Effects of Human Low-Density Lipoproteins on Superoxide Production by Formyl-Methionyl-Leucyl-Phenylalanine Activated Polymorphonuclear Leukocytes

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Summary: Neutrophils play a major role in the host defence by producing reactive oxygen species. These products are liberated by activated cells and are known to cause endothelial cell injury and damage. The present study shows that low-density lipoproteins increase superoxide anion production by twofold in polymorphonuclear leukocytes stimulated by formyl-Met-Leu-Phe in vitro. Moreover, LDL induced a large increase in phosphoinositides and cytosolic-free calcium. Data from experiments performed on neutrophils treated with pertussis toxin, staurosporine, propranolol or niflumic acid suggest that modulation of phospholipase D and A_2 activities could be involved in the modification by LDL of leukocyte response to formyl-Met-Leu-Phe. LDL lipid moiety could play a key role in their action on polymorphonuclear functions because cholesterol was exchanged between lipoproteins and cells that can modify membrane fluidity and interact with the formyl-Met-Leu-Phe receptor.

Introduction

Dyslipoproteinaemia, demonstrating increased blood lipoprotein concentrations, hypertension and tabagism, is often associated with accelerated atherogenesis mechanisms (1, 2). Increased lipoprotein concentrations, and especially high low-density lipoprotein (LDL) concentrations, could modify polymorphonuclear leukocyte (PMN) functions. Some authors have reported the enhanced oxidative metabolism of polymorphonuclear neutrophils isolated from hyperlipoproteinaemic subjects and an inhibition of polymorphonuclear neutrophil phagocytosis following their treatment with LDL in vitro (3, 4).

In a previous study, we demonstrated that LDL stimulates superoxide (O_2^-) generation by human polymorphonuclear neutrophil in vitro (5). This effect is concentration dependent and might mediate venous endothelial structure injury by arachidonic acid metabolites, proteolytic enzymes and reactive oxygen species released by activated polymorphonuclear neutrophils (6−10). Moreover, they might enhance LDL oxidation and contribute to the formation of foam cells mainly implicated in the atherogenic process (11−13).

The oxidative burst results from the assembly and activation of the NADPH oxidase, a transmembrane electron transport chain which reduces oxygen to superoxide (14, 15). This (O_2^- generation is initiated in neutrophils by a variety of agonists (16). Among them is the formyl-Methionyl-Leucyl-Phenylalanine, a tripeptide analogous to bacterial wall constituents, and currently being used for in vitro experiments. Polymorphonuclear neutrophil activation by formyl-Met-Leu-Phe triggers a cascade of tightly controlled biochemical events leading to an oxidative burst (17−19). The first step consists of the ligand binding to specific cell surface receptors. Among them is the formyl-Met-Leu-Phe receptor which is well-characterized and coupled to cellular responses through a pertussis toxin-inhibitable G protein (20). Three phospholipases are mainly involved at different steps of neutrophil activation; phospholipase C, phospholipase A_2 and phospholipase D. Hydrolysis of membrane phospholipids leads to the formation of numerous bioactive lipids. These pathways were implicated in the signal transduction of polymorphonuclear neutrophil activated by formyl-Met-Leu-Phe (18, 21).

In order to clarify LDL effects of formyl-Met-Leu-Phe-induced polymorphonuclear neutrophil oxidative burst, we investigated in vitro the action of pharmacological tools on some steps of transduction leading to the oxidative burst of polymorphonuclear neutrophil.

Materials and Methods

Reagents

Pertussis toxin (lot 72H-0641-1), staurosporine, propranolol, niflumic acid, and formyl-Met-Leu-Phe were purchased from Sigma Chemical Co. (Saint-Louis MO, USA).

Stock solutions of formyl-Met-Leu-Phe (1 mmol/l) and staurosporine (1 mmol/l) were prepared in dimethyl sulphoxide and stored at −20 °C.

Stock solution of propranolol (50 mmol/l) was prepared in phosphate buffered saline without Ca^{2+} and Mg^{2+} and stored at $-20\,°C$.

Dilutions of stock solutions were made in phosphate buffered saline for each experiment.

Lipoprotein isolation

LDL ($1.030 < d < 1.050$ kg/l) was prepared from the serum of normolipaemic subjects (3.5 mmol/l $<$ total cholesterol < 6.5 mmol/l and triacylglyerols < 1.0 mmol/l) in the presence of ethylenediaminetetraacetic acid (2.69 μmol/l) and phenylmethylsulphonyl fluoride (0.1 mmol/l) to prevent both action of lipolytic enzymes and endogenous proteases and to avoid oxidation. Isolation of LDL was achieved through two successive ultracentrifugation steps at $100\,000\,g$ for 18 hours at $4\,°C$. It was then dialyzed exhaustively against the phosphate buffer and stored for less than five days under nitrogen at $4\,°C$ after sterilization by filtration (0.22 μm Millipore). The purity of the LDL fraction was assayed by SDS polyacrylamide gel electrophoresis. Since the main constituent of LDL is cholesterol, LDL concentration was expressed as LDL cholesterol in mmol/l.

Cell preparation

The cells were isolated from heparinized ($10\,000$ U/l) peripheral blood of healthy volunteers from the laboratory population, by successive use of Ficoll-Hypaque and polyvinylalcohol (22). After isolation, the cells were put in a phosphate buffered solution for determination of superoxide generation and viability was assessed through a trypan blue exclusion test.

Superoxide production

Superoxide production was measured in terms of ferricytochrome c reduction as previously described (5, 23). The stimulating agent was formyl-Methionyl-Leucyl-Phenylalanine 100 nmol/l. The results were expressed as O_2^- release in mmol/10^6 cells.

Phospholipid turnover analysis

Polymorphonuclear neutrophil labelling

After isolation, polymorphonuclear neutrophil cell count was adjusted to 10^{10}/l in Hank's balanced salt solution containing 20 mmol/l Hepes, pH = 7.4, supplemented with 250 mg/l bovine serum albumin as previously described (24). Cells were labelled with 7.4 GBq/l (200 mCi/l) of myo-[2-^{3}H]inositol and incubated for 16–20 h at $37\,°C$ with gentle shaking.

At the end of incubation, cells were washed three times, counted and put in the same Hank's solution, supplemented with 1 g/l bovine serum albumin and 10 mmol/l LiCl (for inhibiting the enzymes of inositol phosphate dephosphorylation). Cells were adjusted to a concentration of 10^7 cells per 600 μl aliquots and incubated for 10 min at $37\,°C$ before the stimulation.

Polymorphonuclear neutrophil stimulation

Stimulating agents were applied to aliquots tested in duplicate, as follows:

- phosphate buffered saline for 15 s (controls)
- formyl-Met-Leu-Phe from 10^{-9} to 10^{-6} mol/l for 15 s
- LDL (2.5 mmol/l) for 5 min
- LDL (2.5 mmol/l) for 5 min before the addition of formyl-Met-Leu-Phe for 15 s.

The reaction was stopped by addition of perchloric acid, followed by three cycles of freezing-thawing.

After centrifugation, the hydrosoluble perchloric acid precipitation supernatants containing inositol phosphates were diluted and neutralized. The inositol lipids were extracted from the perchloric acid

insoluble pellets, dried and deacylated by alkaline hydrolysis as described by Creba et al. (25) and Poggioli et al. (26). The [^{3}H]inositol phosphates and [^{3}H]glycerophosphoryl esters were then separated by anion-exchange chromatography on Dowex AG1-X8 columns using the buffer system described by Downes et al. (27) and Berridge et al. (28).

Cytosolic-free calcium concentrations

Changes in cytosolic free calcium concentration were measured in polymorphonuclear neutrophil loaded with 1 μmol/l Fura 2/AM at $37\,°C$ for 45 min (29, 30). Cells were then washed and suspended in 10 mmol/l HEPES/Hank's balanced salt solution. Fura 2 fluorescence assays were performed with aliquots of $5 × 10^6$ polymorphonuclear neutrophil in 2 ml Hank's balanced salt solution, using a fluorimeter (Jobin Yvon 3D, France) equipped with a thermally controlled cuvette holder and a magnetic stirrer. Various concentrations of LDL were tested: 0.01 to 5 mmol/l cholesterol. Excitation and emission wavelengths for Fura 2 fluorescence assays were 340 and 510 nm, respectively. Cytosolic calcium concentrations were calculated as described (30).

Intracellular level of polymorphonuclear neutrophil cholesterol

Polymorphonuclear neutrophils were preincubated in duplicate at $37\,°C$ for 5 min and placed in contact with increasing concentrations of LDL for 15 min. Superoxide generation was measured in half of the tubes and cellular cholesterol content was measured in the other. In these tubes, ferricytochrome c was omitted. After stopping the reaction in an ice-water bath, the cells were washed three times in phosphate buffered saline before freezing.

After lipid extraction from neutrophils, cellular cholesterol measurement was performed by gas chromatography coupled with mass spectrometry. This measurement was performed in the Laboratoire de Biochimie des Lipoprotéines de Dijon (Service du Pr Gambert).

Statistical analysis

The Mann-Whitney's test and the Wilcoxon's test were used for the comparison of unpaired and paired quantitative variables.

Results

After polymorphonuclear neutrophil preincubation for 5 min at $37\,°C$ with buffer or LDL, superoxide production was assayed over the next 5 min in the presence of formyl-Met-Leu-Phe 100 nmol/l.

In the presence of LDL, formyl-Met-Leu-Phe-induced stimulation was increased twofold (Wilcoxon's test: $p \le 0.01$) (control: O_2^- 6.72 ± 1.65 nmol/10^6 cells; with LDL at 2.5 mmol/l: O_2^- 14.92 ± 2.19 nmol/10^6 cells; with LDL at 5 mmol/l: O_2^- 16.59 ± 2.18 nmol/10^6 cells; means $\pm$ SEM, n = 9).

Pertussis toxin action

Preincubation with pertussis toxin (1 μg for $2 × 10^7$ cells) drastically impaired polymorphonuclear neutrophil response by ADP-ribosylation of G-protein (31). There was a variability in the activity of pertussis toxin depending on the batch purchased, but the maximal effect was observed with polymorphonuclear neutrophil treated for 2 h in Hank's solution under shaking, as previously described by Gabig et al. (32) and Omann et al.

(33). After centrifugation, cells were washed before their stimulation by formyl-Met-Leu-Phe with or without LDL. The cells' response to formyl-Met-Leu-Phe was inhibited after pertussis toxin treatment, in the absence (polymorphonuclear neutrophils + formyl-Met-Leu-Phe: O_2^- 14.37 ± 0.83 nmol/10^6 cells; polymorphonuclear neutrophils + pertussis toxin + formyl-Met-Leu-Phe: O_2^- 3.28 ± 1.38 nmol/10^6 cells; means ± SEM, n = 3) or in the presence of LDL (polymorphonuclear neutrophils + LDL + formyl-Met-Leu-Phe: O_2^- 18.45 ± 0.67 nmol/10^6 cells; polymorphonuclear neutrophils + pertussis toxin + LDL + formyl-Met-Leu-Phe: O_2^- 3.72 ± 1.44 nmol/10^6 cells).

Effects of LDL on phosphatidyl turnover

Polymorphonuclear neutrophil incubation with LDL at a concentration of 2.5 mmol/l induced a large increase in phosphoinositides, and particularly in phosphatidylinositol 4,5-bisphosphate along with inositol 1,4,5-trisphosphate (fig. 1). Polymorphonuclear neutrophil incubation with formyl-Met-Leu-Phe induced, as expected, an activation of phosphatidylinositol 4,5-bisphosphate breakdown and formation of inositol 1,4,5-trisphosphate.

Stimulation of polymorphonuclear neutrophils by formyl-Met-Leu-Phe in the presence of LDL also led to an increase of phosphatidylinositol 4,5-bisphosphate in all the LDL or formyl-Met-Leu-Phe concentrations used (tab. 1).

Effect of LDL on calcium mobilization

The intracellular Ca^{2+} mobilization, measured as Fura 2 fluorescence intensity, increased with LDL concentrations (fig. 2). At the lowest LDL concentration, the kinetics were similar to those obtained by formyl-Met-Leu-Phe stimulation. At LDL concentrations above 0.5 mmol/l cholesterol, kinetics were the same as those obtained after polymorphonuclear neutrophil stimulation by a particular agent, such as opsonized zymosan. Bovine albumin at concentrations from 0.1 to 1.0 mg/l induced less than 1/10 of the increase in Fura 2 fluorescence intensity induced by LDL at respective protein concentrations.

Staurosporine effect

At a concentration of 100 nmol/l, staurosporine showed a potentiation of formyl-Met-Leu-Phe-induced polymor-
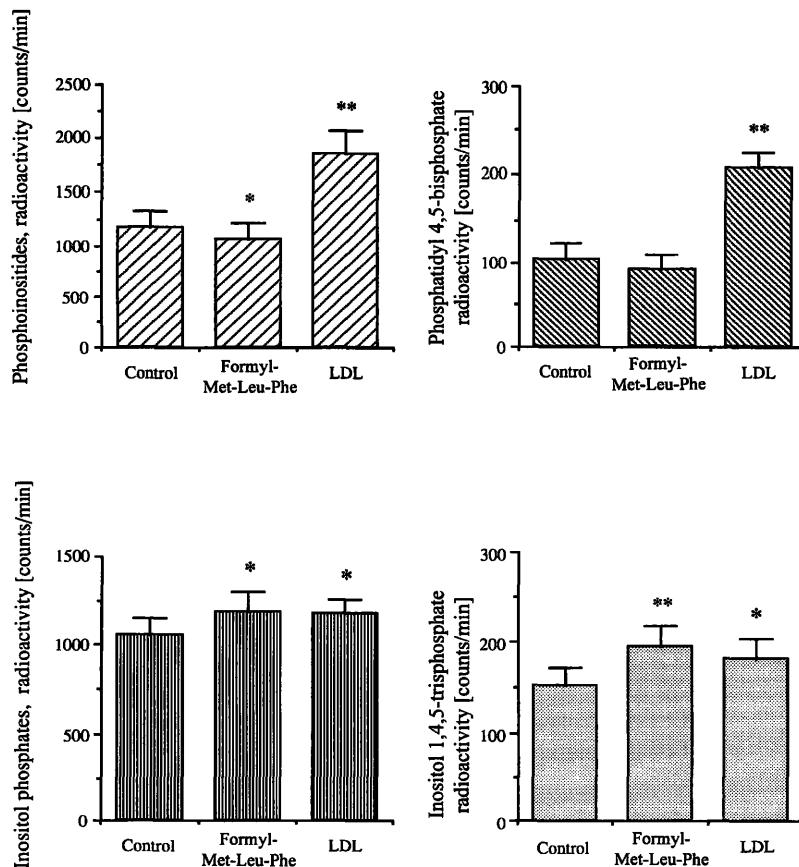

Fig. 1 Variation of phosphoinositides and inositol phosphates of polymorphonuclear neutrophils stimulated with formyl-Met-Leu-Phe or LDL.
Mean ± SEM
Wilcoxon's test for a paired series (n = 10)
* significantly different from control p ≤ 0.05
** significantly different from control p ≤ 0.01

Tab. 1 Effects of LDL on polymorphonuclear neutrophil stimulation by formyl-Met-Leu-Phe. Radioactivity was expressed in counts per minute

LDL (mmol/l)	Formyl-Met-Leu-Phe (mmol/l)	Phosphatidylinositol 4,5-bisphosphate		Inositol 1,4,5-trisphosphate	
		Formyl-Met-Leu-Phe (counts/min)	LDL + formyl-Met-Leu-Phe (counts/min)	Formyl-Met-Leu-Phe (counts/min)	LDL + formyl-Met-Leu-Phe (counts/min)
2.5	10^{-6}	29	65	124	70
2.5	10^{-6}	167	292	164	170
2.5	10^{-6}	70	263	251	202
5.0	10^{-6}	33	136	168	136
5.0	10^{-6}	163	344	167	154
2.5	10^{-7}	77	200	241	220
5.0	10^{-9}	159	340	137	110

phonuclear neutrophil stimulation (polymorphonuclear neutrophils + formyl-Met-Leu-Phe: O_2^- 2.58 ± 0.92 nmol/10^6 cells; polymorphonuclear neutrophils + staurosporine + formyl-Met-Leu-Phe: O_2^- 6.37 ± 2.06 nmol/10^6 cells; means ± SEM, n = 3).

Staurosporine did not significantly modify the LDL effect on the polymorphonuclear neutrophil response to formyl-Met-Leu-Phe (polymorphonuclear neutrophils + LDL + formyl-Met-Leu-Phe: O_2^- 8.12 ± 1.91 nmol/10^6 cells; polymorphonuclear neutrophils + staurosporine + LDL + formyl-Met-Leu-Phe: O_2^- 8.87 ± 2.29 nmol/10^6 cells).

Propranolol effect

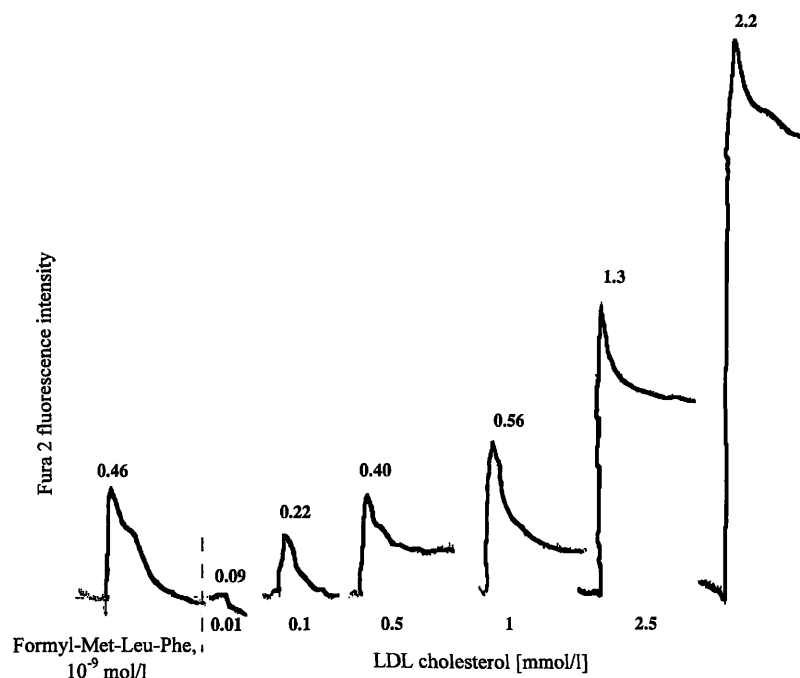Polymorphonuclear neutrophil preincubation for 5 min at 37 °C with phosphate buffer or with various concentrations of propranolol, ranging from 0.05 mmol/l to 5 mmol/l, was performed before the polymorphonuclear neutrophil stimulation with LDL, formyl-Met-Leu-Phe or LDL + formyl-Met-Leu-Phe.

Propanolol exerted a potent stimulating effect at a concentration of 0.5 mmol/l but an inhibiting effect at the highest concentration (5 mmol/l).

LDL added their proper effect to that exerted by formyl-Met-Leu-Phe (fig. 3a).
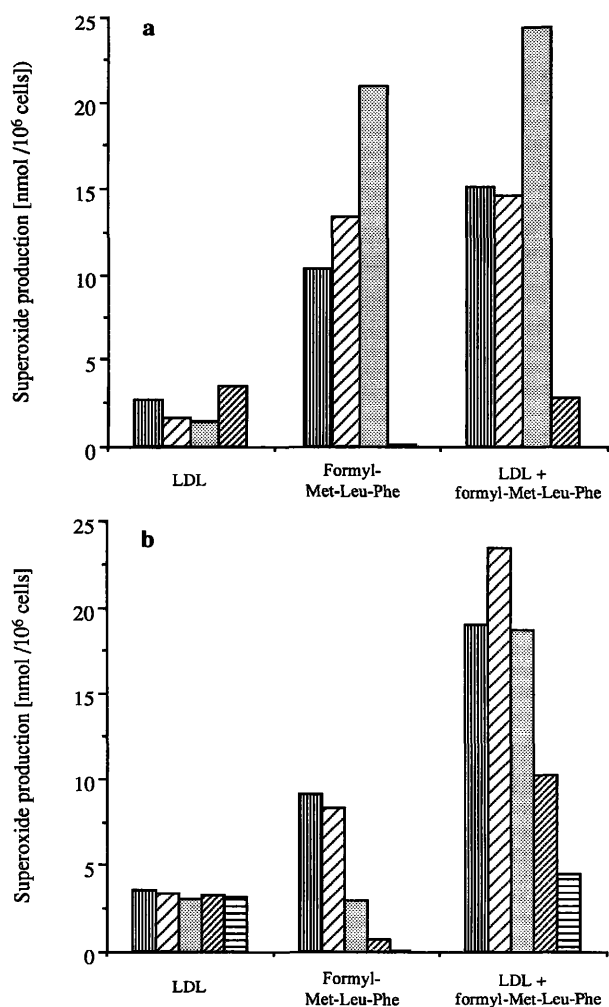
Niflumic acid effect

Niflumic acid had no effect on polymorphonuclear neutrophil stimulation by LDL. When the cells were preincubated for 5 min at 37 °C with various concentrations of niflumic acid (88.6 µmol/l, 443 µmol/l, 886 µmol/l,

Fig. 2 Effect of LDL on cytosolic-free calcium concentration in polymorphonuclear neutrophils. Calcium concentration stated above the peaks was calculated from Fura 2 fluorescence intensity using the formula: $[Ca^{2+}]$ nmol/l = 224 (F − F_{min})/(F_{max} − F) where F_{min} and F_{max} were the fluorescence intensity measured on unstimulated and on Triton X-100 treated polymorphonuclear neutrophils respectively. A representative experiment out of three is shown.

Fig. 3 Effect of propranolol (a) and niflumic acid (b) on polymorphonuclear neutrophil stimulation by formyl-Met-Leu-Phe and LDL.
Concentrations of propranolol used:
▥ control, ▨ 0.05 mmol/l, ▨ 0.5 mmol/l, ▨ 5 mmol/l
Concentrations of niflumic acid used:
▥ control, ▨ 88.6 μmol/l, ▨ 0.5 mmol/l, ▨ 886 μmol/l,
▤ 1772 μmol/l
Result of one representative experiment

1772 μmol/l) before the addition of formyl-Met-Leu-Phe, an inhibiting effect was observed for concentrations higher than 88.6 μmol/l. LDL potentiated the formyl-Met-Leu-Phe stimulating effect and added their proper stimulating effect to the response of control cells incubated in phosphate buffered saline (fig. 3b).

Intracellular cholesterol content

In the presence of LDL (0.25−1 mmol/l cholesterol) a concentration-dependent increase of polymorphonuclear neutrophil cholesterol content was observed (*Mann-Whitney* test: $p \leq 0.05$) (fig. 4).

Discussion

This study has shown that LDL, at physiological concentrations, interacts with polymorphonuclear neutrophil

stimulation either directly by its own stimulating effect or indirectly by modification of the response to formyl-Met-Leu-Phe.

In previous work (5), a slight enhancement of the binding of formyl-Met-Leu-Phe to its specific membrane receptors was observed in the presence of LDL which may induce an increase in O_2^- released in response to formyl-Met-Leu-Phe. Because LDL did not interfere with pertussis toxin inhibitable G proteins, they also might have a "priming" effect which can potentiate the NADPH oxidase stimulation by formyl-Met-Leu-Phe (34) rather than increase the number or affinity of the formyl-Met-Leu-Phe receptors.

Phospholipase C activity was investigated by means of inositol phosphate measurement and a phosphoinositide metabolism study. We observed a highly significant increase of phosphatidylinositol 4,5-bisphosphate content which could not be due to an accelerated inositol turnover because dephosphorylating enzymes were inhibited by the lithium added to the incubation buffer. However, it is possible that tritiated inositol, in excess in the cell medium, conjugated to CMP-activated phosphatidic acid was able to replenish membrane stocks of phosphatidyl inositol (phosphates). This phenomenon might be due to phospholipase D activation by LDL, leading to an increased generation of phosphatidic acid. This activation may be greater than that of phospholipase C inducing a relatively weak enhancement of inositol 1,4,5-trisphosphate release. Inositol 1,4,5-trisphosphate is released into the cytosol, binds to specific receptors on intracellular Ca^{2+} storage organelles and induces the release of calcium. In our study, the intracellular calcium mobilization, measured as Fura 2 fluorescence intensity, increased with LDL concentrations. However at LDL cholesterol concentrations above
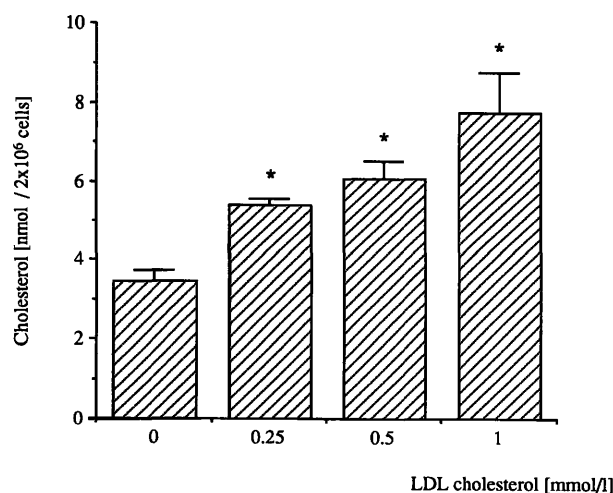
Fig. 4 Cellular cholesterol content of polymorphonuclear neutrophils after contact of 15 min with LDL at different concentrations.
Mean ± SEM; one experiment in triplicate
Mann-Whitney's test for non-paired values
* significantly different from control ($p \leq 0.05$)

0.5 mmo/l, Fura 2 fluorescence was sustained even in an incubation medium without calcium and in the presence of EGTA (data not shown). These data suggest that the calcium efflux could be decreased by LDL. To the best of our knowledge, there is no previous study on the effect of LDL on polymorphonuclear neutrophil phosphoinositide metabolism. However *Block* et al. (35) have already described a stimulation of the phosphatidylinositol cycle by LDL in human platelets, lymphocytes and fibroblasts.

Protein kinase C, a phospholipid/Ca^{2+}-dependent kinase, is a key enzyme in the regulation of intracellular signal transduction. Staurosporine, an alkaloid extracted from *Streptomyces*, was described by *Tamaoki* et al. (36) as a protein kinase C inhibitor. These authors have suggested that staurosporine interacts directly with protein kinase C and this hypothesis has been corroborated by *Nakadate* et al. (37) who have shown that staurosporine interacts at the active site of the catalytic fragment of protein kinase C. Staurosporine is one of the most potent inhibitors, active at nanomolar concentrations, but demonstrates similar affinities for protein kinase C, tyrosine kinases and c-AMP-dependent protein kinase (38, 39). In our previous experiments, polymorphonuclear neutrophil stimulation by phorbol myristate acetate was inhibited 90% by staurosporine, 100 nmol/l. However, the effect of LDL on polymorphonuclear neutrophil does not seem to be directly and exclusively protein kinase C-dependent because in the present study it was not inhibited by staurosporine.

Nevertheless, staurosporine exerts several effects on polymorphonuclear neutrophil activation. This substance at a concentration of 100 nmol/l potentiates polymorphonuclear neutrophil stimulation by formyl-Met-Leu-Phe (40) and inhibits superoxide generation at higher concentrations. These effects might depend on the length of polymorphonuclear neutrophil treatment with staurosporine. *Combadière* et al. reported 125% O_2^- production compared to the control (41) while we found a 200% increase in O_2^- production by polymorphonuclear neutrophil incubation 10 min with staurosporine before the addition of formyl-Met-Leu-Phe. Likewise, an increase of phosphatidic acid release was shown by *Reinhold* et al. (42) and *Perianin* et al. (43). Recently, *Mori* et al. (44) have shown that enhancement of superoxide production by staurosporine 100 nmol/l via phospholipase D activation in formyl-Met-Leu-Phe-stimulated polymorphonuclear neutrophils was the result of an increase in diacylglycerol production through phosphatidic acid.

Considering the phopsholipase D activation already described with staurosporine, we hypothesize that the effects of LDL treatment on polymorphonuclear neutrophil phosphoinositide metabolism could be explained by a similar mechanism.

In formyl-Met-Leu-Phe-activated polymorphonuclear neutrophils, phosphatidylcholine is the phospholipase D substrate generating phosphatidic acid which in turn leads to a diacylglycerol release after its dephosphorylation by phosphatidate phosphohydrolase. *Rossi* et al. have shown that this enzyme was inhibited by propranolol (45). In the same experimental conditions as those used by these authors, we showed an enhancement of O_2^- generation which is for *English* et al. (46) correlated to phosphatidic acid release. However, *Agwu* et al. have shown that polymorphonuclear neutrophil stimulation by formyl-Met-Leu-Phe was counteracted by high a propranolol concentration (5 mmol/l) which inhibited phospholipase D (47). Under the same experimental conditions, we observed that LDL did not modify the propranolol effect.

In formyl-Met-Leu-Phe-stimulated human neutrophils, protein kinase C-independent activation mechanisms have been suggested and *Müller* & *Nigam* (48) have shown that staurosporine enhances arachidonic acid and platelet activation, and leukotriene B_4 release. We have thus tested the activity of niflumic acid, a non-steroidal anti-inflammatory drug, on polymorphonuclear neutrophil activation. According to *Abramson* et al. (49) various concentrations of niflumic acid inhibited formyl-Met-Leu-Phe-induced O_2^- generation in a concentration-dependent manner. Nevertheless, niflumic acid did not inhibit the LDL-induced polymorphonuclear neutrophil oxidative metabolism and even the lowest concentration used (88.5 µmol/l) potentiated an LDL effect, probably by stimulating phospholipase A_2, which leads to arachidonic acid accumulation.

LDL is able to modify in vitro the polymorphonuclear neutrophil oxidative metabolism either by a direct stimulating effect or by alteration of the polymorphonuclear neutrophil response to formyl-Met-Leu-Phe by an action which could involve phospholipase D and phospholipase A_2 activations.

The LDL structure plays a main role in its action of polymorphonuclear neutrophil functions. LDL modifies cell lipid content, since a concentration-dependent increase in cholesterol level was observed after incubation of polymorphonuclear neutrophil with increasing LDL concentrations. We have previously demonstrated (5) that LDL from hypertriglyceridaemic subjects stimulated the polymorphonuclear neutrophil oxidative metabolism and inhibited polymorphonuclear neutrophil chemotaxis less than LDL from normolipaemic subjects whose composition is different. Moreover, LDL trypsination experiments showed that the lipid moiety is mainly responsible for the described effects of LDL on polymorphonuclear neutrophil migration (5). These observations, added to the fact that the polymorphonuclear neutrophil cholesterol amount increases after the incubation of these cells with increasing LDL concentrations,

suggest that lipid exchanges between lipoproteins and cell membranes might appear and modify the membrane fluidity. These modifications could alter formyl-Met-Leu-Phe receptor expression and affinity. This hypothesis was corroborated upon viewing the alteration of formyl-Met-Leu-Phe binding on polymorphonuclear neutrophils extracted from hyperlipidaemic type IIb patients (50).

In spite of their short half-life, polymorphonuclear neutrophils could be involved in atherogenic states because they display, in their excited state, a great source of free radicals involved in endothelial injuries. LDL are transported into the intimal layer through the endothelium (51) and their concentration in the arterial intima is three times greater than in plasma (52). The polymorphonuclear neutrophils present in the intima may be activated by LDL. Activated neutrophils might produce oxygen free radicals and therefore modify LDL but they are found in small numbers in the atherosclerotic intima (53). However it has been shown in rabbits fed a high cholesterol diet that polymorphonuclear neutrophils were the most numerous cells at the beginning of the atheromateous process, and were soon replaced by monocytes and lymphocytes (54). Therefore it seems particularly interesting to study the interaction between polymorphonuclear neutrophils and lipoproteins during the early stages of atherogenesis in animal models.

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