicity are not fully understood. As mention earlier, one of the functions of the ER is calcium homeostasis (Nielsen and Petersen, 1972). Disturbing any of the ER functions may result in ER stress and UPR which tend to restore ER homeostasis but switch to apoptosis when ER stress is prolonged. ER stress may thus contribute to the increased risk of insulin resistance, type 2 diabetes and other cardiovascular complications associated with obesity (Ozcan *et al.,* 2004; Gregor and Hotamisligil, 2007). Calcium homeostasis is also related to the pathophysiology of type 2 diabetes and its complications (Advani *et al.,* 2004). Moreover cholesterol loading to the ER causes ER stress and activation of UPR (Li *et al.,* 2004).

Previous work in our Laboratory has shown that, in addition to its role as a peroxisome proliferator-activated receptor gamma (PPARγ) ligand, the anti-hyperglycaemic drug rosiglitazone causes inhibition of the ER 'housekeeping Ca^{2+} pump' enzyme sarco/endoplasmic reticulum calcium ATPase 2b (SERCA2b), unchecked leakage of Ca^{2+} from the ER lumen and triggering of UPRs (Caddy *et al.,* 2008). It was therefore the aim of this study to examine whether oxLDL, a natural PPARγ ligand could disrupt ER calcium homeostasis and trigger UPRs in two cell types relevant to the progression of type 2 diabetes and its compications: monocytes and macrophages. Because ER stress restores normal cell physiology or induce cell death when stress is prolonged, the effect of oxLDL on apoptosis was also investigated.

MATERIALS & METHODS.

Materials. All reagents were purchased from Sigma-Aldrich (Poole, UK) unless stated otherwise. oxLDL, and rosiglitazone were obtained from Autogen Bioclear (Calne, UK) and GlaxoSmithKline (Uxbridge, UK) respectively. Human monocytic THP-1 cell lines were obtained from the European Collections of Cell Cultures (Salisbury, UK).

Maintenance of cells in culture. Human monocytic leukemia (THP-1Mon) cell lines were used in these studies (Tsuchiya *et al.,* 1980). The cells were maintained in culture under standard conditions. To generate macrophages (THP1MΦ) cells were treated with 100ng/ml porbol myristate acetate (PMA) for 72h. After confirming that the cells were fully differentiated (Figure 1), the media containing PMA was then aspirated and fresh culture media was added.

RNA isolation and real-time quantitative PCR. Total RNA were extracted with Trizol® reagent according to the manufacturer's instructions (Invitrogen, Paisley, UK). RNA samples were converted to cDNA using an Applied Biosystems® High-Capacity cDNA Archive Kit. CD36, PPARγ and β-actin mRNA expressions were assessed using SYBR® Green Assays (Applied Biosystems, Warrington UK) and analysed using an Applied Biosystems 7500 Real-time PCR system. Semi-quantitative comparisons of mRNA expression levels were carried out using the $2^{-\Delta\Delta CT}$ method were ΔCT equals the difference between CT values for target gene and the house-keeping gene, β-actin. The following primers were used:

CD36. Fwd: 5'-GGAAGTGATGATGAACAGCAGC-3'
Rev: 5'-GAGACTGTGTTGTCCTCAGCGT-3'
PPARγ. Fwd: 5'-CGTGGCCGCAGATTTGAA-3'
Rev: 5'-CTTCCATTACGGAGAGATCCAC-3'
β-actin. Fwd: 5'-TCCTGTGGCATCCACGAA-3'
Rev: 5'-GAAGCATTTGCGGTGGAC-3'

In the case of SERCA2b, mRNA expression was investigated using an Applied Biosystems 7500 Real-time PCR system and assessed semi-quantitatively (relative to Glyceraldehyde Phosphate Dehydrogenase (GAPDH)) via Taqman® Gene Expression

Assays (Applied Biosystems, Warrington, UK). In all cases, thermocycling was as follows: initial denaturation (2min; 50°C/10min; 95°C), followed by 50 cycles of denaturation (15sec; 95°C)/annealing-extension (60sec; 60°C).

XBP-1 activation. Activation of the UPR transcription factor x-box binding protein 1 (XBP-1) was assessed in THP-1Mon and THP1MΦ via a method adapted from Shang (Shang *et al.,* 2005) which uses RT-PCR, agarose gel electrophoresis and densitometric analysis to determine XBP-1 activation. Because inositol requiring ER-to-nucleus signal kinase 1's (IRE1) endoribonuclease activity excises a 442bp segment from within exon 4 of the unspliced XBP-1 mRNA species (XBP-1u) under conditions of ER stress, and so generates a spliced mRNA (XBP-1s) encoding the active form of the protein (Xu *et al.,* 2005), XBP-1 activation can be determined via densitometric analysis of banding patterns on 2% agarose gels by means of the following formula:

[XBP-1(s) + 0.5 XBP-1(h)]/[XBP-1(s) + XBP-1(h) + XBP-1(u)]

where XBP-1(s) is a 398bp PCR product, XBP-1(u) is a 442bp PCR product, and XBP-1(h) is an additional PCR product representing a heteroduplex XBP-1 cDNA species (Shang *et al.,* 2005). XBP-1 activation was then expressed relative to that seen in control THP-1Mon cells.

Subcellular fractionation and Ca^{2+}-ATPase Assay. Microsome preparation from THP-1Mon was conducted according to the methods of Maruyama and MacLennan 1988, and Papp *et al.,* 1992 respectively, with minor adaptations. Cells were homogenised, and subjected to differential centrifugation, with the final pellet containing the microsomal fraction being resuspended in ATPase assay buffer and aliquots snap-frozen in liquid nitrogen prior to storage at -80°C.

Ca^{2+}-dependent ATP hydrolysis was measured using a coupled enzyme assay, and free Ca^{2+}concentrations were calculated, as described previously (Webb *et al.,* 2000; Storer *et al.* 1976).

Cell viability and apoptosis Assays. Cell viability and apoptosis levels was determined using 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTS) and Caspase-Glo 3/7 assays (Promega, Southampton, UK), respectively, according to the manufacturers' instructions. The resulting optical density or luminescence data was read via Dynex plate-reading spectrophotometer or luminometer (Worthing, UK), respectively.

Statistical analysis. Data were expressed as mean ± standard error of the mean. Statistical significance was determined with Student's *t*-test comparison between two groups of data sets. Significance levels were set at $P<0.05$.

RESULT

Effect of PMA on THP-1Mon diffferention. THP-1Mon cells were stimulated with 100ng/ml PMA (Silverstein 1996) and left to differentiate for 72 hours. Images captured using fluorescence microscope show that the cells were fully transformed into macrophages after 72 hours as evidence of their extension of cell membrane and increased adherence

Activation of PPARγ by oxLDL. oxLDL has been shown to dose dependently induce PPARγ mRNA expression in macrophage (Taketa *et al.,* 2008). As shown in figure 2, both 1 and 40µg/ml oxLDL up-regulate the expression of PPARγ mRNA compared to untreated cells.

Effect of PPARγ ligands on CD36 mRNA expression. oxLDL entry into monocyte/macrophages occurs via receptor-mediated endocytosis through the lipid scavenger receptor CD36 (Endemann *et al.,* 1993). Moreover, CD36 expression is highly regulated in monocytes/macrophages and it can be upregulated at transcriptional level by PPARγ ligands (Febbraio *et al.,* 2001). Differentiation of THP-1 cells increases their expression of PPARγ (Chinetti *et al.,* 1998). As shown in figure 3, PMA differentiated THP1MΦ expressed approximately 3 fold increase in CD36 compared to 1.5 fold in undifferentiated THP-1 cells.

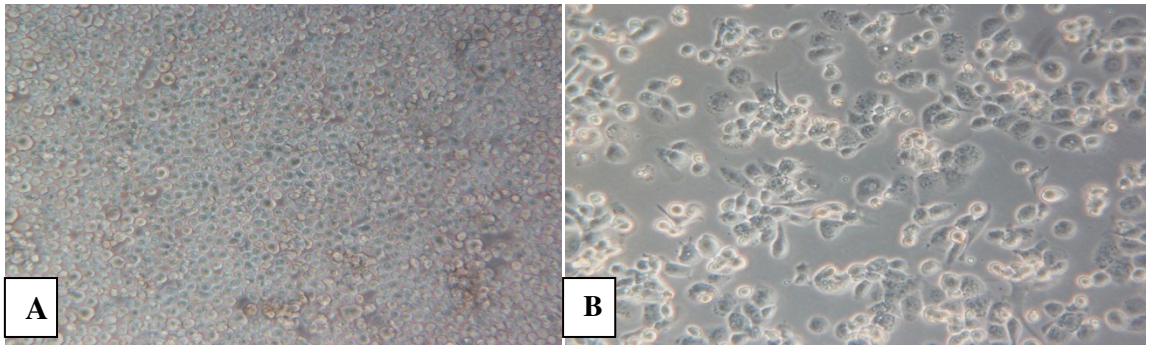

Figure 1: Effect of 100ng/ml PMA on THP-1Mon cells. THP-1Mon cells were incubated without (A) and with (B) 100ng/ml PMA for 72 hours and visualized using fluorescence microscope.

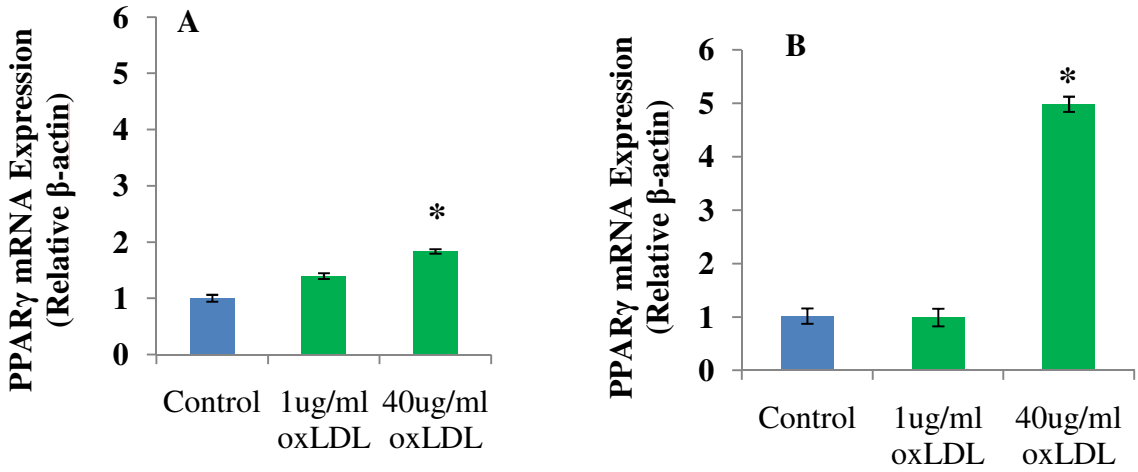

Figure 2: Effect of oxLDL on PPARγ mRNA expression. THP-1Mon (A) and THP1MΦ (B) were incubated for 24 hours with 1 or 40µg/ml oxLDL. PPARγ mRNA expression was quantified by real time-PCR relative to β-actin. Data is expressed as mean ± SEM of three independent experiments (* denotes P<0.05 compared to control cells).

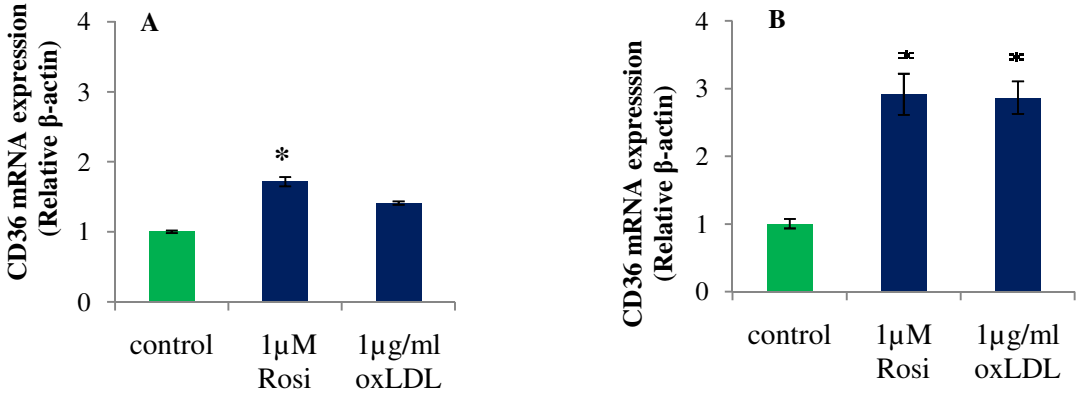

Figure 3: Effect of 1µM rosiglitazone (synthetic PPARγ ligand) and 1µg/ml oxLDL (natural PPARγ ligand) on CD36 mRNA expression in THP-1Mon (A) and THP1MΦ. THP-1Mon and THP1MΦ were incubated with 1µM rosiglitazone or 1µg/ml oxLDL for 24 hours. CD36 mRNA expression was quantified by real time-PCR and is reported as a ratio to β-actin. Data is expressed as mean ± SEM of three independent experiments (* denotes P<0.05 compared to untreated cells).

Effect of PPARγ ligands on ER Ca^{2+}-ATPase activity. In microsomes isolated from untreated THP-1cells, maximal Ca^{2+}-ATPase activity was identified (at ~pCa 4.82). This activity was not inhibited by incubation with 0.1% DMSO alone (21±9 nmol/mg/min; *P*>0.05) or oxLDL 40µg/ml (9±2nmol/mg/min; *P*>0.05). Activity was significantly inhibited by incubation with 3µM $15dPGJ_2$ (1±1nmol/mg/min; *P*<0.05) or 200µg/ml Cholesterol (-1±3nmol/mg/min; *P*<0.05), suggesting that free cholesterol is the oxLDL component that inhibits ER Ca^{2+}-ATPase activity (Li *et al.*, 2004).

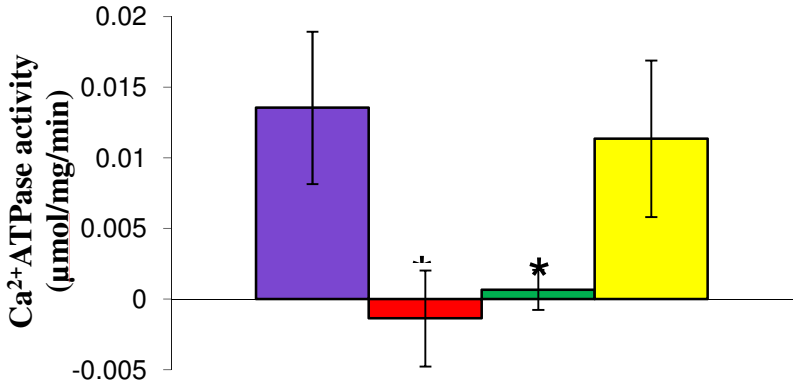

Figure 4: Effects of DMSO (0.1% v/v; blue), cholesterol (200µg/ml; red), 15dPGJ2 (3µM; green) and oxLDL (40µg/ml; yellow) on THP-1Mon ER Ca2+-ATPase activity (*denotes P<0.05 compared to control).

Disruption of ER homeostasis triggers UPR. To determine whether disruption in ER Ca2+ homeostasis trigger UPR, relative expression of XBP-1 spliced variants showed that oxLDL induced XBP-1 splicing in THP1MΦ, but not in THP-1Mon (Fig. 5). Cholesterol did not significantly induce XBP-1 splicing in both cell types.

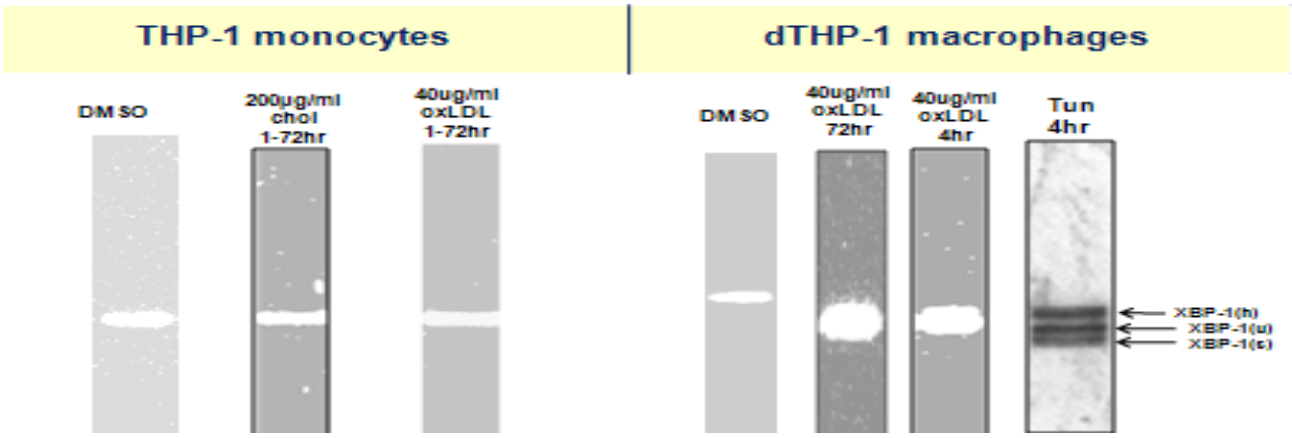

Figure 5: Splicing of XBP-1 mRNA as an indicator of ER stress. Effects of DMSO (0.1% v/v), Cholesterol (200µg/ml), oxLDL (40µg/ml) and Tunicamycin (10ng/ml) on XBP-1 splicing in THP-1Mon and THP1MΦ. (XBP-1(s): active splice variant; XBP-1(u): inactive splice variant; XBP-1(h): hybrid splice variant; representative of >3 separate experiments).

Effect of oxLDL on XBP-1activation. Densitometric analysis of XBP-1 activation showed that oxLDL induced XBP-1 activation in THP1MΦ (Fig 6). However, no XBP-1 activation could be observed in THP-1Mon (data not shown).

PPARγ Ligands induced transcriptional activation of XBP-1 target gene SERCA2b. Studies have demonstrated that the natural PPARγ ligand 15dPGJ2 are specifically traffic to the ER and trigger the UPR (Takashi *et al.*, 1998) and SERCA2b is an ER stress inducible gene (Caspersen *et al*, 2000). SERCA2b

mRNA was significantly upregulated in THP-1 cells treated with 15dPGJ2 but not oxLDL (Figure 7). In contrast, both $15dPGJ_2$ and oxLDL significantly upregulate SERCA2b mRNA in dTHP-1 macrophages (Figure 8).

Effect of oxLDL on THP-1Mon and THP1MΦ apoptosis. Caspase -3 and -7 has been shown to play key effector roles in mammalian apoptosis (Garcio-Calvo *et al.,* 1999; Le *et al.,* 2002; Hitomi *et al.,* 2004) and therefore effect of oxLDL on caspase activation as a marker of apoptosis was investigated in THP-1Mon and THP1MΦ treated with 1 or 40µg/ml oxLDL. As shown in figure 9, both 1 and 40µg/ml oxLDL do not induce apoptosis in THP-1Mon (A). In contrast, both 1 and 40µg/ml oxLDL induced a small non-significant increase in apoptosis in THP1MΦ (B).

Effect of oxLDL on THP-1Mon and THP1MΦ viability. THP-1Mon or THP1MΦ were treated with 1 or 40µg/ml oxLDL for 24 hours. Effect of oxLDL on THP-1Mon and THP1MΦ was investigated. As shown figure 10, both 1 and 40µg/ml oxLDL do not induce decrease cell viability in THP-1Mon (A). In contrast, both 1 and 40µg/ml oxLDL induced a small non-significant decrease cell viability in THP1MΦ (B).

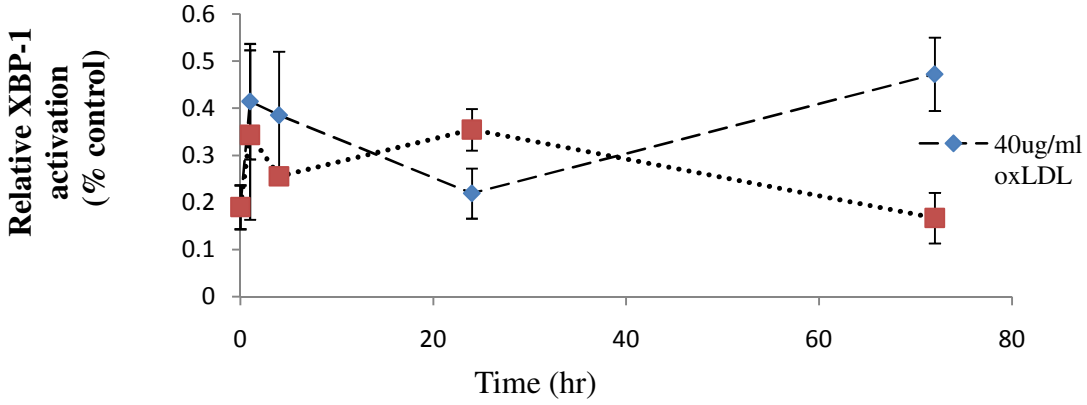

Figure 6: Effect of oxLDL on XBP-1 activation in THP1MΦ. XBP-1 activation was assessed via a method adapted from Shang (Shang 2005). Band intensity of gel images were measured using Quantity One Software (Bio-Rad, UK). After background correction, band intensities were obtained and XBP-1 activation was then determined.

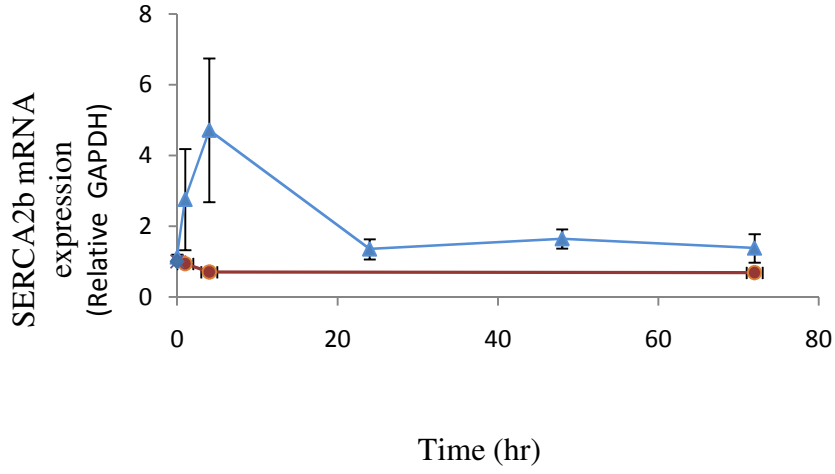

Figure 7: Effect of 15dPGJ2 and oxLDL on SERCA2b mRNA expression in THP-1Mon. THP-1Mon cells were treated with 40µg/ml oxLDL or 3µM 15dPGJ2 for 0 to 72 hours.

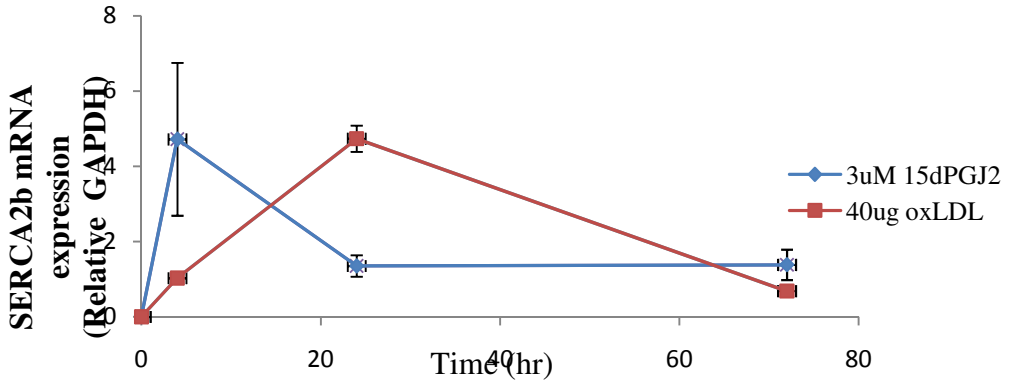

Figure 8: Effect of 15dPGJ2 and oxLDL on SERCA2b mRNA expression in THP1MΦ. THP1MΦ were treated with 40µg/ml oxLDL or 3µM 15dPGJ2 for 0 to 72 hours. SERCA2b mRNA expression was quantified by real time-PCR and is reported as a ratio to GAPDH. Data is expressed as mean ± SEM of three independent experiments.

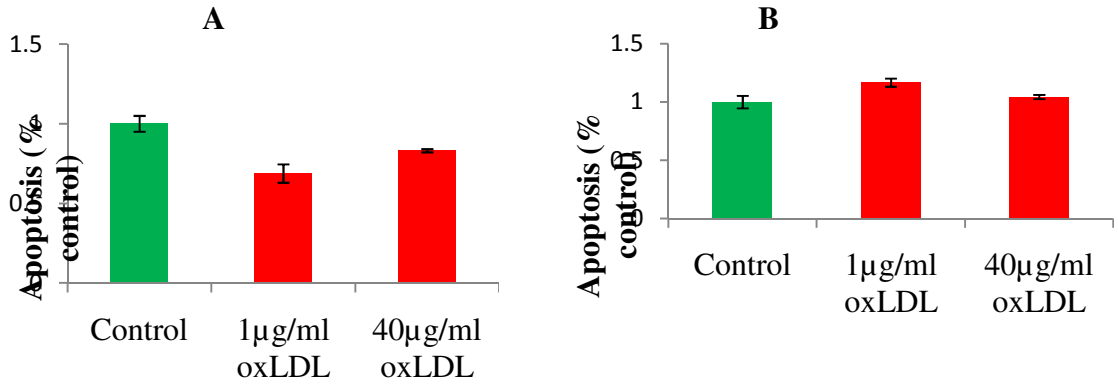

Figure 9: Effect of oxLDL on THP-1Mon and THP1MΦ apoptosis. THP-1Mon cells (A) or THP1MΦ (B) were treated with 1 or 40µg/ml oxLDL for 24 hours. At the end of the incubation, the cells were analyzed for caspase -3/7 activity via Caspase-Glo luminescence-based apoptosis assay using Dynex luminometer. Data are expressed as mean % control.

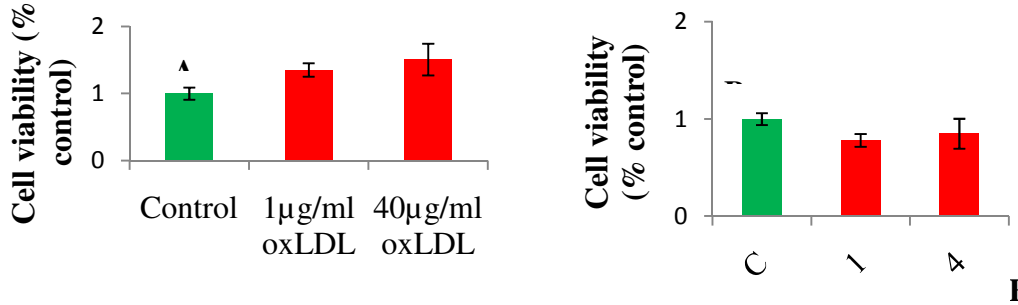

Figure 10: Effect of oxLDL on THP-1Mon or THP1MΦ viability. THP-1Mon (A) or THP1MΦ (B) were treated with 1 or 40µg/ml oxLDL for 24 hours. At the end of the incubation, the number of viable cells was determined using MTS reduction assay. Data are expressed as mean % control.

DISCUSSION

Elevated plasma LDL leads to an increase in the adherence of circulating monocytes to arterial endothelial cells and rate of LDL entry into the intima (Steinberg, 1997). In the intima, LDL can undergo oxidative modification catalyzed by any of the cells of the vasculature, i.e. macrophages, smooth muscle cells or endothelial cells (Steinberg, 1997) forming a modified form of LDL called oxidized LDL (oxLDL). oxLDL has been shown to induce variety of biological and physiological functions in vitro and these may seem to be contradictory in some respects. For example, oxLDL induces macrophage and smooth muscle cell proliferation (Yui *et al.,* 1993; Auge *et al.,* 1995; Biwa *et al.,* 1998) and also macrophage viability and survival (Sakai *et al.,* 1996; Hamilton *et al.,* 2001). While conversely a large body of literature also exists that demonstrates the cytotoxic and/or pro-apoptototic effects of oxLDL (Reid *et al.,* 1993; Dimmiler *et al.,* 1997; Sata *et al.,* 1998; Coles *et al.,* 2001; Martinet & Kockx, 2001; Nahn *et al.,* 2003; Tabas 2005; Seimon *et al.,* 2009; Sanson et al., 2009). The reasons for these contradictory reports might be due to differences in the concentration and oxidation processes of the oxLDL used in these different studies. Thus, the present study showed that oxLDL is cytotoxic at least to macrophages.

The first property of oxLDL to be discovered that makes it more atherogenic than native LDL is the fact that it is recognized by the scavenger receptors leading to accumulation of cholesterol in foam cells (Henriksen *et al.,* 1982). The scavenger receptor CD36 has been shown to bind and internalize oxLDL in macrophage (Nicholson *et al.,* 2000) and increases its functional expression. In line with this study, the induction of CD36 mRNA in THP-1Mon or THP1MΦ was investigated. As shown in figure 3, oxLDL increase the functional expression of CD36 mRNA in both THP-1Mon or THP1MΦ. However, a statistically significant induction was only observed in THP1MΦ. One important feature of atherosclerotic plaques is the intracellular accumulation of oxLDL within macrophages, which is the result of scavenger receptor CD36 recognizing altered molecular patterns present on oxLDL (as distinct from non-oxidised LDL), and mediating the accumulation of cholesterol that is characteristic of macrophage foam cells (Tiwari *et al.,* 2008).

The endoplasmic reticulum (ER) performs several important functions including post-translational modification, folding and assembly of newly synthesized secretory proteins and calcium homeostasis. However, various conditions can disturb any of the ER functions leading to imbalance between protein-folding load and the capacity of the ER, causing unfolded or misfolded proteins to accumulate in the ER lumen, a condition referred to as ER stress (Araki *et al.* 2003; Zhang and Kaufman, 2008). To combat the deleterious effects of ER stress, cells have evolved a protective response called the unfolded protein response (UPR) (Patil and Walter, 2001; Ron and Walter, 2007) which aim to restore normal ER homeostasis but switch to apoptosis when when stress is prolonged (Szegezdi *et al.,* 2006). Interestingly, in macrophages, trafficking of free cholesterol to the ER membrane has been shown to trigger ER stress and the UPR, due to incorporation of cholesterol into the normally cholesterol-poor ER membrane and alteration of its physico-chemical properties, which leads to disruption of ER function (Sanson *et al.,* 2009).

This study showed that oxLDL (1 and 40µg/ml which represent the concentrations seen in sedentary individual (Butcher *et al.,* 2008) and plasma of subjects after a meal rich in fat (Emanuel *et al.,* 1991) respectively) could induce ER stress and so trigger the UPR in monocyte/macrophages. Upregulation of XBP-1 and SERCA2b genes in this study imply that oxLDL was causing ER stress and UPR in these cells. At least three transcription factors are actvated as a direct result of ER stress: double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK), inositol requiring ER-to-nucleus signal kinase 1 (IRE1α), and activating transcription factor 6 (ATF6) (Eizirik *et al.,* 2008). Once activated, the cytosolic domain of IREα acquires endoribonuclease activity and cleaves 26 nucleotides from the mRNA encoding the UPR transcription factor XBP-1 generating an active splice variant, XBP-1s (Eizirk *et al.,* 2008) (Fig 5). XBP-1s is translocated to the nucleus where it binds to ER stress-response elements (ERSE) and/or unfolded protein response elements (UPRE) in the promoters of target genes (Lee *et al.,* 2003). In line with this study, SERCA2b whose promoters contain three ERSE (Thuerauf *et al.,* 2001) was

upregulated in dTHP-1 macrophages. The Ca^{2+}-ATPase activity of SERCA2b enzyme can be affected by the physico-chemical properties of the membrane in which these enzymes are embedded (Starling *et al*. 1996; Li *et al.* 2004). Because Ca^{2+} pumping relies on transfer between E1 and E2 conformations, the domains of the enzyme (including its transmembrane domains) must move relative to each other, and relative to the phospholipid bilayer in which the enzyme is embedded (Toyoshima *et al*., 2000; Toyoshima *et al*., 2002). Increased membrane rigidity due to enrichment of the ER membrane with free cholesterol, as a result of accumulation of oxLDL (Yamada *et al.* 1998), prevents macrophage SERCA2b undergoing such conformational changes (Starling *et al.* 1996). This leads to SERCA2b upregulation (Fig 8) and subsequent initiation of down stream UPR pathways.

Apoptotic cell death has been shown to play an important role in cardiovascular diseases (MaClellan *et al.,* 1997) and apoptosis was virtually absent in non-atherosclerotic plaques (Bjorkerud and Bjorkerud, 1996). Although apoptosis induced by oxLDL has been demonstrated in vascular cells (Bjorkerud and Bjorkerud, 1996; Escargueil-Blanc *et al.,* 1997), the mechanisms are not fully understood. During ER stress, depletion of ER calcium stores causes the release of calcium from the ER to the cytoplasm (Zong *et al.,* 2003). The increase in calcium in the cytoplasm activates m-calpain which cleaves and activates procaspase-12 (Nakagawa and Yuan, 2000). Activated caspase-12 further activates the downstream caspases, which play a key effector role in mammalian apoptosis (Garcia-Calvo *et al.,* 1999; Hitomi *et al.*, 2004). Because Apoptosis mediated by ER stress depends on activation of Caspase, and Caspases -3 and -7 play key effector roles in mammalian apoptosis (Garcia-Calvo *et al.,* 1999; Le 2002; Hitomi *et al.,* 2004), figure 9 showed that oxLDL activate caspase 3/7 thereby inducing apoptosis in macrophage (Fig 9a). To further investigate the physiological significance of the apoptosis assay, cell viability was measure. Moreover, oxLDL induced a decrease in macrophage cell viability (Fig 10b).

In summary, oxLDL particles contain cholesterol, and figure 4 shows that cholesterol causes inhibition of integral ER membrane proteins such as the ER Ca^{2+}-ATPase, possibly via intercalation of large amounts of cholesterol into the normally cholesterol-poor ER membrane. However, cholesterol does not seem to exert effects in intact cells in our experiments. In contrast, oxLDL did induce ER stress, particularly in THP1MΦ. As macrophages express high levels of the oxLDL scavenger receptor CD36, they should take up greater quantities of oxLDL than monocytes. The observations that oxLDL can induce UPRs in macrophages, and that cholesterol inhibit ER Ca^{2+}-ATPase activity, suggest that cholesterol may be the oxLDL component responsible for macrophage lipotoxic ER stress effects as seen in obesity. As disrupted cellular Ca^{2+} homeostasis/ER stress may be linked to macrophage lipotoxicity this data may enhance our understanding of the diverse effects of oxLDL, particularly in the context of obesity, type 2 diabetes and the metabolic syndrome.

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