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# COMPARATIVE CYTOPROTECTIVE EFFECT OF DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKERS AGAINST THE TOXICITY OF OXIDIZED LOW DENSITY LIPOPROTEIN FOR CULTURED LYMPHOID CELLS

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**Abstract**—The ability of dihydropyridine $Ca^{2+}$ channel blockers (nicardipine, nimodipine and nisoldipine) to inhibit low density lipoprotein (LDL) oxidation and to prevent the cytotoxicity of oxidized LDL for lymphoid cells have been compared. The lipid peroxidation of LDL promoted either by UV radiation or by copper ions was inhibited (antioxidant effect) in a dose-dependent manner by nisoldipine ($IC_{50}$ values were evaluated at around 10 $\mu$M), whereas nimodipine was less potent ($IC_{50}$ around 50–100 $\mu$M) and nicardipine almost inactive. The cytotoxicity of LDL treated (by UV or by copper) in the presence of effective antioxidant concentrations of dihydropyridine $Ca^{2+}$ channel blockers was less than that of unprotected oxidized LDL (i.e. LDL oxidized in the absence of any dihydropyridine $Ca^{2+}$ channel blockers). The inhibition of the cytotoxic effect of LDL oxidized in the presence of dihydropyridine $Ca^{2+}$ channel blockers correlated well with protection from oxidation by these compounds. Beside this indirect protective effect, dihydropyridine $Ca^{2+}$ channel blockers exhibit a direct protective effect for cells against the toxicity of previously oxidized LDL. Although complete protection cannot be obtained because of the cytotoxicity of the dihydropyridine compounds *per se*, the $IC_{50}$ values were $6 \pm 2$ and $80 \pm 20$ $\mu$M for nisoldipine and nimodipine, respectively. The potential relevance to the prevention of atherogenesis is discussed.

Modified low density lipoproteins (LDL†), more particularly oxidatively modified LDL, which have been detected in atherosclerotic lesions [1, 2] are thought to play a major role in atherogenesis [3–9]. Extensively oxidized LDL (oxidation promoted by transition metals and/or by cells) are characterized by oxidative attack of lipid, leading to formation of peroxidation products, to loss of polyunsaturated fatty acids and of endogenous antioxidants. In addition reaction with apoB, lead to loss of surface amino groups, modification of charge, fragmentation and decreased affinity for the apoB/E-receptor [6–10]. Oxidized LDL are cleared through the scavenger–receptor pathway of macrophage cells [3, 7, 8] and seem to be involved in the recruitment of monocytes which are rapidly converted to foam cells and trapped in the subendothelial space [7, 10]. Beside the lipid accumulation, oxidized LDL have been shown to be highly cytotoxic to cultured cells [11–13]. LDL minimally modified by mild oxidation have been shown to be biologically active [5, 7, 14]. In order to study their properties, we have developed a new experimental model system consisting of LDL treated by short UV radiation that induces mild lipid peroxidation without major alteration of apoB-100 [15, 16]: this explains why the UV-oxidized LDL are taken up through the apoB/E-receptor and exhibit dose-dependent cytotoxicity to cultured cells [17]. Cell injury due to oxidized LDL is potentially involved in the genesis of atherosclerotic lesions, for instance injury of endothelial cells [7, 10, 11] and necrotic lesions observed in the atheroma plaque [7, 18]. Cells can be protected from the cytotoxic effect of oxidized LDL by at least two mechanisms: (1) extra-cellular inhibition of LDL oxidation; (2) direct protection of cells against injury of cytotoxic lipid peroxidation products contained in oxidized LDL [19]. Three types of compounds exhibiting relatively specifically one or two of these properties are known: probucol inhibits LDL oxidation [19, 20], vitamin E prevents the cytotoxicity of oxidized LDL at the cellular level [19] and some flavonoids (such as catechin or quercetin) are active at both sites and protect LDL as well as cells [19, 21, 22].

Dihydropyridine $Ca^{2+}$ channel blockers (Fig. 1), a group of potent inhibitors of voltage-dependent L-type $Ca^{2+}$ channels [23, 24], have been used in the prevention of ischemic myocardial injury [25] and atherosclerosis [26–28]. Recently, Breugnot *et al.* [29] reported that nifedipine was able to inhibit *in vitro* LDL oxidation. As we have reported that antioxidants are able to protect cells against the cytotoxic effect of oxidized LDL [19], as some dihydropyridines exhibit antiperoxidant activity on

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† Abbreviations: CFDA, carboxyfluorescein diacetate; LDL, low density lipoproteins; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium; TBARS, thiobarbituric acid reactive substances; DMSO, dimethyl sulphoxide.

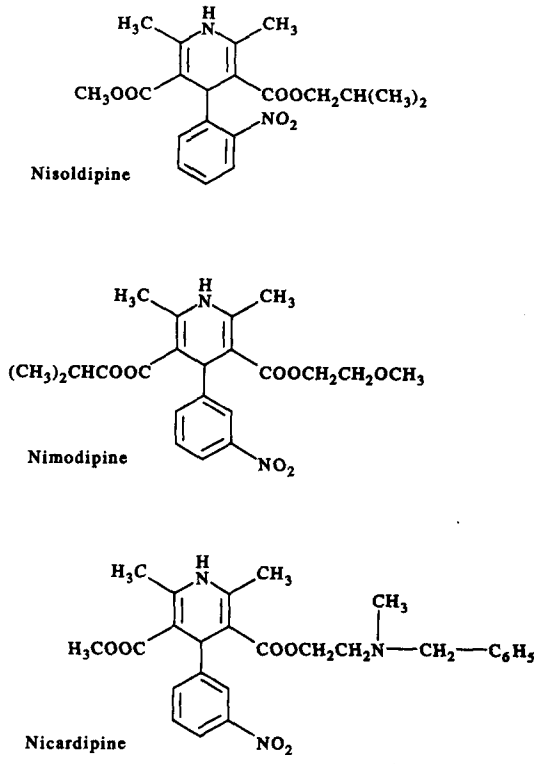


Fig. 1. Structure of the dihydropyridine $Ca^{2+}$ channel blockers used.

liposomal systems [30] and as, to our knowledge, the protective effect of dihydropyridines against the injurious effect of oxidized LDL has never been reported, it was of interest to compare the protective properties of various dihydropyridine $Ca^{2+}$ channel blockers used in the prevention of atherosclerosis [31], in order to understand whether the cyto-protective effect is a general property of all the dihydropyridines or is restricted to some molecules of this chemical group.

We used the experimental model system described previously [17, 19] and compared the protective effect of three dihydropyridine $Ca^{2+}$ channel blockers. We conclude that all the dihydropyridine $Ca^{2+}$ channel blockers do not exhibit the same protective activity (the most active compounds being nifedipine and nisoldipine) and that the protection against the cytotoxic effect of oxidized LDL results from inhibition of extracellular LDL oxidation (at high concentration) and the direct protection of cells against the toxic effect of previously oxidized LDL (this effect being prominent at low concentration).

## MATERIALS AND METHODS

*Chemicals.* Trypan blue dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) bromide and dihydropyridine $Ca^{2+}$ channel blockers nicardipine (and nifedipine) were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.), [$^3$H]-cholesteryl oleyl ether (38 Ci/mmol) and [$^3$H]-thymidine (5 Ci/mmol) from Amersham (Paris, France), carboxyfluorescein diacetate (DFDA) from Molecular Probes (Eugene, OR, U.S.A.), cell culture reagents, RPMI 1640, phenol red-free- or (FRF-) RPMI 1640, foetal calf serum, penicillin, streptomycin, from Seromed (Paris), Ultroser HY from IBF (Villeneuve-la-Garenne, France) and the other chemicals from Merck (Darmstadt, Germany) or Prolabo (Paris). The dihydropyridine $Ca^{2+}$ channel blockers nisoldipine and nimodipine were a generous gift from Bayer A.G. (Wuppertal, Germany).

*Cell culture.* Lymphoid cell lines were established by Epstein–Barr virus transformation and grown in standard medium, i.e. RPMI 1640 supplemented with 10% foetal calf serum, then 48 hr before experiments, the standard medium was replaced by RPMI 1640 containing 2% Ultroser HY (a serum substitute without lipoprotein) as described previously [17, 19].

*LDL isolation, labelling with cholesteryl ether and determination of uptake by cells.* LDL (density 1.01–1.063 g/mL) were isolated by sequential ultracentrifugation [32] from pooled plasma, dialysed, sterilized on 0.2 μm millipore membranes and kept at 4° under nitrogen until use, exactly under the previously used conditions [15, 19].

LDL were labelled with [$^3$H]cholesteryl oleyl ether (around $10^5$ dpm/mg apoB) according to Roberts *et al.* [33], isolated again by ultracentrifugation, dialysed and sterilized on 0.2 μm millipore and kept under nitrogen at 4° until use, under the previously used conditions [17]. Radiolabelled LDL (100 μg apoB/mL) were added to the culture medium for 12 hr, then cells were isolated by centrifugation (400 *g* for 10 min), carefully washed twice in phosphate-buffered saline, homogenized by sonication in 1 mL of distilled water and an aliquot was used for determining the cell-associated radioactivity (using a Packard liquid scintillation counter model Tricarb 4530) under the previously used conditions [17].

*LDL oxidation by UV radiation and protection by dihydropyridine $Ca^{2+}$ channel blockers.* LDL solution (2 mg apoB/mL), mixed with variable concentrations (0–100 μM) of dihydropyridine $Ca^{2+}$ channel blockers (dissolved in dimethyl sulphoxide; 0.5% DMSO final concentration), were submitted to UV-C radiations (254 nm, 0.5 mW/cm$^2$) for 2 hr, under the standard conditions used previously [17, 19]: briefly, the 0.5 mL of LDL solution (0.5 mm thickness, contained in an open glass test tube) was irradiated through the test tube orifice with a vertical UV beam, the UV lamp being situated 10 cm above the level of the test tube orifice, i.e. 15 cm above the level of the LDL solution). Dihydropyridine compounds were mixed with the LDL solution to evaluate their inhibitory effect on UV-induced LDL oxidation. In order to evaluate the "filter effect" of dihydropyridine compounds, the LDL solution (without any dihydropyridine compound) and dihydropyridine compounds were irradiated by UV in two cuvettes in series [the UV radiation passing through a quartz cuvette containing a 100 μM dihydropyridine solution (thickness of the solution: 2.5 mm) placed in

horizontal position on the top of the test tube containing the LDL solution].

Lipid peroxidation of LDL was evaluated by determining thiobarbituric acid reactive substances (TBARS) and $\alpha$-tocopherol content as indicated previously [15, 16]. Non-irradiated LDL were prepared similarly and used as controls.

In the copper-promoted oxidation system, LDL (carefully dialysed against phosphate-buffered saline without EDTA for 48 hr, and used at 2 mg apoB/mL) were submitted to mild oxidation by copper ions (1 $\mu$M $CuSO_4$ at 37° for 1 hr) in the presence of variable concentrations (0–100 $\mu$M) of the tested dihydropyridine $Ca^{2+}$ channel blockers.

*Cytotoxicity of LDL treated by UV in the presence of dihydropyridine $Ca^{2+}$ channel blockers.* A fixed amount (200 $\mu$g apoB/mL) of LDL treated by UV in the presence of variable concentrations of dihydropyridine $Ca^{2+}$ channel blockers as indicated above, were added to the culture medium and cells in 24-microwell tissue culture plates (around $5 \times 10^5$ cells in 1 mL of culture medium per microwell) were incubated with 200 $\mu$g/mL LDL (expressed as $\mu$g apoB/mL). After 48 hr incubation, cell viability was determined on an aliquot of cell culture by using two tests simultaneously, the CFDA test according to McGinnes *et al.* [34] and the MTT test according to Price and McMillan [35].

The "direct" cytoprotective effect of dihydropyridine $Ca^{2+}$ channel blockers was evaluated by adding variable concentrations of these compounds (dissolved in DMSO) to the culture medium simultaneously with the 200 $\mu$g apoB/mL oxidized LDL (UV-treatment in the absence of exogenous $Ca^{2+}$ channel blockers or any additive). After 48 hr incubation, cell viability was determined as indicated above. The potential cytotoxicity of $Ca^{2+}$ channel blockers was evaluated by incorporating increasing concentrations of these compounds (0–100 $\mu$M dissolved in DMSO; 0.5% DMSO final concentration) to the culture medium.

Proteins were determined by the method of Lowry *et al.* [36].

## RESULTS

### *Inhibition of lipid peroxidation of LDL and of their subsequent cytotoxicity by dihydropyridine $Ca^{2+}$ channel blockers (Figs 2 and 3).*

Among the dihydropyridine $Ca^{2+}$ channel blockers tested, nisoldipine inhibited LDL oxidation promoted by UV or copper as demonstrated by the inhibition of lipid peroxidation markers (TBARS) and by the protection of $\alpha$-tocopherol content. In comparison, nimodipine was less active, whereas nicardipine was nearly inactive (Figs 2A and 3A). Under the standard experimental conditions used in the UV system (Fig. 2A), $IC_{50}$ values (calculated on the basis of inhibition of TBARS formation) were $10 \pm 2$ $\mu$M for nisoldipine, $75 \pm 15$ $\mu$M for nimodipine, while nicardipine showed low activity. In order to exclude an inhibitory effect of lipid peroxidation specific to the UV system (e.g. absorption of UV light by $Ca^{2+}$ channel blockers, independent on any antioxidant activity) we evaluated the "filter effect" of these compounds by irradiation LDL solution and dihydropyridine compounds in two cuvettes placed in series (the cuvette containing the dihydropyridine compounds being placed between the test cuvette (containing

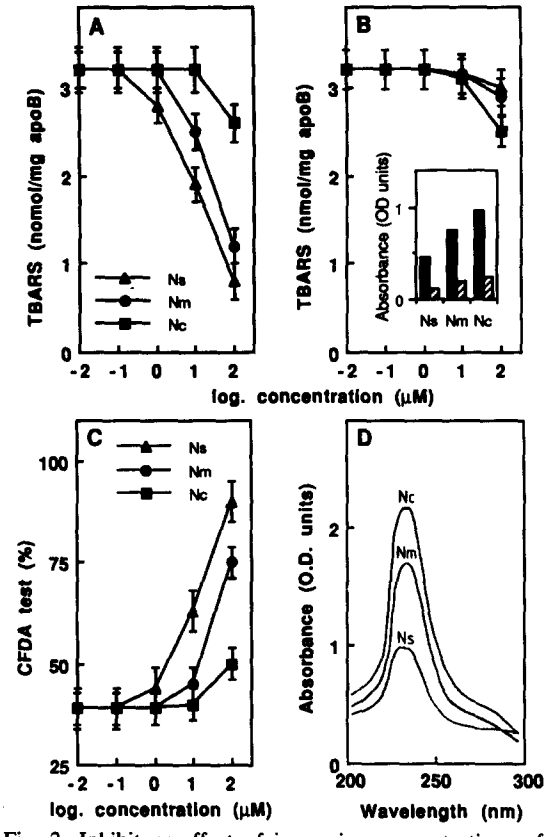


Fig. 2. Inhibitory effect of increasing concentrations of dihydropyridine $Ca^{2+}$ channel blockers, nisoldipine (triangles), nimodipine (circles), nicardipine (squares) against oxidation of LDL (2 g apoB/L) induced by UV radiation (UV-C, 254 nm, 0.5 mW/cm$^2$ for 2 hr). In (A) dihydropyridine $Ca^{2+}$ channel blockers were incorporated into the LDL solution (thickness of the LDL solution irradiated by UV: 0.5 mm). In (B) in order to evaluate the "filter effect" of dihydropyridine $Ca^{2+}$ channel blockers, the LDL solution (without any dihydropyridine compound) and the solution of dihydropyridine $Ca^{2+}$ channel blockers (0–100 $\mu$M) in a cuvette placed between the test tube (containing the LDL solution) and the UV source (as described in Materials and Methods). Inset: absorbance (at 254 nm) of dihydropyridine $Ca^{2+}$ channel blockers (100 $\mu$M) in cuvettes of 10 mm (black bars) or 2.5 mm (hatched bars) path length. (C) Test of the cytotoxicity of LDL irradiated by UV-C in the presence of increasing concentrations of dihydropyridine $Ca^{2+}$ channel blockers (under the conditions as in (A) on cultured lymphoid cell lines. A fixed concentration (200 $\mu$g apoB/mL) of the dihydropyridine/UV-treated LDL was incorporated in the culture medium (RPMI 1640 containing 2% Ultroser) and after 48 hr, the viability of lymphoid cells was assessed by the CFDA test. In A–C, the results are the mean (±SEM) of three separate experiments. (D) Spectra of a 100 $\mu$M of dihydropyridine $Ca^{2+}$ channel blockers, nisoldipine (Ns), nimodipine (Nm), nicardipine (Nc) between 200 and 300 nm, recorded using a Kontron spectrophotometer (model Uvikon 930).

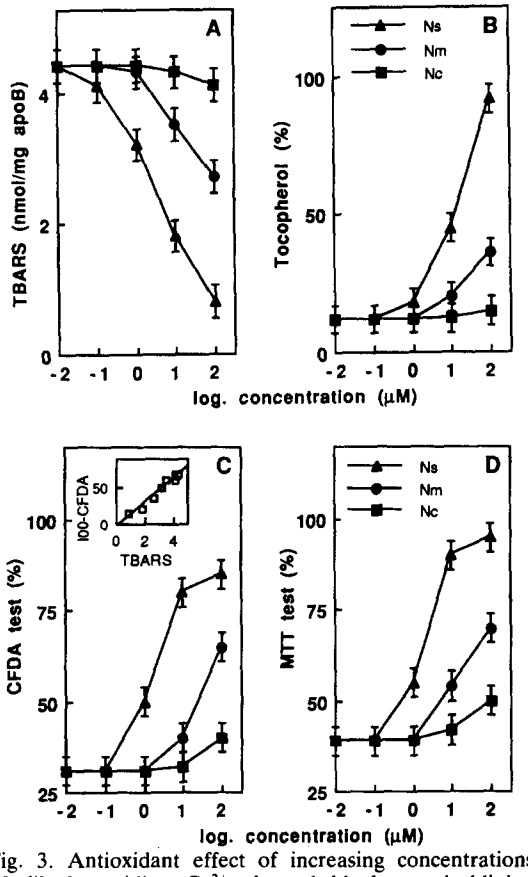


Fig. 3. Antioxidant effect of increasing concentrations of dihydropyridine $Ca^{2+}$ channel blockers, nisoldipine (triangles), nimodipine (circles), nicardipine (squares) against oxidation of LDL (2 g apoB/L) promoted by copper ions (1 $\mu$M $CuSO_4$ for 2 hr at 37° (A and B) and inhibition of the cytotoxicity (C and D). (A) Inhibition of TBARS formation (expressed as nmol TBARS/mg apoB). (B) Protection of endogenous tocopherol content of LDL (expressed as % of level in non-oxidized LDL). (C and D) Cytotoxicity of the dihydropyridine/copper-treated LDL of (A) tested on cultured lymphoid cell lines under the conditions of Fig. 2C, by using the CFDA test (C) and the MTT test (D). In the inset of (C) is reported the study of the correlation between the level of TBARS in the dihydropyridine/copper-treated LDL (Fig. 3A) and their cytotoxic effect (Fig. 3C, expressed as 100 − CFDA%) (regression line and correlation coefficient: $y = 16.6x - 3.8$ and $r = 0.97$). The results are the mean (±SEM) of three separate experiments. Abbreviations as Fig. 2.

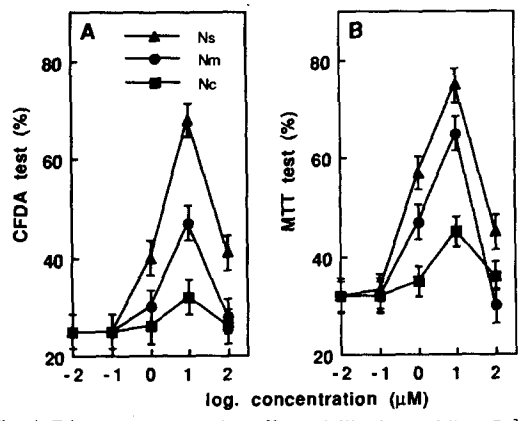


Fig. 4. Direct cytoprotective effect of dihydropyridine $Ca^{2+}$ channel blockers, against the cytotoxicity of previously oxidized LDL. A fixed concentration of oxidized LDL (200 $\mu$g apoB/mL of LDL treated by UV-C under the same conditions as in Fig. 2, but in the absence of any additional compounds) were added simultaneously to the culture medium with increasing concentrations of dihydropyridine $Ca^{2+}$ channel blockers (up to 100 $\mu$M). The cell viability was determined after 48 hr by CFDA (A) and MTT (B) tests. The results are the mean (±SEM) of three separate experiments. Abbreviations as Fig. 2.

the LDL solution) and the UV source. While the absorbance of the dihydropyridine compounds of 254 nm (Fig. 2D and inset of Fig. 2B), was not negligible under the conditions used here, the "filter effect" was relatively low as shown in Fig. 2B. We calculate by comparing Fig. 2A and Fig. 2B) that the relative importance of the "filter effect" of 100 $\mu$M dihydropydine (ratio of the values of Fig. 2B to those of Fig. 2A) is low for nisoldipine and nimodipine (around 10% and 10–15%, respectively), but high for nicardipine (around 80–90%). In order to eliminate any interference specific to the UV system, we evaluated the antioxidant effect of dihydropyridine compounds in a copper-promoted oxidation system. Nisoldipine and nimodipine exhibited a significant antioxidant effect (Fig. 3A and B): $IC_{50}$ values were evaluated (on the basis of TBARS formation) at around $5 \pm 0.2$ $\mu$M for nisoldipine and above 100 $\mu$M for nimodipine. In this experimental system nicardipine was almost ineffective. Comparison of the results obtained in the UV-induced and copper-promoted model systems, demonstrate that nisoldipine exhibits antioxidant properties and is effective in preventing lipid peroxidation of LDL, whereas nimodipine is a less potent antioxidant. Nicardipine is almost ineffective in the copper system: this confirms the above conclusion that the major part of the inhibition of LDL oxidation by nicardipine was due to a "filter effect" (but not to a true antioxidant effect). The tested compounds were generally more effective in inhibiting TBARS formation (Fig. 3A) than in protecting $\alpha$-tocopherol (Fig. 3B). The antioxidant effect of nifedipine (data not shown) was similar to (or slightly better than) that of nisoldipine.

In order to study the potential biological relevance of the protective effect of the dihydropyridine $Ca^{2+}$ channel blockers against LDL oxidation, we tested the cytotoxicity of LDL oxidized by UV or copper in the presence of dihydropyridine compounds (dihydropyridine/UV- or copper-treated LDL). LDL oxidized by UV were severely cytotoxic (we chose conditions of LDL oxidation leading to around 70% dead cells after 48 hr of cell culture in the presence of 200 $\mu$g apoB/mL oxidized LDL). LDL treated by UV or copper in the presence of protective concentrations of nisoldipine or nimodipine were much less cytotoxic than unprotected UV-treated LDL (Figs 2C and 3C and D). The inhibition of

LDL oxidation (Fig. 3A) and that of the subsequent cytotoxicity of the dihydropyridine/copper-treated LDL (Fig. 3C) were well correlated (inset of Fig. 3C). This suggests the existence of a relationship between the extent of LDL lipid peroxidation and their cytotoxicity, as discussed previously [17] and the antioxidant effect of dihydropyridine $Ca^{2+}$ channel blockers inhibiting LDL oxidation and their "indirect" cytoprotective effect.

*"Direct" protection of cells against the cytotoxicity of oxidized LDL (Fig. 4)*

We have tested the "direct" cytoprotective effect of the dihydropyridine $Ca^{2+}$ channel blockers by incorporating into the culture medium of lymphoid cells the tested drug (used at variable concentrations) simultaneously with the previously oxidized LDL (200 $\mu$g apoB/mL of LDL oxidized in the absence of any additive). Nisoldipine increased significantly the resistance of cells against the cytotoxic effect of oxidized LDL whereas nimodipine was less active and nicardipine was only very poorly effective (Fig. 4). Note that 10 $\mu$M was the optimum concentration for protection and that 1 $\mu$M of nisoldipine and nimodipine gave some protection. The protective effect of these dihydropyridine compounds is limited because of their own cytotoxicity at high concentrations (in our experimental model system, the cytotoxicity of the drugs began at around 10 $\mu$M) (Table 1).

As shown in Table 2, the protective effect of dihydropyridine $Ca^{2+}$ channel blockers was not due to an inhibition of uptake of UV-treated LDL (at the used concentration). We used a 12 hr uptake time because under the conditions employed in the cytotoxicity experiments (200 $\mu$g apoB of oxidized LDL), the cytotoxicity begins to rise between 12 and 18 hr of contact with oxidized LDL and after this period the apparent LDL uptake declined (probably because of the loss of cell integrity) and thus cannot be accurately determined over longer periods of time as reported previously [17].

## DISCUSSION

The results reported in this paper clearly demonstrate that some $Ca^{2+}$ channel blockers from the dihydropyridine series exhibit two types of protective effects: (1) a true antioxidant activity which inhibits LDL oxidation outside the cell; (2) a direct cytoprotective effect which prevents the cytotoxicity of oxidized LDL inside the cells. LDL lipid peroxidation can be effectively prevented by adding the dihydropyridines to solutions of LDL just before oxidation by UV radiation or by copper ions. The antioxidant effect of dihydropyridine compounds is well correlated with the protection of endogenous tocopherols, in agreement with the results observed with other antioxidants [9, 43]. In both systems used here, nisoldipine was the most potent compound (nifedipine exhibited a similar or slightly higher effect than nisoldipine), whereas nimodipine was less effective and nicardipine almost ineffective. Similar results were obtained in both systems (UV and copper) suggesting that the protection of LDL against oxidation results from a true antioxidant property of the active dihydropyridine compounds and that the "filter effect" of high concentrations of dihydropyridine compounds is minimized by the experimental conditions employed (i.e. UV-irradiation of relatively thin liquid films). Our results are consistent with the antioxidant effect of nifedipine reported by Ondrias *et al.* [44] in phosphatidylcholine liposomes and by Breugnot *et al.* [29] in copper-induced oxidation of LDL. In contrast to our results, Janero and Burghardt [30] reported that nisoldipine and nicardipine were much more active than nifedipine: these apparently conflicting data are probably due to differences in the experimental conditions, since the xanthine oxidase/liposomes system (generating $O_2^-$ and other free radical oxygen species), visualizes the free radical scavenging activity of the tested compounds, whereas the inhibition of LDL oxidation by nifedipine seems to be independent of any free radical

Table 1. Cytotoxicity of increasing concentrations of dihydropyridine $Ca^{2+}$ channel blockers on cultured lymphoid cells

| | Dihydropyridine $Ca^{2+}$ channel blocker concentration ($\mu$M) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.1 | 1 | 10 | 100 |
| CFDA test | | | | | |
| Nisoldipine | 100 ± 6 | 100 ± 7 | 90 ± 6 | 60 ± 5 | 10 ± 3 |
| Nimodipine | 100 ± 6 | 101 ± 6 | 100 ± 6 | 93 ± 5 | 50 ± 7 |
| Nicardipine | 100 ± 6 | 100 ± 5 | 92 ± 6 | 69 ± 5 | 18 ± 4 |
| MTT test | | | | | |
| Nisoldipine | 100 ± 9 | 99 ± 7 | 100 ± 7 | 70 ± 7 | 15 ± 5 |
| Nimodipine | 100 ± 9 | 99 ± 8 | 102 ± 9 | 94 ± 8 | 40 ± 7 |
| Nicardipine | 100 ± 9 | 100 ± 7 | 99 ± 8 | 74 ± 9 | 20 ± 5 |

Variable concentrations of dihydropyridine $Ca^{2+}$ channel blocker ($\mu$M) were added to the standard culture medium (without any oxidized LDL), cells were grown for 48 hr in the presence of the added compounds and cell viability was assessed (after 48 hr contact) simultaneously by CFDA and MTT tests (expressed as % of the CFDA or MTT content in control cells, i.e. cells grown under standard conditions but without dihydropyridine).

The results are the mean (±SEM) of three separate experiments.

Table 2. Uptake of LDL, expressed as cell associated LDL (μg apoB/mg cell protein), in the presence or absence of a fixed concentration of dihydropyridine $Ca^{2+}$ channel blocker (10 μM)

| LDL concentration (in the culture medium) | Dihydropyridine $Ca^{2+}$ channel blocker added | | | |
|---|---|---|---|---|
| | None | Nisoldipine | Nimodipine | Nicardipine |
| Non-treated LDL | | | | |
| 50 μg/mL | 2.1 ± 0.3 | 2.0 ± 0.3 | 2.2 ± 0.4 | 2.1 ± 0.4 |
| 100 μg/mL | 3.1 ± 0.4 | 3.1 ± 0.4 | 3.2 ± 0.3 | 3.0 ± 0.4 |
| 200 μg/mL | 3.6 ± 0.3 | 3.5 ± 0.4 | 3.7 ± 0.4 | 3.5 ± 0.4 |
| UV-treated LDL | | | | |
| 50 μg/mL | 2.0 ± 0.3 | 1.9 ± 0.2 | 2.0 ± 0.4 | 2.0 ± 0.4 |
| 100 μg/mL | 3.2 ± 0.4 | 3.3 ± 0.3 | 3.2 ± 0.4 | 3.2 ± 0.5 |
| 200 μg/mL | 3.6 ± 0.3 | 3.6 ± 0.4 | 3.5 ± 0.3 | 3.5 ± 0.3 |

Lymphoid cells were grown in the presence of control and UV-treated LDL (previously labelled with [$^3$H]cholesteryl oleyl ether) and in the absence (control) or presence of a fixed concentration of dihydropyridine $Ca^{2+}$ channel blocker (10 μM). After 12 hr pulse, the cell associated radioactivity was measured as described in Materials and Methods.

The results are the mean (±SEM) of two separate experiments.

scavenging activity [29]. Moreover, there is no obligatory relationship between the free radical scavenging activity and the inhibitory effect of LDL oxidation, since vitamin E can scavenge oxygen radicals [29], but is relatively ineffective in inhibiting LDL oxidation in our UV-induced system [19].

The study of the cytotoxicity of LDL treated by UV in the presence of dihydropyridine $Ca^{2+}$ channel blockers demonstrates that, as expected, when these compounds inhibited LDL lipid peroxidation, they also prevented their subsequent cytotoxic effect. These data support the idea that the cytotoxic effect of oxidized LDL is caused by lipid peroxidation products [6, 17] and that their TBARS content can predict their cytotoxicity [17, 19]. Moreover, the protection of LDL-tocopherols could also be involved in the prevention of the cytotoxic effect of oxidized LDL since vitamin E is able to increase the cellular resistance against oxidized LDL [19, 37].

Beside the indirect cytoprotective effect (resulting from the inhibition of LDL oxidation) discussed above, the second part of the results reported in this paper demonstrate that the dihydropyridine $Ca^{2+}$ channel blockers are able to protect cells directly against the cytotoxic effect of LDL previously oxidized in the absence of dihydropyridines. In this case, the dihydropyridines (the most active being nifedipine and nisoldipine) increased the cellular resistance against the cytotoxic effect of oxidized LDL taken up by the cells. As shown by the study of the cellular uptake of oxidized LDL in the presence of dihydropyridine compounds (Table 2), it is excluded that the cytoprotective effect of these compounds could result from an inhibition of LDL uptake. Thus, the direct cytoprotection by active dihydropyridine compounds can be compared to that of vitamin E or catechin which prevent the cytotoxic effects of oxidized LDL by a mechanism completely independent of the inhibition of LDL oxidation [19], probably by blocking the intracellular transduction of the cytotoxic signals which lead finally to membrane lesions [17]. As the exact chemical nature of the cytotoxic compounds (lipid peroxides, oxysterols, aldehydes could be possibly involved) [6, 9, 38–40] and the mechanism of the intracellular "transduction" (probably a multistep process) is largely unknown, the precise molecular mechanism of the protective effect of dihydropyridine compounds is also unknown.

The direct cytoprotective effect of dihydropyridine $Ca^{2+}$ channel blockers ($IC_{50}$ between 1 and 10 μM for nifedipine and nisoldipine) occurred in the same range of concentrations as the inhibition of LDL modification and over-degradation by J774 macrophagic cells [29], the prevention of endothelial cell injury [41] and the inhibition of "synthetic" transformation and proliferation of smooth muscle cells [42]. The apparent $IC_{50}$ is related to the intensity of the oxidative stress (level of LDL oxidation and concentration of oxidized LDL), the dihydropyridine compounds being more effective when the concentration of oxidized LDL was lower. As it is probable that the local concentration of oxidized LDL is low in the early atherosclerotic lesions, we cannot exclude the possibility that the cytoprotective effect of dihydropyridines observed in our model system could be of importance *in vivo* since the oxidized LDL are thought to play a major role in atherogenesis [7, 8].

In conclusion, nisoldipine (and nifedipine) and to a lesser degree nimodipine protect cells against the cytotoxicity of oxidized LDL by acting outside (indirect protection resulting from the inhibition of LDL oxidation) as well as inside the cells (direct protection against the toxic effect of oxidized LDL), similarly to the previously reported properties of catechin and quercetin [19, 22]. Like vitamin E [19], dihydropyridines are more active in direct cytoprotection than in inhibiting LDL oxidation. This could be due to the high intracellular concentration of nifedipine [23] and suggests that the direct protection could be prominent in the antiatherogenic effect of dihydropyridine $Ca^{2+}$ channel blockers.

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