Targeted killing of cultured cells by receptor-dependent photosensitization

(cell surface receptors/low density lipoprotein/pyrene cholesteryl esters/irradiation/familial hypercholesterolemia)

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ABSTRACT This paper describes a method, designated "receptor-dependent photosensitization," by which the receptor-mediated endocytosis of low density lipoprotein (LDL) can be used to deliver a photosensitizing agent, pyrene, to cultured human and animal cells. The hydrophobic core of LDL is extracted and replaced with pyrene covalently coupled to cholesteryl oleate. This reconstituted LDL enters cells in significant amounts only when the cells express LDL receptors, resulting in the accumulation of pyrene cholesteryl oleate within lysosomes. Subsequent exposure of the cells to ultraviolet light leads to cell death. Cells killed by this technique include normal and simian virus 40-transformed human fibroblasts, human A-431 epidermal carcinoma cells, Chinese hamster ovary cells, and mouse L cells, all of which express LDL receptors. Mutant fibroblasts from a patient with homozygous familial hypercholesterolemia, which lack LDL receptors, do not take up significant amounts of the pyrene-containing LDL and are not killed by subsequent exposure to light. The current experiments establish the feasibility of receptor-dependent photosensitization as an efficient and selective method for killing cultured human and animal cells.

By virtue of the extreme selectivity exhibited by cell surface receptors, the process of receptor-mediated endocytosis theoretically should allow the controlled delivery of biologically active molecules to human and animal cells. Two unique properties of plasma low density lipoprotein (LDL) make it a promising vehicle for this purpose. First, LDL contains a lipid core composed of about 1500 molecules of cholesteryl ester. This core can be extracted and replaced with biologically active lipids without affecting the functionally active form of the particle (1). Second, LDL enters animal cells by binding to surface receptors that mediate its cellular uptake (2). Once inside the cell, the LDL is carried to lysosomes where the lipoprotein's protein-phospholipid coat is removed and the cholesteryl ester core is exposed. This uptake process normally functions to supply cholesterol to hepatic and extrahepatic cells. In the steady state, LDL uptake proceeds at a constant rate, each receptor carrying one particle of LDL into the cell approximately once every 10 min (3). After each round of uptake, the receptor is recycled and reutilized (4). Thus, large amounts of LDL are transported into the cell even though a cell may have relatively few receptors on its surface.

Among the biologically active molecules that can be carried in the core of LDL are toxic compounds. Such toxic LDL particles can be used to kill cells that possess LDL receptors (5, 6). Inasmuch as these LDLs will bind to and enter only those cells that have functional LDL receptors, they permit the survival, and hence the selection, of resistant cells that have ac-

quired mutations that disrupt receptor-mediated endocytosis (6).

In order to be effective in killing cells via the LDL receptor pathway, a substance must fulfill at least two criteria: (i) it must be sufficiently hydrophobic so that it can be incorporated into the LDL core rather than its hydrophilic surface; and (ii) it must express its toxic effect after entry into lysosomes (1, 5). One class of agents that meets both of these criteria are the photosensitizing dyes. Many of these dyes are fluorescent, hydrophobic, heterocyclic compounds that absorb photons and trigger a series of free radical reactions, some of which proceed by way of singlet oxygen (7). When cells are allowed to take up photosensitizing dyes that are free in solution, the dyes often accumulate in lysosomes. Subsequent exposure of the cells to light of the appropriate wavelength results in cell death (8-10). The precise mechanism of killing is unknown, but it may involve oxidative damage to the lysosomal membrane with release of toxic lysosomal hydrolases into the cytoplasm (7-9). Killing cells by this approach lacks cellular specificity because all cells will indiscriminately take up photosensitizing agents from the surrounding medium.

In the current studies we have developed a specific receptor-dependent toxic particle by incorporating a photosensitizing compound into the core of LDL. For this purpose, we have used 1-pyrenemethyl-23,24-bisnor-5-cholesterol-22-oate-3 β -yl oleate (PCO), a compound that contains pyrene coupled to a derivative of cholesteryl oleate (6, 11, 12). Previous studies with fluorescence microscopy have shown that this pyrene-containing LDL particle enters cells and accumulates in large amounts within lysosomes, but only when the cells express LDL receptors (6, 12). We now show that cells that take up this compound are quantitatively and selectively killed when exposed to ultraviolet light.

MATERIALS AND METHODS

Materials. [methyl-³H]Thymidine (6.7 Ci/mmol; 1 Ci = 3.7 $\times 10^{10}$ becquerels) was purchased from New England Nuclear. Dulbecco's phosphate-buffered saline was obtained from GIBCO. Cab-o-sil was obtained from Kodak. PCO was synthesized by a modification of a published procedure (11). Chinese hamster ovary (CHO) cells (13) and human A-431 epidermal carcinoma cells (14) were obtained as described in the references. Mouse L cells (tk⁻, aprt⁻) were obtained from Barbara Wold and Richard Axel. Simian virus 40 (SV40)-transformed human fibroblasts

Abbreviations: CHO, Chinese hamster ovary; FH, familial hypercholesterolemia; LDL, low density lipoprotein; PCO, 1-pyrenemethyl-23,24-bisnor-5-cholesterol-22-oate-3 β -yl oleate; r-[PCO]LDL, heptane-extracted LDL reconstituted with PCO; r-[PCO]MeLDL, heptane-extracted methyl-LDL reconstituted with PCO; LPDS, lipoprotein-deficient serum; SV40, simian virus 40.

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(GM-639) were purchased from the Human Genetic Mutant Cell Depository (Camden, NJ). Normal human fibroblasts and mutant familial hypercholesterolemia (FH) homozygote fibroblasts (MC., receptor-negative; J.D., internalization-defective) were derived from skin biopsies (15).

Lipoproteins. Human LDL (ρ , 1.019–1.063 g/ml) was obtained from the plasma of individual healthy subjects and prepared by centrifugation (16). Newborn calf lipoprotein-deficient serum (LPDS) was prepared either by centrifugation (16) or by extracting the lipoproteins with 2% (wt/vol) Cab-o-sil (17). Methylated LDL (MeLDL) was prepared as described (18). r-[PCO]LDL and r-[PCO]MeLDL were prepared by the reconstitution method of Krieger *et al.* (1, 12) in which the endogenous neutral lipids of LDL and MeLDL, respectively, were removed by heptane extraction and replaced with PCO. The mass ratio of PCO to protein (mg/mg) in the final solubilized preparations of r-[PCO]LDL and r-[PCO]MeLDL was 2:1. The concentrations of lipoproteins are expressed in terms of their protein content.

Cells. Cells were grown in monolayer culture in 5% CO₂/95% air in a humidified incubator. Unless otherwise indicated, monolayers of CHO cells, mouse L cells, human A-431 cells, and human SV40-transformed fibroblasts were dissociated with 0.05% trypsin/0.02% EDTA solution and seeded (day 0) at a concentration of 10⁵ cells per 35-mm well in six-well Linbro plates in 2.5 ml of medium A [F-12 Ham's medium or Dulbecco's modified Eagle's medium supplemented with penicillin (100 units/ml), streptomycin (100 μ g/ml), and 1% glutamine] containing 5% LPDS and 0.1% newborn calf serum. On day 1 or 2, additions were made to the wells as indicated in the legends, and the cells were returned to the incubator. Two to 24 hr later, the medium was removed and each cell monolayer was washed with 2 ml of buffer B (phosphate buffered-saline containing 5% LPDS). The cells were then irradiated in 2 ml of fresh buffer B (see below). Immediately after irradiation, buffer B was removed and replaced with 3 ml of medium A containing 10% fetal calf serum. The cells were returned to the incubator, and subsequent growth was assessed by staining or by measurement of [³H]thymidine incorporation (19).

For the experiments with normal and FH homozygote human fibroblasts, cells were seeded (day 0) at a concentration of 4×10^4 cells per 35-mm well in six-well Linbro plates in 2 ml of medium C [Eagle's minimal essential medium supplemented as described (15)] containing 10% fetal calf serum. On day 2, 2 ml of fresh medium C with 10% fetal calf serum was added. On day 3, each monolayer was switched to 2 ml of medium C containing 10% LPDS. On day 4, additions were made to the wells as indicated in the legends, and the cells were returned to the incubator. One to 6 hr later, the cells were washed with buffer B and irradiated as described below. The cells were then incubated for 24 hr in medium C containing 10% fetal calf serum prior to measurement of [³H]thymidine incorporation.

Irradiation. The ultraviolet light source for irradiation consisted of four 40-W black-light-blue lamps (General Electric F40T12/BLB; output, 300–400 nm). The light was filtered through 8-mm plate glass. The dose rate through glass was approximately 800 J/m² per min, and the duration of exposure was varied as indicated in the legends. The light source was 12 cm above the cell monolayers, and the bottom and top surfaces of the chamber were mirrored. The temperature during irradiation was 28°C. The light intensity was measured with a Spectronics DM365N UV meter with calibration traceable to the National Bureau of Standards.

[³H]Thymidine Incorporation. Twenty-four hours after irradiation, each cell monolayer was pulse-labeled with 0.5 or 2.5 μ Ci of [³H]methylthymidine added per ml to the growth me-

dium (19). After incubation for 2 hr at 37°C, each monolayer was washed four times with ice-cold 10% trichloroacetic acid and twice with ethanol/ether, 3:1 (vol/vol). The acid-insoluble material was then solubilized by incubation with 1 ml of 0.5 M NaOH for 30 min at 37°C, after which 100- μ l aliquots were removed for liquid scintillation counting in 6 ml of Dimilume-30 (Packard). For each experiment, blank values were determined by measuring the amount of [³H]thymidine incorporated by untreated cell monolayers kept at 4°C during the 2-hr pulse-labeling period. Blank values, which were routinely <0.5% of values obtained in control cells pulse-labeled at 37°C, were subtracted from all data points. The data are expressed as the dpm of [³H]thymidine incorporated per well. Each value represents the average of two to six replicate dishes. The standard deviation for replicate dishes was less than 6% of the mean values.

Other Assays. Protein was measured by the method of Lowry *et al.* (20). For staining monolayers, the cells were washed with phosphate-buffered saline, fixed at room temperature with 3% formaldehyde, and stained with 0.1% crystal violet for 5 min.

RESULTS

To demonstrate receptor-dependent photosensitization, we grew monolayers of mouse L cell fibroblasts in LPDS for 2 days and incubated them with r-[PCO]LDL for 24 hr. The dishes were then masked so that light reached only selected areas. In Fig. 1 *Left*, the light reached all areas except those masked by the letters "LDL." In Fig. 1 *Right*, the masking was reversed so that the light reached only those areas indicated by the letters "LDL." After exposure to ultraviolet light, the cells were returned to the incubator and allowed to proliferate for 4 days, after which they were fixed and stained. In both dishes the areas that were exposed to light were largely devoid of cells, whereas the masked areas showed abundant growth.

The experiment in Fig. 2 demonstrates that the killing of cultured cells by light requires prior incubation of the cells with pyrene-containing LDL in a form that is capable of binding to the LDL receptor. Three types of cultured cells were tested: human SV40-transformed skin fibroblasts; human A-431 epidermal carcinoma cells; and mouse L cells. When the cells were incubated with native LDL, washed, and then exposed to light,

FIG. 1. Selective killing of treated mouse L cells in regions of light exposure. On day 0, cells were plated at a concentration of 2.5×10^5 cells per 100-mm dish in 7 ml of medium A containing 5% LPDS and 0.1% newborn calf serum. On day 2, the medium was replaced with 7 ml of the same medium containing r-[PCO]LDL at 5 μ g/ml. After incubation for 24 hr (day 3), the medium was removed, and each monolayer was washed with 10 ml of buffer B. (*Left*) Letters "LDL" cut out of black paper were placed between the light source and the dish. (*Right*) Black paper from which the "LDL" letters were cut was placed between the light source and the dish. Both monolayers were irradiated for 40 min in buffer B (7 ml per dish). After irradiation and every second day thereafter, the medium was replaced with 7 ml of medium A containing 10% fetal calf serum. On day 7, the cells were fixed and stained with crystal violet.

FIG. 2. Killing of three types of cultured mammalian cells: requirement for LDL receptor, r-[PCO]LDL, and light. On day 1, each well of SV40-transformed human fibroblasts, human A-431 cells, and mouse L cells received one of the indicated lipoproteins at 10 $\mu\text{g}/\text{ml}$. After incubation for 6 hr, the cells were irradiated for 40 min. After irradiation and every second day thereafter, the medium was replaced with 3 ml of fresh medium A containing 10% fetal calf serum. On day 7, the cells were fixed and stained with crystal violet.

no detectable killing occurred, as indicated by the confluent staining pattern of the cells fixed in the dish. When the cells were incubated with r-[PCO]LDL but not exposed to light, no killing occurred. However, when the cells were incubated with r-[PCO]LDL, washed, and subsequently exposed to light, all of the cells were destroyed. LDL that has been subjected to reductive methylation (MeLDL) no longer binds to the LDL receptor (18). When the three types of cultured cells were incubated with MeLDL that was reconstituted with PCO, there was no killing, even after the cells were irradiated with light.

The relationship between the dose of light and the dose of pyrene-containing LDL is illustrated in Fig. 3. Mouse L cells were incubated with varying concentrations of r-[PCO]LDL ranging from 0 to 20 $\mu\text{g}/\text{ml}$. At each lipoprotein concentration, individual wells of cells were exposed to light for periods ranging from 0 to 80 min. Complete killing of cells was seen only in the dishes that received both the r-[PCO]LDL and light. In general, the higher the concentration of r-[PCO]LDL in the prior incubation medium, the lower the amount of light necessary to kill the cells. When the cells were incubated with r-[PCO]LDL at 2 $\mu\text{g}/\text{ml}$, a 40-min light exposure was sufficient to achieve complete killing. At 20 $\mu\text{g}/\text{ml}$, complete killing was achieved with 20 min of light exposure.

To quantify the toxic effects more precisely, we incubated cells with r-[PCO]LDL, exposed them to light, and then pulse-labeled them with [^3H]thymidine for 2 hr to measure the rate of DNA synthesis. When CHO cells were incubated in the absence of r-[PCO]LDL and then pulse-labeled without exposure to light, the cells incorporated about 80,000 dpm of [^3H]thymidine per well in a 2-hr pulse (Fig. 4). Over the next 15 hr the rate of synthesis of DNA in each 2-hr pulse increased in a logarithmic fashion as the cells divided. When the cells were exposed to ultraviolet light without incubation with r-[PCO]LDL, DNA synthesis was normal at each point during the ensuing 15 hr.

FIG. 3. Effect of varying concentrations of r-[PCO]LDL and varying doses of irradiation on the killing of mouse L cells. On day 0, cells were plated in a 24-well Linbro plate at a concentration of 2×10^3 cells per 15-mm well in 1 ml of medium A containing 5% LPDS and 0.1% newborn calf serum. On day 1, each horizontal row of wells received the indicated concentration of r-[PCO]LDL. On day 2, each vertical row of wells was irradiated for the indicated time. The time of irradiation in individual rows was varied by use of an opaque mask. After irradiation and every second day thereafter, the medium in each well was replaced with 1 ml of medium A containing 10% fetal calf serum. On day 12, the cells were fixed and stained with crystal violet.

When cells were incubated with r-[PCO]LDL and then exposed to light, the incorporation of [^3H]thymidine was reduced by 75% at zero time. This reduced rate of DNA synthesis remained constant over the next 6 hr; by 18 hr DNA synthesis had fallen by more than 99%. In additional studies, we found that, 1 hr after irradiation, 95% of the cells incubated with r-[PCO]LDL excluded trypan blue. The value had declined to 50% by 3 hr after irradiation and to 0 by 5 hr.

FIG. 4. [^3H]Thymidine incorporation by CHO cells at varying periods of time after irradiation. On day 2, each monolayer received either no r-[PCO]LDL (● and ▲) or r-[PCO]LDL at 10 $\mu\text{g}/\text{ml}$ (■). After incubation for 4 hr at 37°C, cells received no irradiation (●) or were irradiated for 32 min (▲ and ■). At the indicated time following irradiation, each monolayer was pulse-labeled for 2 hr with [^3H]thymidine at 0.5 $\mu\text{Ci}/\text{ml}$, after which the amount of [^3H]thymidine incorporated into acid-insoluble material was determined. The protein content in cells receiving no r-[PCO]LDL and no irradiation averaged 230 μg per well at zero time and 450 μg per well at 24 hr.

In a further test of the role of the LDL receptor in photosensitization, we studied the process in cultures of diploid human skin fibroblasts. As measured by [^3H]thymidine incorporation, normal fibroblasts incubated with r-[PCO]LDL were completely killed after a subsequent 30-min exposure to light (Fig. 5A). When cells from a patient with homozygous FH, which have no detectable LDL receptors (receptor-negative mutant) were incubated with r-[PCO]LDL, there was no binding or uptake of the particle detectable by fluorescence microscopy (12). There was also no killing of the cells when they were subsequently exposed to light for periods as long as 50 min (Fig. 5B).

To determine whether internalized r-[PCO]LDL was sufficient to kill cells without a requirement for surface-bound r-[PCO]LDL, we performed a pulse-chase experiment (Table 1). Normal human fibroblasts were incubated for 4 hr at 37°C with r-[PCO]LDL. The monolayers were washed, and one set was irradiated immediately. These cells contained r-[PCO]LDL bound at the surface receptor site as well as r-[PCO]LDL that had been transported into the cell. After washing, a second group of dishes was incubated for an additional 4 hr at 37°C in the absence of r-[PCO]LDL. During this time period all the r-[PCO]LDL that was on the surface entered the cell and accumulated in lysosomes (3). After exposure to light, [^3H]thymidine incorporation was reduced equally in the two groups of cells, whether they were irradiated immediately after incubation with r-[PCO]LDL or after the 4-hr chase period. In contrast, cells from a receptor-negative FH homozygote were not killed when irradiated at either time point.

Fibroblasts from a patient with the internalization-defective form of FH bind about 60% of the normal amount of LDL at the receptor site but internalize only about 5% of the normal amount (15). Table 2 shows that these mutant cells were also resistant to photosensitization with r-[PCO]LDL. After a 1-hr

FIG. 5. [^3H]Thymidine incorporation by normal (A) and receptor-negative FH homozygote (B) fibroblasts incubated without (○) or with (●) r-[PCO]LDL prior to irradiation. On day 4, each monolayer received either no r-[PCO]LDL or r-[PCO]LDL at 20 $\mu\text{g}/\text{ml}$, and, after incubation for 4 hr at 37°C, the cells were irradiated for the indicated time. Twenty-four hours after irradiation, each monolayer was pulse-labeled for 2 hr with [^3H]thymidine (2.5 $\mu\text{Ci}/\text{ml}$), after which the amount of [^3H]thymidine incorporated into acid-insoluble material was determined. The "100% of control" values represent the amount of [^3H]thymidine incorporated in cells receiving no r-[PCO]LDL and no irradiation. These values (dpm/2 hr per well) were 1.1×10^5 and 1.7×10^5 for A and B, respectively. The protein content in cells receiving no r-[PCO]LDL and no irradiation was approximately 70 μg per dish for both cell strains.

Table 1. Sensitization of human fibroblasts by internalized r-[PCO]LDL

Pulse with r-[PCO]LDL	Time of chase	[^3H]Thymidine incorporation, dpm $\times 10^{-3}/2$ hr per well			
		Normal fibroblasts		Receptor-negative FH homozygote	
		No light	With light	No light	With light
—	0	93	94	100	75
+	0	116	0.2	102	95
+	4 hr	123	0.2	72	80

On day 4, each monolayer of normal and mutant human fibroblasts received either no r-[PCO]LDL or r-[PCO]LDL at 10 $\mu\text{g}/\text{ml}$ as indicated. After incubation for 4 hr at 37°C, the monolayers were washed with buffer B and either irradiated immediately as indicated or were incubated for another 4 hr in 2 ml of medium C containing 10% fetal calf serum and then irradiated. The cells were irradiated for 30 min. Twenty-four hours after irradiation, each monolayer was pulse-labeled for 2 hr with [^3H]thymidine (2.5 $\mu\text{Ci}/\text{ml}$), after which the amount of [^3H]thymidine incorporated into acid-insoluble material was determined. The average protein content in cells receiving no r-[PCO]LDL and no irradiation was approximately 100 μg per well for both cell strains.

incubation with r-[PCO]LDL up to 30 $\mu\text{g}/\text{ml}$, the internalization-defective cells were unaffected by irradiation whereas the normal cells were completely killed at a r-[PCO]LDL dose of 10 $\mu\text{g}/\text{ml}$. After a 6-hr incubation, the normal cells were killed at a r-[PCO]LDL concentration of only 1 $\mu\text{g}/\text{ml}$. The internalization-defective cells required r-[PCO]LDL at 10 $\mu\text{g}/\text{ml}$ to achieve complete killing. Thus, the internalization-defective cells were resistant to the r-[PCO]LDL but could be killed after prolonged incubations at relatively high concentrations of the lipoprotein. The differential sensitivity of these mutant cells is consistent with an LDL internalization rate that is about 5% of normal cells (15). In contrast, the receptor-negative mutant cells were completely resistant to killing at r-[PCO]LDL levels as high as 30 $\mu\text{g}/\text{ml}$.

Table 2. [^3H]Thymidine incorporation by normal and internalization-defective FH fibroblasts irradiated after incubation with varying concentrations of r-[PCO]LDL

r-[PCO]LDL in medium, $\mu\text{g}/\text{ml}$	[^3H]Thymidine incorporation, dpm $\times 10^{-3}/2$ hr per well			
	At 1 hr		At 6 hr	
	Normal cells	Internalization-defective cells	Normal cells	Internalization-defective cells
0	247	183	309	215
0.1	300	259	416	239
0.3	331	246	420	242
1	415	193	1.9	62
3	460	208	0	29
10	0	230	0	0
30	0	175	0	0

On day 4, each monolayer received the indicated concentration of r-[PCO]LDL. After incubation for either 1 or 6 hr at 37°C, the cells were irradiated for 50 min. Twenty-four hours after irradiation, each monolayer was pulse-labeled for 2 hr with [^3H]thymidine (2.5 $\mu\text{Ci}/\text{ml}$), after which the amount of [^3H]thymidine incorporated into acid-insoluble material was determined. The protein content in cells receiving no r-[PCO]LDL and no irradiation was approximately 66 μg per well for both cell strains.

DISCUSSION

In the current studies we used the LDL receptor system to establish conditions for the receptor-dependent photosensitization of cultured human and animal cells. The photosensitizing agent, a pyrene derivative of cholesteryl oleate, can be incorporated into LDL in large amounts by using the reconstitution method of Krieger *et al.* (1). Once incorporated into LDL, the PCO molecule will not enter cultured cells in significant amounts unless the LDL binds to receptors and is taken up by receptor-mediated endocytosis (6, 12).

Cells that had taken up r-[PCO]LDL were killed when subsequently exposed to ultraviolet light. A toxic effect was seen in five cell types, all of which express LDL receptors: CHO cells, mouse L cells, human A-431 epidermal carcinoma cells, and human diploid and SV40-transformed fibroblasts. The toxicity of r-[PCO]LDL absolutely required an LDL receptor: there was no killing of receptor-negative FH homozygote fibroblasts. Moreover, when PCO was incorporated into MeLDL, which does not bind to LDL receptors (18), no toxicity was seen.

Toxicity through receptor-dependent photosensitization required uptake of the LDL by cells because no toxicity was observed in cells incubated with r-[PCO]LDL at 4°C and irradiated at 7°C (data not shown). At 4°C, binding to the receptor occurs, but internalization does not take place. Moreover, in fibroblasts from a patient with the internalization-defective form of homozygous FH, which internalize only a small amount of LDL, killing took place only when the cells were exposed for prolonged periods to levels of LDL that were 10-fold above those that killed normal fibroblasts. We do not know whether this requirement for internalization simply reflects the need for the accumulation of large amounts of PCO or whether PCO must reach the lysosome before cells become photosensitive. If it were possible to bind sufficient r-[PCO]LDL to the surface, cells might be killed by this route as well.

Pulse-chase experiments demonstrated that PCO within the cell was sufficient to kill cells without a requirement for surface-bound r-[PCO]LDL. After cells were allowed to bind and take up r-[PCO]LDL and were then washed and incubated in medium without the ligand for 4 hr, all of the visible fluorescence was contained within cellular vacuoles that correspond to lysosomes. When these cells were irradiated, killing occurred (Table 1).

Previous studies, conducted most extensively with porphyrin derivatives, have shown that photosensitizing agents that accumulate within lysosomes can be toxic when the cells are subsequently irradiated (7, 10, 21). Our current results are consistent with this idea in that the PCO was toxic at a time when it was confined to lysosomes. The phototoxicity of porphyrins has been shown to proceed via the generation of singlet oxygen as a result of energy transfer from the dye to O₂ (7, 21). The mechanism by which singlet oxygen or other reactive electron donors damage cells when generated within lysosomes is unknown. It has been speculated that such radicals damage the lysosomal membrane with discharge of toxic lysosomal hydrolases into the cytoplasm (8, 9). This type of toxicity is minimized when cells are exposed to light in a low-oxygen environment and when sodium azide is present and is accelerated when ²H₂O is substituted for H₂O (7).

The methods outlined here should be useful for several other purposes. First, it should be possible to select for cells that have acquired mutations in the receptor-mediated endocytosis of

LDL inasmuch as they will survive exposure to r-[PCO]LDL plus light. Analysis of such mutant cells may clarify the genetic and biochemical steps required for receptor-mediated endocytosis. Second, the method should facilitate studies of the mechanism of free radical-induced killing of cells. Third, the studies raise the remote possibility that LDL particles loaded with dyes can be used to photosensitize malignant cells *in vivo*. Attempts have been made to photosensitize tumor cells *in vivo* by using porphyrin derivatives (22) and acridine orange (23). Targeted delivery of photosensitizers in LDL may enhance the selectivity and efficiency of such killing. Some types of malignant cells are known to express relatively large numbers of LDL receptors (24). These cells should take up large amounts of r-[PCO]LDL. Subsequent irradiation of these tissues with light might aid in the selective killing of such tumor cells.

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1. Krieger, M., Brown, M. S., Faust, J. R. & Goldstein, J. L. (1978) *J. Biol. Chem.* **253**, 4093-4101.
2. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897-930.
3. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279**, 679-685.
4. Basu, S. K., Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1981) *Cell* **24**, 493-502.
5. Krieger, M., Goldstein, J. L. & Brown, M. S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5052-5056.
6. Krieger, M., Brown, M. S. & Goldstein, J. L. (1981) *J. Mol. Biol.*, in press.
7. Spikes, J. D. (1977) in *The Science of Photobiology*, ed. Smith, K. C. (Plenum, New York), pp. 87-112.
8. Slater, T. F. & Riley, P. A. (1966) *Nature (London)* **209**, 151-155.
9. Allison, A. C., Magnus, I. A. & Young, M. R. (1966) *Nature (London)* **209**, 874-878.
10. Schothorst, A., Suurmond, D. & Ploem, J. (1977) *J. Invest. Dermatol.* **69**, 551.
11. Kao, Y. J., Charlton, S. C. & Smith, L. C. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 936.
12. Krieger, M., Smith, L. C., Anderson, R. G. W., Goldstein, J. L., Kao, Y. J., Pownall, H. J., Gotto, A. M., Jr. & Brown, M. S. (1979) *J. Supramol. Struct.* **10**, 467-478.
13. Goldstein, J. L., Helgeson, J. A. S. & Brown, M. S. (1979) *J. Biol. Chem.* **254**, 5403-5409.
14. Anderson, R. G. W., Brown, M. S. & Goldstein, J. L. (1981) *J. Cell Biol.* **88**, 441-452.
15. Goldstein, J. L., Brown, M. S. & Stone, N. J. (1976) *Cell* **12**, 629-641.
16. Brown, M. S., Dana, S. E. & Goldstein, J. L. (1974) *J. Biol. Chem.* **249**, 789-796.
17. Weinstein, D. B. (1979) *Circulation Suppl.* **2**, 60, 54.
18. Weisgraber, K. H., Innerarity, T. L. & Mahley, R. W. (1978) *J. Biol. Chem.* **253**, 9053-9062.
19. Charington, P. V., Smith, B. L. & Pardee, A. B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3937-3941.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
21. Moan, J., Pettersen, E. O. & Christensen, T. (1979) *Br. J. Cancer* **39**, 398-407.
22. Diamond, I., Granelli, S. G., McDonagh, A. F., Neilson, S., Wilson, C. B. & Jaenicke, R. (1972) *Lancet* **ii**, 1175-1177.
23. Thomson, S. H., Emmett, E. A. & Fox, S. H. (1974) *Can. Res.* **34**, 3124-3127.
24. Ho, Y. K., Smith, G., Brown, M. S. & Goldstein, J. L. (1978) *Blood* **52**, 1099-1114.