REVIEWS

Low-Density Lipoprotein as a Vehicle for Targeting Antitumor Compounds to Cancer Cells

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INTRODUCTION

It is difficult to eradicate cancer cells in vivo because they share with normal cells, for the most part, the same biochemical machinery. There is no cytotoxic substance that is completely selective for malignant cells, and all those presently in use cause dose-limiting toxic side effects. For this reason there is a growing emphasis on targeting, i.e., selective delivery of drugs to tumors in ways that bypass normal body tissues.

Among the vehicles that can be used for this purpose is low-density lipoprotein (LDL), a normal blood constituent that is the body's principal means for delivery of cholesterol to tissues. Cholesterol, a major constitutent of mammalian cell membranes, is obtained by cells either by making it themselves or by picking it up from LDL or both. Cancer cells, like all dividing ones, need large amounts of cholesterol because they are making new membrane. There is ample evidence that many types of cancer cells indeed have unusually great LDL requirements. The evidence is 2-fold: measurements of LDL uptake by tumor cells and depletion of LDL in the blood of cancer patients resulting from high uptake by the tumor (*vide infra*). Thus, if LDL could be made to carry antitumor drugs, it would serve as a targeting vehicle. This concept was proposed in 1981–2 (*1,2*) and has been reviewed several times since then (*3–7*).

LDL consists of spherical particles 220 Å in diameter containing a nonpolar core of approximately 1500 molecules of cholesterol esterified with long-chain fatty acids such as oleate or linoleate. Around the core is a phospholipid oriented, like a micelle, with the polar ends outward and the lipophilic fatty acid chains inward. Mixed with the phospholipid is unesterified cholesterol, presumably as a stabilizer, and also a single molecule per LDL particle of apoprotein B which binds to specific cell surface receptors. After binding, the receptors, which are grouped together in coated pits, are internalized by endocytosis and taken to lysosomes, where the cholesteryl esters are hydrolyzed, making free cholesterol available to the cell. The LDL receptors are then recycled to the cell surface. The half-life of LDL in the blood is measured in days, but after binding to fibroblasts, $t_{1/2}$ for internalization is only 10 min. The mechanism of the LDL system was elucidated by Brown, Goldstein, and co-workers (*8–10*).

LDL UPTAKE BY CANCER CELLS

It is not surprising that malignant cells have high LDL requirements because rapidly dividing cells do. Indeed, there is a tendency for growing cells to acquire cholesterol from without and for differentiated cells from within (*11*). The first report regarding cancer and LDL in 1978 was that human acute myeloid leukemia (AML) cells take up 3–100× more LDL than normal cells (*12*). Human AML take up 4–25× more LDL than normal white blood cells (*13*), and the high in vitro uptake of AML correlates with high in vivo uptake (*14*). Human monocytic (FAB-M5) and myelomonocytic (FAB-M4) leukemias and chronic myeloid leukemia in blast crisis (but not acute lymphoblastic leukemia [ALL]) take up much more LDL than normal mononuclear cells, peripheral granulocytes, or nucleated bone marrow cells (*15*).

Some human solid tumors are also avid for LDL. Epidermoid cervical cancer EC-50 absorbs 15× more LDL than fetal adrenal tissue (which has exceptionally high uptake) and 50× more than normal gynecologic tissue. Endometrial adenocarcinoma AC-258 absorbs 10× more than normal (*1*). EC-50 and four other gynecologic cancers have greater LDL uptake than normal cervical tissues (*16*). Gastric carcinoma and parotid adenoma exceed every normal cell type (*17*). Many brain tumors bind 2–3× more LDL than normal brain, especially medulloblastoma, oligodendroglioma, and malignant meningioma (*18*). In most of a group of nine patients, lung tumor tissue's uptake exceeded that of neighboring normal lung by 1.5–3× (*19*). Other tumors, for which quantitative comparisons are lacking, have high LDL uptake: glioma V-251MG (*20*), G2 hepatoma (*21, 22*), squamous lung tumor (*23*), and choriocarcinoma (*24*). Most human tumors have not yet been surveyed, so it is reasonable to suppose that many more will be found to have exceptionally high LDL requirements. This expectation is foreshadowed by the frequent finding of depletion of LDL in the blood of cancer patients, to be discussed subsequently.

Mouse tumors have also been studied. Fibrosarcoma MS-2 takes up several times more LDL than normal tissue (liver, spleen, muscle, fat, heart) (*25*), and similarly for MAC 13 tumors as well as four others (*26*).

The most sinister aspect of cancer is its tendency to spread, or metastasize, throughout the body. This is most often the cause of death, even after resection of the primary tumor, because mets are not only difficult to find but also to kill with standard chemotherapy. Therefore, particularly noteworthy is the small but growing body of evidence that tumor cells that are exceptionally metastatic (*27, 28*), aggressive (*29–31*), or undifferentiated (*11, 32, 33*) are also exceptional in their LDL requirements. If this is borne out in future studies, LDL-based therapy will be even more valuable than it presently appears.

Some tumors, however, do not internalize great amounts of LDL, e.g., ALL (mentioned above) (*15*), chronic lymphocytic leukemia (CLL) (*34*), several colon adenocarcinomas (*35*), Lewis rat renal carcinoma (*36*), cervical cancer EC-168 (*37*), epitheloid carcinoma A-431 (*38*), and guinea pig leukemic lymphocytes (*39*). The latter three have plenty of LDL receptors, but internalization is deficient. Thus, it is important to show not only binding

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but also internalization of LDL before concluding that a given cell type is ripe for LDL targeting.

Some noncancerous pathogens have also been found to have high LDL requirements and therefore are candidates for LDL-based therapy. These will be discussed at the end of this paper.

DEPLETION OF LDL IN THE BLOOD OF CANCER PATIENTS

If tumor cells sequester abnormally large amounts of LDL, one would expect to see reduced levels of plasma cholesterol in cancer patients. This is indeed a well-documented phenomenon, first reported in 1939 when it was observed that leukemia patients had unusually low blood cholesterol (*40*).

There was then a hiatus before the recent spate of papers, which was prompted by the question of what overall effects would ensue if people were to lower their cholesterol to improve cardiovascular health. As expected, comparison of blood cholesterol with mortality in large cohorts revealed a positive association, i.e., high cholesterol correlates with high mortality, but a frequent and unexpected observation was a *negative* association, i.e., rise in mortality at the lowest cholesterol levels. Thus, there is an overall U-shaped relationship between cholesterol and mortality. The excess deaths at very low cholesterol turned out to be due to previously undiagnosed cancer. Cause and effect were not immediately obvious, but it is now abundantly clear that it is cancer that brings about a reduction in cholesterol, and not low cholesterol that causes cancer.

In the 1980 Whitehall study of 17 718 men followed 7.5 years, a U-shaped curve was observed whose anomaly (the upward shift in mortality at lowest cholesterol levels) disappeared after subtraction of cancer cases (lung, stomach, colon) diagnosed within 2 years after the study began, suggesting that these men had undiagnosed early cancer which caused a drop in cholesterol level (*41*). In a similar study of 7603 French men followed for 6.6 years, cancer patients undiagnosed at entry had reduced cholesterol (170 mg/dL), lowest with fastest-growing tumors, especially pancreas, larynx, esophagus, leukemia, stomach, and colon (*42*). A 10-year study of a 39 000 patient cohort in Finland found an inverse cancer–cholesterol correlation, especially stomach and lung, during the first years of followup (*43*). The lowest quintile of serum cholesterol among 160 000 men and women corresponded to elevated cancer of the lung, prostate, and colon for men and breast, uterus, colon, and lung for women during the first two years after cholesterol measurement (*44*). Among 3805 hyperlipidemic men there was a drop in serum cholesterol between 8 months and 2 years prior to diagnosis (*45*). Among 10 295 men and women followed for 14 years, there was a sharp rise in cancer mortality at the lowest cholesterol level, greatest at <5 years followup and for older people (*46*). A similar observation was made in a cohort of 7478 men followed 17–20 years in which the inverse cancer–cholesterol correlation was diminished but not completely eliminated when deaths during the first five, and even more so 10, years were excluded (*47*). An inverse association was observed between serum cholesterol and colon, but not rectal, cancer in 7926 Japanese–American men over a 20-year span, more pronounced <10 years than >10 years before diagnosis, in the order Dukes B > C > A, D. The risk doubled for cholesterol <180 vs >200 mg/dL (*48*). In the MRFIT trial, 350 977 men were followed 12 years after cholesterol determination. Low cholesterol was associated with cancer of the liver, pancreas, lung, lymphatic, and hemopoietic systems, the association fading with time (*49*). Among 3091 Dutch men and women followed 28 years, the risk factor for gastrointestinal cancer (men only), years 3–15, was 4.2 for the lowest vis-à-vis the highest cholesterol quintile, falling to *ca.* 1.0 after that. For lung cancer in men and GI cancer in women there was no association (*50*). In other cohorts of 7716 (colon, pharyngeal, oral, esophagal, liver) (*51*) and 1910 (*52*) patients, as well as several smaller studies, lowering of cholesterol close to diagnosis was observed for cervical (*53*), colon (*54*), prostate (*55*), stomach, bladder, CNS, and colorectal cancers (*56*).

A more direct connection between cholesterol depletion in the patient and high LDL uptake by the cancer was made in a few studies. Lowered serum cholesterol in AML patients was closely correlated with high uptake by the AML cells of ^{14}C-sucrose-loaded LDL in vivo and of ^{125}I-LDL in vitro (*14*). In 59 leukemia patients, blood cholesterol was inversely correlated with the rate of high affinity degradation of LDL by the leukemia cells (*57*).

Inverse cancer–cholesterol correlations can be reversed. In the study just cited (*57*), when the leukemia cell burden was reduced by chemotherapy, blood cholesterol rose. Similarly, only in AML patients with high leukemia cell counts was low cholesterol observed (*47/85*), which rose during remission (*58*). Patients with a variety of leukemias and lymphomas had reduced cholesterol levels which rose to that of normal controls after chemotherapy-induced response, but not if the therapy failed (*59*). In cancer patients who responded to chemotherapy, LDL increased as follows (no. of patients, % LDL increase): malignant lymphoma (17, 32%); small cell lung cancer (11, 7%); transitional cell carcinoma (7, 7%); breast (16, no change) (*60*).

Of special importance is the fact that the effect of cancer in lowering blood cholesterol is greatest with the most aggressive, metastasizing tumors, in accord with their greater LDL requirements (*vide supra*). In the cited study of hyperlipidemic men, the drop in serum cholesterol was seen especially with nonlocalized rather than localized cancer (*45*). In another study, 83% of cancer patients presented with hypocholesterolemia, which was most severe with the most undifferentiated morphology (*61*). An inverse colon cancer–cholesterol correlation was most marked with the most advanced cases, particularly for women (*62*). Plasma LDL was lowered with acute leukemia, bladder, and breast carcinoma, especially the most aggressively metastasizing cases (*30*). LDL receptor activity in breast (*29*) and other (*11, 34*) cancers is higher the greater the degree of malignancy. Tumors of low but not high differentiation have high LDL uptake (*11,34*) and reduce plasma cholesterol the most (*32, 61*). Survival time, a measure of tumor aggressiveness, is lowest with highest LDL uptake in breast cancer (*29, 30*) and with lowest serum LDL in AML, chronic myeloid leukemia (CML) (*31*), and other types (*42*). Metastatic prostate cancer is associated with lower cholesterol levels than nonmetastatic (*55*).

There are many additional studies in which were seen an inverse relationship between plasma cholesterol and cancer of many types (*63–69*), as well as a few reporting no relationship (*71–73*). Diet is not responsible (*74*). The great preponderance of the evidence therefore favors the theory that the reduced cholesterol often observed in cancer patients is the result of uptake of LDL by the tumor and not the cause of the cancer (*75*).

RECONSTITUTION OF LDL WITH CYTOTOXIC DRUGS

In order to kill tumors with drugs that are targeted in LDL, the drugs must somehow be bound to the LDL in such a way that (1) they cannot escape from it while traveling in the blood enroute to the tumor, (2) their cytotoxicity is chemically or physically masked while LDL-bound, and then restored after entering the target cells, (3) in quantity × killing power there is enough drug to kill cancer cells contained in the reconstituted LDL (r-LDL), whose uptake is limited by the number of LDL receptors on the tumor cells and their rate of internalization, and (4) the presence of Apo B and its binding power to LDL receptors are retained. The ability of the drug, once released from its carrier, to escape from lysosomes must also be taken into account (*76*).

Association of a drug with LDL may be stable or unstable. Any or several of the following experiments must therefore be done to ensure that the drug enters cells only via LDL receptors. (1) Receptor-positive but not receptor-negative cells should be killed. (2) r-Me-LDL should be taken up by scavenger receptors (e.g., on macrophages) but not normal ones. Scavenger receptors take up damaged (methylated or acetylated) rather than normal LDL. Macrophages have many more scavenger than normal LDL receptors. (3) Saturability of uptake can be demonstrated by blockading LDL receptors with native LDL. It should reduce r-LDL uptake and cytotoxicity, while Me-LDL should not. (4) Cytotoxicity in vitro may be stronger with r-LDL than with free drug, although this is not necessarily the case. In vivo results must be interpreted with care, since even leaky r-LDL might be superior to free drug given as a bolus, if leakage is slow and steady (depot effect). (5) Benzyl alcohol inhibits LDL uptake and is therefore a possible test for the mechanism of killing (*77*).

The first published procedure was that of Krieger, in which LDL is isolated, lyophilized on potato starch, stripped of its core by heptane extraction, and reconstituted by adding the drug in a nonpolar solvent which is then evaporated and replaced with aqueous buffer (*78*). Lipophilic groups ("LDL anchors") on the drug, such as oleyl, linoleyl, retinyl, or cholesteryl, are required for successful, stable reconstitution (*79*). Since the entire core (which stabilizes the particle) is replaced, there is little tolerance for nonoptimal (i.e., poorly anchored) structures, but the carrying power of the r-LDL is high. The principal problems are imperfect anchoring, which leads to leakage of the drug from the r-LDL or failure to reconstitute altogether, and the creation of aggregated r-LDL, which is rapidly cleared by the reticuloendothelial system (RES). Contamination by aggregate therefore requires an extra step to remove it and lowers the yield (*80*).

The Masquelier procedure (*81*) differs in that sucrose is substituted for starch, and the core is not discarded. This makes for easier reconstitution without aggregation, and the r-LDL's pharmacokinetics are close to that of native LDL, but its carrying power for drug is lower.

In a more drastic process, LDL is taken apart with detergent (sodium deoxycholate, SDOC) and the Apo B reconstituted with drug and EPC (egg phosphatidyl choline) into a microemulsion. Yields and carrying power are high (*82–86*). In a comparison of these three procedures by another group, the first was favored (*87*).

A modified microemulsion technique has been described by Samadi-Boboli and co-workers (*88*). 9-Methoxyellipticine–cholesteryl oleate–DMPC (dimyristoyl phosphatidyl choline) microemulsion is incubated with LDL, a simple procedure which, however, gives lower carrying power (5–10 molecules drug/LDL particle) and drug yield (2.5%). There is greater carrying power (70 molecules/LDL) with the oleyl *N*-methylellipticinium 9-ester (*89*) and still greater (400 molecules) with improvements in reconstitution technique(*90*).

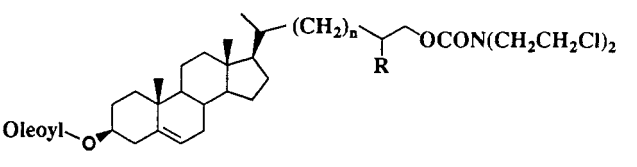
Drugs can be chemically linked to LDL (*81*, *91*). This has the advantage that leakage will not occur. However, the carrying power is limited, and increasing the load reduces binding to the LDL receptor, presumably because derivatization of Apo B alters it. Also, unless the drug is released efficiently after endocytosis, its cytotoxic power may be lowered (*91*). Boronated LDL has been made, carrying enough boron for boron neutron capture therapy (*92*), but there are no data on receptor-negative cells or competition with native LDL.

Finally, drugs can be associated with LDL by mixing them together in various ways. The advantage is that these procedures are very simple. The disadvantages can be low drug loading and leakage of drug from the complex, probably because some of it remains in the surface layer of the LDL and does not reach the core. LDL can be mixed directly with the drug (*23*, *93–95*), with the drug as a dry film (*96*) (40 drug molecules/LDL can be incorporated but 78% is easily released in serum) (*97*), or with a drug–cholesteryl ester microemulsion (*88*, *89*, *98*). Facilitated transfer (*99*) using natural transfer proteins has been tried (*5*, *100*), giving 18 molecules/LDL (*87*).

CYTOTOXICITY OF LDL–DRUG CONJUGATES

The first cytotoxic compound incorporated into LDL was 25-hydroxycholesteryl oleate, in 1978 (*101*). This was followed by pyrene–cholesteryl oleate, a photosensitizer (*102*).

We reported in 1984 a series of antitumor compounds reconstituted with LDL by the original method (*103*). Some compounds failed to reconstitute, others leaked out of the r-LDL, and still others reconstituted stably but had insufficient killing power. Two LDL anchors were required to prevent leakage. One drug, known as "compound 25", met all criteria in that it afforded stable r-LDL that killed all the target cells at reasonable r-LDL concentrations, while r-Me-LDL was not cytotoxic. Excess native LDL abolished cytotoxicity. The results were the same in other hands using reconstitution by the microemulsion (*104*), but not the modified sucrose technique (*105*), presumably because less drug is carried when not all the core is removed. An increase in cytotoxicity was needed, and this has now been achieved in compounds **1** and **2** (*106*), which have twice the number of warheads and twice the killing power. Apparently, in compound 25 the limit of enzymic carbamate-hydrolyzing power had been reached.



"Cpd 25": n=1, R=H

1 : n=1, R=$CH_2OCON(CH_2CH_2Cl)_2$

2 : n=3, R=$CH_2OCON(CH_2CH_2Cl)_2$

In addition to compound 25, Lundberg has also reconstituted prednimustine in a microemulsion, achieving 338 molecules/Apo B in particles 2.5× the diameter of native LDL that were stable for months (*86*). Activity was *ca.*

1.5× free drug, with the difference greatest at low concentration. Receptor-negative cells were not used.

Using their own modified method (Masquelier's) (*81*), Vitols et al. reconstituted LDL with the water-insoluble drug WB 4291 (1-[bis(2-chloroethyl)amino]-3-methylnaphthalene), incorporating 1500 molecules/LDL (*107*). This r-LDL showed receptor-mediated cytotoxicity in vitro in that native but not methylated LDL abolished cytotoxicity. Normal (receptor-positive) more than mutant (receptor-negative) cells were killed, but since the ID_{50} ratio was only 1:3, some leakage of drug out of the r-LDL was indicated. Clearance time in rabbits was longer for this r-LDL than that made by the Krieger (*78*) method. Median survival time (MST) of leukemic mice was prolonged 2.5× ip-ip with 40% survivors, but MST was increased only 1.42× iv-iv. LDL reconstituted with AD-32 (400–500 molecules/LDL) by Krieger's method was taken up via LDL receptors, but leaky (*108*). Stability of the r-LDL was improved with combined linoleyl and retinyl anchors (100–200 molecules/LDL), giving rise to selectivity and cytotoxicity (*109*).

Soula's group reported binding and degradation of r-LDL bearing *N*-methylellipticinium-9-oleate to be normal, saturable, and blocked by native but not Me-LDL (*89*). Macrophages absorbed r-LDL and LDL equally, and much less than acetyl-LDL, indicating normal behavior for the r-LDL. Drug uptake in r-LDL exceeded that of free drug. Cytotoxicity to L1210 was dose-dependent, greater than that of free drug, and blocked by native but not Me-LDL. Receptor-negative cells were not used. Loss of free drug in human serum plateaued at 10%. These results show uptake of r-LDL via LDL receptors, but do not rule out some leakage.

Uptake, cytotoxicity, and intracellular distribution of an LDL–daunomycin (DNM) complex (made by simple mixing) by lung cancer cells was equal to that of free DNM, although intracellular metabolism of DNM from the complex was slower, possibly because the presence of LDL upset lysosomal digestion of DNM; comparison with free DNM + LDL was not made (*23*). Receptor-mediated uptake of LDL–DNM was not demonstrated, either by suppression with free LDL or by lack of uptake by receptor-negative cells, so that leakage of DNM from the complex before uptake is possible.

An estramustine–LDL complex (*110*) made by Masquelier's procedure (*81*) (modified), containing 143 drug molecules/LDL, was 100× less active than free drug against two cell lines, with no effect of native LDL (*110*). Dose–response persisted above LDL receptor saturation. Thus, nonspecific uptake of the complex or free drug played a major role. Similarly, prednimustine–LDL (163 molecules/LDL) had 10× less cytotoxicity than free drug, not affected by native LDL (*111*). Conclusions are similar.

Dioleyl floxuridine, incorporated into r-LDL by Krieger's method (50–70 molecules/LDL), was taken up by Hep G2 cells via LDL receptors (although leakage was not ruled out) and had a serum half-life shorter than native but >6–9× longer than free drug (*87*).

Vincristine–LDL (VC/LDL), prepared by Masquelier's method, has been given to nine patients with ovarian or endometrial carcinoma (*112*). Uptake of r-LDL by the liver and adrenals was suppressed with chenodeoxycholic acid and prednisolone, respectively (*vide infra*). Side effects were peripheral neuropathy/paraesthesia (mild to severe, said to be less than with $VCSO_4$ alone), abdominal pain, and significant alopecia, showing that some if not most of the VC probably leaked out of the r-LDL. This is not surprising since VC has no good LDL anchors.

LDL can also be used to target photosensitizing drugs (*113,114*). Cytotoxicity then ensues only after irradiation. This has two potential advantages: toxicity can be postponed until after non-tumor-bound drug has been cleared from the body, and if the sensitizer operates catalytically, less drug is needed. In the first example, pyrene–cholesteryl oleate was targeted in r-LDL, exclusively via LDL receptors since receptor-negative cells were not harmed, inducing cell death after irradiation at 300–400 nm (*115*). Porphyrins can be associated with LDL (130–250 molecules/LDL) without inhibiting LDL receptor binding (*116*) or processing (*117*), and uptake can be enhanced 1.5–1.8× by lovastatin, which upregulates LDL receptors by inhibiting cholesterol biosynthesis (*118*). A porphyrin derivative (BPD), associated with LDL by simple mixing, catalyzed photodynamic killing of cells 7× better than BPD alone at 37 °C, and 6× better than at 4 °C showing the importance of internalization (*119*). However, porphyrins can exchange between LDL, other phospholipids, and cell membranes, reducing the targeting specificity. This might lead to long-lasting photosensitivity in patients (*113*). In vivo, hematoporphyrin is carried to a mouse tumor by LDL (*120*). Mixing LDL with BPD before injection improves the antitumor effect of light (*121*).

UP- AND DOWNREGULATION OF LDL RECEPTORS

It is desirable to deliver the drug only to the tumor and to exclude it from nontumor sites. However, competing with the tumor for r-LDL are normal tissues and organs, particularly the liver because of its size and LDL receptor content, and the adrenals whose uptake/g is particularly high. Since the drugs to be targeted have some selectivity toward cancer cells, normal organs can take some hits, but protection of the liver and adrenals is nevertheless desirable. Bile acid feeding suppresses hepatic LDL receptor activity in dogs (*122*), and a key paper reports that feeding of sodium taurocholate and hydrocortisone sodium succinate to tumor-bearing mice reduces LDL uptake in the liver by 36% and in the adrenals by 59%, with insignificant reduction in uptake by the tumor (*123*). Also reported to downregulate the liver's uptake of LDL are feeding saturated fats (*124*), cholesterol with hydrogenated coconut oil (*124*), and fasting (*125*) which is said to downregulate LDL receptors on healthy but not tumor cells (*5*).

Another strategy for protecting the liver is based on the fact that normal LDL receptors do not bind to Ac-LDL, Me-LDL, or oxidized LDL (Ox-LDL), which instead are avidly taken up via scavenger LDL receptors, e.g., by macrophages (*vide supra*). Cholesterol liberated from Ac-LDL after endocytosis via scavenger receptors enters the metabolically active pool (*126*) and thus presumably functions normally in downregulating LDL receptor expression. Therefore, I propose that the liver, which is rich in scavenger (on sinusoidal epithelial and Kupffer cells) (*127*) as well as normal LDL receptors, might be downregulated by cholesterol-loaded Ac-LDL, Me-LDL, or Ox-LDL which would not downregulate LDL receptor expression on tumor cells. Sinusoidal epithelial cells of the spleen, bone marrow, adrenals, and ovaries also take up Ac-LDL (*127*) and might be downregulated at the same time.

Another way of protecting the adrenals arises from the fact that angiotensin II increases their LDL uptake and receptor number (*128*). I suggest that ACE inhibitors, which prevent angiotensin II formation, might then reduce LDL uptake by the adrenals.

Increasing the activity of LDL-mediated endocytosis in tumor cells would also be beneficial, provided normal cells were not concomitantly upregulated. LDL receptor activity is indeed stimulated in normal cells by HMGCoA-reductase inhibitors (*129, 130*) or bile acid sequestrants (*129–131*), verapamil (*132*), cachectin, and some growth factors (*133*), TGF-β (134), TNF-α, and IL-1 (*135*). There is one report that HMGCoA reductase inhibitors upregulate normal rat hepatocytes more than Hep-G2 cells (*136*) (bad news if confirmed). On the other hand, compactin is reported to upregulate LDL receptors on Hep-G2 cells with little effect on normal human fibroblasts (*137*). Oncostatin M potently upregulates LDL uptake by Hep-G2 cells, more than normal cells (*138*). The upshot is that we cannot yet tell whether upregulation of tumor LDL receptor activity by the above methods would or would not be therapeutically beneficial.

REMOVAL OF LDL FROM THE PATIENT BEFORE TREATMENT

During treatment, drug-bearing r-LDL must compete with native LDL for access to LDL receptors on the tumor cells, requiring elevated doses of r-LDL. This can be countered by removing LDL from the patients' blood (delipidation) prior to treatment (*139–141*). Although restoration of normal LDL levels takes days (*141*), it might be best to delipidate immediately prior to treatment because it induces upregulation of LDL receptors throughout the body (*142*), and it is unknown whether upregulation in this way would be greater for tumor or normal cells.

However, in hepatocytes, unlike fibroblasts, cholesterol derived from LDL only weakly downregulates LDL receptor activity (*143*), so that one might expect that LDL depletion will upregulate liver uptake in only a small way. In low-LDL media, ACAT inhibitors block time-dependent upregulation in normal hepatocytes more than in Hep-G2 cells (*144*). I suggest, then, that this is a way to prevent liver upregulation during delipidation.

Numerous methods have been developed for extracorporeal removal (*145*) of LDL for the sake of hypercholesterolemic patients. Macroporous bead cellulose removes LDL but not HDL (*146*). The following methods remove the indicated amounts of LDL: plasmapheresis 73% (*147*), apheresis with dextran sulfate cellulose 62–64% (*139*), continuous adsorption on Apo B-antibody column 82% (*140*), anti-LDL antibody/Sepharose 80% (*141*), and apheresis 76% (*148*). Intravenous infusion of liposomes removes >90% of plasma cholesterol from both normal and Watanabe rabbits (*149*). Continuous intraoperative separation of red blood cells by machine in 3–4 min for autologous transfusion has been reported to be safe and efficient (*150*), opening the possibility that LDL could be extracted from the patient's own blood, returning his delipidated plasma and blood cells, and making the drug-bearing r-LDL from his own LDL. LDL obtained via apheresis is also suitable for reconstitution with drugs (*151*).

LDL UPTAKE BY PARASITES

Although this topic is not cancer-related, it is worthwhile noting that diseases other than cancer might possibly be treated with drugs carried in r-LDL. Trypanosoma brucei has LDL receptors that are stable despite surface antigen variation (*152*) and requires LDL, having 800 high- and 52 000 low-affinity LDL receptors (*153*) which carry the LDL to intracellular acidic vacuoles for proteolysis and then recycle (*154*). Schistosoma mansonii parasites take up LDL via specific and saturable binding, breaking it down in the lumen or gut rather than lysosomes (*155*). Trichomonas vaginalis, a common human parasite, has high LDL requirements (*156*). Leishmania amastigotes residing in macrophages are killed 3× more efficiently by methotrexate (MTX)-maleyl BSA, via the Ac-LDL receptor, than by free MTX (*157*).

CONCLUSION

In order to successfully treat cancer by means of LDL-mediated drug targeting, it is necessary to develop a method of securely associating drugs with LDL in such a way that they are released inside, and only inside, the target cells, find drugs potent enough to kill tumor cells when delivered in this way, and work out reliable and convenient ways to delipidate patients. All these things separately have been accomplished, if not to perfection at least in a practical way. Therefore, the path is now open to human trials. Although numerous potential tumor targets have already been identified, and presumably others yet unidentified will be uncovered, it seems to me that the best opportunity for the first strike is against a hematological malignancy such as AML because cure is presently very difficult, access of r-LDL to the target cells is unimpeded, and LDL uptake by this particular tumor is especially high.

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