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Drug targeting

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Abstract

The main problems currently associated with systemic drug administration are: even biodistribution of pharmaceuticals throughout the body; the lack of drug specific affinity toward a pathological site; the necessity of a large total dose of a drug to achieve high local concentration; non-specific toxicity and other adverse side-effects due to high drug doses. Drug targeting, i.e. predominant drug accumulation in the target zone independently on the method and route of drug administration, may resolve many of these problems. Currently, the principal schemes of drug targeting include *direct application of a drug* into the affected zone, *passive drug targeting* (spontaneous drug accumulation in the areas with leaky vasculature, or Enhanced Permeability and Retention-EPR-effect), *'physical' targeting* (based on abnormal pH value and/or temperature in the pathological zone), *magnetic targeting* (or targeting of a drug immobilized on paramagnetic materials under the action of an external magnetic field), and *targeting using a specific 'vector' molecules* (ligands having an increased affinity toward the area of interest). The last approach provides the widest opportunities. Such pharmaceutical carriers as soluble polymers, microcapsules, microparticles, cells, cell ghosts, liposomes, and micelles have been successfully used for targeted drug delivery in vivo. Though the direct conjugation of a drug molecule with a targeted moiety is also possible (immunotoxin), the use of microreservoir-type systems provides clear advantages, such as high loading capacity, possibility to control size and permeability of drug carrier systems and use relatively small number of vector molecules to deliver substantial quantities of a drug to the target. The practical use of the listed systems and approaches for the delivery of therapeutic and diagnostic agents will be considered.

Keywords: Drug targeting; Drug carriers; Cardio-vascular targeting; Monoclonal antibody

1. Introduction

For the majority of pharmaceuticals currently in use, the activity against certain diseases or disease sites is not based on their ability to accumulate selectively in the pathological organ, tissue or cell. Usually, the pharmaceutical agent is rather evenly distributed within the body. Moreover, to reach the site of action, the drug has to cross many biological barriers, such as other organs, cells and intracellular compartments, where it can be inactivated or express undesirable influence on organs and tissues that are not involved in the pathological process. As a result, to achieve a required therapeutic concentration of a drug in a certain body compartment, one has to administer the drug in large quantities, the great part of which is just wasted in normal tissues. In addition, under these circumstances, cytotoxic and/or antigenic drugs can become the cause of many negative side effects.

Drug targeting can bring a solution to all these problems. In a very general sense, one understands drug targeting as the ability of the drug to accumulate in the target organ or tissue selectively and quantitatively, independent of the site and methods of its administration. Ideally, under such conditions, the local concentration of the drug at the disease site(s) should be high, while its concentration in other non-target organs and tissues should be below certain minimal level to prevent any negative side-reactions. The following advantages of drug targeting are evident: (a) drug administration protocols may be simplified; (b) drug quantity required to achieve a therapeutic effect may be greatly reduced as well as the cost of therapy; (c) drug concentration in the required sites can be sharply increased without negative effects on non-target compartments. The same is, for the great extent, true for the use of many diagnostic agents.

The concept of drug targeting, suggested by Paul Ehrlich almost a century ago, considered a hypothetical 'magic bullet' as an entity consisting of two components — the first one should recognize and bind the target, while

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the second one should provide a therapeutic action in this target. Currently, the concept of magic bullet includes a coordinated behavior of three components: (a) drug; (b) targeting moiety; and (c) pharmaceutical carrier used to multiply the number of drug molecules per single targeting moiety. Pharmaceutical carriers include soluble polymers, microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes, and micelles. All of them can be made targeted in one way or another.

The recognition of the target can occur on the level of a whole organ, on the level of certain cells specific for a given organ, or even on the level of individual components characteristic of these cells, such as cell surface antigens. The most universal form of target recognition is the recognition on the molecular level, based on the fact that every organ or tissue certain compounds (antigens) can be found that are specific only for the organ of interest. For successful targeting, another compound can be used as a transporting unit, which is capable of the specific interaction with the specific target component (for example, a monoclonal antibody against the target antigen). Basing on this principle, numerous systems for drug targeting have been constructed capable of the delivery of pharmaceuticals to the variety of tissues and organs (Gregoriadis, 1977; Poste and Kirsh, 1983; Francis and Delgado, 2000).

Currently, the whole set of targeting protocols is under development that includes many different approaches to targeted drug delivery. Not necessarily these approaches involve the use of specific targeting moieties. In certain cases various physical principles and/or some physiological features of the target area may be utilized for a successful targeting of pharmaceuticals and pharmaceutical carriers.

2. Principal schemes of targeted drug delivery

Principal schemes of drug targeting currently investigated in various experimental and clinical settings include: (a) direct application of the drug into the affected zone (organ, tissue); (b) passive accumulation of the drug through leaky vasculature (tumors, infarcts, inflammation); (c) 'physical' targeting based on abnormal pH and/or temperature in the target zone, such as tumor or inflammation (pH- and temperature-sensitive drug carriers); (d) magnetic targeting of drugs attached to paramagnetic carriers under the action of external magnetic field; (e) use of vector molecules possessing high specific affinity toward the affected zone. In a certain sense, cases (c) and (d) can be considered together as a 'physical targeting'.

2.1. Direct application of drugs

In certain cases, drug targeting may be achieved by a very simple way — a drug is administered directly into the affected area (organ or tissue). The successful examples of this approach include the intra-articular administration of hormonal drugs in the therapy of arthritis (Williams et al., 1996) or intracoronary infusion of thrombolytic enzymes in the therapy of thrombus-induced myocardial infarction (Chazov et al., 1976). However, the applicability of such a straightforward approach is rather limited.

2.2. Spontaneous drug accumulation in 'leaky' areas

It was found that under certain circumstances blood vessel wall might become leaky. The ability of vascular endothelium to increase its permeability was noticed, for example, in tumors (Jain, 1987) and hypoxic areas of infarcted myocardium (Palmer et al., 1984a). In such areas with increased vascular permeability even relatively large particles, such as micelles and liposomes ranging from 10 to 500 nm in size, can extravasate and accumulate inside the interstitial space. If these particles are loaded with a certain drug, they can bring this drug into the 'leaky' zone where the drug can be released as a result of normal carrier degradation. Since the 'cut off' size of the permeabilized vasculature can vary from case to case, the size of a drug carrying particle may be used to control the efficacy of such spontaneous or 'passive' drug delivery know also as an 'enhanced permeability and retention' (EPR) effect (Fig. 1).

The evident requirement toward this type of targeted delivery is that drug carriers used should demonstrate the ability to circulate long in the blood to provide a sufficient level of target accumulation. Anticancer drugs (such as doxorubicin) incorporated into long-circulating polyethylene glycol(PEG)-coated liposomes demonstrated excellent effects in EPR-based tumor therapy (Gabizon, 1992) and diminished side-effects, and are already used in clinical conditions. Long-circulating micelles may be used as carriers for drug delivery into tumors with a small 'cut-off' size (Weissig et al., 1998). Long-circulating liposomes were also shown to accumulate in the area of experimental myocardial infarction (Torchilin et al., 1992, 1996). In general, there are several important advantages of prolonged circulation of drugs and drug carriers in the blood flow: (a) one can maintain a certain required concentration of a drug or drug carrier in the blood for a long time after a single administration; (b) one can achieve the accumulation of drugs and drug carriers in areas with affected (leaky) vasculature; (c) one can enhance targeting (immunotargeting) of drugs and drug carriers into areas with diminished blood supply and/or low concentration of a target ligand (antigen)

2.3. Physical targeting of drugs

Physical factors able to mediate targeted drug delivery may be of both, endogenous and exogenous origin. In the first case, the targeting effect is based on the fact that pathological area differs from normal tissues in certain

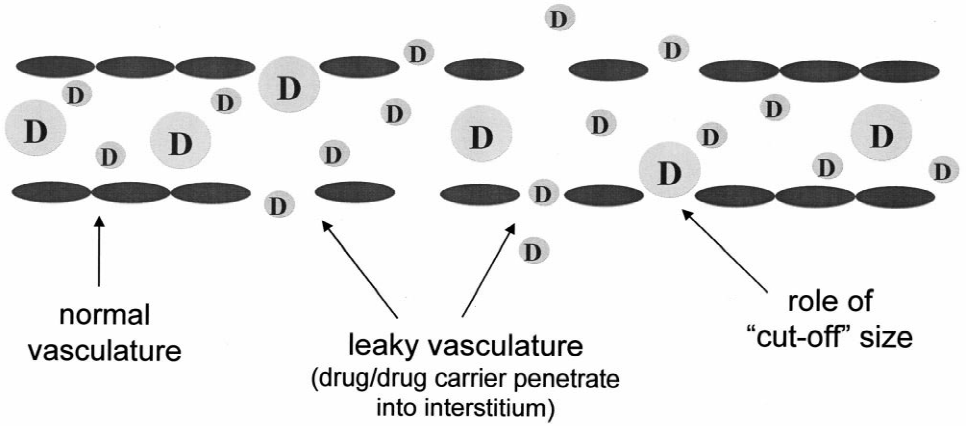

Fig. 1. The schematic representation of the ‘passive’ targeting (interstitial accumulation) of drugs and drug carriers through the leaky vasculature and the role of the ‘cut-off’ size.

properties, such as temperature and/or pH. Thus, it is known that inflamed or neoplastic areas usually demonstrate some acidosis and hyperthermia. This makes it possible to use certain stimuli-responsive drug carriers that can disintegrate at lower pH values or higher temperature than in normal tissues releasing the entrapped drug. Even though the carrier is evenly distributed within the circulation, it still will be degraded only in the target area, where the drug will release and accumulate (Fig. 2). It has been already shown that anti-cancer drug methotrexate incorporated into temperature-sensitive liposomes and injected intravenously into mice with inoculated tumors was accumulated in tumors several times faster, especially, under conditions when the localized external heat was applied onto tumor area (Weinstein et al., 1979). Similar effect was also achieved with pH-sensitive liposomes, which are currently used for experimental delivery of drug and genetic material (see review in Ref. Torchilin et al., 1993).

An interesting example of targeted drug delivery by external physical force is the magnetic drug transport. For this purpose, the drug is immobilized on a microparticulate carrier possessing ferromagnetic properties. Upon the intravenous administration, the carrier can accumulate within the area to which an external magnetic field is applied. Naturally, the ability of magnetic particles to concentrate will depend on both, the blood flow rate and the intensity of the magnetic field (Widder et al., 1983), so the chance of efficient drug accumulation in smaller blood vessels with the lower blood flow rate is higher that in central vessels (aorta) with a very fast blood flow. In animal experiments, the local prevention of thrombosis in arteries of dogs and rabbits was achieved by the intravenous application of the autologous red blood cells loaded with ferromagnetic colloid compound and aspirin, if strong SmCo magnet was secured externally to the artery, where the thrombus was initiated (Orekhova et al., 1990). The authors (Torchilin et al., 1988) obtained streptokinase immobilized on dextran-coated microparticles of iron

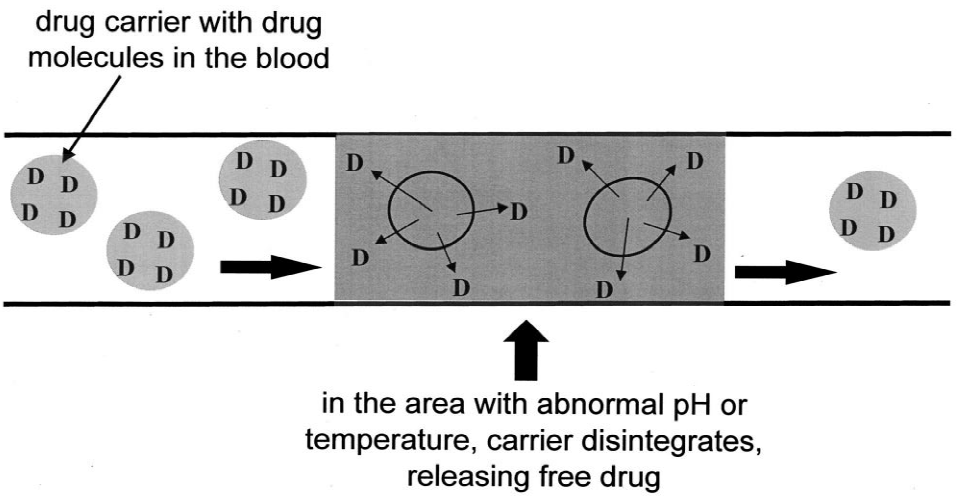

Fig. 2. The schematic representation of the physical targeting of drugs/drug carriers. Stimuli-sensitive drug carrier disintegrates in the target zone and releases the drug.

oxide, and used the preparation obtained for targeted thrombus lysis in carotid arteries of experimental dogs. Thrombus formation in both carotid arteries of each dog was induced by surgical interposing of a trapezium-like vessel wall segment into the lumen. Small permanent SmCo magnet was implanted into the tissues next to one of the vessels in the region of vascular surgery and potential thrombus formation. The thrombolytic preparation containing immobilized streptokinase was administered via the back leg vein of a dog. The experiment is shown in Fig. 3. Immediately after the operation the blood flow in both arteries (control artery and artery with the magnet) decreased as a result of the spasm caused by the surgery. After partial restoration of the blood flow, it was gradually decreased in the artery without the magnet, but increased in the artery with magnet until returned practically to the initial level. Histological analysis of arterial sections obtained 5 h after the operation revealed the existence of the red thrombi in control arteries with complete occlusion, whereas no thrombus formation was observed in the artery with the magnet.

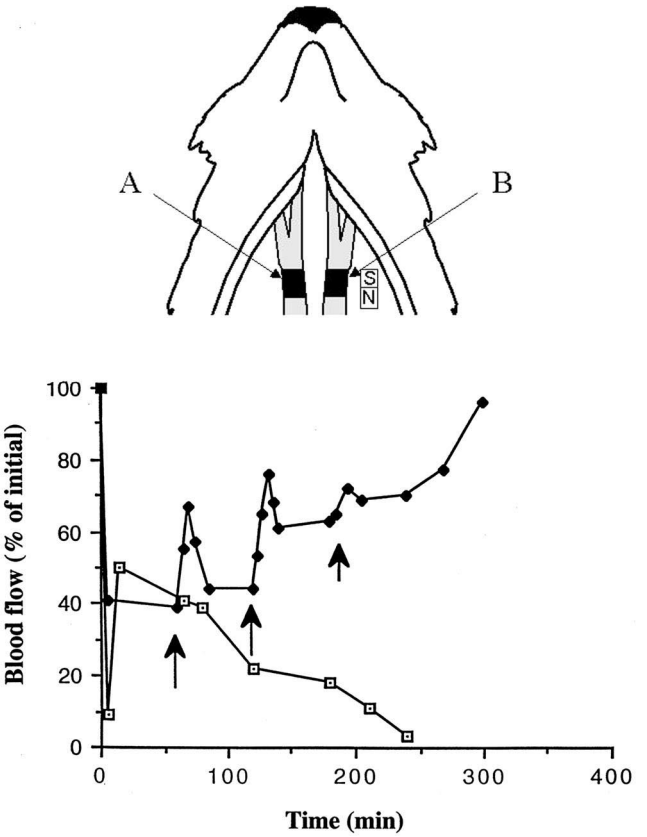

Fig. 3. Magnetic targeting of the thrombolytic enzyme streptokinase. Upper panel: the scheme of the experiment; both carotid arteries (A and B) of the experimental dog were surgically embolized, SmCo magnet implanted near one artery (B) at the occlusion site, 'magnetic' streptokinase was injected repeatedly, and the blood flow in both arteries was registered. Lower panel: blood flow in both arteries; the flow gradually decreases in control artery (empty squares) but is completely restored in the artery with the magnet (filled diamonds) after triple injection of the enzyme on magnetic carrier. Arrows indicate time of enzyme administration. Adapted from Torchilin et al., 1988.

2.4. Targeting moieties

The approaches to drug targeting described so far are not universal. Direct administration of a drug into an affected organ or tissue may be technically difficult, or the disease site may be delocalized. Often, the affected area does not differ much from normal tissues in terms of vascular permeability, temperature of local pH value. Magnetic drug delivery also has limitations connected with the blood flow rate in the target. The most natural and universal way to impart the affinity toward the target to a non-specific drug is the binding of this drug with another molecule (usually referred to as a targeting moiety of vector molecule) capable of specific recognition and binding to a target site. The following substances can be used as targeting moieties: antibodies and their fragments, lectins, other proteins, lipoproteins, hormones, charged molecules, mono-, oligo- and polysaccharides, some low-molecular-weight ligands, such as folate. Monoclonal antibodies against characteristic components of target organs or tissues are the most frequently used vector molecules.

A direct coupling of a drug to a targeting moiety looks as the simplest way to prepare a targeted drug. Immunotoxins represent the most vivid examples of this approach (Vitetta et al., 1983). As shown in Fig. 4, a natural toxin can be 'cut' into active moiety (toxic one) and recognizing moiety, and then the latter is separated and the former is conjugated with an antibody. As a result, a toxic unit may delivered only in those cells that express and appropriate antigen (usually, cancer cells), while antigen-free cells will not be recognized by the immunotoxin and damaged. However, in this case every single antibody molecule is able to carry just one active moiety. Since toxic moieties of toxins/immunotoxins are extremely active (just one such moiety can kill a cell if gets inside and destroys numerous ribosomes), immunoxin may find clinical application. However, in general, the load of a pharmaceutical agent onto targeting moiety should be much higher that simple 1:1 ratio.

Alternatively, some soluble or insoluble carrier can be loaded with multiple active moieties and than conjugated additionally with the targeting unit according to the scheme suggested by Ringsdorf in mid-70s (Ringsdorf, 1975), Fig. 5. Different reactive and biocompatible soluble polymers can be used as soluble carriers, whereas the family of insoluble carriers includes microcapsules, nanoparticles, liposomes, micelles and cell ghosts. Various reservoir-type systems, such as liposomes or microcapsules, demonstrate the following important advantages over other drug carriers: (a) maximum volume at a given surface (i.e. maximum load of the drug); (b) few targeting

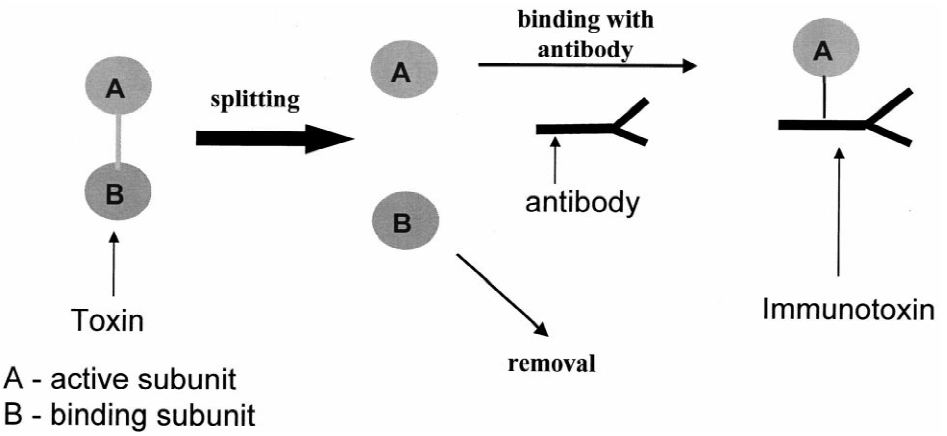

Fig. 4. The scheme of immunotoxin preparation. A natural toxin is splitted into active and cell-binding subunits, and the active subunit is recombined with a target-specific monoclonal antibody yielding the immunotoxin.

moieties can carry multiple drug moieties loaded into the reservoir; (c) possibility to control size and permeability.

By now, it is already shown that body compartments and pathologies that can be successfully targeted via different mechanisms include components of cardiovascular system (blood pool, vascular walls, lungs, heart), reticulo-endothelial system (liver and spleen); lymphatic system (lymph nodes and lymphatic vessels), tumors, infarcts, inflammations, infections, transplants. The parameters determining the efficacy of drug targeting include: the size of the target, blood flow through the target, number of binding sites for the targeted drug/drug carrier within the target, number and affinity of targeting moieties on a drug molecule (drug carrier particle), multipoint interaction of a drug/drug carrier with the target.

To illustrate opportunities and challenges of drug targeting, further, we will consider several examples of targeted drug delivery within the cardiovascular system, since this system demonstrates a broad variety of targets that can be reached by various targeting methods.

3. Targeting of pharmaceuticals within the cardiovascular system

Possible targets within the cardiovascular system may include blood elements, damaged areas of vascular wall, vascular bed itself, thrombi, atherosclerotic plaques, infarcts, damaged cardiomyocytes. Tumors are usually also accessed from the blood flow.

3.1. Antibody-mediated targeted diagnostics and therapy of thromboses

The natural and most important intravascular target is the blood clot. Targeted visualization (imaging) of thrombi and targeted thrombolytic therapy are now among the hottest areas in both drug delivery and thrombolysis fields. The most popular construct to be used for targeting thrombi is a conjugate between a diagnostic label (radioactive metal, first of all) or thrombolytic enzyme (urokinase, streptokinase, tissue plasminogen activator) and thrombus-

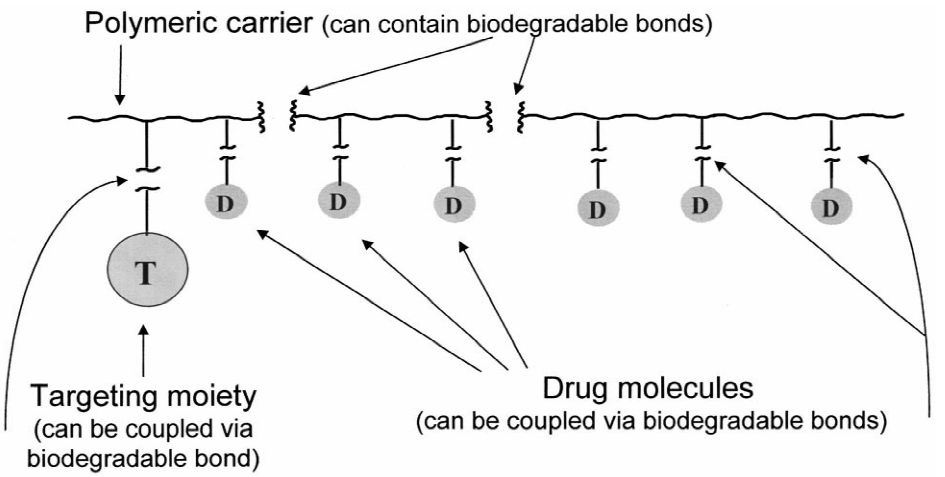

Fig. 5. The schematic structure of polymeric drug. According to Ringsdorf, 1975.

specific monoclonal antibody (see the schematic representation of the targeted thrombolytic therapy in Fig. 6).

The localization and visualization of pulmonary emboli with radiolabeled fibrin-specific monoclonal antibody can serve as a good example for this approach (Kanke et al., 1991). ^{111}In- and ^{99m}Tc-labeled antifibrin antibodies have been also successfully used for the diagnosis of deep vein thrombosis (Knight, 1994). Good review on the radioimmunodetection (targeted visualization) of thrombi utilizing monoclonal antibodies against different thrombus components can be found in (Knight, 1994; Wasser and Pauwels, 1990; Lee et al., 1995).

The special interest toward the targeted thrombolytic therapy, as pointed in (Bos and Nieuwenhuizen, 1992), came from the fact, that the use of some recently developed thrombolytic agents, such as single-chain urokinase-type plasminogen activator and tissue-type plasminogen activator (TPA), was disappointing, mainly due to some of their negative properties in vivo (i.e. rapid inhibition and clearance). Naturally, fibrin — the main non-cellular thrombus constituent which does not present in the blood or in normal tissues — has been considered as a promising antigen from the very beginning. Thus, it was shown in (Bode et al., 1985, 1987) that antifibrin antibodies could be conjugated with urokinase via SPDP without affecting the specific properties of the enzyme or antibody. The fibrinolytic activity of the specific conjugate is about two orders of magnitude higher than that of the native enzyme. After correction for spontaneous lysis, TPA–antibody 59D8 conjugate was shown to be 3 to 10 times more potent than free TPA or its conjugate with non-specific antibody.

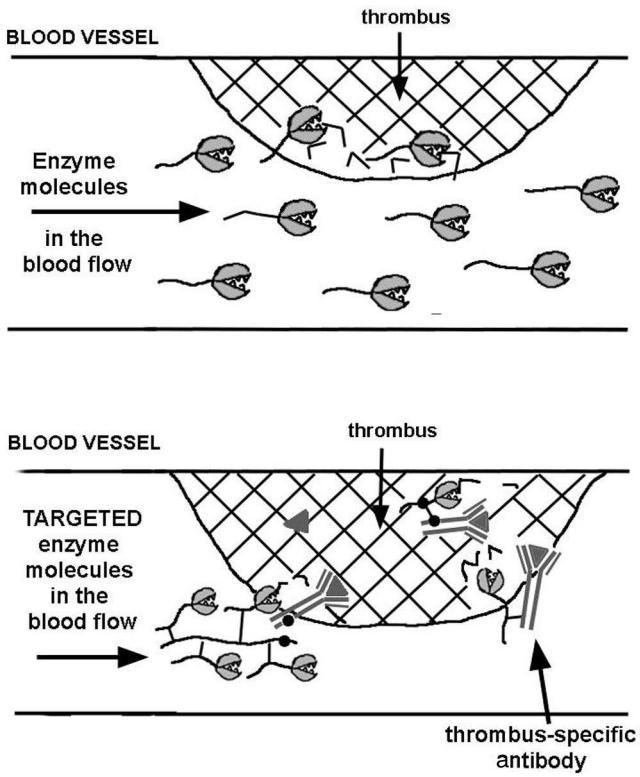

Fig. 6. The schematic pattern of the thrombus lysis under the action of non-targeted (upper panel) and targeted (conjugated with thrombus-specific antibody, lower panel) thrombolytic enzyme.

The more sophisticated method of an antibody use for the targeting of thrombolytics is connected with the application of bifunctional (chimeric) antibodies. Such antibodies possess double specificity — toward thrombus component and toward activating or lytic component of fibrinolytic system of the organism itself (usually, toward plasminogen or urokinase) (Schnee et al., 1987). Being injected intravenously, these antibodies can perform 'targeting in situ' — one part of their molecule binds with the thrombus, whereas another part 'catches' fibrinolytic molecules from the blood and increases their concentration on the surface of the thrombus. Chimeric antibodies can be prepared both by chemical coupling (Charpie et al., 1990) and by cell fusion (Branscomb et al., 1990). Chemical coupling via bis(maleimido methyl) ester was used, in particular, to prepare 7E3×P4B6 bispecific $(Fab')_2$ which recognizes both the platelet GPIIb/IIIa receptor and human tissue plasminogen activator (Neblock et al., 1992). In all the cases mentioned chimeric antibodies provoked sharp increase in the thrombolysis rate in vitro and in vivo. It was proved, for example, in baboons and hamsters, when effective thrombolysis was performed by the conjugate between single-chain urokinase-type plasminogen activator and a bispecific monoclonal antibody against this activator and fibrin (Imura et al., 1992). The data on the antibody use for targeted thrombolysis as well as on various thrombus-specific antibodies themselves are numerous and well reviewed (Torchilin, 1992; Haber, 1994).

3.2. Targeting of atherosclerotic lesions

Another important target within the cardiovascular system is atherosclerotic lesions. Both diagnostic (imaging) and therapeutic agents might be targeted to these lesions. Possible targets within the lesion include low density lipoproteins (LDL), cells of monocyte–macrophage lineage, activated platelets and platelet aggregates, proliferating smooth muscle cells, collagen and other proteins of the extracellular matrix (Narula et al., 1994). LDL are accumulating in the lesion through the permeabilized regenerated endothelium. With this in mind, LDL themselves can be radiolabeled to follow their accumulation in the affected zone, of the accumulation of radiolabeled antibodies against LDL can be followed (Lees et al., 1988). It is also known that all metabolically active atherosclerotic plaques contain proliferating smooth muscle cells (Narula et al., 1994). So, the antigens typical for such cells can also be used for targeted delivery of imaging or therapeutic agents into the plaque. Specific monoclonal Z2D3 IgM antibody labeled with ^{111}In was shown to be

effective in imaging areas of experimental atherosclerosis in rabbits (Narula et al., 1994, 1995).

3.3. Targeted liposomal carriers

To obtain targeted liposomes, different methods have been developed to bind corresponding vectors to the liposome surface. Immunoglobulins, primarily of IgG class, are the most promising and widely used targeting moieties. Numerous methods for antibody coupling to liposomes are reviewed in (Torchilin, 1984; Torchilin and Klibanov, 1993). They meet the following most important requirements: (a) antibody specificity and affinity should not change upon the binding; (b) a sufficient quantity of transport molecules should be firmly bound to the liposome surface; (c) liposomal integrity during binding procedure has to be preserved; (d) the binding should be simple and with high yield. At present as much as 60 to 100 antibody molecules can be attached to a single 200 nm liposome, which provides firm multi-point liposome binding with a target.

The initial stage of many vessel injuries, including atherosclerosis and thrombosis (coronary ones among them), is a disruption of the vessel wall endothelial cover integrity leading to subendothelial denudation, serving as a strong stimulator of platelet activation and adhesion (Ross, 1993). Naturally, it is tempting to think of early detection of such disruptions of endothelium, and direct action at these sites to promote endothelium growth or prevent platelet adhesion onto the exposed collagen. To prove the possibility of using targeted immunoliposomes as specific drug carriers to such areas, conjugates have been obtained between liposomes and antibodies against extracellular matrix antigens — collagen, laminin, fibronectin (Chazov et al., 1981; Smirnov et al., 1986). The data obtained clearly demonstrated that anti-collagen-liposomes and other matrix-specific liposomes could specifically recognize and bind collagen gaps between endothelial cells both in cell cultures and in perfused blood vessels (Fig. 7).

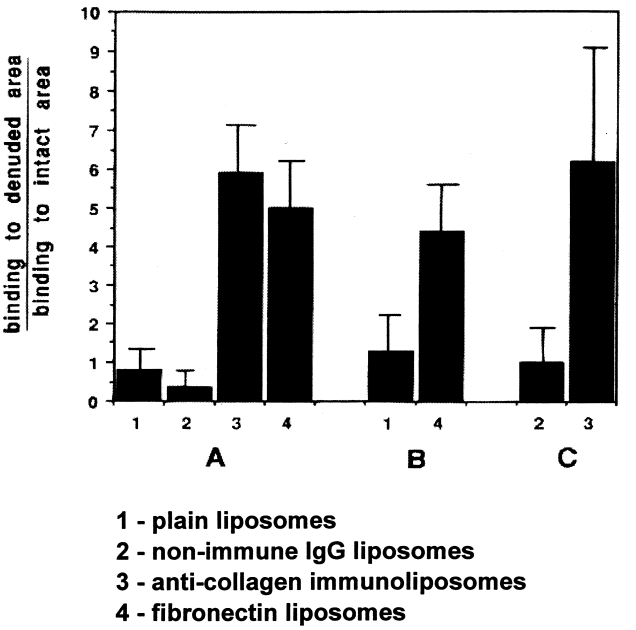

Fig. 7. The binding of various liposomal preparations with intact and denuded areas of perfused human (A), bovine (B) and rabbit (C) blood vessels. Plain liposomes and liposomes bearing non-specific antibody were used as controls that did nod differentiate between normal and denuded areas. Specific liposomes (liposomes bearing anti-collagen antibody or protein fibronectin possessing high affinity toward collagen) clearly demonstrated preferential binding to denuded areas of the vessel wall. Adapted from Smirnov et al., 1986.

3.4. Blood pool targeting

An interesting case of intravascular targeting is the targeting of the blood pool itself. This case includes, mainly, diagnostic (imaging) agents able to stay in the circulation long enough and to provide the information about the status of blood flow in different body compartments. The imaging of the blood pool and determination of functional data of the blood flow are especially important in the diagnosis of cardiovascular and thromboembolic diseases. It may be also useful in the detection of abnormal vascular permeability. Blood pool imaging can be performed in different imaging modalities, such as magnetic resonance imaging, gamma-scintigraphic imaging, and computer tomography. The appropriate imaging agents have to possess long circulation in the blood, low toxicity and immunogenicity, and good excretability. Various macromolecular contrast agents have been already suggested for the blood pool imaging, such as isotope-labeled polymers, label-loaded liposomes, stabilized magnetite colloids, and iodine-containing nanocrystals (Gazelle et al., 1993; Niemi, 1995).

3.5. Targeting of pharmaceuticals to the lungs

Drug targeting to the lungs, as well as carriers and methods for such targeting have been already well reviewed in the literature (see for example Gonda, 2000). The physiology of lungs makes it possible to target them via two different routes — through the circulation and through the respiratory tract (by parenteral or inhalatory administration, respectively). It is interesting, however, that the same drug carriers might be utilized in both cases, such as, for example, liposomes.

Numerous attempts have been made to use immunotoxins for targeted treatment of malignant lung diseases, first of all, human small cell lung cancer (SCLC). Thus, murine monoclonal antibodies recognizing the cluster 2 antigen associated with human SCLC was shown to mediate the toxic effect of ricin A chain in an indirect assay of immunotoxin cytotoxicity (Derbyshire and Wawrzynczak, 1991). Similar experiments were performed with murine antibodies against human SCLC cell line

SW2. Ricin A chain conjugated with these antibodies recognized cluster w4 and cluster 5A antigens and expresses cytotoxic effect (Wawrzynczak et al., 1991). Some other antibodies against SCLC antigens were also used for the preparation of cytotoxic immunotoxins, mainly, with ricin A chain (Zangemeister-Wittke et al., 1994). Antibody 174H.64-DM against the proliferative compartment of mammalian squamous carcinomas was conjugated with daunomycin and sharply enhanced the potency of the latter in the murine model involving the use of KLN-205 cell line, which metastasizes to the lungs following intravenous injection and shows a pattern of growth similar to those of spontaneous squamous carcinoma (Ding et al., 1990). Murine monoclonal antibody NCC-LU-243 was conjugated with mitomycin C and used for the targeted therapy of nude mice with transplanted antigen-positive cell line of human SCLC (Kubota et al., 1992). Radiolabeled antibodies specifically recognizing SCLC cells were suggested as potential targeted agents for diagnostic visualization of this form of cancer, as well as for its radiotherapy (Okabe and Takaku, 1986).

3.6. Targeting of pharmaceuticals to the heart

Like for any other organ of interest, the targeting of pharmaceuticals to the heart has two main objectives: diagnostic imaging of cardiac pathologies and delivery of therapeutics to affected areas.

Monoclonal antibodies provide an effective method for noninvasive detection and visualization of different cardiac disorders, acute myocardial infarction being among them. Taking into account high frequency of this disease, its fast and specific diagnostics is the matter of primary importance. The general strategy toward monoclonal antibody-mediated infarct visualization was discussed recently by Khaw (Khaw, 1994). The approach used is based on the fact that following myocardial cell death as a result of ischemia, an antibody against intracellular antigen will be able to differentiate between viable cells with intact membranes and necrotic cells with disrupted membranes. Cardiac myosin, which is not washed away following cell disintegration, was chosen as the target antigen characteristic of infarcted myocardium (Khaw et al., 1979, 1982). The efficacy of anti-myosin antibodies labeled with I^{131}, ^{111}In and ^{99m}Tc for gamma-visualization of myocardial infarction was proved in rabbit and dog experiments (Khaw et al., 1978). Radiolabeled Fab fragment of anti-myosin R11D10 antibody is already successfully used in clinical condition (Johnson et al., 1989).

3.7. Immunoliposome targeting to the heart

Caride and Zaret (1977) described spontaneous accumulation of positively charged liposomes in regions of experimental myocardial infarction. Later, it was found that liposome accumulation in ischemic tissues is a rather general phenomenon and might be explained by impaired filtration in ischemic areas, which results in trapping of liposomes there (Cole et al., 1982; Palmer et al., 1981). This observation lead to the conclusion, that drug-loaded liposomes can be used for ‘passive’ drug delivery into the ischemic tissues, primarily, into the infarcted myocardium (Palmer et al., 1984b). However, the efficacy of the approach was rather low.

Attempts have been also made to use antimyosin antibody for ‘active’ targeting of liposomes to infarcted myocardium (Torchilin et al., 1979, 1992). First, the preservation of antimyosin antibody activity after coupling to liposomes was proved by unchanged in vitro binding of antimyosin-liposomes to canine cardiac myosin. In vivo studies were performed on dogs with experimental myocardial infarction. Antimyosin-liposomes containing inside $^{111}InCl_3$ demonstrated good accumulation in the infarct (Torchilin et al., 1979). Later, liposomes were prepared, containing on their surface both antimyosin and polyethylene glycol (PEG), and possessing with both abilities — to recognize and bind the target and to circulate long enough providing high target accumulation (Torchilin et al., 1992). Two different ways have been found for liposome accumulation in the infarcted heart (and, probably, in any ischemic area) — the specific one, which requires the presence of antibodies on the surface of liposomes, and the non-specific one, which proceeds via impaired filtration mechanism in affected tissues and requires many passages of liposomes through the target, i.e. prolonged circulation. The combination of Fab and PEG on the liposome surface gives absolutely maximal radioactivity accumulation in the infarct, because both accumulation mechanisms are working in this particular case resulting in additive effect (Torchilin et al., 1996).

3.8. Targeting of ischemic cardiocytes with immunoliposomes

Various pathological conditions including hypoxia and inflammation induce cell membrane lesions. The presence of these lesions which represent microscopic holes in the sarcolemma permits washout of intracellular macromolecules into the circulation. On the other hand, certain intracellular proteins of the cytoskeleton (myosin, vimentin) become exposed through these holes to the extracellular milieu. Appropriately radiolabeled antibodies against intracellular cytoskeletal antigens have been described to delineate cell membrane lesions. Moreover, if these antibodies are coupled to liposomes, such reagents acquire an ability of targeted delivery of these phospholipid vesicles to the sites of membrane lesions of the affected cells and anchor (‘plug’) them directly into (over) the holes (lesions); the principal scheme of the approach is shown in Fig. 8. The phenomenon of ‘‘plug and seal’’ to prevent necrotic cell death was demonstrated using myosin as the cytoskeletal target antigen and the corresponding an-

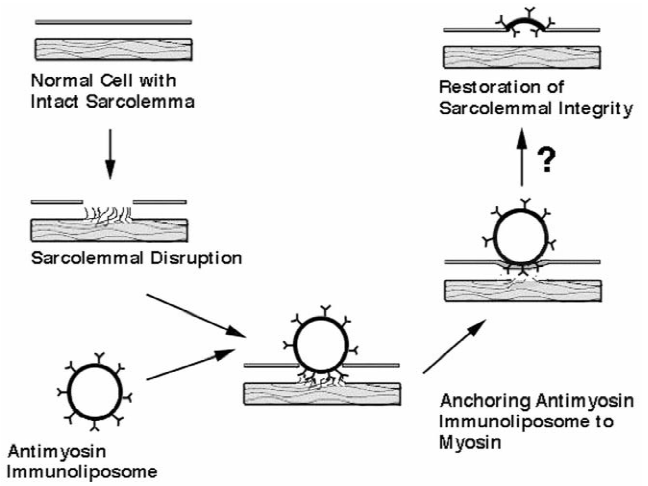

Fig. 8. Diagrammatic representation of the hypothesized mechanism of cell membrane sealing and salvage by antimyosin-liposomes. Modified from Khaw et al., 1995.

timyosin antibody as the anchoring device incorporated in the liposomes, and tested in a hypoxic model of injury of H9C2 rat embryonic cardiocytes (Khaw et al., 1995). H9C2 cardiocytes in hypoxic culture conditions were incubated with antimyosin-immunoliposomes (IL), plain liposomes (PL) and control non-specific IgG liposomes (IgL). Hypoxic and normoxic cardiocytes without liposome treatment were used as additional controls.

Assessment of the viability of the cells was performed after 24 h of hypoxia by trypan-blue exclusion method or by immediate further incubation of the cells with [^{3}H]-thymidine (3HT), see Table 1. According to the data obtained, almost all control hypoxic cardiocytes were non-viable. Plain liposomes provided some protection from hypoxic injury, probably, by nonspecifically sticking to cell surfaces and fortuitously 'seal' some of the cell membrane breaches. Immunoliposomes almost completely prevented cell death with cell viability similar to that of normoxic cells. Hypoxic cells treated with non-specific IgG-liposomes demonstrated viability at the level of PL-treated cells. IL-treated hypoxic cells were growing normally for more than 7 days after the hypoxic event when subsequently cultured under normoxic conditions. These treated cells were observed to be able to replicate normally. Prevention of cell death by the targeted sealing of cell membrane lesions as described could have significant clinical utility.

4. Conclusion

Targeted delivery of pharmaceuticals could bring along a sharp improvement of existing approaches to diagnosis and treatment of various diseases. Currently, clinical drug targeting is mainly used for antibody-mediated diagnostic procedures. Therapeutic application of targeted drug delivery is so far limited to EPR effect (passive accumulation of doxorubicin-loaded long-circulating liposomes in tumors), though clinical trials of some immunotoxins and other antibody–drug conjugates are also under way. Still, numerous studies on various drugs targeting systems are believed to result in the development of new and effective clinical protocols in the near future. The examples presented in this brief review, show broad potential opportunities in using various strategies of drug targeting for resolving important clinical problems.

References

Bode, C., Matsueda, G.R., Hui, K.Y., Haber, E., 1985. Antibody-directed urokinase: a specific fibrinolytic agent. Science 229, 765–766.

Bode, C., Runge, M.S., Newell, J.B., Matsueda, G.R., Haber, E., 1987. Characterization of an antibody–urokinase conjugate. A plasminogen activator targeted to fibrin. J. Biol. Chem. 262, 10819–10824.

Bos, R., Nieuwenhuizen, W., 1992. The potential improvement of thrombolytic therapy by targeting with bispecific monoclonal antibodies: why they are used and how they are made. Biotherapy 5, 187–199.

Branscomb, E.E., Runge, M.S., Savard, C.E., Adams, K.M., Matsueda, G.R., Haber, E., 1990. Bispecific monoclonal antibodies produced by somatic cell fusion increase the potency of tissue plasminogen activator. Thromb. Haemost. 64, 260–266.

Caride, V.J., Zaret, B.L., 1977. Liposome accumulation in regions of experimental myocardial infarction. Science 198, 735–738.

Charpie, J.R., Runge, M.S., Matsueda, G.R., Haber, E., 1990. A bispecific antibody enhances the fibrinolytic potency of single-chain urokinase. Biochemistry 29, 6374–6378.

Chazov, E.I., Matveeva, L.S., Mazaev, A.V., Sargin, K.E., Sadovskaya,

Table 1
Assessment of hypoxic cell viability in the presence of different liposomal preparations by trypan blue exclusion test and by ^{3}H-thymidine uptake[a]

Cells and treatment conditions	Viability by trypan blue (% of initial cell number)	Viability by ^{3}H-thymidine (% of control)
Normoxic cardiocytes (control)	98	100
Hypoxic cardiocytes	13	3
Hypoxic cardiocytes+PL	42	31
Hypoxic cardiocytes+IgL	43	–
Hypoxic cardiocytes+IL	96	89

[a] Viability by Trypan Blue was assessed utilizing all cells in each culture flask in triplicates. Viability by ^{3}H-T uptake was assessed by incubating myocytes cultured under hypoxic or normoxic conditions for 24 h with or without liposomes with 5 μCi of ^{3}H-T under normal culture conditions for another 24 h.

G.V., Ruda, M.Ya, 1976. Intracoronary administration of fibrinolysin in acute myocardial infarct. Ter. Arkh. (Russ.) 48 (4), 8–19.

Chazov, E.I., Alexeev, A.V., Antonov, A.S., Koteliansky, V.E., Leytin, V.L., Ljubimova, E.V., Repin, V.S., Sviridov, D.D., Torchilin, V.P., Smirnov, V.N., 1981. Endothelial cell culture on fibrillar collagen: Model to study platelet adhesion and liposome targeting to intercellular collagen matrix. Proc. Natl. Acad. Sci. USA 78, 5603–5607.

Cole, A.W., Kingaby, R.O., Lab, M.J., Palmer, T.N., 1982. Myocardial liposome uptake in the early stages of myocardial infarction. Cardiovasc. Res. 16, 516–523.

Derbyshire, E.J., Wawrzynczak, E.J., 1991. Monoclonal antibodies recognizing the cluster 2 antigen associated with human small cell lung cancer mediate the toxic effects of ricin A chain in an indirect assay of immunotoxin cytotoxicity. Br. J. Cancer (Suppl.) 14, 74–77.

Ding, L., Samuel, J., MacLean, G.D., Noujaim, A.A., Diener, E., Longenecker, B.M., 1990. Effective drug-antibody targeting using a novel monoclonal antibody against the proliferative compartment of mammalian squamous carcinomas. Cancer Immunol. Immunother. 32, 105–109.

Francis, G.E., Delgado, C. (Eds.), 2000. Drug Targeting. Strategies, Principles, and Applications. Humana Press, Totwa, NJ.

Gabizon, A.A., 1992. Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res. 52, 891–896.

Gazelle, G.S., Wolf, G.L., McIntire, G.L., Bacon, E.R., Cooper, E.R., Toner, J.L., 1993. Nanocristalline CT contrast agents for blood pool and liver-spleen imaging. Radiology 189 (P), 296–297.

2000. Delivery to the lungs. Gonda, I. (Ed.), Adv. Drug Delivery Rev 5.

Gregoriadis, G., 1977. Targeting of Drugs. Nature 165, 407–411.

Haber, E., 1994. Antibody targeting as a strategy in thrombolysis. In: Khaw, B.A., Narula, J., Strauss, H.W. (Eds.), Monoclonal Antibodies in Cardiovascular Diseases. Lea and Febiger, Malvern, pp. 187–197.

Imura, Y., Stassen, J.M., Kurokawa, T., Iwasa, S., Lijnen, H.R., Collen, D., 1992. Thrombolytic and pharmacokinetic properties of an immunoconjugate of single-chain urokinase-type plasminogen activator (u-PA) and a bispecific monoclonal antibody against fibrin and against u-PA in baboons. Blood 79, 2322–2329.

Jain, R.K., 1987. Transport of molecules across tumor vasculature. Cancer Metastasis Rev. 6, 559–593.

Johnson, L.L., Seldin, D.W., Becker, L.C., LaFrance, N.D., Liberman, H.A., James, C., Mattis, J.A., Dean, R.T., Brown, J., Reiter, A., Arneson, V., Cannon, P.J., Berger, H.J., 1989. Antimyosin imaging in acute transmural myocardial infarction: results of a multicenter clinical trial. J. Am. Coll. Cardiol. 13, 27–35.

Kanke, M., Matsueda, G.R., Strauss, H.W., Yasuda, T., Liau, C.-S., Khaw, B.A., 1991. Localization and visualization of pulmonary emboli with radiolabeled fibrin-specific monoclonal antibody. J. Nucl. Med. 32, 1254–1260.

Khaw, B.A., Beller, G.A., Haber, E., 1978. Experimental myocardial infarct imaging following intravenous administration of iodine-131 labeled antibody $(Fab')_2$ fragments specific for cardiac myosin. Circulation 57, 743–750.

Khaw, B.A., Fallon, J.T., Beller, G.A., Haber, E., 1979. Specificity of localization of myosin specific antibody fragments in experimental myocardial infarction: histologic, histochemical, autoradiographic and scintigraphic studies. Circulation 60, 1527–1531.

Khaw, B.A., Scott, J., Fallon, J.T., Haber, E., Homcy, C., 1982. Myocardial injury: quantitation by cell sorting initiated with antimyosin fluorescent spheres. Science 217, 1050–1053.

Khaw, B.A., 1994. Antimyosin antibody for the diagnosis of acute myocardial infarction: experimental validation. In: Khaw, B.A., Narula, J., Strauss, H.W. (Eds.), Monoclonal Antibodies in Cardiovascular Diseases. Lea and Febiger, Malvern, pp. 15–29.

Khaw, B.A., Torchilin, V.P., Vural, I., Narula, J., 1995. Plug and seal: prevention of hypoxic cardyocyte death by sealing membrane lesions with antimyosin-liposomes. Nat. Med. 1, 1195–1198.

Knight, L.C., 1994. Antifibrin antibody for detection of deep vein thrombosis. In: Khaw, B.A., Narula, J., Strauss, H.W. (Eds.), Monoclonal Antibodies in Cardiovascular Diseases. Lea and Febiger, Malvern, pp. 171–186.

Kubota, T., Yamamoto, T., Takahara, T., Furukawa, T., Ishibiki, K., Kitajima, M., Shida, Y., Nakasubo, H., 1992. Targeting cancer chemotherapy using a monoclonal antibody (NCC-LU-243) conjugated with mitomycin C. J. Surg. Oncol. 51, 75–80.

Lee, F.T., Scott, A., Bautovich, G.J., 1995. Radioimmunodetection of thrombi. In: Torchilin, V.P. (Ed.), Handbook of Targeted Delivery of Imaging Agents. CRC Press, Boca Raton, pp. 445–464.

Lees, A.M., Lees, R.S., Schoen, F.J., Isaacson, J.L., Fischman, A.J., McKusick, K.A., Strauss, H.W., 1988. Imaging human atherosclerosis with ^{99m}Tc-labeled LDL. Atherosclerosis 8, 461–470.

Narula, J., Ditlow, C., Chen, F., Khaw, B.A., 1994. Monoclonal antibodies for the detection of atherosclerotic lesions. In: Khaw, B.A., Narula, J., Strauss, H.W. (Eds.), Monoclonal Antibodies in Cardiovascular Diseases. Lea and Febiger, Malvern, pp. 206–215.

Narula, J., Petrov, A., Bianchi, C., Ditlow, C.C., Dilley, J., Pieslak, I., Chen, F.W., Torchilin, V.P., Khaw, B.A., 1995. Noninvasive localization of atherosclerotic lesions with mouse/human chimeric Z2D3 antibody specific for the proliferating smooth muscle cells of human ahteroma: imaging with conventional antibody and image enhancement with negative charge-modified antibody. Circulation 92, 474–484.

Neblock, D.S., Chang, C.H., Mascelli, M.A., Fleek, M., Stumpo, L., Cullen, M.M., Daddona, P.E., 1992. Conjugation and evaluation of 7E3×P4B6, a chemically cross-linked bispecific F(ab′)2 antibody which inhibits platelet aggregation and localized tissue plasminogen activator to a platelet surface. Bioconjugate Chem. 3, 126–131.

Niemi, P., 1995. Intravascular magnetic resonance imaging contrast media. In: Torchilin, V.P. (Ed.), Handbook of Targeted Delivery of Imaging Agents. CRC Press, Boca Raton, pp. 487–500.

Okabe, T., Takaku, F., 1986. Diagnostic and therapeutic applications of monoclonal antibodies to small cell carcinoma of the lung. Jpn. J. Clin. Oncol. 16, 243–252.

Orekhova, N.M., Akchurin, R.S., Belyaev, A.A., Smirnov, M.D., Ragimov, S.E., Orekhov, A.N., 1990. Local prevention of thrombosis in animal areteries by means of magnetic targeting of aspirin-loaded red cells. Thromb. Res. 57, 611–616.

Palmer, T.N., Caride, V.J., Fernandez, L.A., Twickler, J., 1981. Liposome accumulation in ischaemic intestine following experimental mesenteric occlusion. Biosci. Rep. 1, 337–344.

Palmer, T.N., Caldercourt, M.A., Kingaby, R.O., 1984a. Liposome drug delivery in chronic ischemia. Biochem. Soc. Trans. 12, 344–345.

Palmer, T.N., Caride, V.J., Caldecourt, M.A., Twicjler, J., Abdullah, V., 1984b. The mechanism of liposome accumulation in infarction. Biochim. Biophys. Acta 797, 363–368.

Poste, G., Kirsh, R., 1983. Site-specific (targeted) drug delivery in cancer therapy. Biotechnology 1, 869–878.

Ringsdorf, H., 1975. Structure and properties of pharmacologically active polymers. J. Polym. Sci. 51, 135–153.

Ross, R., 1993. The pathogenesis of atherosclerosis: a perspective for 1990s. Nature 362, 801–809.

Schnee, J.M., Runge, M.S., Matsueda, G.R., Hudson, N.W., Seidman, J.G., Haber, E., Quertermous, T., 1987. Construction and expression of a recombinant antibody-targeted plasminogen activator. Proc. Natl. Acad. Sci. USA 84, 6904–6908.

Smirnov, V.N., Domogatsky, S.P., Dolgov, V.V., Hvatov, V.B., Klibanov, A.L., Koteliansky, V.E., Muzykantov, V.R., Repin, V.S., Samokhin, G.P., Shekhonin, B.V., Smirnov, M.D., Sviridov, D.D., Torchilin, V.P., Chazov, E.I., 1986. Carrier-directed targeting of liposomes and erythrocytes to denuded areas of vessel wall. Proc. Natl. Acad. Sci. USA 83, 6603–6607.

Torchilin, V.P., Khaw, B.A., Smirnov, V.N., Haber, E., 1979. Preservation of antimyosin antibody activity after covalent coupling to liposomes. Biochem. Biophys. Res. Commun. 89, 1114–1119.

Torchilin, V.P., 1984. Immobilization of specific proteins on liposome

surface: systems for drug targeting. In: Gregoriadis, G. (Ed.), 1st Edition. Liposome Technology, Vol. 3. CRC Press, Boca Raton, pp. 75–94.

Torchilin, V.P., Papisov, M.I., Orekhova, N.M., Belyaev, A.A., Petrov, A.D., Ragimov, S.E., 1988. Magnetically driven thrombolytic preparation containing immobilized streptokinase — targeted transport and action. Haemostasis 18, 113–116.

Torchilin, V.P., 1992. Targeting of thrombolytic agents: current state of knowledge and perspectives. Ann. NY Acad Sci. 667, 404–416.

Torchilin, V.P., Klibanov, A.L., Huang, L., O'Donnell, S., Nossiff, N.D., Khaw, B.A., 1992. Targeted accumulation of polyethylene glycol-coated immunoliposomes in infarcted rabbit myocardium. FASEB J. 6, 2716–2719.

Torchilin, V.P., Klibanov, A.L., 1993. Coupling and labeling of phospholipids. In: Cevc, G. (Ed.), Phospholipids Handbook. Marcel Dekker, New York, pp. 293–321.

Torchilin, V.P., Zhou, F., Huang, L., 1993. pH-Sensitive liposomes. J. Liposome Res. 3, 201–255.

Torchilin, V.P., Narula, J., Halpern, E., Khaw, B.A., 1996. Poly(ethylene glycol)-coated anti-cardiac myosin immunoliposomes: factors influencing targeted accumulation in the infarcted myocardium. Biochim. Biophys. Acta 1279, 75–83.

Vitetta, E.S., Krolik, K.A., Miyama-Inaba, M., Cushley, W., Uhr, J.W., 1983. Immunotoxins: a new approach to cancer therapy. Science 219, 644–650.

Wasser, M.N.J.M., Pauwels, E.K.J., 1990. Immunoscintigraphy of thrombosis. Eur. J. Nucl. Med. 16, 583–588.

Wawrzynczak, E.J., Derbyshire, E.J., Henry, R.V., Pamell, G.D., Smith, A., Waibel, R., Stahel, R.A., 1991. Cytotoxic activity of ricin A chain immunotoxins recognising cluster 1, w4 and 5A antigens associated with human small cell lung cancer. Br. J. Cancer (Suppl.) 14, 71–73.

Weinstein, J.N., Magin, R.L., Yatvin, M.B., Saharko, D., 1979. Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. Science 204, 188–191.

Weissig, V., Whiteman, K.R., Torchilin, V.P., 1998. Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous Lewis lung carcinoma in mice. Pharm. Res. 15, 1552–1556.

Widder, K.J., Marino, P.A., Morris, R.M., Senyei, A.E., 1983. Targeting antineoplastic agents using magnetic albumin microspheres. In: Goldberg, E. (Ed.), Targeted Drugs. John Wiley and Sons, New York, pp. 201–230.

Williams, A.S., Camilleri, J.P., Goodfellow, R.M., Williams, B.D., 1996. A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis. Br. J. Rheumatol. 35, 719–724.

Zangemeister-Wittke, U., Collinson, A.R., Frosch, B., Waibel, R., Schenker, T., Stahel, R.A., 1994. Immunotoxins recognising a new epitope on the neural cell adhesion molecule have potent cytotoxic effects against small cell lung cancer. Br. J. Cancer 69, 32–39.