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DRUG CARRIER SYSTEMS

Edited by
F. H. Roerdink
A. M. Kroon

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Preface

SOME CONSIDERATIONS AND PROPOSALS

Detailed understanding of cell- and molecular biological processes has opened new ways for the interference with these processes. It is possible to test a wide variety of compounds as to their effects on enzymatic reactions, transport mechanisms, cell proliferation and cell differentiation-related activities in either subcellular systems or whole cells. Synthetic agents and also natural substances, although foreign to the organism under study, can be investigated. In addition, body-specific macromolecules such as hormones, plasma proteins and even intracellular enzymes or cell constituents can be isolated and purified or produced at large scale with the aid of modern biotechnological methods. Also these agents can be used for therapeutic purposes. Not to speak of the remote possibilities for causal treatments of inherited diseases by gene replacement or gene correction.

The availability of these therapeutic possibilities has once more pointed to the necessity that the relevant tissues, cells or subcellular targets should be reached. The beneficial effects fully depend on this prerequisite, especially since the therapeutic potential for certain cells and tissues coincides in most cases with toxicity of the same agents for other cells and tissues. The most striking example of this dilemma is shown by the anticancer drugs, which in general match a cytocidal effect with severe side effects on unaffected organs, not necessarily only those organs characterized by a high turnover rate of part of their cells. Another point of interest is that drug therapy is often more effective if the drug levels remain more or less constant over a large period of time. Extreme initial levels, which provoke severe side effects without improving the therapeutic potential, should be avoided.

These considerations call for drug carrier systems, which address the drugs specifically to the tissues and cells they should interfere with and/or release the active drug in a sustained way.

In the present volume the main developments of the last 20 years in the field of drug carrier systems and drug targeting are reviewed. Attention is paid to antibodies, polymers, liposomes, erythrocytes and erythrocyte ghosts and to implantable infusion pumps. From the data presented it is clear that considerable progress has been made over the past years. This progress not only led to a number of clearcut examples of the potency of the drug-targeting concept, but also pointed out the limitations of the available systems. The natural barriers which have to be overcome before the sensitive targets are really reached, are significant. Particle traffic and transport in the whole body is much more complicated than was expected when the various carrier systems were firstly introduced. In this volume problems of biodistribution and biodegradation are discussed. Moreover, the selectivity and the toxicity of the drugs carried by the various systems is given much attention. This especially holds for a variety of anticancer drugs.

From the data presented it follows that the cells of the reticuloendothelial system (RES) preferentially capture macromolecular drug-carrier conjugates. On the one hand this explains some disappointing results in reaching target organs other than the RES. On the other hand this circumstance has led to powerful approaches to the treatment of diseases specifically localized in the RES. The fight against a number of parasitic infections, which form a major healthcare problem worldwide, may benefit from these new developments. The odds are certainly warranting the onset of clinical trials in this field on a larger scale.

With respect to the various aspects of sustained release the developments are highly promising. Both implantable pumps, which can be refilled, and erythrocytes, in vitro loaded with certain drugs, resealed and then reinfused, appear strong tools in this respect.

In the near future progress may be expected in the field of immunotargeting. Reagents like immunotoxins and antibody-drug conjugates are macromolecules with molecular weights and dimensions, which are relatively low and small as compared to particle-type carriers such as liposomes and the various microspheres. For this reason they will escape easier from the phagocytotic activity of the macrophages in the various body compartments. Also the endothelial lining of the blood vessels can be passed easier by such molecules. The availability of monoclonal antibodies against a growing number of tissue-specific antigenic determinants is an important development in this respect. The binding of drugs such as cytostatic agents to the antibodies can be accomplished by various methods and the mechanisms for internationalization and liberation of the drugs in the target cells are currently under investigation. Encouraging results have already been obtained. As far as peptide drugs or enzymes are concerned future developments on hybrid proteins seem promising. Also the use of ternary complexes, which combine a drug with a targeting principle and a carrier system, may be expected to be developed further in the coming years.

Relatively little attention has yet been aid to the oral route of administration. In the near future this route too may benefit from the knowledge obtained in the various areas of drug targeting research described in this volume. It is obvious that carrier systems are able to protect drugs from hydrolytic breakdown in the gastrointestinal tract. By combining these carriers with appropriate bioadhesive reagents, it can be envisaged that proteins and other macromolecules prone to hydrolysis by the digestive enzymes, can be transported to the distal parts of the intestinal tract and can interfere with the cells of the immune system in that area of the bowel. This approach may open new possibilities for vaccination.

The volume editors: Frits H. Roerdink
Albert M. Kroon

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TARGETING OF DRUGS: IMPLICATIONS IN MEDICINE

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INTRODUCTION

It is widely accepted that the usefulness of many drugs in therapeutic, diagnostic and preventive medicine would be enhanced if drugs were to exert their desired effect selectively on the target site. With few exceptions, however (eg. interference of antibacterial agents with metabolic pathways in bacteria, not shared by the host), drug selectivity ranges from modest to nil. The consequences of poor selectivity in pharmacological action are clearly reflected in cancer treatment where cytostatic agents will also kill or damage normal rapidly proliferating cells. Lack of selectivity is only one, albeit major, of the obstacles to optimizing drug action. Others are inaccessibility of target where, for instance, antimicrobial drugs fail to enter intracellular sites harbouring microorganisms, drug vulnerability in certain biological milieus leading to or surrounding the target, premature drug secretion or allergic reactions due to the molecular nature of drugs.

In spite of the very considerable recent developments in molecular and cell biology, progress in the design of selective drugs has been meagre. On the other hand, some of these developments including the advent of monoclonal antibodies and better understanding of ligand-receptor interactions and ensuing intracellular events, have been the protagonists in the rapid progress of an alternative approach to conferring selectivity on drugs, namely targeting. The drug targeting concept is based on the use of carrier systems to deliver drugs to the intended area of action. Carriers can do so because of an inherent or acquired ability to interact selectively with respective cells (Gregoriadis, 1981). Thus, glycoproteins bind through specific terminal groups to receptors expressed on cell surfaces, antibodies interact with cell surface antigens and colloid particles (eg. liposomes) are endocytosed avidly by macrophages. Loading of carriers with drugs can occur through covalent and other types of bonding, or

passive entrapment as is usually the case with colloids. Injected drug-loaded carriers are expected, once in the vicinity of the target, to associate with it on contact. Subsequently, through a variety of scenarios, the drug is freed to act. However, accumulated experience (Gregoriadis, 1988a; Davis et al, 1984; Tirrell et al, 1985; Gregoriadis and Poste, 1988) during the last decade or so has shown that targeting in vivo can be interfered with by the biological milieu within which targeting occurs.

THE ROLE OF THE BIOLOGICAL MILIEU

The biological milieu, for the purpose of this discussion, includes the body fluids and tissues which the carrier may encounter en route to its destination and the target. Thus, blood which is normally where the carrier finds itself after injection can, through some of its cell or plasma components, alter the circulating carrier to a state incompatible with drug retention or divert it to irrelevant normal tissues which may suffer as a result. Colloid systems for instance, are treated as foreign by the body, opsonized and end up in the reticuloendothelial system (RES) (Davis et al, 1984; Gregoriadis and Poste, 1988). Further, natural macromolecular carriers, such as antibodies, lipoproteins, glycoproteins, etc. will be taken up by tissues (eg. liver) and catabolized during the normal process of the protein turnover (Allison, 1976). These events may be so rapid as not to allow the carrier to associate with the target significantly. In addition, foreign or altered protein carriers (eg. mouse monoclonal antibodies and partly denatured proteins respectively) may elicit immune responses which could, on repeated treatment, lead to allergic reactions or neutralization of the carriers (Gregoriadis and Poste, 1988) (It is anticipated that use of monoclonal antibodies of human origin will remove the threat of immunological response; Crawford et al, 1983). On the other hand, there may be slow, limited or no access of the carrier to a target separated from the blood by capillary and other (eg. basement) membranes. This is especially true for some of the larger colloid or polymeric type of carriers (Gregoriadis and Poste, 1988; Poste and Kirsh, 1983), although it also applies to some extent to proteins such as antibodies (Cobb et al, 1987).

A potential source of problems is also the way by which the carrier moiety mediates drug action on association with the target. When cell death or some kind of modification of its metabolic function are required, it may be necessary for the carrier to enter the cell. For ligands this is usually achieved by receptor-mediated endocytosis (Gregoriadis et al, 1984;

Kolata, 1983) which introduces the ligand and drug moiety into cell compartments where the drug is freed to an active state through the action of the microenvironment. A good example is the use of asialoglycoproteins for the delivery of drugs (Fiume et al, 1984) to the hepatic parenchymal cells expressing the galactose receptor (Ashwell and Harford, 1982). Here, uptake of the glycoprotein by the cells is rapid, highly specific and leads to its localization in the lysosomal apparatus (Gregoriadis et al, 1970) within which lysosomal hydrolases will sever the bond between the drug and the carrier. Depending on the case, the drug could either act locally or, following diffusion, in other cell compartments or even extracellularly. It is possible however, that some cells will not interiorize bound ligands, bonds will not be hydrolysed and drugs will lose their activity or fail to reach their target. Most importantly, problems relating to the biological milieu could be aggravated in the presence of disease where blood composition, tissue microanatomy, membrane structure, receptors and other entities could be altered in ways that are detrimental to effective carrier function as established under normal conditions (McIntyre, 1986).

Appropriate choice of the carrier-drug unit and/or structural manipulations of it, may help to circumvent difficulties presented by the biological milieu. In terms of blood interference with carrier behaviour, the following examples come into mind: It has been shown (McIntosh and Thorpe, 1984) that terminal galactose residues of plasma or cell surface glycoproteins bind antibody-toxin conjugates via the galactose-recognizing sites of the B chain of the toxin (eg. ricin). Such non-specific binding can induce toxicity or severely limit the number of conjugate molecules available for distribution to tumour tissues. This can be now abbrogated through competitive antagonism with excess free galactose or lactose, chemical modification of the toxin to delete its galactose recognizing properties and also by using conjugates with A chains only (McIntosh and Thorpe, 1984). Plasma high density lipoproteins (HDL) on the other hand, destabilize liposomes (Krupp et al, 1976; Scherphof et al, 1978) by removing phospholipid molecules from their bilayers (Kirby et al, 1980). HDL action on liposomes however, can be reduced or abolished altogether by enriching the bilayers with cholesterol and/or phospholipids with high gel-liquid crystalline transition temperatures (Tc) (Kirby et al, 1980; Allen, 1981; Senior and Gregoriadis, 1982). These lipids promote bilayer packing and rigidity, in turn inhibiting HDL insertion (Gregoriadis, 1985).

A number of approaches have been adopted for the reduction of carrier interception by the RES. Recent

examples are the deglycosylation of the A chains of ricin in immunotoxins, which enables the latter to avoid the relevant sugar receptors in the RES (Blakey et al, 1987) and the co-administration of (ricin) immunotoxin with yeast mannan which competes for uptake by the liver (Bourrie et al, 1986). Generally, protein carriers must obviously be prepared in a purified, non-denatured form. Also, the load and type of linked drug must be such that there is only minimal change of the protein's native state (Matzku et al, 1985). To that end, conventional cytotoxic drugs, of which relatively large quantities must be linked to the protein so as to achieve the required dosage, have been replaced with highly potent toxins (eg. ricin, abrin; for reviews see Gregoriadis et al, 1984). With colloid and polymeric carriers, effective RES avoidance is more problematic. Since recognition of most colloids by the RES is the result of their opsoninization, attempts have been made to reduce this by rendering colloids refractory to protein adsorption (eg. a highly hydrophilic surface). As an example, the rate of clearance of injected polystyrene microspheres coated with the hydrophilic block co-polymer poloxamine 908 was significantly reduced (Illum et al, 1987). An even greater reduction of clearance has been achieved in the case of liposomes by simply diminishing vesicle size. Thus, small unilamellar liposomes (SUV) with a 30-60um diameter exhibit half-lives of several hours (Gregoriadis, 1988b). Interestingly, half-lives can be extended greatly (up to 20 hours in mice) by incorporating into the SUV large proportions of cholesterol and phospholipids (Senior and Gregoriadis, 1982) with high Tc. It has been suggested (Gregoriadis, 1985) that in addition to preventing HDL insertion, a packed, rigid bilayer may also interfere with opsonin adsorption (for further discussion see Gregoriadis, 1988b).

As already stated, interception of (altered) protein and colloid carriers by the RES reduces their value in terms of quantitative contact with alternative targets. This, however, is far from being a disadvantage when drugs are to be delivered to the RES which is involved in many microbial and other diseases and also constitutes an important component of the immunological response (for a discussion and relevant applications, see later). It should also be pointed out here that, as in most cases receptors on macrophages recognize foreign carriers through plasma "ligand" components adsorbing onto their surface, interaction of such carriers with the RES is a form of receptor-mediated targeting (commonly referred to as "passive"), in the sense that selectivity is acquired on contact of carriers with the biological milieu.

Although receptor-mediated targeting is potentially a most effective approach to optimizing drug action, some of the problems mentioned earlier, especially target inaccessibility, may prove insurmountable thus cancelling out sophisticated carrier-drug chemistry or ways of controlling other aspects of carrier behaviour in vivo. In addition, not all sites in need of treatment possess (or are known to possess) specific receptors and when they do, corresponding ligands may not be readily available. From the practical point of view, intravenous targeting which is considered ideal because blood will transport carriers to, or near, all areas in the body, is not a favoured route of drug administration in chronic treatment. For these and other reasons, an auxillary "targeting" concept has emerged, now generally known as the controlled release approach. Controlled release, as challenging as and in some ways perhaps more realistic than receptor-mediated targeting, employs a variety of microscopic or macroscopic implantable devices which will release their drug content, often at predetermined rates. Drug release is effected either by continuous degradation of the implant or by the efflux of the drug from the intact implant. Microscopic devices in the form of colloids can also be injected directly into the circulation to be subsequently "implanted" in tissues. It is thought (Storm et al, 1987) that the considerable success of certain liposomal cytostatic drugs in experimental cancer chemotherapy is probably the result of slow drug release from the liver and spleen where liposomes end up after injection. For further discussion on controlled release, which falls outside the scope of this review, see Leong et al, 1986.

IMPLICATIONS IN MEDICINE

Remarkable successes with drug targeting in the treatment or prevention of a wide spectrum of diseases in experimental animals and small clinical trials (eg. Gregoriadis, 1981, 1988a; Davis et al, 1984; Tirrell et al 1985; Gregoriadis and Poste, 1988) suggest that routine clinical applications may be forthcoming. To that end, the first and obvious consideration is that a carrier-drug unit designed to treat a particular disease has clear advantages over the conventional use of the therapeutic agent. Advantages may include a lower dosage at which the agent is effective (preferably in a single bolus thus diminishing both cost and toxicity), prevention of drug loss through premature excretion or inactivation as a result of carrier-mediated altered pharmacokinetics and improved access to the target because of the ability of the carrier to, for instance, enter intracellular sites. Also, novel drug toxicities as a result of altered pharmakinetics should, if

present, be minimal as not to outweigh advantages gained from targeted drug delivery. All these must be proven at the various experimental and clinical stages necessary for the final approval by regulatory bodies.

Historically, initial evidence for the effectiveness of a drug-carrier system in the treatment of a particular disease has been typically provided by academic researchers usually "disillusioned" with the performance of drugs available for this purpose. In some cases, however, researchers are unfamiliar with the clinical and/or industrial demands which must be satisfied before the system can be applied clinically. As a result, some of the ideas and concepts propagated are rather unrealistic and eventually fall by the wayside. This can be accelerated by premature claims which generate scepticism in those to whom the claims are targeted or are not substantiated when tested clinically. In others, carriers are solely designed to succeed in an in-vitro environment which has very little to do with the behaviour of the carrier *in vivo* or with the properties of the target as expressed *in situ*. Thus, fine work producing elaborate drug-carrier systems can become redundant when the systems are tried *in vivo* and their effectiveness shown to be disappointing.

Much of the success with drug targeting has originated from experiments performed with the biological milieu in mind and with the simultaneous involvement of key staff (eg. physicians, cell and molecular biologists, pharmacists, immunologists and biophysicists), occasionally including industrial workers who will infuse into the work their point of view. Recently, progress toward clinical applications of targeted drug delivery has gained new momentum thanks to the efforts of drug delivery research units set up by pharmaceutical industries and of new biotechnology companies the survival of which depends on the early development of drug delivery products. Such companies will employ a wealth of specialist scientists, have close links with hospital physicians and be advised by individuals knowledgeable of intricate legal, financial and marketing aspects. In this way, the combined efforts of academic and industrial workers are likely to lead in the near future to a number of products expected to optimize drug action in a variety of clinical uses. Some of these, together with applications of longer-term promise will be discussed here briefly and the role of individual carriers evaluated. More detailed treatment of ideas will no doubt be given in the various chapters of this book devoted to specific topics.

CANCER TREATMENT

Much of the research on targeted drug delivery has been focussed on cancer treatment not only because

cancer affects great numbers of people but also because it is a major disease in the western world where science is most developed and resources most concentrated. Yet, conventional cancer treatment (chemotherapy, radiation and surgery, alone or combined), especially of solid tumours has been less than satisfactory for a large number of patients: None of the drugs developed so far is selective enough and as a result dosages cannot be adjusted to levels required to kill all tumour cells without being toxic at the same time. The discovery of antigenic sites specific for some tumour cells raised hopes (Bagshawe, 1983) that antibodies against such antigens could serve to deliver cytostatic drugs (including radionuclides) to the cells. Indeed, related experimental work (see Bagshawe, 1985; Gregoriadis and Poste, 1988; Tirrell et al, 1985; Gregoriadis et al, 1984, for numerous examples) with animals bearing tumours established the feasibility of the approach. Enhanced progress in this area, however, did not occur until monoclonal antibodies became available and the replacement of antibody-bound drugs with immunotoxins (Marx, 1982; Vitetta and Uhr, 1985). The first, provided an unlimited source of highly specific molecules for tumour antigenic sites and the second, extremely potent cell killer molecules (Olsnes and Pihl, 1982). Many laboratories have produced evidence of therapeutic advantages (Baldwin and Byers, 1986) for immunotoxins in animals, for instance nude mice bearing human tumours (Griffin et al, 1987; Johnson and Laguzza, 1987; Gregoriadis, et al, 1984) and encouraging results have been obtained in patients (Baldwin and Byers, 1986; Beverley and Riethmuller, 1987). Clinical work (Frankel, 1985; Douay et al, 1985) also suggests that allergic reactions from mouse monoclonal antibodies are either not apparent or can be tolerated reasonably well. There is, nonetheless, considerable uneasiness (Garnett and Baldwin, 1986) regarding the potential toxicity of toxins to normal cells, such as those of the RES which will take up much of the injected immunotoxins. Moreover, toxins being foreign, will elicit immune responses and are likely, on chronic treatment, to be neutralized by the antibodies formed (Marx, 1982). Researchers are, therefore, re-examining the use of antibody-bound conventional cytostatic drugs (Garnett and Baldwin, 1986; Diener et al, 1986; Manabe et al, 1984). Even more promising results in animals and patients (Bagshawe, 1985; Chan et al, 1986; Carrasquillo et al, 1984; Buraggi et al, 1985) have been obtained with radiolabelled monoclonal antibodies in tumour imaging where problems (Epenetos et al, 1986) are of lesser magnitude compared to those encountered in therapy.

The use of colloids, in cancer treatment has also been considered (eg. Gregoriadis, 1988a; Tirrell et al, 1985; Davis et al, 1984) in spite of the obvious difficulties (Poste and Kirsh, 1983) of particle penetration to areas where tumours reside. However, there is indirect evidence from animal work that tumour cells might take up small liposomes (eg. Gregoriadis et al, 1977; Ogihara et al, 1986; Patel et al, 1985) or nanoparticles (Grislain et al, 1983) to a greater extent than cells of neighbouring normal tissue. Whether these results can be explained on the basis of a higher endocytic activity of some tumour cells combined with increased local permeability of capillaries, marker diffusion from circulating vesicles or nanoparticles followed by marker localization in the tumour area because of increased blood flow in that area, or indeed, as a result of migration of monocytes (with the engulfed carrier) to tumours, remains an open question (Gregoriadis, 1980). Unfortunately, preferential uptake of (liposomal) markers by tumours in man has not as yet been convincingly demonstrated (Gregoriadis et al, 1974; Ryman and Tyrrell, 1980; Perez-Soler et al, 1985).

The case of using colloids in cancer chemotherapy appears stronger in work aimed at reducing toxicity while at the same time maintaining the tumourcidal effect of the drug. Experiments with liposomes containing anthracycline cytostatics (eg. Gregoriadis, 1988a; Forssen and Tokes, 1983; Fichtner et al, 1984; Gabizon, et al, 1986; Mayhew et al, 1987) have unequivocally shown reduction of cardiotoxicity and dermal toxicity and prolonged survival of tumour-bearing animals compared to controls receiving the free drug. Interestingly, there have been already promising results with at least two of the ongoing related clinical trials. In phase I work, for instance, the maximum tolerated close for daunomycin-containing liposomes was much greater (90 mg drug per m^2) than that of the free drug (30-45 mg/m^2 body surface). When the same liposomes were used to treat, in a limited phase II trial, five patients with recurrent breast cancer, three of the patients became complete responders at 75 mg daunomycin/m^2 (A. Rahman, personal communication). In a second study with liposomal daunomycin (Sells et al, 1987) the drug was also well tolerated by six patients with hepatic metastases from primary gastrointestinal adenocarcinomas and there was evidence of significant reduction of malignant hepatomegaly in one of them. It has been postulated (for discussion see Gregoriadis, 1988a) that liposomal drug taken up by the RES tissues is released to penetrate malignant cells and exert its effect. If so, it is possible that other drug-loaded colloid carriers including albumin microspheres, nanoparticles, and erythrocyte ghosts (all of which