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Mini-Review

Drug targeting systems for antiviral agents: options and limitations

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1. General considerations

The design of new antiviral agents is often merely based on considerations concerning their desired antiviral activity. Only after the usual in vitro screening the question arises how the particular compound can be administered in vivo and delivered to its site of action. It is obvious that the physicochemical features that determine the antiviral activity will intrinsically dictate its fate in the body. Unfavorable pharmacokinetic characteristics can be encountered in this stage of development. For instance, poor oral absorption or bioavailability may occur. In addition, accumulation in tissues leading to overt toxicity can be found. Very rapid elimination from the body and formation of toxic metabolites may imply other drawbacks. Consequently, major problems may be encountered in the clinical phase of testing: severe side effects, large interindividual variations in bioavailability as well as an unpractical frequency of administration.

Therefore, it is preferable to perform *early pharmacokinetic screening* in the development of antivirals in order to find an optimal combination of pharmacodynamic and pharmacokinetic features.

However, even if a satisfactory bioavailability of antiviral agents can be attained, only a very small fraction of the bioavailable drug will be directly

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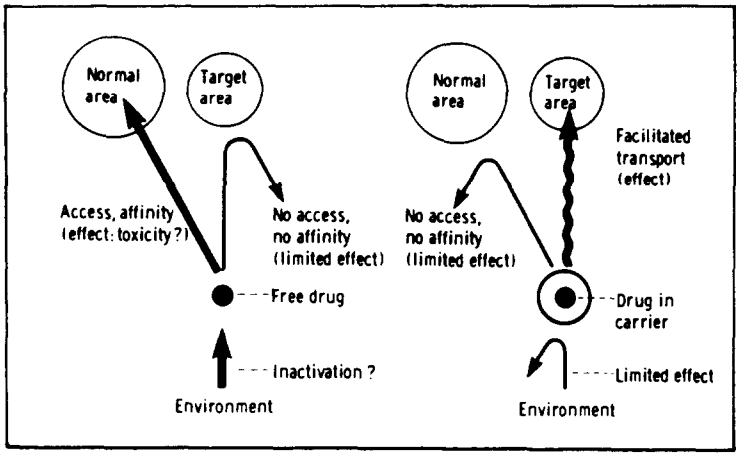

Fig. 1. The principle of drug targeting: the disposition of a conjugated drug in the body is determined by the physicochemical properties of the carrier instead of the characteristics of the free drug. This leads to higher drug concentrations at the side of action combined with lower concentrations at the non-target tissue where toxicity might occur (according to Gregoriádes, 1983).

involved in the antiviral effect. The drug will be mainly distributed to tissues where its presence is not strictly required, or even worse, where it may produce severe side effects. Obviously it would be safer and more economic to deliver the antiviral drug mainly to the cell type where virus replication takes place. This approach is usually called *drug targeting* (Figs. 1 and 2).

Basically two approaches have been taken to obtain a more optimal delivery: (1) The *carrier approach*: the antiviral drug is covalently coupled to soluble macromolecules or included in a particular type of drug carriers (nanoparticles and liposomes). The fate of the drug in the body is now dictated by the chosen carrier. The drug concentration in the target tissue is a result of the relative rates of cellular uptake of the drug conjugate, liberation of the drug, and efflux rate of free drug from the target tissue (Body and Aarons, 1991; Siegel et al., 1991) (Fig. 3). The advantage of targeting is only significant if the target compartment is different from the compartment where toxicity is occurring and also if the active drug is released predominantly at the target site (Stella and Himmelstein, 1992). The larger the distribution of the parent drug to the non-target tissue and the more efficient the distribution of this drug in the coupled form to the target tissue, the higher the benefits of targeting will be.

(2) The *prodrug approach*: chemical derivatives of the drugs are prepared that have more favorable kinetic features and/or are specifically activated at the site of action. Since the latter aspect has been reviewed quite extensively (Canonico et al., 1988), this review will mainly deal with the design of soluble and particle-type of drug carriers for cell-specific delivery of antivirals.

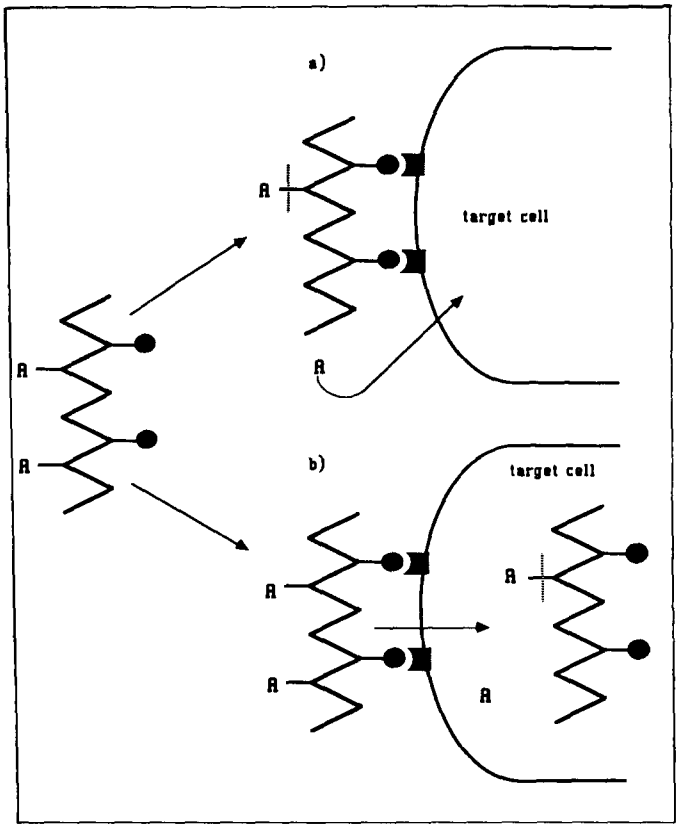

Fig. 2. Release patterns of an active agent (A) after reaching the target tissue: (a) release of A outside the target cell; (b) release of A inside the target cell. Preferably the release rate should be controllable. (From Crommelin and Storm, 1988.)

2. Research aims in drug delivery

The four major strategies in drug targeting are:

(a) *Slow release targeting*. Through association with a suitable carrier, the antiviral drug is delivered to circulating or fixed macrophages. The drug is gradually released from the carrier and diffuses out of the cells, leading to an increased residence time in the body (Storm et al., 1991).

(b) *Passive (side effect avoidance) targeting*. Through the association of the drug with a suitable carrier it is prevented from distribution to sites of toxicity.

(c) *Active targeting*. Through coupling to a macromolecular, cell-specific carrier the drug reaches higher therapeutic concentrations at the site of virus replication. Consequently, the dose can be reduced and side effects will be minimized.

(d) *Dual targeting*. This implies the use of carrier molecules that have their own intrinsic antiviral effect. With this approach drug-conjugates can be

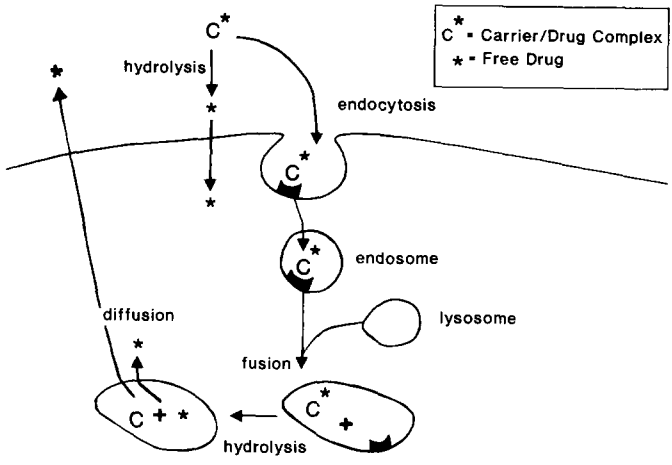

Fig. 3. After binding of the target-device of the carrier to a specific receptor on the target cell, the complex enters the cell via receptor-mediated endocytosis and finally ends up in lysosomes. Here the drug is released, diffuses to the cytoplasm and can perform its therapeutic action. The targeting concept assumes cell-specific recognition of the carrier, intracellular release and cellular retention of the active drug.

prepared with a dual mode of action on the viral replication process: that of the coupled drug in addition to that of the carrier itself (Jansen et al., 1991c). A major advantage is that the virus replication process can be attacked at multiple points, potentially leading to synergistic effects and prevention of the development of resistant virus strains.

It is important to note that especially the last three approaches provided new perspectives in drug screening. Very potent antiviral compounds with a severe toxicity that were dropped for further testing became serious candidates again because their distribution in the body could be optimized and their therapeutic index largely improved.

Coupling of drugs to macromolecular carrier systems a priori implies that parenteral formulations have to be used. Although parenteral dosing is quite accepted for short-term and even for long-term clinical use (e.g. insulin and other hormone preparations), it is clear that such drug-targeting preparations should have major advantages compared with the parent drug to justify their development. Such advantages could include curing of the disease instead of only slowing down the infection process, the therapy of intracellular infections for poorly penetrating drugs or a major reduction of the dosing frequency.

Site-specific drug delivery does not prevent the development of steady state plasma concentrations of the parent drug: even if the release rate in the target cells is slow, a part of the drug will tend to enter the general circulation. However, the plasma levels will be generally lower and the local concentration in the target tissue higher. Drug-targeting preparations can also be used combined with lower doses of the parent drug. This approach could be used if, for instance, steady state levels of the parent drug after administration of a

drug-delivery form would be too low to provide sufficient therapeutic concentrations in the central nervous system (CNS).

Generally, macromolecular carriers will be unable to deliver drugs into the brain, because of lack of passage to the blood–brain barrier (BBB). However, there is recent evidence that some macromolecules can pass the BBB by transcytotic processes. For transferrin and anti-transferrin receptor antibodies, this process has been clearly established and it was shown that methotrexate can be delivered to the brain in the form of an antibody conjugate (Friden et al., 1991). Mannose-labeled liposomes were recently claimed to pass the BBB and delivered to and digested by glial cells (Umezawa and Eto, 1988).

3. General guidelines in drug delivery research

A number of essential aspects should be mentioned in the design of drug-targeting preparations (Meijer et al., 1990a,b; Poznansky and Juliano, 1984; Tomlinson, 1990):

(a) It is preferable to test drug-delivery preparations as soon as possible in vivo since promising in vitro results certainly do not guarantee a similar efficiency in vivo.

(b) It is advisable to test drug-delivery preparations with regard to possible immunogenicity in an early stage of development, since major problems may occur especially with chronic dosing.

(c) Cell-specific distribution of the drug-targeting preparations as well as the rate of drug release from the carrier should be studied in vivo both in the normal and the pathological situation. It is of prime importance to check if the chosen drug-targeting concept is also valid in the diseased state. Also, variable sites of viral replication in the various stages of the disease as well as the chronicity of the infection should be taken into account.

(d) Drug loading of the carrier should be carefully considered: enough drug molecules should be internalized with the carrier to obtain adequate cellular levels of the drug. However, the coupling of too many drug molecules to the macromolecular carrier may largely perturb its physico-chemical features and corrupt the carrier selectivity for the particular receptor system.

(e) A type of carrier should be chosen that is relatively non-toxic also with regard to its degradation products.

(f) The chosen carrier should be able to pass anatomical barriers in the body en route to the target tissues.

Generally the development of a drug-targeting preparation requires an integrated multidisciplinary approach: cell biology should go hand-in-hand with medicinal chemistry, pharmaceutical technology and clinical medicine. In this respect potential problems of large-scale preparation and clinical phase I and phase II testing should be anticipated.

4. Reasons for drug delivery of antivirals

Antiviral drugs are designed with the aim to act on virus-specific processes differing from biological events in the host cells. Nevertheless complete virus-specificity is never obtained and manipulation of the whole body disposition may be necessary to obtain a sufficient therapeutic index.

Features prompting the design of a drug-targeting formulation for an antiviral compound are:

- Extremely rapid excretion or metabolism, endangering the building up of therapeutic concentrations at practical dosage regimens.
- Severe toxicity in non-target tissue. Examples are bone marrow depression and severe neurotoxicity of vidarabine (ara-A) or formation of very toxic metabolites as recently reported for zidovudine (AZT) (Cretton et al., 1991).
- Poor penetration into the target cells, e.g. poly-anionic agents that are potent reverse transcriptase inhibitors and anti-herpes drugs that poorly penetrate the BBB.
- Inadequate phosphorylation of nucleoside analogues into their active triphosphate forms.
- Poor water-solubility leading to limitation in topical administration or precipitation of the drug in the renal tubuli (acyclovir (ACV)).

5. The opportunities in drug delivery: carriers, barriers and cell-specific receptors

5.1. Antiviral prodrugs

This approach consists of the preparation of chemical derivatives leading to prodrugs that have altered pharmacokinetics and/or become activated in virus-infected cells. A well-known example is acyclovir that is activated by HSV-derived kinases, thereby obtaining an impressive selectivity. Nevertheless, its rather low bioavailability, poor penetration of the BBB and poor water-solubility are still innate disadvantages. Various esters of ara-A (Canonico et al., 1988) have improved water-solubility and in vivo efficacy against herpes infections. For instance, the 2′,3′-di-*O*-acetylester is considered a promising prodrug, combining an optimal balance of water and lipid solubility, deaminase resistance and bioavailability. The tri-acetate of ribavirin has an improved penetration of the BBB and is superior in the treatment of arena virus-induced hemorrhagic fever in monkeys and of various other viruses (Canonico et al., 1988). Improved penetration of the BBB was also reported for more lipophylic methyl-substituted dideoxypurines. They may be specifically activated by adenosinedeaminase, an enzyme with increased levels in the CNS during some infective diseases (Barchi et al., 1991). Various groups have worked on lipid-derivatives of nucleoside analogues, such as AZT (Hostetler and Richman, 1989; MacCoss et al., 1983; Piantadosi et al., 1991; Stein et al., 1989, 1990; Welch et al., 1985), as well as foscarnet (Neto et al., 1990), in a

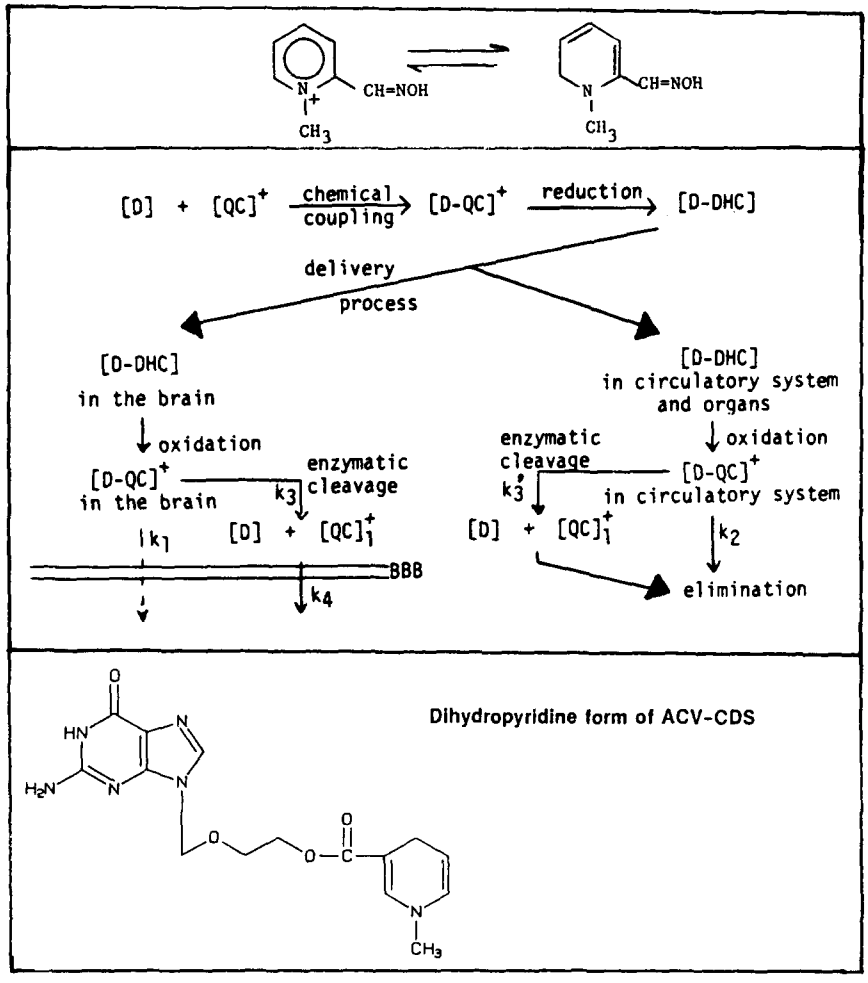

Fig. 4. Synthesis and schematic representation of the disposition in the body of dihydropyridine derivatives of nucleoside analogues. After the prodrug is delivered to the brain, the dihydropyridine moiety is oxidized to a permanently positively charged pyridinium group, which is entrapped in the brain. The active drug is then slowly released by enzymatic conversion.

number of cases showing a markedly improved bioavailability, residence time in the body and antiviral efficacy. An appealing modality is the coupling of various nucleoside analogues to dihydropyridine moieties (Bodor et al., 1989). Such lipophilic prodrugs (so-called chemical delivery systems (CDS)) of ACV, Ara-A and AZT rapidly pass the BBB after which the prodrug is oxidized to the hydrophilic pyridinium salt. Since the pyridinium group contains a permanently charged quaternary nitrogen and diffusion out of the CNS, at least by passive processes, is greatly retarded (Fig. 4), the prodrug is trapped in the brain. Subsequent enzymatic ester cleavage of the prodrug is supposed to provide a slow local release of the active antiviral component. For ACV, this

leads to 20-fold higher total concentrations as well as a 20-fold increase in residence time in the brain. Although the ACV-CDS complex is also concentrated in other tissues, it disappears more rapidly from these sites compared with the CNS, because these barriers are less absolute. However, the efficacy in the treatment of, for instance, herpes simplex encephalitis and CMV pneumonia, being promising therapeutic objects here, has yet to be studied. A similar prodrug approach has been taken for AZT, showing a 10-fold increase in brain exposure and a 30-fold increase in CNS half-life, while the potency of the nucleoside prodrug in human lymphocytes was similar to AZT itself (Chu et al., 1990). In general, the acute and chronic toxicity of the redox products remain to be studied, as well as the relative rates at which the active drug is released intracellularly and extracellularly in target and non-target tissues.

Another innovative approach is the chemical coupling of two different antivirally active compounds to yield a prodrug with more favourable properties. Zidovudine and phosphonoformic acid were coupled with the rational to obtain intracellular release of the substrate and product analogue that occupy adjacent but not identical binding sites on the RT template. Although the expected synergistic effect was not found, the combination had clearly decreased toxicity, probably related to the gradual intracellular cleavage of the phosphate ester bond.

In the design and testing of antiviral prodrugs, it should be realized that efficacy may largely depend on the time of administration after viral challenge (Canonico et al., 1988). The replicative cycle of the particular virus as well as the activity stage of the disease should be taken into account in relation to the rates of cellular uptake, activation and degradation of the prodrug preparation.

5.2. The microparticle-type of carrier

This category of targeting devices includes liposomes, natural occurring lipid particles, cellular carriers as well as nanoparticles. An advantage of these type of carriers is that drugs can be simply incorporated in their parent form, without covalent linkage. Either they dissolve in the aqueous phase or become associated with the lipid or polymeric material. To obtain sufficient drug loading, lipophilic derivatives of polar drugs with biodegradable bonds can be synthesized for inclusion in the lipoid phase of liposomes and lipid-particles (Bijsterbosch and Van Berkel, 1990a). A major disadvantage of microparticles is that they cannot pass the endothelial lining and that extravasation is generally poor (Crommelin and Storm, 1988; Gregoriadis, 1983; Sullivan et al., 1986; Szoka, 1990; Weinstein and Leserman, 1984). Although some investigators claim that slow transcellular (vesicular) transport of liposomes and microspheres is possible in endothelia, their practical application will be largely restricted to intravascular targets. Exceptions to this rule may be well perfused solid tumors and inflamed tissues (Tomlinson, 1990) in which increased extravasation was reported. The mononuclear phagocyte system (MPS), also called the reticulo-endothelial system (RES), efficiently captures

exogenous particles either in the parent or in the opsonized form. This process may markedly restrict the contact time of liposomes and nano-particles with the target cells. If the phagocytosed material is not degraded rapidly enough, it may also block MPS function and give rise to chronic toxicity, especially at repeated administration (Allen, 1988). Various coatings or protectants on the particles, such as negatively charged glycolipids (e.g. phosphatidylinositol or monosialoganglioside), have been used to slow down capturing by the MPS and markedly increase the residence time in the circulation (Gabizon and Papahadjopoulos, 1988).

5.2.1. Liposomes

Liposomes are small vesicles composed of unilamellar or multilamellar arrays of phospholipid bilayers surrounding one or several aqueous compartments (Fig. 5). Charge, lipid composition and size (ranging from 20 to 10,000 nm) of liposomes can be varied and such factors may strongly affect the elimination from the circulation (Papahadjopoulos and Gabison, 1990; Weinstein and Leserman, 1984). Repeated extrusion through polycarbonate filters with appropriate pore size and microfluidizing techniques can yield very small liposomes (< 100 nm) while most of the entrapped solute is maintained. Sialic acid groups or other polar moieties at the liposomal surface may decrease phagocytic uptake and thereby increase circulation time of liposomes (so-called stealth liposomes) (Gabizon and Papahadjopoulos, 1988). By choosing a proper composition of the liposomes, for instance by including cholesterol and

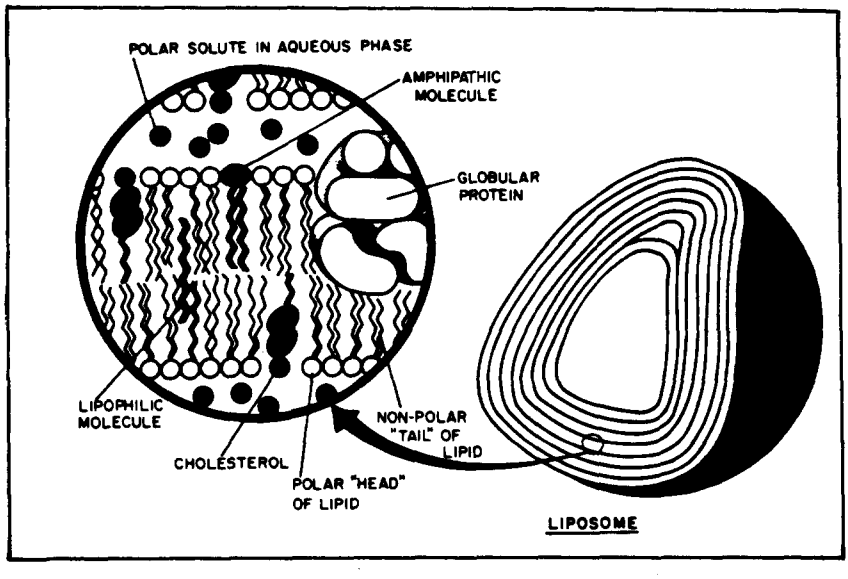

Fig. 5. A simplified representation of the structure and composition of liposomes. Potential sites of inclusion of hydrophilic, lipophilic and amphipatic molecules, cholesterol and proteins with multilamellar liposomes are shown (according to Crommelin and Storm, 1988).

distearoyl phosphatidyl choline, circulation time is increased and up to 5% of the injected dose can be found in tumors in mice (Papahadjopoulos and Gabizon, 1990). Yet the tendency of liposomes to be phagocytosed by the MPS can also be positively exploited in drug delivery: drugs in micro-particles that are accumulated in MPS cells can be slowly released through the rate-limiting biodegradation of the carrier. This results in a 'systemic slow release compartment' that can have major advantages in limiting interdose fluctuation in plasma concentrations of drugs with a small therapeutic index (Crommelin and Storm, 1988). In addition, accumulation at sites of toxicity can be favorably decreased. Important examples are liposome preparations of the anti-mycotic agent amphotericine B and of the antineoplastic drug doxorubicin, leading to a 10-fold increase in the therapeutic index [see Szoka (1990) for refs.]. Drug-containing liposomes may also be more effective in the case of intracellular infections with parasites and bacteria as shown for antileishmaniasis and antimalaria drugs as well as for certain antibodies active against salmonella, mycobacteria and listeria infections (Weinstein and Leserman, 1984). An enhancement of antiviral efficacy has been demonstrated for liposomes containing ribavirin, showing a major therapeutic effect 24 h after normally lethal inoculation with Rift Valley fever virus as well as HSV I and influenza A virus (Szoka, 1990; Weinstein and Leserman, 1984). Improved antiviral therapy with liposomes has also been reported for murine interferon in a hepatitis model, for zidovudine in a murine immunosuppression model (Phillips et al., 1991), for iodoxuridine in a rabbit HSV model, for phosphonoformate (foscarnet) in the case of HSV 2 infections [see Szoka (1990) for review] as well as for ganciclovir and foscarnet against CMV fibroblast infections (Bakker-Woudenberg et al., 1991). Foscarnet could be an excellent candidate since after initial delivery into the cells it will only minimally redistribute from the cells due to its polar character (Szoka, 1990). An alternative antiviral therapy with liposomes is the activation of macrophages with immunomodulators such as muramyl peptides (Roerdink et al., 1988; Weinstein and Leserman, 1984). Macrophages have been shown to restrict the replication of many different viruses in vitro (Weinstein and Leserman, 1984). In vivo activation of these cells is not easy because of extremely short half-lives and insufficient exposure to the particular lymphokines. Liposomes containing immunomodulators such as *N*-muramyldipeptide (MDP) and *N*-muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE) can activate human monocytes to kill HSV-2 infected cells without lysing the uninfected cells. The conversion to a cytolytic state by MTP-PE liposomes occurs at a 800-times lower concentration of muramyl peptide compared with MDP itself as was clearly demonstrated in vivo against various other virus infections. For HIV infections in vitro it was shown that liposome-containing MTP-PE was not only effective immediately after infection, but also reduced virus replication during an established infection (Lazdins et al., 1990). One single treatment was sufficient to obtain maximal effect. Recent clinical studies with liposome-encapsulated MTP-PE clearly demonstrated macrophage activation and

antitumor activity. Tolerance was generally good although dose-finding was essential because of a quite narrow therapeutic window (Lazdins et al., 1990).

In some of these studies (Konopka et al., 1990), the control liposomes without the drug also had an effect on virus levels. They reduced the fusogenic ability of HIV I and also, to some extent, prevented giant cell formation. Interestingly, this effect was most pronounced for negatively charged liposomes while in contrast positively charged liposomes enhanced virus infectivity (Konopka et al., 1990). The variable effects of the drug carrier emphasize the importance of liposome composition and also demonstrate that drug carriers should not a priori be seen as inert, unreactive modalities.

Since many human viruses infect and replicate in monocyte-macrophages (Weinstein and Leserman, 1984) this cell type is an important target for antiviral compounds. The question arises whether the liposome-included drug is really delivered intact to the cytoplasm of the cells and whether it is resistant to the degradative lysosomal route after phagocytosis. In fact, a cytoplasmic distribution is essential for the effects of reverse transcriptase and protease inhibitors as well as for antisense nucleotides and glycosylation inhibitors (De Clercq, 1990). Antibody-bearing liposomes containing antisense oligomers would provide a double specificity: the antibody-mediated selection of the particular cell type as well as the selectivity of the chosen mRNA sequence complementary with the liposome-delivered oligomer (Leonetti et al., 1990b; Milhaud et al., 1989). Yet the antisense component should become available in the cytoplasm. Final delivery of chemically labile components to the aggressive lysosomes could be prevented, at least partially, by the use of pH-sensitive liposomes that are composed such that the liposome membranes are destabilized or become fusogenic with regard to the endosomal membranes at an acidic pH (Szoka, 1990).

Another innovative approach is to mimic the action of enveloped viruses: liposomes could be equipped with virus-derived polypeptides. Such proteins undergo a conformational change at the endosomal pH, resulting in exposure of their hydrophobic residues and fusion with the membranes (Szoka, 1990). Proteins from sendai virus, VSV, SFV, HSV and influenza virus have been reconstituted in liposomes. These 'virosomes' have been mainly tested in vitro. It is anticipated, however, that in vivo distribution will not be sufficiently cell-specific, and that problems of immunogenicity and preparative difficulties will be encountered (Machy and Leserman, 1987).

By manipulating the size, charge and membrane lipid composition of liposomes, their availability for the infected cells can be improved. Nevertheless, a more active type of targeting could be achieved by including a target-device at the external surface of the carrier. Examples of such targeting moieties are tissue-specific antibodies (Weinstein and Fujimori, 1990), glycoproteins (Ishihara et al., 1990) and glycolipids. An example of the latter is cetylmannoside for the delivery to human blood monocytes (Yamashita et al., 1991). Lactosylceramide (Spanjer and Scherphof, 1983) and tris-gal-chol (a cholesterol derivative with terminal clustered galactose groups (Fig. 6)) were

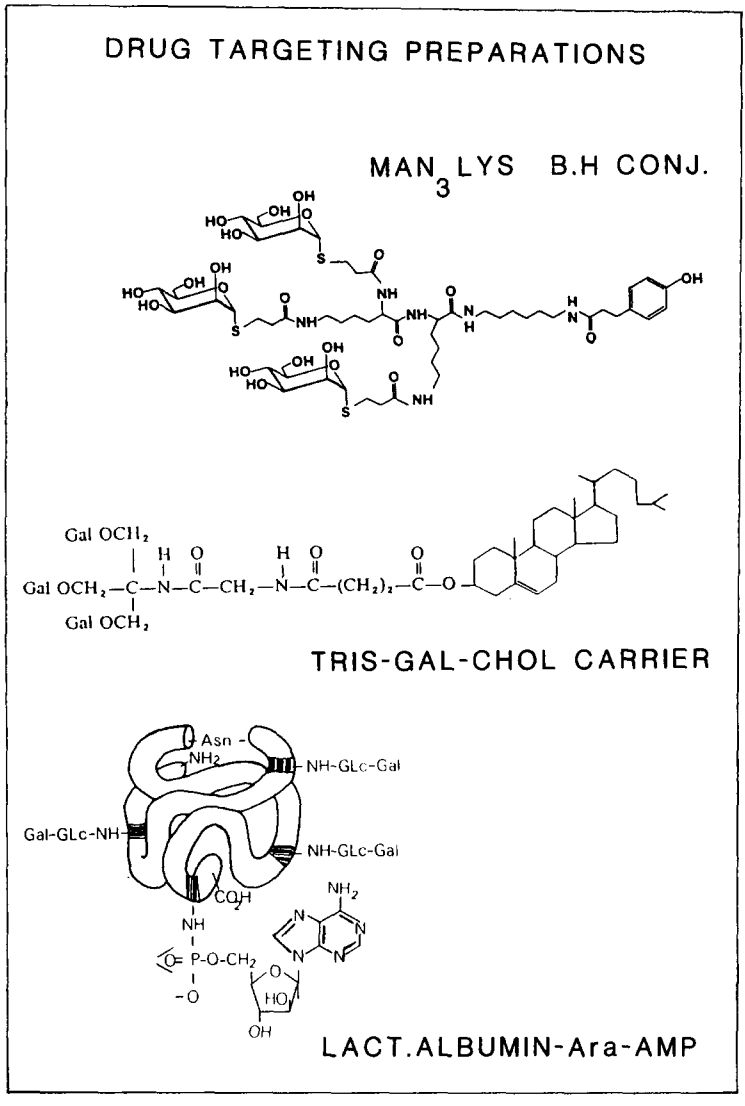

Fig. 6. Artificial drug carriers based on sugar recognition. Polylysine can be derivatized with clustered mannose groups and provides a carrier for Bolton-Hunter reagent (BH) to macrophages (top). Cholesterol can be connected via a Tris-spacer to three clustered galactose groups and can be included in LDL and HDL particles, targeting them to sinusoidal cells and hepatocytes through recognition by the galactose particle receptor and the galactose/*N*-acetyl galactosamine receptor (hepatic lectin), respectively. Ara-AMP is covalently linked via an acid labile phosphoamide bond to randomly lactosaminated albumin and is thereby targeted to hepatocytes.

employed to target small liposomes to hepatocytes and larger ones to Kupffer cells (Bijsterbosch and Van Berkel, 1990b; Bijsterbosch et al., 1989). Another example is the targeting to peritoneal macrophages with galactosylated liposomes, which were prepared by including a triantennary galactosyl-lysyl-

lysine dipeptide (Haensler and Schuber, 1991). This study clearly demonstrates the importance of optimal geometrics in the recognition of the sugar moiety. An increased tissue specificity can also be obtained by coupling epidermal growth factor (Ishii et al., 1989) or asialoglycoproteins to the liposomes (Storm et al., 1991; Sullivan et al., 1986; Weinstein and Leserman, 1984). An alternative is reductive lactosamination of the apolipoprotein portion of LDL and HDL particles (Attie et al., 1980).

An important tool in site-specific delivery of liposomes may be the use of immunoliposomes: liposomes to which antibodies or fragments of antibodies are attached using various hetero bifunctional cross-linking reagents (Gregoriadis, 1983; Tomlinson, 1987; Storm et al., 1991; Sullivan et al., 1986; Suzuki et al., 1991; Weinstein and Leserman, 1984). However, in vivo studies with immunoliposomes that are presently available still exhibit major uptake by cells of the MPS. This process can only be reduced in favor of the target cells by prior administration of lysosome inhibitory agents or large amounts of 'dummy liposomes'. The latter aspect also implies that whole body distribution of liposomes is in principle dose-dependent (Juliano, 1988; Tomlinson, 1987).

A recent development is the design of target-sensitive immunoliposomes (Ho et al., 1987a,b). They are composed of palmitoyl antibody-stabilized phosphatidyl ethanolamine bilayers that are destabilized by binding to an antigen on the target cell leading to a local release into the cytoplasm of the target cells. In vitro this resulted in a 1000-fold increase in uptake of ACV compared with the free drug. In vivo testing of the target-sensitive liposomes should give more definite clues as to the applicability.

Finally, a number of potential drawbacks for the practical use of liposome preparations should be mentioned. First of all it should be realized that intracellular delivery of some nucleoside analogues with liposomes may not be effective since the drugs are rapidly degraded in lysosomes (Machy and Leserman, 1987; Szoka, 1990). Other formidable obstacles for liposome-mediated delivery are opsonisation by circulating antibodies (or by antigens in the case of immunoliposomes), leading to clearance by Fc-receptors. In addition, heterogeneity of the target cells may corrupt the immunoliposome concept (Juliano, 1988). Subcutaneous, intraperitoneal, pulmonary or topical application of liposomes leading to transport to lymphatics and lymph nodes may circumvent some of the above-mentioned problems (Machy and Leserman, 1987; Weinstein and Leserman, 1984).

A general warning should be put forward with regard to the design of site-directed drug-targeting preparations: if endocytosis is required for cellular delivery, virus-infected cells may be much less active in endocytosis, for instance due to depletion of energy-rich metabolites or decreased expression of cell surface receptors. The delivery concept may thus be severely invalidated in case of some particular viral diseases (Ho et al., 1987b).

In spite of these potential drawbacks, liposome science is flourishing. Innovations include physical modalities such as heat-sensitive liposomes to be activated locally by application of heat and immunoliposomes containing

phototoxic drugs (Devanathan et al., 1990; Yemul et al., 1987). The latter type of liposomes can be activated by irradiation after arrival in the target tissue, offering a second degree of specificity. Liposomes can also be used in extracorporeal circuits to selectively affect subsets of T-lymphocytes (Yemul et al., 1987). A recent finding of considerable interest is that T-lymphocytes and fibroblasts can be enticed to endocytose liposomes by including antibodies directed to histocompatibility antigens on these cell types. In contrast, B-cells did not internalize such carriers although they became extensively bound to the surface (Machy and Leserman, 1990; Suzuki et al., 1991). Size of the liposomes is again essential here: T-cells do not endocytose liposomes larger than 100–150 nm.

5.2.2. LDL and HDL particles

Endogenous lipid particles such as LDL (25 μm) and HDL (10 μm), containing a lipid and apoprotein part, can be viewed upon as a sort of 'natural targeted liposome': the lipoid core can be used to incorporate lipophilic drugs or lipophilic prodrugs. The apolipoprotein part of the particles can be glycosylated. Consequently, cell-specific recognition by receptors other than the physiological LDL and HDL receptors can be attained (Bijsterbosch and Van Berkel, 1990; Bijsterbosch et al., 1989; Van Berkel et al., 1985). Alternatively, the lipid component of these particles can be equipped with glycolipids that expose their sugar groups and are instrumental in delivery to sugar-recognizing lectins on various cells types in the body (Table I).

The dimensions of LDL and HDL particles predict that they will not easily pass normal endothelial barriers apart from the fenestrated linings in liver and spleen and possibly endothelia in tumors.

5.2.3. Microspheres and nanoparticles

This type of carrier often consists of biocompatible polymers. Both soluble and particulate carriers have been produced. The soluble carriers have the advantage that large amounts of drug can be incorporated. Up to 80% (w/w) of these soluble carriers can be used for drug-loading (Duncan, 1987; Duncan and Lloyd, 1991; Tomlinson, 1987). Dextrans, ficoll, sepharose, heteropolysaccharides, poly (L-lysine) and *N*-hydroxy propyl methacrylamide (HPMA) copolymers are well-known examples. They have been applied for instance for delivery of amantidine and interferons (Canonico, 1988). The polymeric backbone of the carrier can also be provided with specific sugars aimed at the various surface lectins of cells in the body (Duncan et al., 1989; Midoux et al., 1990).

Nanoparticles (0.2–0.5 μm) have been prepared from polyalkyl-cyanoacrylates (Couvreur, 1988), and microspheres from denatured albumin (diameter of 30–200 μm). They have a lower payload than the soluble polymers. Drug-loading may occur by simple absorption or real incorporation (Speiser, 1991). An advantage is the non-covalent binding of the drug. Nanoparticles can also be equipped with antibodies or their F_{ab} fragments (Couvreur, 1988). Yet, after

in vivo administration, there is rapid clearance by cells of the MPS. Therefore, especially intracellular infections in Kupffer cells and other macrophages may be a useful target. Even coating of these particles with polyethylene glycols (PEGs) does not divert such polymeric microspheres from the MPS cells of liver and spleen (Juliano, 1988; Tomlinson, 1987). Opsonisation of nanoparticles with immunoglobulins, fibronectin or complement factors in the intact organism may be at least partially responsible for the in vivo routing to the cells of the MPS (Juliano, 1988). Phagocytosis or adsorptive endocytosis of nanoparticles into macrophages will certainly activate the immune system and may largely increase the immunogenic response toward the drug and/or its carrier, representing a potential drawback with chronic dosing of such preparations. Albumin microspheres with a size exceeding 100 μm may become entrapped within capillary networks that they encounter after i.v. administration (Codde et al., 1990; Friend and Pangburn, 1987). The mechanical filtration by the lungs should be taken into account and could be exploited for delivery of antivirals to this organ.

5.2.4. Cellular carriers

These have been extensively studied (Friend and Pangburn, 1987; Poznansky and Juliano, 1984) but relatively few practical applications are available to date. Blood-derived cells such as (modified) red blood cells, leucocytes, lymphocytes and fibroblasts have been employed as storage sites for drugs or cellular transplants (Machy and Leserman, 1987; Poznansky and Juliano, 1984). Cellular carriers may have the advantage of the natural biocompatibility. However, they will fully encounter the endothelial barriers and are unable to fuse with other cells. Nicolau and Cudd (1989) designed long-lived CD_4-bearing RBC's by electro-insertion of CD_4 molecules. These cells were claimed to be capable of clearing free HIV and gp 120 and allow binding of HIV-infected cells. The latter may lead to cellular aggregates that should be easily removed by phagocytosis, but in principle may also lead to severe toxicity.

5.3. Soluble type of carriers

5.3.1. Receptor-based drug targeting in vivo

The success of drug targeting with macromolecular carriers is intimately dependent upon the selectivity in the distribution of the cellular receptor targets in the body. Other crucial factors are the anatomical and/or pathological barriers that have to be passed en route to these recognition sites (Duncan et al., 1989; Meijer et al., 1990b; Roche et al., 1990; Tomlinson, 1990). Table 1 lists a large number of receptors for macromolecules that have been identified and that are more or less specific for the cell type indicated. Many such receptors are lectins that recognize oligosaccharide chains in a specific geometric arrangement, with a pronounced role for the type of the terminal sugar (Fig. 7). In addition, such receptors may differentiate on the basis of

TABLE 1

Protein receptors on various cell types in the body

Cell	Receptor
Hepatocytes	Galactose-t(n)GP (high density), HDL, LDL, EGF, IgA, Transferrin.
Kupffer cells	Galactose particles, mannose-t(n)GP, LDL, AMPC, Polymeric negatively charged proteins, Complement factors.
Fibroblasts	Mannose 6-phosphate-t(n)GP, Transferrin, LDL, EGF, Transcobalamin II, AMPC.
Mammary acinar cells	Growth factor.
Endothelia	
– Blood/brain	Transferrin, insulin.
– Diaphragma, lung, heart	Albumin.
– Liver	Monomeric negatively charged proteins, Man/GlcNAc-t(n)GP, Fc receptor.
Enterocytes	Maternal IgG, dimeric IgG, transcobalamin II.
Monocytes	Mannose 6-phosphate-t(n)GP.
Macrophages	Man/GlcNAc-t(n)GP, β-Galactose-t(n)GP, Mannose 6-phosphate-t(n)GP, AMPC.
T4 cells	Galactose-t(n)GP (low density), CD4, Interleukin, transferrin.
Renal tubular cells	Low molecular weight proteins (cationic).

t(n)GP = terminated (neo)-glycoproteins; IgA = Immunoglobulin A; EGF = Epidermal growth factor; AMPC = α_2-macroglobulin protease complex.

overall charge and the charge density of macromolecules (Jansen et al., 1991a). Often these receptors also provide mechanisms for internalization followed by intracellular transport to degradative compartments. At these sites, the coupled drug molecules should be liberated through biodegradation of the carrier. In some cases, however, only external binding is offered. A local release of drug from the carrier at the microclimate of the cell membrane should then provide a sufficient driving force for uptake into the target cell (Shen and Ryser, 1981). Both receptor affinity and density as well as the presence of competing endogenous ligands determine the extent of carrier-receptor occupation and thereby the extraction of the carrier-drug complex by the target tissue.

At least five mammalian receptor systems with a predominant hepatic distribution are available for the clearance of glycoproteins (Table 1). Most of these processes have been studied in detail with regard to cellular kinetics. Depending on the rate and site of ligand-receptor dissociation within the cells, intracellular trafficking can occur to endosomes and lysosomes but also (albeit to a lesser extent) to other cell organelles such as Golgi-apparatus, mitochondria and the cell nucleus. The relative rates of acidification of the intracellular vesicular compartments may largely determine intracellular

sorting and routing (Stoorvogel et al., 1989). It is important to note that continuous exposition of certain receptors to their macromolecular ligands can lead to rapid downregulation of cell surface receptors since receptor recycling within the cells is not complete (Sugiyama and Hanano, 1989). This downregulation should be taken into account in predicting pharmacokinetics of glycoprotein carriers. For instance, when the particular target receptors are present on more than one cell type in the body, and downregulation in these cells occurs at different rates, tissue specificity for drug-carrier complexes in the body may change in time during chronic dosing.

Similar types of sugar-recognizing receptors for glycoproteins are also present (albeit to a smaller extent) on blood cell types (Gabius et al., 1990; Midoux et al., 1990). More knowledge is required to map such receptors with regard to their favorite type of sugar recognition (i.e. number and density of sugars and geometric organization of the sugar groups) as well as with regard to humoral and pathological stimuli that determine receptor expression and up- and down-regulation.

Depending on the stage of the disease, the expression of normal surface receptors can vary greatly. For instance the galactosyl-receptor on hepatocytes is poorly expressed in primary hepatic cancer cells (Keegan-Rogers and Wu, 1990) and receptor density or the efficiency of receptor-mediated endocytosis can also be affected by virus infection of the particular cell type (Meijer et al., 1990b).

In the scope of this review it is interesting to note that many viruses have envelope glycoproteins that use well-defined cell surface receptors for virus binding and entry. In fact, viruses can be regarded as delivery systems for genetic material and could be employed as modalities for cell-specific gene- and drug-targeting (Tomlinson, 1987; Wilson, 1986). Polypeptides or proteoglycan-carriers could be designed that would mimic such virus-specific recognition and could be exploited to target antiviral drugs to those cells where virus replication would take place (Szoka, 1990). Along these lines, carriers could be employed that specifically react with viral envelope proteins exposed at the surface of the infected cells.

5.3.2. Properties of soluble macromolecular carriers

The major difference between the soluble type of carriers and the particle type (see 5.2) is their size. The size of the particles ranges from 0.1 μm to 100 μm, whereas the soluble carriers are much smaller, with a molecular weight of less than 200 kDa. The small size may provide some advantages but also certain disadvantages:

Loading capacity. Soluble carriers usually have a relatively low loading capacity of less than 50 drug molecules per carrier, whereas liposomes and LDL particles may carry up to 500 molecules per particle. Therefore mainly very potent drugs can be used. These drugs have to be covalently linked to the carriers and to assure release of the active drug at the target site, biodegradable

or acid-labile spacers can be used (Shen and Ryser, 1981; Trouet et al., 1982b). We recently developed acid labile spacers between drug and carrier for release in intracellular acidic compartments and also specific peptide spacers that can only be cleaved by enzymes present in the target cells (Franssen et al., 1991).

Extravasation and uptake by the MPS. One of the major advantages of soluble carriers over the particle type is that the former can easily leave the systemic circulation. Consequently they can be used to target drugs to cells that are not in direct contact with the blood. Another advantage is that they are not necessarily removed by the MPS.

5.3.3. Glycoproteins

Many enzymes, acute-phase proteins and most plasma proteins (apart from albumin and lysozyme) are glycoproteins. The principal sugars forming the oligosaccharide chains are mannose, *N*-acetylglucosamine, galactose and sialic acid (*N*-acetyl-neuraminic acid) (Forgac, 1988; Steer and Ashwell, 1986). Fig. 7 gives a schematic representation of the oligosaccharide side chains of orosomucoid, a glycoprotein used in many experimental studies (Poznansky and Juliano, 1984; Wu, 1988).

The classic publication of Ashwell and Morell in 1974 (Ashwell and Morell, 1974), describing the rapid plasma clearance of desialylated ceruloplasmin in rabbits, disclosed a large field of research in studying the disposition of circulating glycoproteins (Steer and Ashwell, 1986).

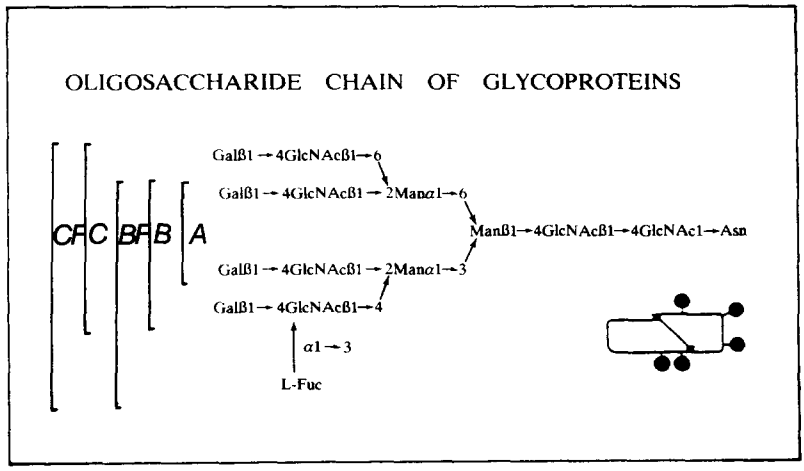

Fig. 7. Characteristic structure of an oligosaccharide side chain of natural occurring glycoproteins, such as α1-acid glycoprotein (orosomucoid) and fetuin. Normally the major part of the galactose groups is connected to N-acetyl-neuraminic acid (sialic acid). Microheterogeneity in the antennary structure as well as in sialic acid content exists under pathological conditions. A, B and C denote bi-, tri- and tetraantennary structures, respectively. In the CF and BF forms L-fucose is present at the indicated site. The inset shows localization of five of these chains at the orosomucoid polypeptide moiety, having two subunits connected with disulfide bridges.

The terminal sialic acid residues on the carbohydrate moieties of glycoproteins are regarded essential for the normal survival of these compounds in the circulation. A glycoprotein which is desialylated exposes terminal galactose groups and is cleared much more rapidly from the circulation than the corresponding native glycoprotein. The galactose groups are recognized by a specific receptor on the plasma membrane of the hepatocyte. It is called the asialoglycoprotein receptor (ASGPR) and has been studied with a large number of desialylated plasma glycoproteins like, for instance, orosomucoid, fetuin, lactoferrin, α_2-macroglobulin and haptoglobulin (Forgac, 1988; Sasaki et al., 1987; Steer and Ashwell, 1986).

After this first discovery of a galactose-recognizing (lectin-like) receptor on hepatocytes, several authors have reported receptors on other cell types with an affinity for other sugars (Table 1). For example, a Mannose/GlucNAc receptor on alveolar macrophages and sinusoidal liver cells was discovered (Hoppe and Lee, 1983; Schlesinger et al., 1976; Summerfield et al., 1982) and a galactose terminal biantennary oligosaccharide-recognizing receptor on T-lymphocytes (Bezouska et al., 1985b).

5.3.3.1. Potential problems in the preparation of glycoproteins and their drug conjugates

Glycoproteins represent excellent objects for drug targeting (Bodmer and Dean, 1988; Poznansky and Juliano, 1984). Preparation is relatively cheap compared with liposomes or antibody-drug conjugates. Moreover, the structure can be easily modified with regard to the protein backbone as well as the functional sugar groups. So far naturally occurring plasma proteins such as orosomucoid (α_1-acid glycoprotein) and fetuin were often used. They display a relatively high affinity for the asialoglycoprotein receptor probably due to the clustered arrangement of the antennary oligosaccharide side chains (Stowell and Lee, 1980). A major problem of naturally occurring plasma proteins is the presence of the non-terminal sugars in the oligosaccharide chain that invite interactions with other receptors in the body. Enzymatic cleavage of sugars from the chain in order to expose the required terminal sugar is often incomplete. With this procedure, glycoproteins with a mixture of terminal sugars are produced and consequently a loss of cell specificity should be anticipated.

Some artificial carrier systems mimic this geometric organization of sugar groups (Fig. 6). Plasma proteins such as albumin and apoprotein B can be randomly derivatized with various kinds of sugar molecules. In this manner *neo*glycoproteins are produced with a well-defined amount and kind of exposed sugar. One method for sugar-coupling to peptides is the reductive amination using boronhydride and a disaccharide, e.g. lactose (Schwartz and Gray, 1977). In this reaction the aldehyde moiety of glucose reacts with ε-NH_2 groups of lysine, resulting in a terminal galactose moiety. The nitrogen-atom in the protein-sugar linkage can still be protonated and consequently no positive charge is lost in this coupling reaction. Nevertheless, conformational changes

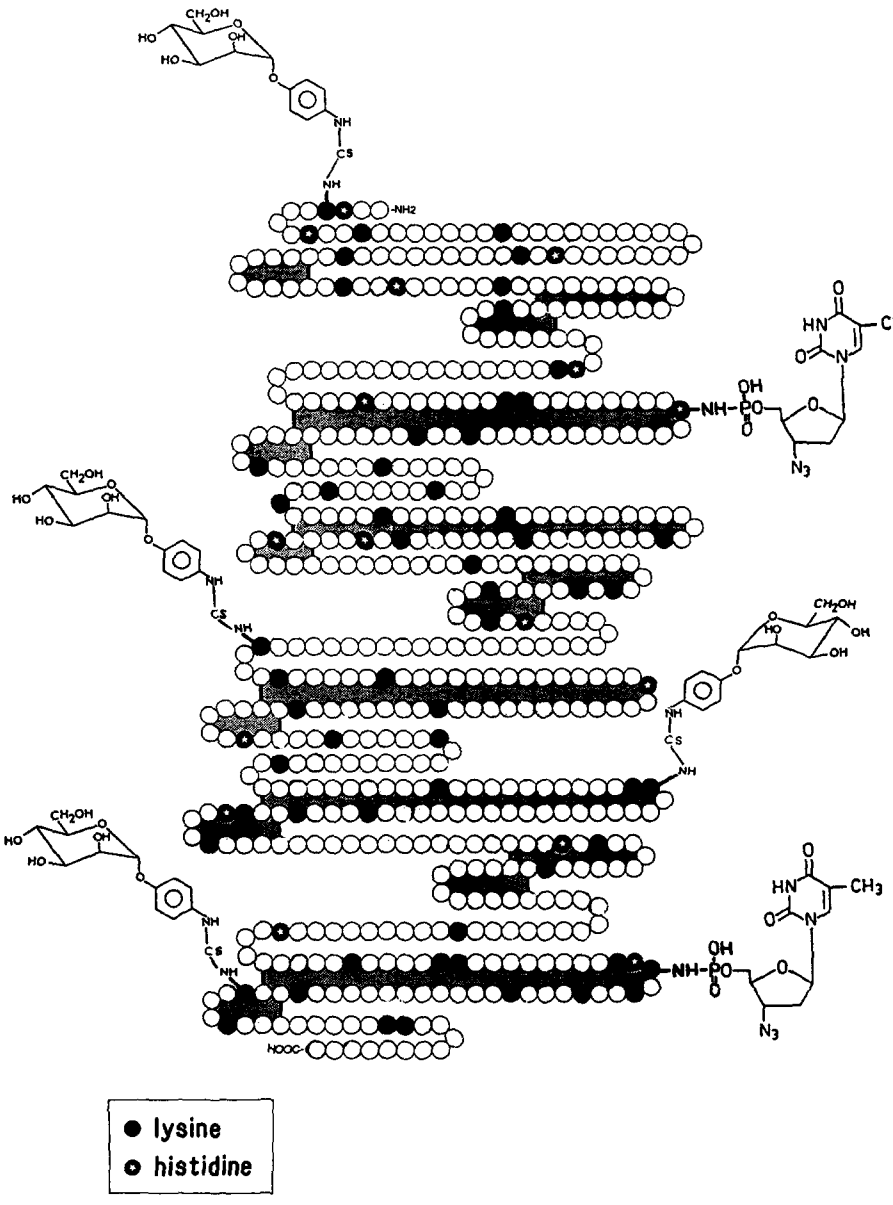

Fig. 8. Schematic representation of a neoglycoprotein-AZTMP conjugate: para-aminophenyl-mannose is coupled to lysine groups of human serum albumin, AZTMP is bound to lysine and histidine (Molema et al., 1991).

and loss of flexibility in the protein molecule due to sugar derivatization can occur. Other methods to connect sugars include the thioglycoside method (Stowell and Lee, 1980) and coupling via thiophosgene activation of *para*-aminophenyl sugars (Kataoka and Tavassoli, 1985). The latter method yields

negatively charged proteins since the nitrogen-atom in the linking moiety cannot be protonized in contrast to the lysine ε-NH_2 groups in the parent protein. FPLC chromatograms using an anion exchange column indeed showed that the net negative charge of the neoglycoproteins was positively correlated to the amount of sugars coupled (Jansen et al., 1991a). Competition studies and immunohistochemistry indicated that these polyanionic compounds are no longer solely recognized on the basis of the connected sugar groups, but are endocytosed via other receptors on sinusoidal liver cells, for example a receptor on Kupffer cells that recognizes both the particular terminal sugars and the net negative charge (Jansen et al., 1991a). In addition, a scavenger receptor is described mediating the removal of polyanionic proteins, which is mainly present on endothelial liver cells (Jansen et al., 1991b). Also, subsequent covalent coupling of drugs to the remaining ε-NH_2 groups may further increase the negative charge. Drugs with acidic functional groups will amplify this problem. For instance, coupling of fluorescein-isothiocyanate (FITC) will add two negative charges per coupled molecule. The coupling of three FITC molecules to one albumin molecule converts albumin to a proper ligand for the above-mentioned scavenger receptor (Van der Sluijs et al., 1986).

Chemical moieties used to link drugs to carrier proteins should be stable in the bloodstream to prevent premature degradation. Such linkages should be labile in acidic compartments within the target cells. For instance, antiviral nucleoside analogues in their mono- or diphosphate forms can be linked to lysine ε-NH_2 and histidine nitrogen via a pH-sensitive phosphoamide group (Fiume et al., 1980; Molema et al., 1990, 1991) (Figs. 6 and 8). In this synthesis, the phosphate group of the antiviral drug is activated at pH 7.5 with a water-soluble carbodiimide (ECDI). It should be noted that in a side reaction, the carbodiimide can also react with carboxylic groups of albumin and cross-link them with NH_2-lysine of another albumin molecule, leading to polymer formation. This may very well explain the observations of Fiume et al. (1987) that ara-AMP coupled to lactosaminated albumin, apart from being targeted to hepatocytes, is also delivered to sinusoidal cell types in the liver. These cell types preferentially take up polymerized proteins (Jansen et al., 1991b). It was found that if the drug is activated by ECDI at pH 4.5 and the coupling to albumin is performed at pH 7.5, polymer formation is prevented and the conjugate is specifically endocytosed by hepatocytes (Jansen et al., 1992).

We have recently shown that after cellular delivery, ara-AMP is released in the polar monophosphate form (Jansen et al., 1992). This may be crucial for keeping the drug inside the cells (Fiume et al., 1982). Non-phosphorylated nucleosides such as trifluorothymidine were shown to rapidly leak out of the liver after delivery with glycoprotein carriers (Fiume et al., 1982). Another advantage of introducing phosphorylated nucleoside derivatives in the cell via covalent binding to carrier proteins may be an improved rate of cellular activation into the triphosphate form. In addition, coupling of3393 phosphorylated derivatives may also circumvent resistance at the level of thymidilate kinase, which is a serious drawback in the case of AZT.

In general, caution is warranted in the synthesis of drug–protein complexes. Mild methods should be used that conserve the spatial conformation of the protein as much as possible. It should be taken into account that deviation from the normal charge distribution in the protein may lead to immunogenicity and loss of cell specificity. Prominent hydrophobic features of the connected drugs can lead to aggregation of the carrier molecules and capturing by phagocytotic systems. The higher the drug load, the more artefacts will be introduced with regard to cell specificity. In order to control such factors more easily, it has been advocated to replace the naturally occurring plasma-proteins with polypeptides carriers with a more simple structure. For instance, polylysines with different charge and molecular weight can be tailor-made and provided with clustered sugar groups (Arnold, 1985; Ponpipon et al., 1984). Other examples are dextrans of various molecular weights (Molteni, 1979), galactosylated Ficoll (Rifai et al., 1982) and poly-hydroxy-methyl-acrylamide material (Duncan, 1987).

5.3.3.2. rCD4-toxin conjugates

In the search for more effective anti-HIV agents, several groups reported on the use of recombinant CD4 (rCD4) as a carrier for toxins. In principle, rCD4 can exhibit the same degree of cell specificity and affinity as antibodies do. rCD4 was chosen because of its high affinity for the HIV envelope gp120. Although gp120 shows a distinct variability among different HIV strains, its CD4 binding site is highly conserved. Therefore, rCD4 may exhibit the same high affinity to gp120 as cellular CD4 and would potentially target coupled antiviral drugs to HIV-infected cells.

The loss of CD4+ cells in HIV patients is partly due to the interaction of gp120 in the membrane of infected cells with CD4 molecules on non-infected cells. This leads to cell membrane fusion and syncytium formation and implies that rCD4 carriers will have an additional intrinsic therapeutic effect. A pronounced anti-HIV activity of a conjugate of rCD4 with deglycosylated ricin A chain was found in a HIV-infected human T-cell line. Non-infected cells expressing MHC II antigens (the natural ligand for CD4) were not affected (Till et al., 1988). A recombinant protein, containing the HIV-binding portion of the human CD4 molecule, was linked to active regions of *Pseudomonas* exotoxin A (PE-A) and displayed selective toxicity towards cells expressing the gp 120 (Chaudhary et al., 1988). A combination of this CD4–PE-A and reverse transcriptase inhibitors resulted in highly synergistic effects and led to a complete elimination of infectious HIV-1 from human T-cell lines in vitro (Ashorn et al., 1990). In spite of these promising in vitro effects, further in vivo testing is awaited. Major problems may be encountered in the immunogenicity of the rCD4 products as well as in harmful effects caused by interactions with elements of the immune system. Furthermore, destruction of HIV-infected cells and the sudden release of particles may lead to massive infections of previously uninfected (CD4-positive) cells.

5.3.4. (Monoclonal) antibodies

The use of (monoclonal) antibodies to target drugs to specific cell types is a promising approach in view of the large extent of tissue specificity that is obtained. Drugs are coupled to an antibody (Fig. 9), thereby creating a hybrid molecule with the specificity of the immunological ligand that retains the therapeutic activity of the drug (Vitetta and Uhr, 1985).

Serious disadvantages that can be anticipated using immunoconjugates include:

(a) specific toxicity of the antibodies (Abs) due to their cross-reactivity with non-target cells (Vitetta and Uhr, 1985),
(b) limited access of the Ab preparations to the target cell type (Hwang et al., 1984),
(c) cell heterogeneity with respect to the determinant to which the antibody is directed (Hwang et al., 1984; Olsnes et al., 1989),
(d) immunogenicity of the Ab and especially that of the Ab-drug conjugate.
(e) 'opsonisation' of injected Abs and complex formation with circulating antigens.

The importance of the latter point was clearly demonstrated by the observation that tumor-specific antibodies have largely different pharmacokinetics in tumor-bearing individuals compared with normals because of a rapid clearance of the immune-complexes by the liver (Shea et al., 1989). Immunotoxins may also be partly inactivated and cleared via the α_2-macroglobulin system and thereby accumulate in non-target tissue such as liver and monocytes (Gheti et al., 1991).

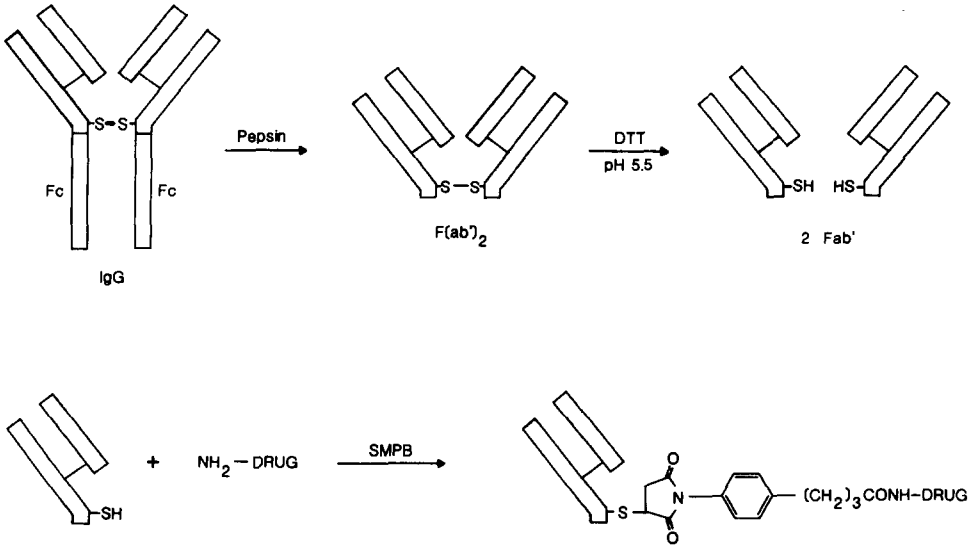

Fig. 9. Preparation of $F(ab')_2$ fragments by pepsin digestion of IgG molecules. Fab' monomers are generated from these fragments by reduction with dithiotreitol (DTT) at low pH. The $-NH_2$ group of the drug molecule is covalently attached to the Fab' monomer using the cross-linking reagent SMPB.

The potential problems with the Ab carriers can be tackled in various ways. Purification of the antibody, using affinity techniques, can be used to extract the cross-reacting fraction (Ghose et al., 1982). Pretolerisation of patients by immuno-suppresive monomethoxypolyethyleneglycol (mPEG) conjugates prior to the treatment with the immunoconjugate itself may reduce the immunogenic reactions (Sehon, 1991). The access of Abs to the cells can be improved by employing immunologically active fragments ($F_{(ab)}$ and $F_{(ab)2}$) instead of the complete IgG protein (Ghose et al., 1982). Transcapillary and cell wall passage of these fragments is much better than that of the intact immune products (Ghose et al., 1982; Olsnes et al., 1989). In addition, such fragments display a diminished immunogenicity compared to the entire protein (Ghose et al., 1982). Yet, the most attractive targets for immunoconjugates remain intravascular cell types such as lymphocytes, monocytes and macrophages.

5.3.4.1. Immunotoxins

Toxins are molecules that inactivate vital cytosolic components of the protein synthesis machinery in a catalytic manner. A major requirement for a therapeutic effect of toxins, therefore, is to reach the cytosol of the target cell (Olsnes et al., 1989; Vitetta, 1990). Immunotoxins (ITs) are conjugates of antibodies and toxins in which the cell binding moiety of the toxin is replaced by the binding specificity of an Ab. Although ITs were originally developed for the treatment of various tumors, the concept is also applicable in antiviral therapy (Vitetta, 1990). Ricin toxin is one of the most commonly applied and studied compounds. It consists of two chains, the A- and B-chain. The A-chain is responsible for the cellular activity, whereas the B-chain is in fact a galactose-specific lectin, responsible for the binding to virtually all eukaryotic cells (Vitetta and Uhr, 1985). Replacing the B-chain with a monoclonal antibody (MoAb) therefore provides a greatly improved cell specificity. Yet, studies in rats and mice (Blakey and Thorpe, 1988), showed that ITs of Abs and Ricin A-chain are rapidly taken up by various liver cell types, due to the presence of mannose-rich oligosaccharides in the A-chain. Deglycosylation of the A-chain resulted in an IT-dgA with a much longer half-life in vivo (Blakey and Thorpe, 1988).

Investigations by Hwang et al. (1984), using the more potent Abrin Toxin A-chain, showed that different cell types expressing the same antigen are not necessarily equally susceptible to the cytotoxicity of a conjugate consisting of the same MoAb.

Mice with ongoing graft-versus-host disease were treated with MoAbs coupled to Ricin A-chain, resulting in a significantly increased survival time. However, protection lasted only as long as the IT-A was administered (Vallera et al., 1991).

For treatment of HIV infections, highly specific ITs can be developed directed to specific viral antigens on the cell surface of infected cells or aimed at the highly conserved CD4 molecule on T4-lymphocytes or on several other cell types (Kim et al., 1990; Uckun et al., 1989; Zarling et al., 1990). However, the

therapeutic aim is to specifically destroy infected cells. Although MoAbs directed against HIV-1 envelope gp 120 have a high selectivity for infected cells, this approach may be corrupted due to the heterogeneity of gp 120 among the various HIV isolates. Furthermore, circulating free gp 120 may bind to healthy CD4+ cells and convert them to target cells for the MoAbs (Till et al., 1989). In contrast to gp 120, HIV fusion glycoprotein gp 41 is only expressed on infected cells. Its structure is highly conserved, resulting in highly specific anti-gp 41 MoAbs (Till et al., 1989).

Immunotoxins of Ricin A-chain or *Pseudomonas* exotoxin and MoAbs directed against gp 120 inhibited production of infectious virus in infected cells, and did not affect non-infected cells in vitro (Matsushita et al., 1990; Pincus et al., 1989). However, the authors recognized the problem of gp 120 heterogeneity and proposed to use panels of broadly reactive ITs in vivo to avoid variant virus selection.

Barbieri et al. (1989) used an IT composed of anti-CD4 MoAb and Saporin, a ribosome inactivating protein. This Saporin-IT was effective and selective in killing CD4+ cells. In contrast anti-T4 and anti-T8 Ricin A-chain ITs were found to be ineffective against CD4+ and CD8+ cells, probably due to a poor internalization. The authors concluded that internalization of ITs may occur through the toxin moiety rather than through the antibody part and that Saporin is taken up by cells through a mechanism different from that operating in the case of Ricin A-chain. Immunoconjugates, recognizing the CD4-binding region or a variable region of gp 120 and containing pokeweed antiviral protein, were able to kill H9 cells infected with various HIV-strains in vitro without affecting non-infected cells. They retained their ability to neutralize HIV virions and to prevent syncytium formation (Kim et al., 1990).

Anti-gp41-dgA ITs were shown to have a very selective and potent toxicity towards HIV-infected H9 and U937 cells (Till et al., 1989). The reason for this remarkable cytotoxic potency may be the proximity of the target epitope relative to the plasma membrane. This may facilitate internalization of dgA and routing of dgA to intracellular compartments that are instrumental in translocation of the A-chain into the cytosol. Experiments performed in the presence of an excess of unconjugated anti-gp41 Ab only partially inhibited IT-induced cytotoxicity. This may suggest that the presence of anti-gp41 Abs in the serum of patients may not be an obstacle for therapy with this type of immunoconjugates (Till et al., 1989).

5.3.4.2. Antibody-enzyme conjugates

A novel strategy for the delivery of cytotoxic or antiviral agents to specific cell types is the prodrug activation by Ab-enzyme conjugates. The enzymes to be targeted are chosen for their ability to convert relatively non-toxic drug precursors into their active form. Such antibody coupled enzymes can then be specifically delivered to the cell type that expresses the antigenic determinant (Senter, 1990). The formation of active drugs in the close proximity of the target cells could lead to higher cellular and lower systemic concentrations of

the active drug. In fact, the need for Ab internalization, one of the major problems in immunoconjugate application, is eliminated in this concept. Enzymes that can be used are for instance alkaline phosphatase (for the conversion of phosphated prodrugs) and carboxypeptidase (an enzyme converting some inactive carboxy compounds into their active carboxylic counterparts). Although this strategy has only been described in cancer therapy so far (Bagshawe et al., 1988; Senter et al., 1988), it may also be applicable to the therapy of viral infections. A potential drawback forms the presence of related (endogenous) enzymes in the general circulation of the treated individual that may lead to premature activation of the particular product.

5.3.4.3. Bifunctional antibodies

An important development is the targeting of bifunctional antibodies. For instance linking an antitumor antibody with an antilymphocyte antibody produces a bifunctional protein that can redirect T-lymphocytes to lyse tumor cells (Nelson, 1991). A similar procedure was proposed for toxic T-lymphocytes killing HIV-infected cells (Berg et al., 1991).

5.3.5. Soluble polymers

Soluble synthetic polymers have been widely employed to design versatile drug carrier preparations. Polymer chemistry allows the introduction of target devices into the backbone of the polymeric carrier molecule essential for cell-specific drug delivery (Duncan and Lloyd, 1991). A larger number of potential target sites in the body can be reached compared with the microparticulate types (Lloyd, 1991).

Such polymeric carriers should not adhere non-specifically to cells and therefore excessive charge or hydrophobicity of such polymers has to be avoided. In addition, the molecular weight should be large enough to avoid glomerular filtration in the kidneys and small enough to reach the target cells (Lloyd, 1991).

N-(2-hydroxypropyl)methacrylamide (HPMA) polymers have been extensively studied as drug carriers. However, they have the disadvantage that the main polymer chain is not biodegradable (Cartlidge et al., 1987). Cross-linking of relatively small HPMA polymers with oligopeptides, however, leads to intracellular degradation of the cross-links and excretion of the polymer breakdown products (Cartlidge et al., 1987). The introduction of oligopeptide structures also provides suitable attachment moieties for drugs (Copecek et al., 1984). The immunogenicity of conjugates based on copolymers of HPMA are claimed to be low (Copecek et al., 1984).

One important application of soluble peptide or polymeric carriers would be the delivery of antiviral antisense oligo(deoxy)nucleotides. Multiplication of rous sarcoma virus, vesicular stomatitis virus, herpes simplex virus, influenza virus and HIV-1 can be inhibited using synthetic oligodeoxyribonucleotides that specifically hybridize to complementary DNA or RNA sequences. However, the antisense concept has one implicit drawback: sensitivity of

oligomers to circulating nucleases and poor penetration into the target cells (Agrawal and Sarin, 1991; Jaroszewski and Cohen, 1991). One way to improve the cellular uptake of the polyanionic oligomers is the coupling to hydrophobic moieties. For instance, oligodeoxynucleotides containing methylphosphonate are rapidly taken up in CHRC5 cells (Shoji et al., 1991). Furthermore, linking an undecyl group to the 5′ terminal phosphate of the antisense oligomer effectively inhibited influenza virus reproduction, while the non-modified antisense oligomer had no effect (Kabanov et al., 1990). Conjugation to cell-specific macromolecules could also provide a potential solution to this problem. Degols et al. (1989) reported a 90–99% inhibition of VSV multiplication in L929 cells with an antisense oligomer-*poly*-L-lysine conjugate at concentrations far below those needed for the uncoupled oligomers. Stevenson and Iversen showed that at concentrations as low as 200 nM, *poly*-L-lysine-modified oligomers prevented MT4 cells from HIV-1-induced cytopathic effects (Stevenson and Iversen, 1989). Although this delivery system is useful for increasing the cellular availability (Leonetti et al., 1990a) and the metabolic stability of the oligomers, *poly*-L-lysine is rather toxic at high concentrations and also ineffective in some cell lines (Degols et al., 1989). Therefore other types of soluble macromolecular carriers are worthwhile considering.

Transferrin-*poly*-L-lysine and transferrin-protamine were complexed with plasmid DNA and shown to be efficiently bound to and endocytosed by hematopoietic cells, leading to the expression of the transferred genes (Zenke et al., 1990). Expression of transferrin receptors on proliferating cells could render these cells susceptible for intracellular delivery of antivirally active drugs or antisense oligomers.

6. Application of drug targeting for the delivery of antivirals

6.1. Blood cells

The presence of blood cells in the systemic circulation makes them prime targets for infiltrating viruses. For instance, monocytes and other antigen-presenting cells are able to internalize all kinds of viruses as an essential part of their role in the immune system. In some cases, however, this feature makes them also vulnerable to viral infections.

A number of viruses exhibit a specific blood cell tropism. HTLV-III (HIV), the causative agent of AIDS, infects T4 lymphocytes, but also monocytes and macrophages, cells that can act as HIV reservoirs in the body (Collman et al., 1989; Pauza, 1988). MHC class I antigens are efficiently endocytosed in numerous cell types. They are suspected to be receptors for the Semliki forest virus rendering MHC I-positive cells potential target cells for SFV (Roux et al., 1989). Epstein-Barr Virus shows specific tropism for B-lymphocytes (Sommerfelt et al., 1988; Tanner et al., 1988) and HSV-1 is able to infect

monocyte-derived macrophages (Midoux et al., 1990).

Agents like AZT, ddI, ddC, and ara-A are well known for their potent antiviral activity. Unfortunately, they also may display considerable toxicity and are often rapidly cleared from the body. Bringing such drugs to the close proximity of the target cells, or, even better, an intracellular delivery, could be of importance in decreasing the overall toxicity and inhibiting the rapid metabolism and/or elimination.

Determinants that may act as recognition sites on blood cells can be divided in four groups: (a) receptors for lymphokines and hormones, (b) immunological receptors, (c) sugar-recognizing lectins, and (d) lymphocyte-homing receptors.

Transferrin is a major mammalian serum glycoprotein, transporting iron from sites of absorption and storage to tissue cells. Transferrin cell-surface receptors are found on all growing cells (Dautry-Varsat et al., 1983), making these cells target cells for delivery of drugs using transferrin as a carrier molecule.

Activated lymphocytes express high affinity Interleukin-2 receptors mediating the transmission of signals for lymphocyte proliferation (Lee and Mookerjee, 1989). Interleukin-6 induces acute-phase protein synthesis and augments cytotoxic T-cell generation in addition to B-cell Ig secretion. IL-6 receptors were shown to be present on human mononuclear lymphocytes, granulocytes and monocytes/macrophages (Munck Petersen et al., 1990). Various other IL receptors are present on blood cells and are able to endocytose bound ligand (Peters and Norback, 1990). Successful targeting using IL receptors was achieved through the diphteria toxin-related IL-2 fusion protein (DAB_{486}-IL-2). It was demonstrated to selectively inhibit protein synthesis in IL-2 receptor bearing T cells and cell lines. DAB_{486}-IL-2 bound to the high affinity form of the IL-2 receptor and was rapidly internalized into acidified vesicles (Bacha et al., 1988; Kiyokawa et al., 1991; Waters et al., 1990).

CD4 molecules are expressed on various blood cell types, being involved in MHC class II restricted T-cell recognition phenomena. They also have a functional role in transducing signals with respect to immune response (Lifson and Engleman, 1989). The CD4 molecule is a target molecule for HIV gp120 and oligosaccharide side chains in gp120 and CD4 are helpful in the recognition phenomena. CD4 could also serve as a target molecule for, e.g. (mannose terminating) *neoglycoproteins* or *recombinant gp120* (rgp120) conjugated with antiviral drugs. Endocytosis and processing of gp120 has been demonstrated in activated lymphocytes by Lanzavecchia et al. (1988).

One of the functional roles for sugar-recognizing lectins on the lymphocyte surface is the recognition of bacteria by their membrane-bound sugar moieties (Lee et al., 1983). Lectins on blood cells are also important for cell–cell recognition phenomena as well as for endocytosis and intracellular transport of ligands (Monsigny et al., 1988). Table 2 shows the distribution of lectins on the particular blood cell types including their sugar-recognizing properties. The use of these lectins for purposes of drug targeting is currently under investigation.

TABLE 2

Lectin-like receptors on varions bloodcell types

Blood cell type	Species	Sugar specificity	Reference
Macrophages			
Macrophages	rat, pig	galactose	Bezouska, 1985
	rat	mannose	Simmons, 1986
	human	mannose	Midoux, 1990; Ezekowitz, 1990
Macrophages, peritoneal	mouse	mannose, GlucNAc	Roche, 1990
Macrophages, bone marrow	mouse	mannose, fucose	Roche, 1990
Monocytes			
freshly isolated	human	mannose-6-P	Monsigny, 1988; Roche, 1985
	human	beta-glucan	Czop, 1985
in culture	human	mannose-6-P, mannose	Monsigny, 1988; Roche, 1985
Lymphocytes			
B-lymphocytes	human	mannose	Barzilay, 1982
	human	mannose, glucose	Lee, 1983
	human	mannose	Monsigny, 1988
	pig	galactose	Bezouska, 1985
	mouse	sulphated polysacch.	Parish, 1985
T-lymphocytes	mouse	lactose, lactose + sialic acid	Decker, 1980
	pig	galactose	Bezouska, 1985
	mouse	galactose, galactose + mannose	Kieda, 1978 and 1979
	mouse	sialic acid, galactose, GlucNAc, mannose	Boldt, 1976
	mouse	sulphated polysacch.	Parish, 1985
T8-lymphocytes	human	L-rhamnose	Kieda 1982 and 1985; Monsigny 1988

Apgar et al. (1982) reported binding of glycoprotein-containing micelles to lymphocytes only when the cells are in an activated state. Only complex oligosaccharide chains were recognized by the lectins, whereas simple carbohydrates were not. Biantennary branched oligosaccharide chains seem to have a preference for binding to lymphocytes, whereas tri- and tetraantennary branches display a higher binding affinity for hepatic recognition systems (Bezouska et al., 1985a,b). Sugar density on the glycoproteins is an important factor in view of hepatic recognition. Particularly glycoconjugates having more than 20 mannose molecules per protein molecule were shown to be high affinity ligands for a variety of receptors (Ezekowitz et al., 1990) (Table 1). Receptors for sulphated polysaccharides are present on lymphocytes, macrophages, polymorph nuclear leucocytes, mast cells and fibroblasts (Chong and Parish, 1986). This broad distribution pattern renders this type of potential carrier molecules unsuitable for targeting to specific blood cell types. The degradation of endocytosed

mannose-6-phosphate-BSA in freshly isolated monocytes is very low, whereas cultured monocytes do show appreciable degradation of the ligand (Monsigny et al., 1988). Grillon et al. recently reported that mitogenic stimulation of T-lymphocytes yields a 4-fold increase in galactosyl binding cells, representing mainly T-helper cells, whereas rhamnose binding cells (T-suppressor cells) only moderately increased (1991).

The presence of sugar-recognizing lectins on blood cells was proposed by us as a rationale for the design of neoglycoproteins for targeting of AZT to HIV-infected lymphocytes and monocytes/macrophages. Some neoglycoprotein-AZT monophosphate (AZTMP) conjugates, differing in type of sugar and sugar density, were found to be at least as potent against HIV-1 cytopathicity in vitro in MT4 cells as AZTMP itself (Molema et al., 1990). Possible mechanisms to explain this remarkable potency were postulated: (1) slow but ongoing extracellular release of the drug during the antiviral assay, (2) binding of the conjugates to the MT4 cells, followed by release of the drug in the proximity of the cells and/or (3) endocytosis of the conjugates and intracellular release of the drug. Interestingly, covalent attachment of AZTMP to the neoglycoproteins also resulted in diminished cytotoxicity of the drug. In the course of these investigations it was observed that some of the developed neoglycoproteins have a potent in vitro anti-HIV-1 activity by themselves. The antiviral activity was positively correlated to the net negative charge of the modified albumins, introduced by the sugar coupling (Janssen et al., 1991a,c). The sugars themselves did not seem to contribute to the antiviral activity. Some of the modified albumins showed an anti-HIV-1 potency equal to that of AZT (on a molar basis). They were shown to inhibit the fusion process (Janssen et al., 1991c), responsible for virus entry and syncytium formation. This specific mode of action was not reported before. This observation enabled the design of drug carrier conjugates with a dual action: the intrinsic activity of the carrier combined with the antiviral activity of a coupled drug acting at various levels of the replication process.

Monsigny and coworkers derivatized a poly-L-lysine macromolecule with mannosyl and the antiviral drug PMEA through glycyl-glycyl spacer arms. The resulting neutral mannosylated polymer, containing a molar ratio drug to protein of 20:1, was shown to be more potent in inhibiting HSV-1 replication in vitro in human macrophages than free PMEA (Midoux et al., 1990). A similar carrier was also used to target muramyl-dipeptide to macrophages (Davidson, 1973).

Lymphocyte homing receptors are lectin-like receptors on the cell membrane surface of various lymphocyte subsets (Rosen et al., 1990), functionally indispensable to the recirculation of lymphocytes from the blood to lymphoid organs (Stoolman and Rosen, 1983). High endothelial venules (HEV) express tissue-specific carbohydrate containing determinants for lymphocyte recognition and so far, at least three distinct lymphocyte-HEV recognition systems have been characterized (Berg et al., 1990). Determinants specifically recognized by these lectins consist of a mannose, fructose or fucose moiety

and some negative charge, e.g. mannose-6-phosphate (Brandley et al., 1990; Butcher, 1984; Jutila et al., 1990; Rosen et al., 1990; Stoolman, 1989), fructose-1-phosphate (Rosen et al., 1990; Stoolman, 1989), fucose-4-sulphate (Brandley et al., 1990), fucoidin (Butcher, 1984; Stoolman, 1989; Stoolman and Rosen, 1983) and phosphomannan (Jutila et al., 1990; Stoolman, 1989). Lymphocyte homing receptors are believed to belong to the family of lectin-like proteins, which includes the serum mannose-binding protein and the mannose receptor on various tissue macrophages. Among these homing receptors, the mannose receptor is thought to be the only member that can mediate phagocytosis (Ezekowitz et al., 1990). Therefore, homing receptor lectins on lymphocytes can probably only serve to mediate the extracellular release of drugs bound to suitable carriers.

6.2. Liver cells

Using receptor-mediated endocytosis of drug-protein conjugates in the liver, selective drug delivery has been accomplished in vivo, resulting in an increase in drug potency and in therapeutic safety. Examples are the delivery of the antiviral drugs trifluorothymidine (Fiume et al., 1984) and adenine-9-β-D-arabinoside monophosphate (ara-AMP) (Fiume et al., 1986). In the case of ara-

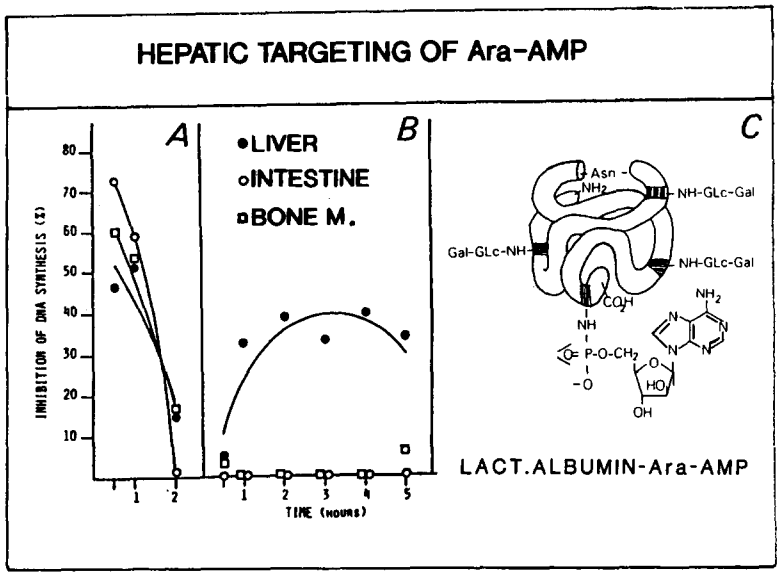

Fig. 10. Increased efficiency of ara-AMP through coupling to lactosaminated HSA (L-HSA) on the inhibition of DNA synthesis in the liver (closed circles) and decreased side effects on intestinal mucosa (open circles) and bone marrow (squares). Ectromelia viral hepatitis yielded (mainly viral) DNA synthesis and was quantified by [^{3}H]thymidine incorporation 24 h after infection. The L-HSA-ara-AMP conjugate, in a three times lower dose compared with uncoupled ara-AMP, shows a prolonged inhibitory pattern (B) compared with ara-AMP itself (A) but does not suppress DNA synthesis in the other tissues (according to Fiume et al., 1982, 1986).

TABLE 3

Example of antiviral drug targeting

Drug	Carrier	Virus	Cell type	Recep.	Exp. Meth.	Ref.
ara-AMP	Lac-HSA	Ectrom.	Hepat	Gal	in mouse	Fiume, 1986
ara-AMP	Gal-Poly-Lysine	Ectrom.	Hepat	Gal	in mouse	Fiume, 1982
ara-AMP	Lac-HSA	Hep.B	Hepat	Gal	in man woodchuck	Fiume, 1988 Ponzetto, 1991
Trifluorthymidin	Asialo-Fetuin	Ectrom.	Hepat	Gal	in mouse	Fiume, 1982
AZTMP	Man-HSA Gal-HSA Fuc-HSA	HIV-1	MT4	?	in vitro	Molema, 1990 Molema, 1991
PMEA	mannose-poly-lys.	HSV-1	Human Macroph.	?	in vitro	Midoux, 1991
Ricin A	rCD4	HIV	T4	gp120	in vitro	Till, 1988
Pseudom. Exotox. A	hCD4	HIV	T4	gp120	in vitro	Ashorn, 1990 Chaudhary, 1988
Ricin A Pseudom. Exotox. A	anti-gp120 mAb	HIV	T4	gp120	in vitro	Pincus, 1989 Matsushita, 1990
Saporin	anti-CD4 mAb	HIV	T4	CD4	in vitro	Barbieri, 1989
Ricin A	anti-gp41 mAb	HIV	H9 U937	gp41	in vitro	Till, 1989
Oligodeoxyribo-nucleotides.	poly-L-lysine	VSV	L929	none	in vitro	Degols, 1990
Ribavarin	liposome	RVF HSV-1 Influenza	?	none	in vivo	Weinstein, 1984
Interferon	liposome	Hep. B	Hepat	none	in vivo	Szoka, 1990
Foscarnet	liposome	HSV-2	?	none	in vivo	Bakker-Woudenberg, 1991
		CMV	Fibrobl.	none	in vivo	
MTP-PE	liposome	HSV-2	?	none	in vivo	Lazdins, 1990
Ricin	anti-CD4	–	CD4+	CD4	in vitro	Uckun, 1989
	anti-CD8	–	CD8+	CD8	in vitro	Uckun, 1989
Pokeweed antiviral protein	anti-gp120	HIV	H9	gp120	in vitro	Kim, 1990
	anti-CD4/ CD5/CD7	HIV	CD4+/ CD5+ CD7+	CD4, CD5, CD7	in vitro	Zarling, 1990
DAB_{486}	IL-2	–	IL-2R+	IL-2R	in vitro	Bacha, 1988 Waters, 1990 Kiyokawa, 1991

AMP, single administration of a conjugate with lactosylated albumin (L_{30}-HSA) (Fiume et al., 1986) yielded a stronger and longer-lasting inhibition of ectromelia virus DNA replication in the liver compared to the parent drug. Virtually no effects of the ara-AMP conjugate on bone marrow and intestinal mucosa cells were found, as shown in Fig. 10. Studies in mice revealed minimal immunogenicity of the L-HSA-ara-AMP conjugates (Fiume et al., 1987b). Recently, Ponzetto et al. showed a clear reduction of Woodchuck hepatitis virus DNA replication in vivo after treatment with ara-AMP and acyclovir-MP conjugated to lactosaminated albumin. These effects occurred at a significantly lower dose compared to that of the free drugs (Ponzetto et al., 1991). In a clinical study, 5 chronic HBV-infected patients were treated with L_{30}-HSA-ara-AMP (1–3 courses 1.5 mg coupled ara-AMP/kg/day for 3–7 days). In all

patients, the plasma levels of HBV DNA dropped significantly. Yet in 4 patients they returned to pretreatment values when the administration was discontinued. In one patient, plasma HBV DNA remained undetectable during the follow-up period of one year (Fiume et al., 1988). After kinetic analysis of the plasma conjugate concentrations, using the data of Fiume et al. (1988), we calculated a volume of distribution of 7 l/70 kg but a surprisingly low plasma clearance of 4.6 l/h per 70 kg ($t_{½}$ = 1.5 h). In healthy volunteers, the $t_{½}$ of technetium-99m-labeled galactose-terminated albumin was reported to be only several minutes (Vera et al., 1989). A number of factors could (partly) account for the abnormal low clearance. First: in the latter study galactose-terminated albumin was used in which the galactose was attached to the albumin via an amidino group (Lee et al., 1976), whereas in the study with the ara-AMP conjugate the galactoses were coupled via a reductive amination (Schwartz and Gray, 1977). Second: the ara-AMP conjugate consisted partly of polymeric material, whereas the technetium-labeled galactose amidino derivative was likely to be monomeric. However, these chemical factors are unlikely to be the sole cause for the abnormal kinetics. More importantly, there could be a downregulation of the hepatic galactose receptor in the case of HBV infection. Although human hepatoma cells such as HepG2 cells normally expose the galactose receptor, preliminary data from our laboratory show that HBV DNA-transfected cells (HepG2 2.2.15) have a much lower expression of this receptor. Thus hepatitis B-infected cells may have altered receptor characteristics depending on the state of the disease.

Apart from antiviral therapy, successful targeting to the liver was reported for the antineoplastic drug daunorubicin (Trouet et al., 1982a) and the antiparasitary drug primaquine (Hofsteenge et al., 1986). Drug targeting by receptor-mediated endocytosis via the ASGP receptor has also been employed to inhibit (Furuno et al., 1983) or to monitor (Van der Sluijs et al., 1985) hepatocyte proteolytic activity and to increase delivery of various proteins to liver cells. The thiolprotease inhibitor pepstatin, a peptide that does not easily pass membranes, could be successfully targeted to rat liver lysosomes in vivo and was very rapidly released from the asialofetuin carrier (Furuno et al., 1983) and excreted from the cells. This demonstrates the important point that targeted drugs that are efficiently released from the protein carriers can be rapidly removed from the target cell. In such cases, more slowly degraded carriers, or programmed release via peptide or lactic acid spacers, connecting the drug to the macromolecular carriers, may be preferred for sustained therapeutic effects in the target cells. An efficient but unfortunate redistribution of a targeted drug was earlier reported for the antiviral drug trifluorothymidine coupled to asialofetuin. Release of the drug from the liver after carrier degradation resulted in severe bone marrow depression, in spite of the primary successful targeting. Such a redistribution does not occur in the case of ara-AMP probably due to its polar character and/or rapid phosphorylation to the di- and triphosphate forms. The possibility to introduce phosphorylated forms of nucleoside analogues via covalent linking to suitable carriers, may be crucial

in cases where inefficient phosphorylation of potential antiviral agents in hepatitis B-infected cells occurs (Lofgren et al., 1990).

As mentioned under section 5.3.5., alternatives for natural glycoproteins and neoglycoproteins as drug carriers are polymers such as galactosylated poly-L-lysine (Ryser and Shen, 1986), galactosylated Ficoll (Rifai et al., 1982) (a polycarbohydrate), and polyhydroxymethylacrylamide material (Duncan, 1987).

Recently, a combination of poly-L-lysine coupled to asialoorosomucoid was used to target various exogenous genes to hepatocytes (Wu and Wu, 1988). Introduction of the particular plasmid DNAs induced hepatocytes to synthesize proteins that are coded by the introduced genes. However, the delivered DNA is mainly routed to and degraded in lysosomes, while the rest of the genetic material remained extrachromosomal and was degraded within two weeks (Wu et al., 1988). More persistent expression of the transferred gene was obtained by inducing artificial liver regeneration either by partial hepatectomy or treatment with the hyperplasia-inducing agent nafenopin (Wu et al., 1990). The rapid cell division probably increases the chance of integrating in the genome of the cells. Recently gene delivery was described using a carbohydrate-linked transferrin-poly-L-lysine conjugate for efficient gene introduction in eukariotic cells expressing the transferrin receptor. The oligosaccharide group of transferrin acts as a natural spacer between the protein and the DNA binding poly-lysine moiety. Transferrin can also be replaced by gp 120 to create a CD4-tropic gene delivery system (Wagner et al., 1991).

Many authors describe the use of retroviral vectors for gene therapy in liver (Kaleko et al., 1991; Wilson, 1986). Retrovirus-mediated gene transfer provides a tool for efficient and persistent gene therapy. For instance (neo)-CD4-genes and recombinant defective HIV systems were successfully targeted to kidney Cos cells (Poznansky et al., 1991; Shimada et al., 1991). This method can be used to express antiviral sequences in T-lymphocytes to provide an intracellular immunization or to transfect genes coding for the synthesis of cytokines. Cells could be engineered to produce sCD4 in order to inhibit HIV entry to scavenger HIV particles or to mitigate the toxic effects of soluble HIV gp 120 (Morgan et al., 1990). Other delivery systems for DNA that have been successfully applied are cationic liposomes (Gao and Huang, 1991; Rose et al., 1991), and various other types of liposome preparations (Leibiger et al., 1990, 1991; Nicolau and Cudd, 1989) that were made suitable for targeting to various cell types in the liver.

Targeting of genes and antisense oligomers to various cell types in vivo may become an important item in the manipulation of pathophysiological processes including viral infections.

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