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Activation of human blood monocytes by oxidized polyunsaturated fatty acids: a possible mechanism for the generation of lipid peroxides in the circulation

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Summary. The ability of native and oxidized lipids and lipoproteins to stimulate production of reactive oxygen species (ROS; superoxide and hydrogen peroxide) by human blood monocytes has been studied *in vitro*. Neither native human low density lipoprotein (LDL), 'altered' LDL (oxidized either by lipoxygenase, activated human monocytes or air) nor oxidized cholesterol had any significant effect on ROS production of monocytes. However, different oxidation products of a lipid emulsion (Lipofundin; largely consisting of linoleic acid oxidized either by lipoxygenase, Fe^{3+} or ultraviolet irradiation) greatly enhanced ROS production of monocytes. A hypothesis that activation of circulating leucocytes by oxidized fatty acids may generate oxidized plasma LDL, was tested in rabbits. Characteristics of LDL, separated from rabbit plasma 6 h after intravenous injection of an oxidized lipid emulsion, was compared to that of LDL isolated before the lipid treatment. Post-treatment LDL-fraction of plasma had increased lipid peroxide content and compared to the pretreatment LDL, caused a threefold increase in the incorporation of cholesterol into cultured (rat aortic) endothelial cells. The observed intense and lasting stimulation of monocytes by oxidized polyunsaturated fatty acids *in vitro*, and the generation of 'altered' LDL by these oxidized lipids *in vivo* suggests a mechanism by which atherogenic oxidized LDL could form in the circulation.

Keywords: lipid peroxidation, phagocytosis, atherosclerosis

Recently, evidence has accumulated suggesting that 'modified or altered' lipoproteins (Carew 1989; Steinberg *et al.* 1989) and cholesterol oxidation products (Morin & Peng 1989) play an important role in atherogenesis. The common initiating step of modification is the peroxidation of polyunsaturated fatty acids and cholesterol in the low density lipoprotein (LDL) lipids. In the vessel wall, interaction of oxidatively modified LDL (m-LDL) with the resident macrophages results in foam cell formation, and the chemotactic effect of m-LDL helps in the recruitment of more circulating monocytes. Oxidation of lipids and hence the interaction between oxidized lipids and cells (macrophages, endothelial and smooth muscle cells) have been suggested to occur only within the

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artery wall and not in the circulation (Steinberg *et al.* 1989). This view is based on the assumption that 'altered' LDL disappears from the circulation in a few minutes (Nagelkerke *et al.* 1984), and that the abundant antioxidant defences in the plasma prevent the occurrence of such oxidation. However, another line of evidence suggests that plasma lipid hydroperoxides are significantly increased in patients with severe atherosclerosis (Stringer *et al.* 1989a) and disorders associated with vascular damage (Nishigaki *et al.* 1981; Yagi 1987; Uysal *et al.* 1986; Davies *et al.* 1990). Further, monocyte-altered LDL was shown to disappear from the circulation only twice as quickly as native LDL (plasma half-life 3.0 h *vs* 6.2 h respectively) (Görög *et al.* 1989). As lipid peroxides are cytotoxic to endothelial cells (Yagi *et al.* 1981; Morin & Peng 1989) and induce proliferation of smooth muscle cells (Stringer *et al.* 1989b), an increase in lipid peroxide level in blood could provoke atherosclerotic lesion of the vessel wall.

In our earlier studies, intravenous injection of oxidized lipid emulsion in rabbit resulted in a rapid decline followed by a sustained secondary rise in plasma lipid peroxide levels (Görög *et al.* 1989). This intriguing finding suggested an amplifying mechanism by which the intravascular lipid hydroperoxides would trigger more intense peroxidation of the lipid components of plasma. This hypothesis has been tested in this paper, by studying the effect of oxidized lipids and m-LDL on the production of reactive oxygen species (ROS) by human peripheral blood monocytes. The ability of oxidized polyunsaturated fatty acids to stimulate monocytes and trigger their ROS production, may have potential importance in atherogenesis, by generating and maintaining a sustained level of lipid hydroperoxides in the circulation.

Materials and methods

Lipid emulsion (Lipofundin)

Lipofundin 20% (B. Braun, FRG) is a stable fat emulsion prepared from soybean oil and emulsified with soybean lecithin. The emulsion, which has a particle size of 1 μm (chylomicron size), is largely composed of linoleic and oleic acids and used clinically for parenteral nutrition.

Preparation of LDL

LDL ($d = 1.019–1.063$ g/ml) was prepared from freshly drawn human plasma containing disodium ethylenediamine tetraacetate (EDTA, 1 mg/ml) by differential ultracentrifugation (Havel *et al.* 1955). LDL was dialysed at 4°C for 48 h against 2 × 500 vol of 0.15 M NaCl and then sterilized by passage through a 0.45 μm Millipore filter.

Isolation of human monocytes

Mononuclear cells were isolated from heparinized venous blood (5 U/ml) by centrifugation on Ficoll-Hypaque gradients (400*g* for 40 min at 20°C) and incubated in RPMI-1640 medium with 10% foetal calf serum (complete medium) for 3 h at 37°C in a plastic dish. After incubation, the non-adherent cells were removed from the dish by rinsing (× 3) with cold Hank's balanced solution (HBSS). Adherent cells were then detached by incubation for 15 min with 0.02% EDTA at room temperature and by gentle scraping with a plastic policeman. These cells (>96% monocytes) were then washed, resuspended in complete medium and kept non-adherent in a Teflon dish for 3–4 days (in a CO_2 incubator at 37°C, changing the medium daily) to allow them to differentiate into mature macrophages (Andreesen *et al.* 1983).

Culture of endothelial cells

Endothelial cells were cultured from the thoracic aorta of rat by a primary explant technique as described by McGuire and Orkin (1987). For incubation with LDL, cells (4th passage) were seeded in 35-mm dishes and cultured in RPMI-1640 medium con-

taining antibiotics and 20% foetal calf serum, until confluency ($5.5–6 \times 10^5$ cells/dish) at 37°C in a humidified CO_2 incubator. The confluent monolayers were washed with medium without serum and incubated with complete medium containing 2.5% bovine serum albumin instead of calf serum for 24 h to deplete cholesterol content of cells. The medium was then changed for a fresh one containing 400 μg LDL protein/dish and the cells were incubated (two dishes for each LDL preparation) for 8 h in the CO_2 incubator. Cells were then washed with buffered saline, detached from the dish with 0.25% trypsin-EDTA, collected by centrifugation, and resuspended in 500 μl 0.1% triton X-100. The repeatedly frozen and thawed samples were tested for total cholesterol content, by the CHOD-iodide enzymatic assay (Merc, Darmstadt). Calibration curve was obtained by cholesterol (Sigma) and was linear in the 1–100 μg range.

LDL modification

By phagocytosing monocytes. The technique has been described in detail (Görög & Kakkar 1987). LDL was incubated with monocytes (1 mg LDL protein/10^7 cells/ml) at 37°C for 1 h. At the beginning of the incubation, cells were activated by the addition of 100 μl of a 2.5% suspension of polystyrene microspheres/ml culture medium (microspheres, 3 μm diameter, Polysciences, UK). At the end of the incubation, cells and the free beads were centrifuged (12 000*g* for 5 min) and the supernatant was called 'monocyte-modified LDL'.

By lipoxygenase. LDL was incubated with lipoxygenase (EC 1.13.11.12; Type I, Sigma; 10 μg/mg LDL protein/ml) for 18 h at 37°C. After incubation, density was adjusted to 1.08 g/ml by solid KBr, the solution (18 ml) was overlayered with 2 ml density solution of 1.063 g/ml and centrifuged at 140 000*g* for 20 h. The top (LDL) fraction was dialysed against EDTA/saline for 24 h and called 'lipoxygenase-modified LDL'.

By air or Fe^{3+}. LDL was incubated in 95% air-5% CO_2 at 37°C for 5 days (air-modified LDL) or under the same conditions but added 10 μM Fe^{3+} (Fe^{3+}-modified LDL), for 1 day.

Oxidation of lipofundin

Lipofundin (20%) was incubated with lipoxygenase (100 μg/ml) for 18 h at 37°C, as described for LDL. After centrifugation at 140 000*g* for 4 h, the top layer was collected, diluted in 0.15 M saline and the lipofundin content was determined by measuring the light-scattering index in a Perkin-Elmer fluorimeter (Ohkawa *et al.* 1978).

Lipofundin was oxidized with Fe^{3+}, as described for LDL.

Lipofundin was oxidized by ultraviolet light irradiation. Light from a 150 W xenon lamp was thrown onto the sample by a surface mirror. Lipofundin was placed in an open dish and irradiated for 6 h.

Oxidation of cholesterol

A suspension of cholesterol in buffered saline was incubated with cholesterol oxidase (EC 1.1.3.6; Sigma) at pH 7.4/25°C for 18 h. The enzyme was removed by repeated centrifugation and washing of the oxidized cholesterol.

Characterization of oxidatively modified LDL

Lipid peroxides were estimated as thiobarbituric acid reactive substances (TBARS), using a modification of Yagi's fluorimetric method at pH 3.2–3.4 (Ohkawa *et al.* 1978), as described in our earlier work (Stringer *et al.* 1989a). Thiobarbituric acid reaction was carried out directly by adding 10–20 μl of the sample (LDL or lipofundin) to the acetic acid–TBA mixture containing 10 μl of 5 mM Fe^{3+} as a catalyst to decompose lipid peroxides and 50 μl of 7.0 M butylated hydroxytoluene (BHT) as antioxidant to prevent auto-oxidation during the assay (Janero & Burghardt 1989). The mixture was incubated at 90°C

for 60 min, the tubes then placed on ice, centrifuged at 12000*g* for 3 min, and the fluorescence was read at 515 nm excitation and 553 nm emission wavelengths. Fluorescence was expressed in nmol malondialdehyde (MDA), using a standard curve obtained with 1,1,3,3,-tetramethoxypropane.

Lipid peroxide content of LDL separated from rabbit plasma was measured by a highly sensitive iodometric assay, using CHOD-iodide (Merc, Darmstadt, FRG), as described by El-Saadani *et al.* (1989). Concentrations of lipid peroxides were calculated by a molar absorptivity of I_3^- measured at 365 nm (2.46×10^4/M/cm).

Modification of LDL was also characterized by electrophoresis in agarose gel (Noble 1968).

Modified LDL was further characterized by localization of lipid peroxides in the separated lipid classes. Lipids were extracted from LDL with chloroform–methanol 2:1 (v/v) and applied to a thin-layer chromatography plate (TLC). Neutral lipids (NL) were separated on Whatman LK6D plates in a solvent system consisting of hexane–ether–acetic acid 80:20:1 by volume. Phospholipids (PL) were separated on Whatman LK5D plates; the developing solvent consisted of chloroform–methanol–water 70:30:5 (Smith & Seakins 1976). Lipids were visualized with iodine vapour. Lipid peroxides were visualized by a starch–iodide spray (Huttner *et al.* 1977).

Treatment of rabbits with oxidized lipofundin

Five adult male New Zealand White rabbits, weighing between 3.2 and 3.5 kg were used. Ultraviolet light-oxidized lipofundin (20%, 1 ml/kg; TBARS 47 nmol/ml) was administered intravenously. Blood samples (15 ml) were taken from the ear vein into tubes with EDTA (1.5 mg/ml) prior to and 6 h after the lipofundin treatment. LDL fractions of the plasma samples were separated by differential ultracentrifugation, as described above.

Superoxide assay

Light emission generated by activated leucocytes was measured. Lucigenin (10,10′-dimethyl-bis-9,9′-biacridinium nitrate from Sigma) was used to amplify the chemiluminescence (CL), as the specificity of lucigenin for $O_2^{\cdot}$ has been documented (Corbisier *et al.* 1987). A custom-built chemiluminometer was used, in which the sample was kept at 37°C and stirred by a built-in magnetic stirrer. A cooled photomultiplier tube (Products for Research Inc., Danvers, Mass.) continuously detected the light, the count of photons per second was digitized and averaged over 3-s intervals by a Panax-Reigate interface, displayed by a Packard scaler, and also plotted by a chart-recorder. The standard CL assay was performed in a 0.1 M glycine–NaOH buffer at pH 9.0, containing 1 mM EDTA, 0.1 M lucigenin and cells 2×10^7/ml; the final volume of the test was 0.6 ml.

Hydrogen peroxide assay

The fluorimetric assay described by Bass *et al.* (1983) was used. Leucocytes were incubated with 2′,7′-dichlorofluorescein diacetate (DCFH-DA). During incubation, DCFH-DA diffused into the cells, was hydrolysed to 2′7′-dichlorofluorescein (DCFH) and was thereby trapped within the cells. Due to H_2O_2 production by the cell, the intracellular and nonfluorescent DCFH was oxidized to highly fluorescent 2′,7′-dichlorofluorescein (DCF). Intracellular oxidation of DCFH is mediated by H_2O_2 generated by the cell. The standard assay was performed by preincubating cells (10^6/ml) for 15 min with 5 μM DCFH-DA in PBS without Ca^{2+} and Mg^{2+} in a final volume of 10 ml in a water bath at 37°C. 0.5 ml of the cell suspension was placed in a microcuvette, EDTA was added (2 mM final concentration), and the fluorescence of the cells stirred with a magnetic stirring flea was measured in a Perkin-Elmer spectrofluorimeter (495 nm excitation, 545 nm emission).

Results

The effects of native, 'altered' LDL preparations, natural and oxidized lipid emulsion and oxidized cholesterol on superoxide-specific CL of human blood monocytes are shown in Table 1. CL induced by lipids was expressed as percentage of response of cells to a standard stimulus (bead phagocytoses). Of the LDL preparations, only the monocytes-modified LDL induced slight, statistically non-significant increase in CL. Oxidized cholesterol also avoided stimulation of cells. However, all the different oxidized lipid emulsions (oxidized by lipoxidase, Fe^{3+} or UV-irradiation), were highly active in inducing CL from monocytes (Fig. 1). Measurement of the duration of the induced CL revealed long-lasting effects. Although the peak effect of UV-oxidized lipofundin on CL was less than half of the standard phagocytosing stimulus, it lasted 2.3 times longer. No apparent relationship was observed between TBAS reactivity and the CL induced by oxidized lipids. Monocytes altered LDL and lipoxygenase-oxidized lipofundin showed close TBAS-reactivity, while the latter produced 11-fold more CL than the former.

Effects of native and oxidized lipids/lipoproteins on hydrogen peroxide production by monocytes, expressed as percentage of the response to a standard PMA, is shown in Table 2. Lipoxidase and monocyte-altered LDL induced slightly more H_2O_2 production than native LDL, but statistically the differences were not significant. All oxidized lipofundin preparations greatly enhanced H_2O_2 production by monocytes. No correlation was found between lipid peroxide content and hydrogen peroxide production. Although the concentration of TBARS in monocytes-altered LDL and UV-oxidized lipofundin were similar, the latter was much more effective in stimulating H_2O_2 production of cells.

The effect of intravenous administration of

Table 1. Superoxide production of human blood monocytes stimulated by oxidized lipid products

		Chemiluminescence (mean ± s.e.m.)	
Lipid	TBARS*	Peak (%)†	Integral (30 min)§
None (spontaneous)		1.45 ± 0.38	5.83 ± 3.16
None (phagocytosis)		100	100
LDL (native)	1.22	2.50 ± 0.89	10.10 ± 2.03
LDL-Ox (lipoxygenase)	5.45	2.63 ± 0.99	10.12 ± 4.78
LDL-Ox (monocytes)	6.25	4.10 ± 1.56	12.55 ± 1.87‡
LDL-Ox (air)	2.25	1.89 ± 0.95	7.93 ± 4.60
Lipofundin	1.48	5.70 ± 2.08‡	18.70 ± 5.69‡
Lipof-Ox (lipoxygenase)	7.84	30.62 ± 8.61‡	164.60 ± 25.26‡
Lipof-Ox (Fe^{3+})	9.42	24.88 ± 5.82‡	121.05 ± 53.91‡
Lipof-Ox (UV-light)	8.60	41.60 ± 8.12‡	232.65 ± 76.66‡
Cholesterol-Ox	2.10	2.30 ± 1.19	15.20 ± 1.99‡

Mean of four series of experiments.

* nmol MDA/mg LDL; ml lipofundin; 100 μg cholesterol.

† Arbitrary units, i.e. height of the chart-recording in mm, expressed in the % of the recording of beads-phagocytosed monocytes (100%).

‡ Difference from the spontaneous chemiluminescence is significant, $P < 0.001$.

§ Integrated area of the increased chemiluminescence on the chart-recording.

Table 2. Effect of oxidized lipid products on hydrogen peroxide formation by human monocytes

Lipid	Increase of fluorescence (10 min)	
	TBARS*	% (mean ± s.e.m.)
None (spontaneous)		2.83 ± 1.11
PMA (20 ng/ml)		100
LDL (native)	1.60	5.25 ± 1.74
LDL-Ox (lipoxygenase)	4.82	8.93 ± 2.43
LDL-Ox (monocytes)	6.45	9.18 ± 3.35
LDL-Ox (air)	1.90	2.68 ± 1.49
Lipofundin	2.22	8.98 ± 4.58
Lipof-Ox (lipoxygenase)	11.40	48.80 ± 9.96†
Lipof-Ox (Fe^{3+})	13.20	61.20 ± 14.20†
Lipof-Ox (UV)	8.60	61.15 ± 19.93†
Cholesterol-Ox	2.25	3.77 ± 1.79

Mean of four separate experiments.

* nmol MDA/mg LDL; ml lipofundin; 100 μg cholesterol.

† Difference from the spontaneous fluorescence is significant: $P < 0.001$. Arbitrary units, mm deflection of the recordings on the chart.

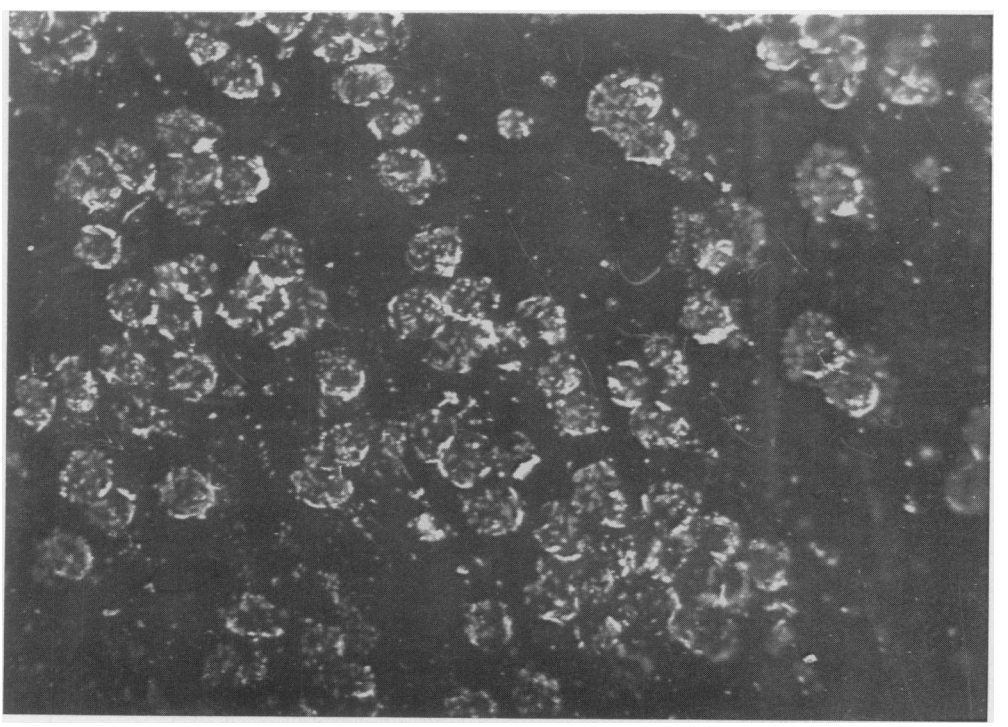

Fig. 1. Fluorescence micrograph of unfixed monocytes shows intense fluorescence of cells after incubation with CTC and oxidized lipofundin. × 380.

Table 3. Altered characteristics of LDL separated from rabbit plasma 6 h after intravenous injection of an ultraviolet light oxidized lipofundin ($n = 5$)

No. of rabbit	Lipid peroxide content of LDL* (nmol/mg LDL)		Cholesterol content of endothelial cells† (μg/dish)	
	LDL-Pre	LDL-Post	LDL-Pre	LDL-Post
1	<10	27	4.29	6.56
2	<10	36	3.96	8.78
3	<10	54	2.32	12.72
4	<10	71	6.91	17.99
5	<10	61	3.49	13.53
Mean	<10	49	4.19	11.92
s.e.m.		±8.1	±0.76	±1.98
P (pre *vs* post)			0.012	

* Measured by iodometric assay.

† Confluent cells (about 6×10^5 cells/dish) were incubated with LDL separated from rabbit plasma collected prior to or 6 h after the oxidized lipofundin treatment (Pre/Post-LDL, respectively). Significance was calculated by the paired Student's *t*-test.

(UV) oxidized lipofundin to rabbits on the characteristics of plasma LDL isolated 6 h after the treatment is shown in Table 3. Lipid peroxide content was significantly increased in LDL preparations separated 6 h after injection of oxidized lipofundin. Lipid classes separated by TLC from native and monocyte oxidized human LDL, as well as from rabbit LDL obtained before and after lipofundin treatment, are shown in Fig. 2. From the neutral lipids (NE), TLC separated cholesterol (C), triglycerides (TG) and cholesterol esters (CE). In addition, monocyte-oxidized human LDL contained four additional spots. None of the neutral lipid classes stained for lipid peroxide in native LDL, but TG, CE and all additional spots of the monocyte-oxidized human LDL did. No difference was observed in the pattern of phospholipid classes (PL) between native and monocyte-oxidized LDL and none of the spots stained for lipid peroxides.

Neutral lipids of LDL separated from rabbit plasma after treatment with lipofundin showed a similar pattern to the monocyte-oxidized human LDL. TG, CE and the three extra spots stained for lipid peroxides. No difference was found in the pattern and staining of phospholipid classes between rabbit LDL preparations collected before and after lipofundin treatment.

Electrophoretic mobility of monocyte-oxidized human LDL was slightly increased (relative mobility to native LDL was 1.16). There was no difference in the electrophoretic mobilities between rabbit LDL preparations collected before and after lipofundin treatment.

When this post-treatment LDL was incubated with cultured endothelium for 24 h, a threefold increase in cellular cholesterol content was observed, as compared to incubation of cells with LDL separated before the oxidized lipofundin treatment (Table 3). A correlation was found between the efficacy of delivering cholesterol to the cells and lipid peroxide content of different LDL preparations.

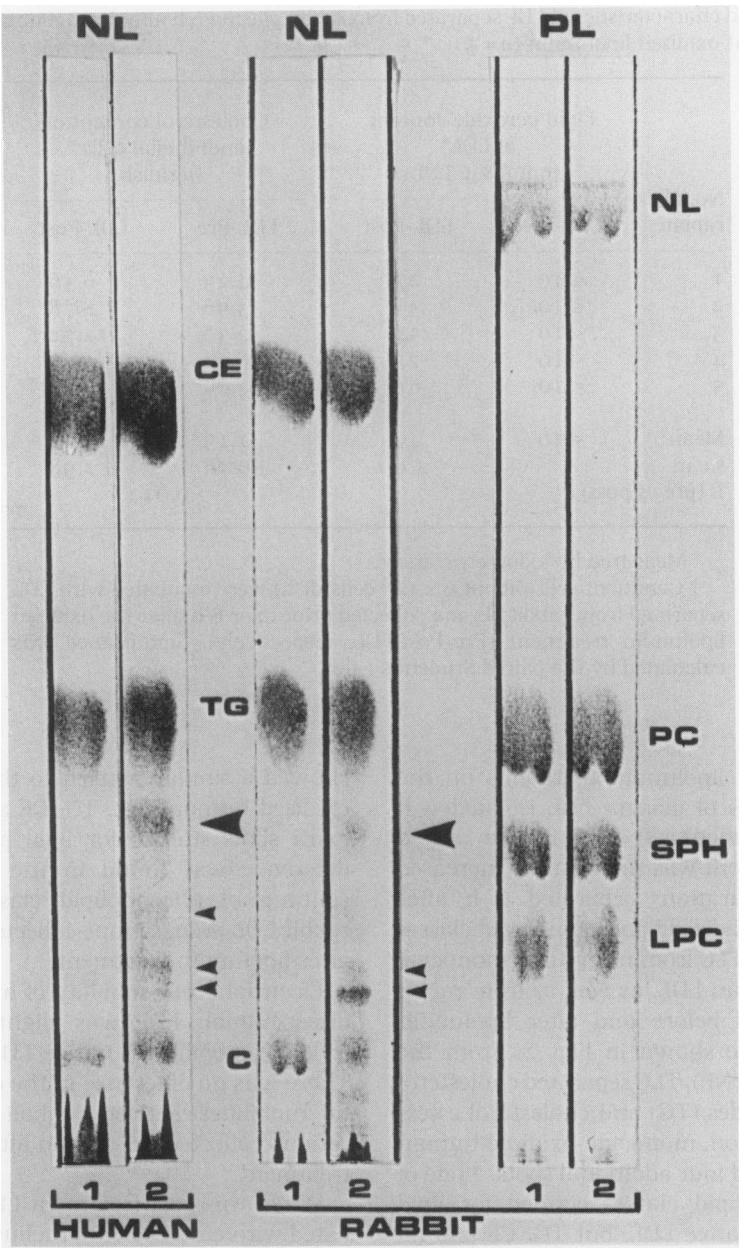

Fig. 2. Neutral lipids (NL) and phospholipids (PL) from human and rabbit LDL preparations. Lane 1: native human LDL and rabbit LDL prior to treatment; Lane 2: monocyte-modified human LDL and rabbit LDL after lipofundin treatment. (NL) contained cholesterol (C), triglycerides (TG) and cholesterol esters (CE). The additional spots which appeared in the monocyte-modified human LDL or in LDL collected from rabbit plasma after lipofundin treatment are indicated by arrows. (CE), (TG) and the four extra spots stained for lipid peroxides in monocyte-modified human LDL; (CE), (TG) and three extra spots stained for lipid peroxides in rabbit LDL after lipofundin. (PL) classes were separated into lysophosphatidylcholine (LPC), sphingomyelin (SPH), phosphatidylcholine (PC).

Rabbit plasma (8 ml) was incubated with (UV) oxidized lipofundin (0.2 ml, 20%) and with saline (0.2 ml) at 37°C for 6 h. At the end of the incubation, plasma samples were filtered through a 0.2 μm filter (to remove lipid emulsion) and LDL was isolated in the presence of 20 μM butylated hydroxytoluene, by differential ultracentrifugation. No significant difference was found between lipid peroxide content of LDL preparations incubated with or without oxidized lipofundin (16 and 19 nmol/mg protein, mean, $n=4$).

Discussion

Incubation of human monocytes with oxidized polyunsaturated fatty acids induced a significant and sustained release of $O_2^{\cdot}$ and H_2O_2 from the cells. Practically no increase in either superoxide or hydrogen peroxide production was observed when cells were incubated with LDL (native or oxidized/altered) or with cholesterol (native or oxidized). This is in conflict with previous findings showing LDL to be a general activator of different cells (Block *et al.* 1988) and with those studies where both native and modified LDL (acetylated or malondialdehyde-modified) stimulated lysosomal enzyme secretion (Kelley *et al.* 1988) or ROS release from monocytes (Hartung *et al.* 1986). Poor correlation was found between potency to stimulate ROS production of monocytes and lipid peroxide content of different lipid preparations. Both monocyte-altered and lipoxidase-treated LDL had increased lipid peroxide levels while neither of them triggered monocytes to release ROS. Peroxidized fatty acids in lipofundin seem to be the components which activate cells. The fact that TBARS reactivity in oxidized LDL is attributed mainly to lipid peroxides in esterified neutral lipids such as cholesteryl esters and triglycerides, as fatty acid hydroperoxides were not identified (Zhang *et al.* 1989), explains the lack of stimulation of ROS production from monocytes, by oxidized LDL.

Recent findings provide evidence that lipid peroxidation could take place in the circulation, especially if levels of the antioxidant ascorbate are reduced (Frei *et al.* 1989). The assumption that plasma LDL could be oxidized or 'altered' as a result of stimulation of leucocytes was tested *in vivo*.

Intravenous administration of oxidized lipofundin resulted in a significant increase in lipid peroxide content of plasma LDL isolated after the treatment. In view of our findings, the possibility of a simple partition of lipid peroxides between the injected lipids and plasma LDL is unlikely: cell-free incubation of oxidized lipid with plasma caused no change in lipid peroxide content of the separated LDL. It is rather an oxidation-coupled 'alteration' of LDL, probably by the contribution of leucocytes, which accounts for the increased lipid peroxide content of circulating LDL. Appearance of extra spots in the neutral lipid classes of rabbit LDL separated after lipofundin treatment, which stained for lipid peroxides, supports this assumption. Similar extra spots of neutral lipids containing lipid peroxides were observed in human LDL oxidized by monocytes (present study) or by air (Zhang *et al.* 1989).

An important characteristic of 'altered' LDL is its ability to be taken up rapidly by cells though the scavenger receptor pathway. The existence of the latter in endothelial cells was demonstrated by Voyta *et al.* (1984). Earlier, we observed a 9.3-fold increase in the uptake of monocyte-altered LDL by rat aortic endothelium *in vivo*, as compared with the uptake of native LDL (Görög & Kakkar 1987). In cultured human vein and rat aortic endothelial cells, a 4.4-fold (Görög & Kakkar 1987) and 6.3-fold increase (unpublished) in the uptake of monocyte-altered LDL was measured, respectively. Thus, an increased cholesterol delivery to endothelial cells by an LDL preparation is evidence for the 'altered' nature of the lipoprotein. This 'altered' nature of LDL generated by oxidized lipid emulsion in rabbit *in vivo* was demonstrated by its increased uptake by cultured endothelial cell, resulting in a three-

fold increase in cellular cholesterol content, compared with the effect of the pretreatment (native) LDL.

Stimulation of monocytes by oxidized polyunsaturated fatty acids could play an important role in atherogenesis. There is a general consensus that alteration (oxidation) of lipoprotein is a prerequisite for massive deposition of cholesteryl esters within the foam cells of atherosclerotic lesions, with the restriction that foam cell formation takes place only in the vessel wall (Steinberg *et al.* 1989). Some studies however suggest that monocytes can be transformed into foam cells while still in the circulation and penetrate as such into the arterial intima (Leary 1941; Poole & Florey 1958; Still & O'Neal 1962). Further, activation of the phagocytic system (PMN and monocytes) by BCG had a profound effect on atherogenesis (Gaton *et al.* 1988). We now suggest a mechanism by which highly atherogenic oxidized LDL could be generated in the circulation.

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