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Controlled Release of Antiretroviral Drugs

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Abstract

The treatment of AIDS using combinations of antiretroviral drugs has highly reduced the HIV-1 related morbidity and mortality, provided that the plasma viral load can be maintained as low as possible. However, eradication of the virus does not seem attainable with the present strategies of interventions which is due to two major obstacles: If resistant mutations appear the virus will escape further treatment, and latent virus reservoirs exist which cannot be reached with the current treatment regimens. One of these sanctuaries is the mononuclear phagocyte system (MPS) with its HIV-1 target cells, such as monocytes/ macrophages (MO/MAC), dendritic cells (DC) and Langerhans cells which can be considered as primary cells for viral entry, and subsequently are responsible for distribution of the virus throughout the organism into various tissues. Colloidal drug carriers are easily phagocytosed by MO/MAK. Therefore, they can facilitate the uptake of antiviral drugs by these cells and may enable a considerably improved AIDS therapy. The present article summarises strategies which allow the targeting of antiviral drugs to these cells by the use of carrier systems including nanoparticles, liposomes, immunoliposomes or red blood cells.

Key words

Drug targeting. Nanoparticles. Liposomes. Antiretroviral drugs. Monocytes/macrophages.

Introduction

Many promising antiviral agents against HIV unfortunately have disadvantageous physicochemical properties which lead to a poor bio-distribution and insufficient cellular uptake. One presupposition for a therapeutic approach is to maintain adequate drug levels at the sites of viral replication over extended periods of time. Moreover, the well-known adverse reactions and side effects of antiviral treatment are often related to the accumulation of the drug at inappropriate sites. Drug carrier systems and dosage forms, such as nanoparticles, liposomes and others, hold the promise of overcoming these pharmacokinetic obstacles[1-5]. Colloidal drug delivery systems differ fundamentally from other pharmaceutical dosage forms. They represent disperse systems in which polymeric (e.g. nanoparticles) or lipid particles [e.g. liposomes or solid lipid nanoparticles (SLN)] are dispersed in a liquid phase. The size of these particles is in the nanometer range. This size range offers the decisive advantage of this class of pharmaceutical dosage forms as it allows the so-called drug targeting which often is not possible with free drug. Drug targeting is defined as the delivery of drugs to specific organs or tissues in the body. Besides colloidal drug carriers, other pharmaceutical dosage forms as well as chemically modified drugs (prodrugs) can be used for this purpose. After access of the carrier system or of a prodrug to the target site, the release can be

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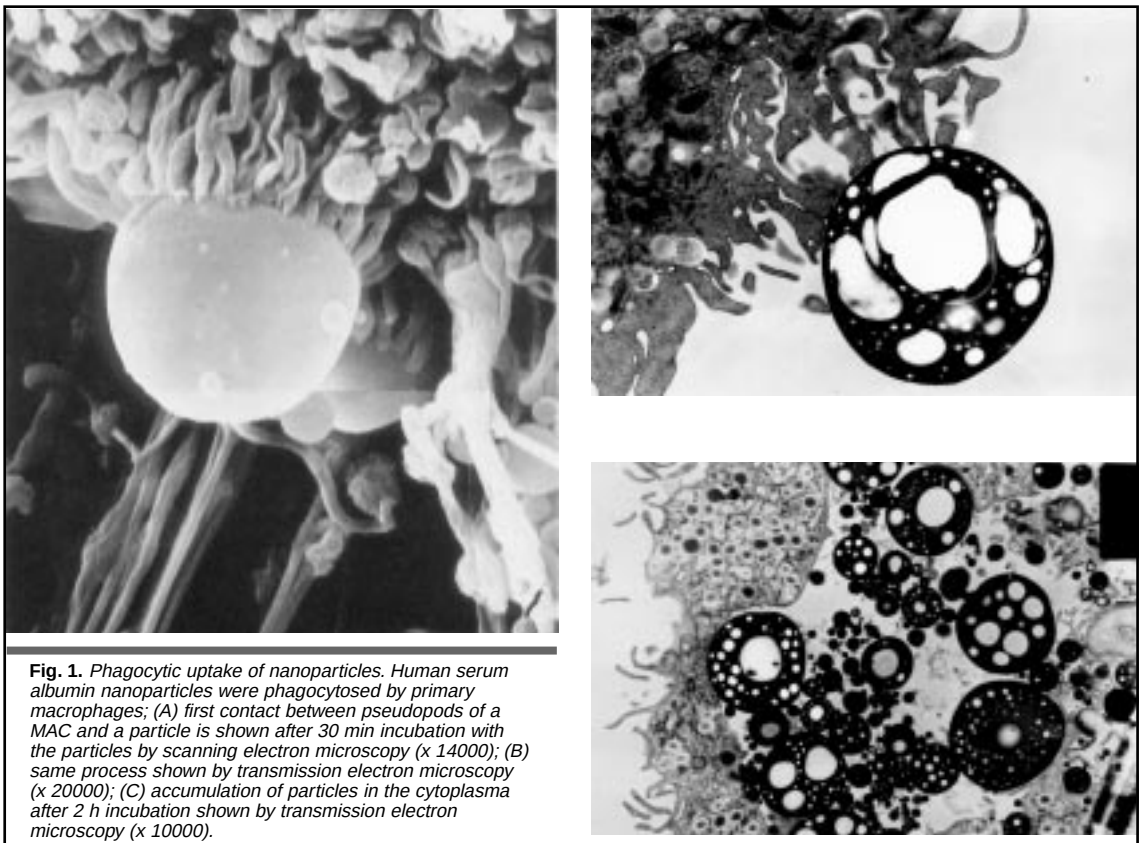

Fig. 1. *Phagocytic uptake of nanoparticles. Human serum albumin nanoparticles were phagocytosed by primary macrophages; (A) first contact between pseudopods of a MAC and a particle is shown after 30 min incubation with the particles by scanning electron microscopy (x 14000); (B) same process shown by transmission electron microscopy (x 20000); (C) accumulation of particles in the cytoplasma after 2 h incubation shown by transmission electron microscopy (x 10000).*

controlled by the delivery system[6]. The major advantage of targeted delivery is the ability to lower the necessary dosage, which enables a reduction in side effects.

Due to their structure, colloidal particles such as nanoparticles and liposomes are recognised and taken up in larger amounts by the mononuclear phagocyte system (MPS) after intravenous injection and are deposited in certain organs (liver, lymphoid tissue) that are rich in macrophages. Apart from CD4 T lymphocytes, cells of the MPS (i.e., monocytes/macrophages) play a decisive role in the pathogenesis of AIDS and have to be considered as reservoir for HIV. In tissues like the lung and the brain, HIV is located primarily in macrophage-like cells (i.e., alveolar macrophages and microglia, respectively). *in vitro*, the incorporation of nanoparticles in primary monocytes/macrophages can be visualised by electron microscopy. In Fig. 1 different steps of phagocytic uptake of nanoparticles made from human serum albumin in these cells is demonstrated. Consequently, specific drug carrier systems represent promising vehicles for the transport of antiviral agents to monocytes/macrophages.

Nanoparticles

Nanoparticles are solid polymeric colloidal drug carriers ranging in size between 10 and 1000 nm[2, 4,5]. The polymers employed for their preparation can be of artificial or natural origin. Substances can either be adsorbed onto the surface or be incorporated into these particles. An overview of the different production methods and materials is given by Kreuter[2] and Alleman *et al.*[3]. Due to the particular structure and surface characteristics of nanoparticles, the cells of the reticuloendothelial system (RES), like monocytes/macrophages, are a prime target since they are taken up preferentially by these phagocytosing cells. The active uptake of rhodamine 6G-labelled HSA nanoparticles is shown in Fig. 2. The process is found to be temperature-dependent, since at 37 °C a homogeneous staining of the whole cytoplasm was observed, whereas at 4 °C only minor fluorescence at the cell membrane occurred without phagocytosis of the fluorescent drug carriers into the cytoplasm.

In a study of Schäfer *et al.*[7], phagocytosis of nanoparticles in monocyte-derived macrophages *in vitro* was investigated with respect to particle material, size, surface properties and other parameters. Phagocytosis is highly dependent on the type of material used for the preparation of particles and on particle size (Table 1). Nanoparticles made from polyhexylcyanoacrylate (PHCA) or human serum albumin with a diameter of 200 nm were found to be most suitable for targeting of antiviral substances to macrophages.

Nanoparticles made out of human serum albumin (HSA-NP) and polyhexylcyanoacrylate (PHCA-NP) loaded with AZT and ddC were used to examine their potential in inhibiting HIV-1 replication *in vitro*[8]. Cultured monocyte/macrophage cells were pre-incubated with drug-loaded nanoparticles or with the

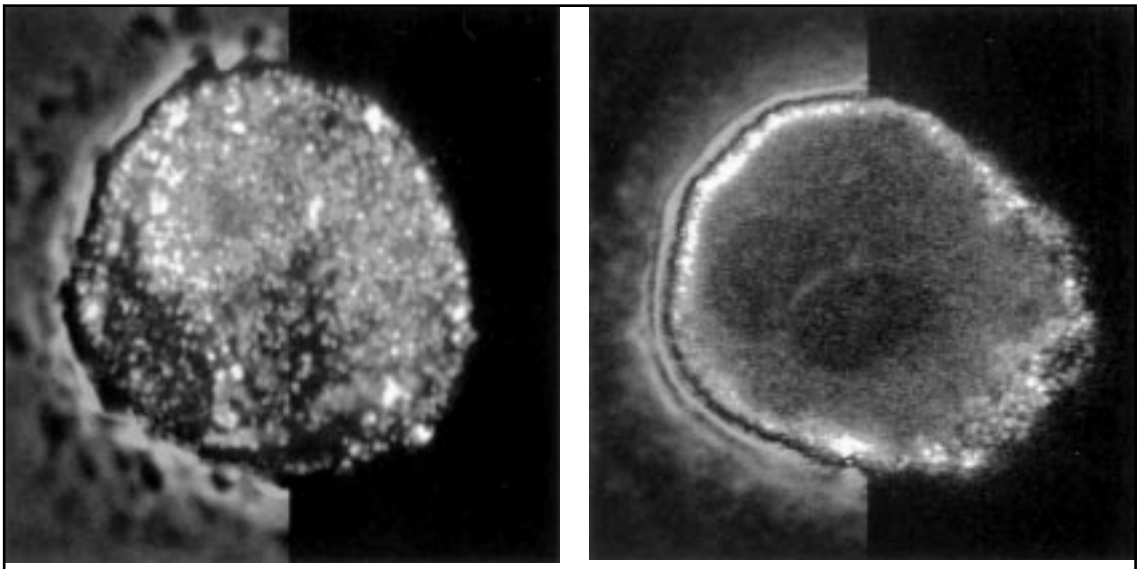

Fig. 2. *Confocal laser scanning micro-graphs of primary monocytes/macrophages incubated for 4 h with rhodamine 6G labelled human serum albumin (HSA) nanoparticles (A) at 37 °C, (B) at 4 °C.*

free drug before infection with HIV-1. The antiviral effect was monitored by evaluating HIV replication. HSA- and PHCA-NP loaded either with AZT or with ddC were effective against HIV-1 infection. However, in relation to free drug, they showed no superiority. As AZT and ddC can easily diffuse into the cells, these substances do not require drug delivery by a carrier system. More lipophilic compounds or larger drug molecules with poor bio-availability, however, could profit from delivery by these particles.

This assumption was proven by the following investigations by the same authors. The lipophilic HIV protease inhibitor saquinavir was incorporated into PHCA-NP and tested on acutely and chronically HIV-1 infected monocyte/macrophage cells. Again, the prophylactic effect of the preparations was evaluated in comparison to the free drug solution and to a simple mixture of nanoparticles and drug solution[9]. Efficiency of the preparations was measured by HIV-1 production. In both acutely and chronically infected cells, the nanoparticle preparation showed superior effects compared to the free solution and the simple mixture. A more than 10-fold increase in antiviral activity was shown in acutely infected MO/MAC. An IC50 of 0.39 nM was determined for the nanoparticle preparation, whereas the free-drug solution and the simple mixture of free drug and unloaded nanoparticles had an IC50 of 4.23 and 5.31 nM, respectively (Fig. 3). These findings indicate that the cellular uptake of protease inhibitors, in contrast to nucleoside analogs, is the limiting factor for their antiviral effectiveness.

Although in the above cell culture experiments with human MO/MAC no difference in the inhibition of HIV replication between AZT or ddC bound to nanoparticles or in free form was observed, it has to be considered that a totally different situation in the whole organism could be expected due to the advantageous body distribution of the particle-bound drug. Indeed that was observable: Löbenberg and Kreuter[10,11] bound ^{14}C-labeled AZT to nanoparticles using the surfactant bis(2-ethylhexyl) sulphosuccinate sodium (DOSS). After intravenous injection in rats, the liver concentrations of the [^{14}C]-AZT label were about 2.5 to 18-fold higher after binding to nanoparticles than after injection of the solution, with differences increasing with time. In other organs of the RES, i.e. the lungs and in the spleen, the difference between the two preparations was about 10-fold in favour of the nanoparticles after 480 min.

Table 1. *Influence of particle size and material on phagocytosis of manoparticles by monocytes/macropgaes.*

Particle material	**PHCA**	**PBCA**	**PMMA**	**HSA**	**HSA**
Particle size (nm)	200	200	130	200	1,500
Pahbocytozed manoparticles (mg/mL ± SD	3.01 ± 0.45	6.56 ± 0.41	19.28 ± 2.82	4.38 ± 0.29	15.84 ± 2.79

PHCA: polyhexylcyanoacrylate
PBCA: polybuthylcyanoacrylate
PMMA: polymethylmethacrylate
HSA: human serum albumin

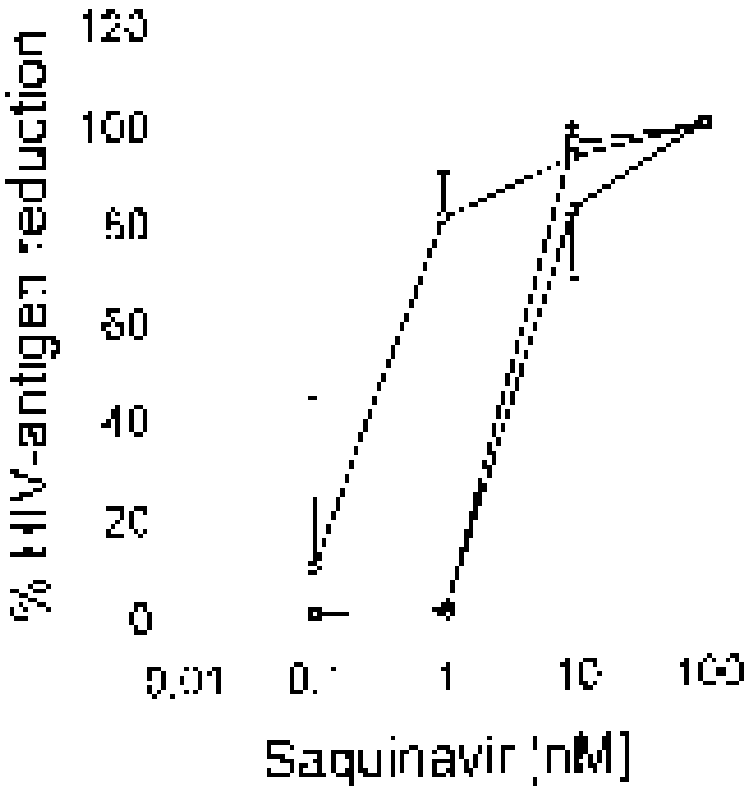

Fig. 3. *Antiviral activity of saquinavir-loaded hexylcyanoacrylate nanoparticles in acutely HIV-1 infected monocytes/macrophages. Three days after infection cells were exposed to various concentrations of drug-loaded nanoparticles (m), an aqueous solution of saquinavir (o), or a simple mixture of unloaded nanoparticles and an aqueous drug solution (D). HIV-1 p24 antigen production was measured in cell culture supernatant by ELISA. The results are presented as percent reduction of HIV-1 antigen compared with that in infected but untreated control.*

Auto-radiographs after i.v. injection of the solution showed a rather homogeneous although sparse distribution of the AZT label in liver and lungs, whereas after i.v. administration of the nanoparticle preparation the radioactive concentrations were much higher and showed a spotted appearance, indicating their accumulation in the macrophages of these organs[10,12].

These authors also investigated the oral administration of nanoparticles in rats[10,13]. Nanoparticles again led to an accumulation of AZT in tissues containing a large number of macrophages. In the liver, the area under the drug concentration versus time curve (AUC) of ^{14}C-labeled AZT was 30% higher when the drug was bound to nanoparticles. In the brain, the uptake of AZT also was significantly higher when using nanoparticles as the drug-targeting system. Oral absorption of AZT from the solution was very rapid whereas binding of drug to nanoparticles delayed oral absorption. After administration of nanoparticles, 60 min were required to reach the highest concentrations in the RES organs, whereas with a solution the maximum was reached already after 30 min.

pH-sensitive nanoparticles produced from the methacrylic acid copolymers Eudragit L100-55 and S100 were used to increase the bio-availability of the highly lipophilic compound CGP 57813, which is almost non-ionisable and nearly insoluble in water, after oral delivery to dogs[14]. This compound was shown to be a strong inhibitor of the HIV/protease, which was developed by Ciba-Geigy (Basel, Switzerland). pH-sensitive nanoparticles can be used to specifically control the liberation of incorporated substances in the gastrointestinal tract. Thus, a controlled release can be achieved and higher plasma levels obtained due to an optimal liberation in the gut. In the above study by Leroux *et al.*[15] no plasma levels were detected after the substance was given in amorphous form. With nanoparticles, a significant plasma profile was observable, although it was not as high as was expected from previous results with mice[15]. Furthermore, the area under the plasma concentration-time curve (AUC) was highly dependent on the feeding state of the animals. A further optimisation of the process seems to be possible since the pH-responsiveness of the nanoparticles may be optimised and adjusted leading to an increase in AUC.

Liposomes

Liposomes consist of one or more lipid bilayers surrounding a hydrophilic core. The main constituents of the liquid layers are phospholipids and in most cases also cholesterol. Lipophilic drugs can be incorporated into the lipid double layers, hydrophilic drugs into the aqueous layers between the lipids as well as into the aqueous core. Amphilphilic drugs may be incorporated into both types of layers, but frequently dissolve the liposomes. Depending on the number of layers uni- or multi-lamellar systems can be distinguished. The size range of liposomes is comparable to that of nanoparticles[6,16]. A major disadvantage of liposomes is their relatively lower physical stability. Due to their similarity to bio-membranes, some physiological enzyme systems may lead to their destruction. For an overview of the different production methods and applications see a review by Commelin and Schreier[17].

A combined *in vitro* and *in vivo* study was performed by Hostetler *et al.* using AZT and ddC[18]. AZT coupled to a dipalmitoylphosphatidic acid (DPP) and ddC coupled to a dioleoylphosphatidic acid (DOP) were employed in the form of free substances and incorporated into liposomes. As a cell model, HT4-6C cells were used, derived from transformed HeLa cells infected with HIV-1, and the antiviral effect was measured by the plaque reduction assay. Additionally, they used the human lymphoblastoid cell line CEM-CCRF which was infected with HIV-1. In the second case, p24 antigen production was determined to evaluate the efficacy. AZT-DPP-liposome formulation was compared to the free drug in RLV infected mice. The authors found contradictory results *in vitro* and *in vivo*. In the cell systems the phospholipid liposomal preparations were both active in HIV-infected cells but substantially less effective compared to their free substances. Moreover, marked differences in the antiviral activities of AZT and ddC in both cell lines were observed. Both liponucleotides were slightly more active in HT4-6C cells. In contrast, the mouse model gave a clear indication that the liposome formulation was more effective. For liposomal DOP-ddC formulation, Hostetler *et al.* found a 107-fold increased AUC relative to free ddC in the spleen and a 3.8-fold increased AUC in lymph nodes. Although the conjugate as well as the liposome formulation both need to be optimised, the authors expect that phospholipid prodrugs encapsulated into liposomes may improve HIV therapy by enhancing the efficacy and reducing the toxicity of antiviral agents.

The potential use of liposomes coupled with derived human serum albumin (HSA) (cis-aconitic anhydride; Aco-HSA) may also lead to an improved bio-distribution and efficacy in HIV therapy[19]. The actual mechanism of action of Aco-HSA is not presently clear but it is suspected that it plays a major part in inhibiting the virus-cell binding and the fusion of the virus with the cell membrane. Aco-HSA was shown to have an antiviral effect by itself. PEG was employed to further influence the *in vivo* distribution of the liposomes. MT-4 cells, a T4 lymphocyte cell line, were infected with HIV-1 and used as a cell model. The antiviral assay was measured by the viability of the cells. Although free Aco-HSA showed the best results, the encapsulated formulation also achieved considerable effects in the antiviral assay. In a body distribution study with rats, the liposome formulation showed the most promising results since most of the injected dose clearly was taken up by liver and spleen. These are the main organs containing macrophages that represent one of the most important targets for AIDS therapy. The authors speculated that with Aco-HSA-PEG-liposomes a dual attack on HIV replication might have been accomplished: Firstly, an inhibition of binding/fusion of the virus with the cell membrane due to coupled Aco-HSA and, secondly, on the reverse transcriptase by including nucleoside analogues.

Other researchers incorporated foscarnet into liposomes[20]. Foscarnet is effective against CMV and other forms of the herpes virus family. It also inhibits the reverse transcriptase of HIV-1 and has a synergistic therapeutic effect with zidovudine. Dussere *et al.* investigated the antiviral effect of the liposomal formulation *in vitro* in HIV-1 infected human promonocytoid U937 cells and, additionally, the accumulation of foscarnet in murine monocytic RAW 264.7 cells. The *in vivo* biodistribution of radioactively labelled liposomes was evaluated in rats. The objective was to find a liposomal formulation for the specific macrophage targeting of foscarnet in order to achieve the required high therapeutical concentrations. Depending on the specific phagocytotic activity, the liposomal formulation was taken up to a much higher extent by both macrophage-related cell lines RAW cells and U937 cells, than the free compound. The antiviral activity of foscarnet in human U937 cells was slightly increased by the liposomal formulation compared to free foscarnet. *in vivo*, the liposomal foscarnet showed a distribution pattern that was distinctively different from the free solution. Especially an accumulation in the brain and in the eyes was found that was much higher with the liposomal formulation. Altogether, encapsulation of foscarnet in liposomes improved drug pharmacologic parameters since the plasma half-life was greatly enhanced by suppressing the rapid phase of renal elimination. Higher drug concentrations with the liposomes were also found in the lymph nodes and in the lungs. Both organs are important reservoirs of HIV or CMV, respectively. Liposomal delivery of foscarnet thus seems to increase the efficiency of the therapy of the HIV infection and also that of viral co-infections such as with CMV, as foscarnet also possesses anti-CMV activity.

Two studies on the acute toxicity and body distribution of loaded liposomes containing anti-HIV drugs including ddC, ddI, foscarnet, and AZT were performed in mice[21,22]. The liposome concentrations in these studies were 10 and 100 times higher than the dosage suggested for human use. No acute toxicity was found after i.v. injection of the animals. Increased drug concentrations were found in the targeted lymphoid tissue such as lymph nodes and other organs of the reticulo-endothelial system (RES). In a recent study ddCTP was encapsulated

in liposomes and tested in the murine acquired immunodeficiency syndrome (MAIDS) model. Results with this formulation indicated the reduction of proviral DNA in cells of the MPS in both spleen and bone marrow[23].

Lipophilic alkyl/acyl dinucleoside phosphate derivatives of AZT were incorporated into liposomes by Schwendener *et al.*[24] and their *in vitro* and *in vivo* effects were tested using HIV-1 infected CD4+ HeLa and H9 cells and RLV-infected mice, respectively. Large differences between the two derivatives (N4-hexadecyldC-AZT and N4-palmitoyldC-AZT) were observed, and no correlation between the cell and animal models was obtained. The antiviral activity in the cell cultures was evaluated by plaque reduction in the HeLa cells and by the measurement of HIV-1 p24 antigen production in H9 cells. The measurement of spleen size and weight was performed to show the effects of the treatment *in vivo*. *in vitro* the lipophilic AZT derivatives inhibited HIV replication in a dose-dependent manner, however, higher concentrations were required to obtain a similar antiviral effect as with free AZT. In contrast to these findings, the results of animal testing were very favourable for the lipophilic derivatives as they showed a highly improved therapeutic profile. The non-correlation of the effects was attributed to the low phagoycytotic activity of the above cell lines.

Duzgunes *et al*. recently could show that the experimental HIV-1 protease inhibitor L-689,502 encapsulated in negatively charged, multilamellar liposomes was about 10-fold more effective than the free drug in inhibiting HIV-1 in monocytes/macrophages[25], confirming earlier studies of Bender *et al*. who obtained similar results with nanoparticle-bound saquinavir (see above, Fig. 3)[9]. The same was found when Duzgunes *et al*. tested the reverse transcriptase inhibitor 9-(2-phosphonylmethoxyethyl)adenine (PMEA) which had reduced EC50 values by an order of magnitude when delivered to HIV-1 infected macrophages in pH-sensitive liposomes[25].

Immunoliposomes

Immunoliposomes enable an even more specific targeting of certain cell types. Monoclonal antibodies directed against cell surface molecules can be attached covalently to the surface of the liposomes. Immuoliposomes enter into lymphoid cells by a receptor-mediated endocytotic pathway. Analogues of 2',5'-oligoadenylates (2-5A), cordycepin (3'-deoxyadenosine) core trimer (Co3) and its 5'-monophosphate derivative (pCo3), were shown to inhibit viral replication by nearly 100% if applied with immunoliposomes[26]. H9 human T-cells were infected with HIV-1 one hour after the application of the liposomes, and the RT-activity and p24-antigen production was measured. H9-cells were treated before with anti-CD3 antibodies with which protein A coupled to the surface of the liposomes could react. Protein A binds with high affinity to the Fc region of several antibody classes.

ddU is one of the most potent inhibitors of RT of the HIV *in vitro*. However, ddU itself is a poor substrate for cellular nucleoside kinases; phosphorylated derivatives are not able to cross the cell membrane. For this reason protein A was coated to drug-loaded liposomes to increase the ability of phoshorylated ddU to enter cells[27]. HIV-1 infected CCRF-CEM and MT-4 cell cultures were used to examine the effects of the liposome preparations. On CEM cells, antibodies against the CD7 and HLA class I were used; on MT-4 cells anti-HLA class I as well as II antibodies were employed to direct protein A bearing liposomes to the cells and RT-activity and p24 antigen expression was measured. The phosphorylated derivatives of ddU encapsulated in these liposomes were able to inhibit replication of HIV *in vitro*, the efficacy of which was dependent on the antibody and ddU derivative used. No inhibition was observed with non-targeted liposomes containing phosphorylated ddU, or with empty liposomes, whether targeted or not. In another approach using liposomes bearing anti-HLA-DR Fab' fragments, the targeting of these carriers to cells expressing the MCH class II complex was evaluated and compared to that of conventional liposomes. Anti-HLA-DR immunoliposomes were found to accumulate to a higher degree in cervical and brachial lymph nodes compared to normal liposomes, indicating the targeting of HLA-DR positive cells (i.e. monocytes/macrophages and activated CD4+ T lymphocytes) which are the primary cell reservoirs of HIV-1[28].

Red blood cells (RBCs)

The possibility of using red blood cells (RBC) to target drugs against HIV and HSV in *in vitro* and *in vivo* systems was demonstrated by several groups. RBCs are treated by the organism as a physiological part in the biochemical cycle and, therefore, are of large interest as a drug carrier. RBCs are mostly taken up by macrophages that are, as mentioned above, a prime target for HIV-therapy. Usually, erythrocytes are loaded with drugs via hypotonic dialysis, and then resealed and re-annealed. The recognition by macrophages can be enhanced by band 3 clustering through treatment with ZnCl2 and BS3 (bis-sulfosuccinimidyl-suberate) and binding of autologeous IgG. Band 3 is the predominant anion transport system in erythrocytes. This system has to be switched off if RBC encapsulated compounds is not to be pumped out of these cells.

ddCyd in its 5'-triphosphate form (ddCTP) was encapsulated into autologous RBC to overcome the poor phosphorylation activity of the RES cells. The efficacy of this formulation was tested on HIV-1 infected human monocytes/macrophages[29]. Additionally, the *in vivo* effects were evaluated in murine leukaemia virus infected mice[30]. In both models an increased efficacy of treatment with ddCTP encapsulated in RBC was shown. The p24-antigen production of the HIV-1 infected monocytes/macrophages clearly was reduced. *in vivo*, the RBC-treated mice exhibited a significant reduction in spleenomegaly, lymphoadenopathy and hypergammaglobulinemy as signs for viral reduction.

In another publication the same group also was able to show the efficacy of the treatment in a feline model[31]. The feline immunodeficiency virus (FIV) causes similar symptoms in cats as HIV in humans. Thus it is an ideal model to evaluate the treatment with autologeous RBCs. *in vitro* a co-cultivation model of FIV-infected monocyte-derived macrophages with peripheral blood lymphocytes from healthy cats was established. As described before, ddCTP-loaded RBCs were used against the infection of FIV. ddCTP-loaded erythrocytes were able to reduce FIV in macrophages infected *in vitro* or obtained from naturally or experimentally infected cats.

Another drug investigated for its use in anti-HIV therapy incorporated into RBCs[32] was 9-(2-phosphonyl-methoxyethyl)adenine (PMEA). It is a prototype of the family of acyclic nucleoside phosphonates and exhibits strong inhibitory effects on replication of HIV and HSV. PMEA encapsulated in RBCs was shown to be 500 times more effective than the free substance in inhibiting virus replication in human primary monocyte/macrophage cells, as determined by p24 antigen measurements. Even though empty RBCs showed an effect on their own, the drug-loaded RBCs were the most effective preparation. It can be concluded from the above experiments that red blood cells are efficient transporters of drugs into the RES, both *in vitro* and *in vivo*. The biggest advantage of this carrier is its physiological nature. However, a problem might be the up-scaling required for the therapy in humans.

Conjugates

Low sensity lipoprotein (LDL) particles represent an important part in the metabolisation cycle of lipids in the human organism. These particles carry mostly cholesterol to the cells throughout the body. Additionally, they carry apolipoprotein fractions (Apo) on their surface which are able to dock with different receptors on the cell surfaces. Native LDL is cleared from plasma through a regulated pathway by binding to the apo B/E receptors present on hepatocytes. Chemical modification of apo B like glucosylation or oxidation changes the affinity from the apo B receptor towards the scavenger receptor. Scavenger receptors are expressed on the surface of cells of the monocyte/macrophage lineage and on the endothelium. For example derivatisation of the e-amino of the apoB-protein abolishes binding to the LDL receptor and initiates the affinity to the scavenger receptor. Thus coupling antiretroviral substances such as inhibitors of HIV reverse transcriptase to the e-amino group of the lysine side chain creates a high affinity for monocytes/macrophages. This effect offers the chance to target selectively these cells which are an important reservoir of HIV.

Radioactively labelled thymidine and AZT were coupled to the e-amino groups of lysines of LDL-Apo B and their uptake behaviour was studied in human hepatocytes (Hep G2) and murine macrophages (P388)[33]. Hep G2 cells do not exhibit a scavenger receptor in contrast to the murine macrophages used in the experiments. Instead they possess the normal apo B receptor. It was shown that the coupling of the nucleosides induced a shift in affinity to the scavenger receptor of the macrophage cells. By auto-radiography the uptake, lysosomal cleavage from LDL and triphosphorylation of 3H-labelled thymidine-LDL in murine macrophages was demonstrated.

In a later work the antiretroviral effects of these AZT-coupled LDL particles were shown[34]. Human macrophage cultures were infected with HIV-1 and subsequently treated with LDL-coupled AZT and with free AZT serving as a control. Additionally the scavenger-receptor free T-lymphocyte Molt 4/8 cells were infected with HIV-1 and subsequently treated the same way. It was demonstrated that LDL-coupled AZT was internalised specifically by macrophages via scavenger receptor and had anti-HIV activity comparable to free AZT, whereas the free substance had a better effect on the Molt 4/8 cells. The same results were also found with fluorothymidine, a potent inhibitor of the HIV-1 reverse transcriptase[35]. The optimisation of the coupling procedures for substances to LDL particles was also described[36]. The authors propose a better efficiency of antiretroviral therapy with the LDL-couples nucleosides during early stages of infection.

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