Essential properties of drug-targeting delivery systems

Karel Petrak

How, if at all, can drug delivery help to create ideal drugs? After four decades of trying, an effective site-specific drug-delivery system has not yet been developed. This review draws attention to the pharmacokinetic conditions that must be met to achieve a successful performance by site-selective drug–carrier delivery systems. In a drug–carrier approach, a drug is attached to a macromolecular carrier via a chemically labile linker. The carrier transports the drug to its site of action and releases it at the target site. For this simple approach to work, several fundamental conditions (nonspecific interactions, target site access, drug release and drug suitability) must be satisfied. The importance of these essential requirements, not always recognized in the development of drug-delivery systems, is discussed and illustrated by recent examples selected from the literature.

Can drug delivery help to create ideal drugs? Over a century ago, Paul Ehrlich [1] described a drug that is aimed precisely at a disease site and that would not harm healthy tissues as a 'magic bullet'. However, at therapeutic concentrations, very few drugs bind solely to their intended therapeutic target. A concept of site-specific drug-delivery systems was formed and, according to this concept, a drug would be attached to a carrier that would take the 'pay-load' (the drug) to the target (attached to the carrier via a targeting ligand) and release it at the target site. The practical realization of this concept has fascinated and eluded scientists ever since.

After early attempts by Wade *et al.* [2], four decades of research have not yet produced an effective, generally applicable, site-specific drug-delivery system [3]. Only 'target-homing' drugs that specifically recognize their pharmacological target have achieved any degree of site-selective delivery. For example, Rituxan® was the first therapeutic antibody approved by the FDA (in November 1997) for treating cancer. Rituxan® works by binding to a particular protein (the CD20 antigen), located on the surface of normal and malignant B cells, that recruits the body's natural defenses to attack and kill the marked B cells. Stem cells (B-cell progenitors) in bone marrow lack the CD20 antigen, allowing healthy B cells to regenerate after treatment, returning to normal levels within months. So, although this antibody does not differentiate between normal and malignant cells, its action is limited to one particular, renewable cell type. Serious adverse events (fatal infusion reactions, tumor lysis syndrome and severe mucocutaneous reactions) are, nevertheless, still associated with this antibody treatment.

Essential properties of drug-targeting delivery systems

For a drug to exert its desired effect it needs to be in physical contact with its physiological target, such as a receptor. Site-selective drug delivery ensures

Karel Petrak
PJP Innovations,
707 Knox Street,
Houston,
Texas 77007,
USA
e-mail: kpetrak@houston.rr.com

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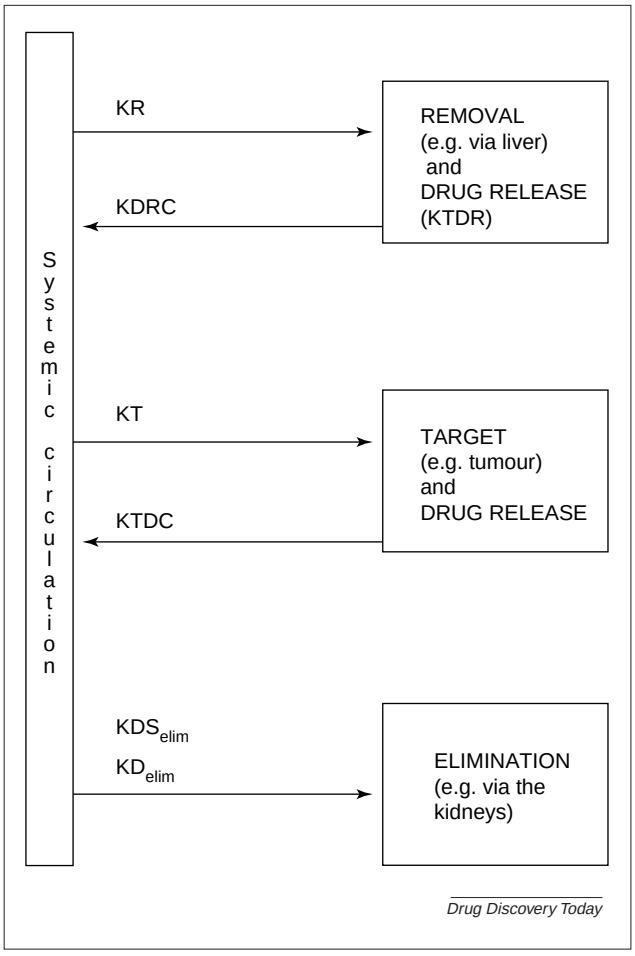

FIGURE 1

After systemic administration of a drug–carrier conjugate into the central (blood–lymph) compartment of the body, the access, retention, release and elimination of the conjugate and its individual elements to the relevant organ, tissue and, ultimately, to the drug target are determined (in the main) by the following rate processes: elimination of drug–carrier conjugate (KR), release of free drug at the non-target site (KDRC), delivery of drug–carrier conjugate to the target site (KT), release of free drug at the target site (KTDR), removal of free drug from the target site (KTDC), elimination of the drug–carrier conjugate (KDS$_{elim}$) and the free drug (KD$_{elim}$) from the body.

that such interactions take place only in the desired anatomical location of the body. Although, in principle, every drug might benefit from site-selective delivery not every drug is equally suitable for the process. Drugs that are not retained at the site of action for a long enough period of time will not benefit from site-specific release. Also, drugs that have the same site for efficacy and toxicity will not improve through site-selective delivery and their effect:side-effect ratio could even get worse. Evidently, drugs that already have an inherently high specificity for reaching and interacting with their targets, for example therapeutic antibodies, do not need to be considered for targeting. Several publications offer mathematical analyses of targeted delivery kinetics [4–7] and specify the properties needed for the site-specific delivery approach to work. It appears, however, that realizing these requirements has, to date, proved to be elusive.

The minimum requirements for a targeted drug-delivery system to have any chance of returning the 'wished for' performance must be considered. Focusing on the drug–carrier conjugate to be administered into the systemic compartment of the body, the key events governing such drug delivery are shown in Figure 1. The events are discussed in terms of rate constants corresponding to each depicted event. In an ideal delivery system the pharmacokinetic processes would progress at a rate that would maximize the chances of the eventual physical delivery of the drug to its target.

Rate of elimination of drug–carrier conjugate (KR)

It is essential that the drug–carrier conjugate is not removed too rapidly from the circulation. If it is eliminated from systemic circulation more rapidly than it is delivered to the target site, the amount of conjugate at the target site might never be enough to provide the required concentration of free (unbound) drug. The design and the production of the delivery system need to eliminate all nonspecific interactions occurring between the drug–carrier conjugate and the environment of the systemic compartment [8,9]. The central compartment of the body (blood and lymph) is essentially an aqueous, polar medium. Van Oss [10] noted that at least 17 different types of noncovalent interactions in polar media were reported in scientific literature and demonstrated that it is always possible to represent them (for aqueous media) by a composite of the three primary forces: electrodynamic, electrostatic and hydrogen bonding. In the design of a drug-delivery carrier, one needs to consider the contributions of these three primary forces to the carrier's overall properties. The most frequently employed approach is to use water-soluble, inert macromolecules as drug carriers, or to attach them (covalently or by adsorption) to the surface of drug-carrying particles. The function of the carrier is to mask all unwanted interactions between the drug and the environment until the drug is released from the carrier at the target site.

Rate of release of free drug at the non-target site (KDRC)

Depending on the amount of drug, the release of drug away from the target site could nullify any benefits that might potentially come from delivering the drug to the target site. This could be because the amount of drug reaching sites of systemic toxicity might become too high or, second, the amount of free drug that reaches the target site after it has been released from the conjugate at non-target sites might be greater than the amount of drug actually being delivered to the target using the delivery system. Every claim that a drug-delivery conjugate delivers the drug preferentially to the target site should be documented by measuring the actual amount of drug delivered; it should not be inferred from an observed change in the apparent efficacy of the drug conjugate. Examples of this will be given later in this review.

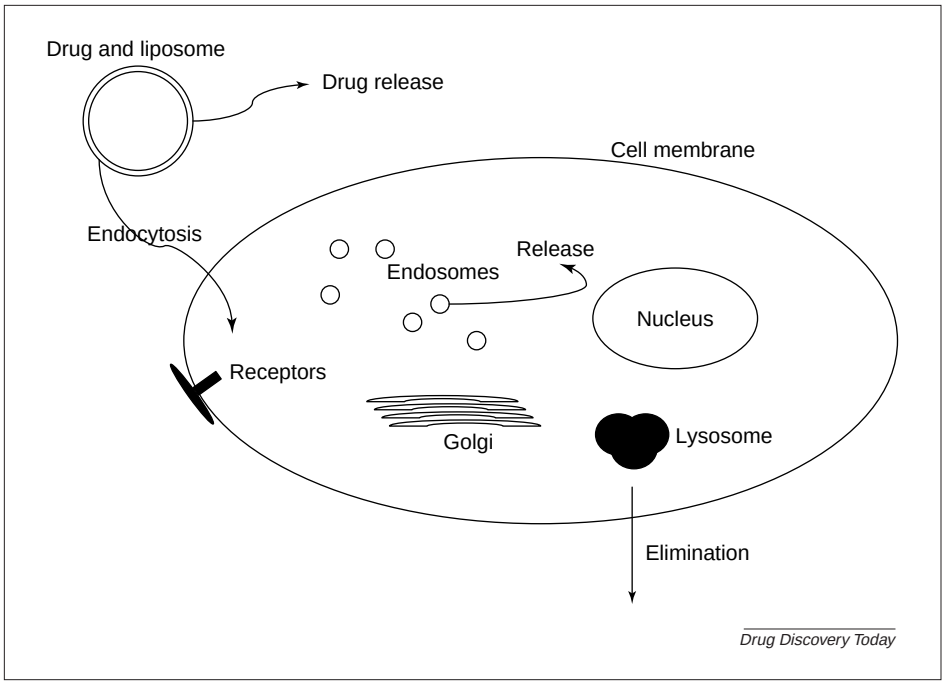

FIGURE 2

After a drug–carrier conjugate or liposome reaches the target cell, several additional processes, each controlled by their own rate constants, need to take place before the drug reaches the surface of its target cell (e.g. drug release, drug elimination, drug–receptor binding) or its intracellular target (e.g. receptor binding, endocytosis, lysosome uptake, nuclear targeting, drug release, drug elimination, drug binding, etc.).

Rate of delivery of drug–carrier conjugate to the target site (KT)

If the drug conjugate reaches the target site too slowly, the supply of free drug (as governed by the KTDR rate constant) might never be sufficient to generate the concentration required to elicit the desired therapeutic effect at the site of action. The total amount of drug delivered (i.e. the area under the curve in a drug concentration versus time plot for the target site) is irrelevant if, at any time, the free-drug concentration at the target site does not reach its pharmacologically effective level. Delivery of the drug–carrier conjugate to the target organ might not guarantee that an adequate amount of the free drug will be available at the actual target (in particular intracellular targets, such as the nucleus, mitochondria and Golgi) because additional rates of transport (e.g. passive or active across the cell membrane, release from vesicles and transport to the nucleus) and processing (drug release, drug metabolism, etc.) will influence the overall outcome (Figure 2). A similar mathematical machinery, as used for the overall compartmentalized body pharmacokinetics, should be applied to the drug delivery at the cellular level but this is beyond the scope of this review. Intuitively it would not be expected that, for example, a drug (such as DNA plasmid), delivered to the surface of a cell via a ligand–receptor targeting liposome, would necessarily reach its intracellular target. Even DNA plasmid, released intracellularly from an endocytosed liposome, will need to overcome several intracellular hurdles (e.g. degradation, elimination, nuclear localization, etc.) to reach its intracellular nuclear destination efficiently (Figure 2). A novel lipopolymer for gene delivery – branched methoxy-poly(ethylene glycol)-poly(ethyleimine)-cholesterol – has recently been reported by Fewell *et al.* [11]. However, to deliver the lipopolymer DNA to tumor cells, direct intratumoral administration of the drug–carrier conjugate was required, and all of the other requirements discussed in this review would need to be met for the system to work systemically.

Rate of release of free drug at the target site (KTDR)

The capacity of the system selected for the release of free drug from the conjugate should be considered. It needs to be suitable for processing the entirety of the drug–carrier conjugate arriving at the target site, doing so at a rate that also ensures drug accumulation at this site.

Rate of removal of free drug from the target site (KTDC)

Drugs that benefit most from target-selective delivery are those that are retained at the site while acting on their target of action. Therefore, drugs need to be specifically designed to be used with target-selective delivery systems and drug delivery should not be used for rescuing poorly performing, existing drugs. Certain drugs (e.g. DNA in gene therapy) will need to be delivered into the cytoplasm; therefore, it would be preferential for the release of the drug to take place within the cells. This could lead to the enhanced retention of the drug in close proximity to its target. As shown previously [7], an increased rate of elimination of free drug from the central compartment tends to increase the advantage brought about as a result of drug targeting, but also increases the required rate of input (of the drug carrier) to maintain a therapeutic effect.

Rate of elimination of the drug–carrier conjugate and free drug from the body (KDS_{elim} and KD_{elim})

For optimal targeting, elimination of the complete drug–carrier system should be minimal. These systems are frequently too large to be eliminated via the kidneys [12]. Consequently, the liver is mainly responsible for the removal of drug conjugates from the circulation. The rate of elimination of free drug from the systemic circulation should be rapid relative to its rate of transfer from the target site to the central compartment of the body. This way, the drug-delivery system will at least achieve a decrease in the drug-associated toxicity (in cases when the site of toxicity is different from the site of therapeutic effect).

BOX 1

Passive carriers can deliver drugs by responding to the local conditions in the body.

Recently, acid- and salt-triggered multifunctional poly(propylene imine) dendrimers have been offered as a prospective drug-delivery system [38] employing poly(ethylene glycol) chains for stability and protection, as well as guanidium groups for targeting. The release of drugs is achieved through a change in pH. The *in vivo* applicability remains to be demonstrated.

Another polymeric system claiming site-specific distribution is a water-soluble poly(vinylpyrrolidone-co-dimethyl maleic anhydride) [39]. Poly(VP-co-DMMAn)-modified superoxide dismutase has been claimed to accumulate in the kidneys after intravenous administration and accelerated recovery from acute renal failure in a mouse model more effectively than superoxide dismutase alone. This example utilizes the natural route by which an inert macromolecule is eliminated from the body via the kidneys.

BOX 2

A human clinical study with a macromolecular prodrug.

Studies in humans provide the ultimate test for all drug delivery systems. The macromolecular prodrug, DE-310, studied by Wente *et al.* [40] is composed of the topoisomerase-I-inhibitor DX-8951 (exatecan) and a biodegradable macromolecular carrier, covalently linked by a peptidyl spacer. Six patients with different, solid tumor types received 6 mg/m^2 DE-310 (equivalent to DX-8951) as a single, three-hour infusion, administered seven days before scheduled tumor resection. No preferential accumulation of DE-310, DX-8951 and G-DX-8951 in human tumor tissues was observed. It is, however, questionable as to whether drug measurements (at a single time-point) taken seven days after administration are representative of the whole drug-distribution process.

Details of these brief conclusions, derived using the tools of mathematical modeling, can be found in the reference by Boddy *et al.* [7]. It would be prudent to pay attention to these considerations at the start of any drug–carrier-system development. It might also be worth determining some of the key characteristics of the drug-delivery system and the drug to be delivered *in vitro*, before using them *in vivo* [13]. For example, opsonizing protein–drug–carrier interactions should be determined *in vitro* [8]. The opsonization of foreign particles, such as bacteria, with so-called opsonins (e.g. IgG antibody molecules, fibrinogen and complement protein C3b) by phagocytes is a method of marking them for destruction. Phagocytes contain surface receptors that bind to these opsonins and the invading particle is engulfed, surrounded and phagocytozed. To test the opsonization of putative drug-delivery particles with fibrinogen *in vitro*, measurement of the isothermal adsorption of ^{125}I-fibrinogen onto the particles can be used. The lower the plateau adsorption of fibrinogen, the less likely the particles will be opsonized *in vivo* and the more likely it is that they will remain in the circulation [14].

How has recent work addressed the demands of site-specific drug delivery?

Many proposed drug-delivery systems, such as liposomes and nanoparticles, have been linked to the promise of new, advanced therapies [15–17]. The systems have been referred to as 'Trojan horses', capable of overcoming the barriers presented by the body (e.g. opsonization, clearance, blood–brain barrier, etc.) and disease biology (e.g. tumor access). However, not all of these strategies have lived up to their expectations. Following examples will illustrate recent experimentation.

Synthetic carriers for drugs

Many passive carriers (macromolecules that, upon injection, remain in the circulation for an extended time and are removed from the body mainly by glomerural filtration), expected to be suitable for drug delivery, have been generated in the past. In most cases, the property of the carrier changes when the drug is attached to it, this can severely limit the carrier's utility. For example, the amount of passive drug–carrier construct that can access an inflamed site will be determined by the volume of plasma extravasating into the site of inflammation and by the concentration of the construct in the circulation. In this case it is not only the rate, but also the capacity of the process that limits a successful site-selective drug delivery. It is unlikely that passive carriers will substantially advance the field. Examples of pH- and salt-responsive carriers, and the use of passive biodistribution, are given in Box 1.

Micelles prepared from polyethyleneglycol and/or phosphatidyl-ethanolamine conjugates accumulated in the infarction zone eightfold more than in a nondamaged part of the heart muscle [18]. Ultimately, delivery systems need to be shown to work in humans. This can be done using studies like the example described in Box 2.

Passive carriers can be attached to ligands that specifically interact with a biological target. This issue is discussed in greater detail in the targeting part of this review.

Linkers and drug release

Carriers protect the drug while transporting it to its intended target. In most cases only a fraction of the drug is effectively delivered. Further, the eventual availability of the drug will depend on the manner, rate and efficiency of release of the drug from the linker [19]. As shown by our theoretical analysis, the timing of the release of the drug must ensure that a therapeutic level of the drug is maintained at the site long enough for the drug's effect to take place. Safavy *et al.* [20] demonstrated (using a paclitaxel–antibody conjugate) that changing the rate of linker cleavage could alter drug efficacy. A succinate (SX) linker (PTXSXC225) was compared with a glutamate (GL) linker (PTXGLC225). Under the same experimental conditions, PTXGLC225 showed a 16-fold increase in the half-life of the drug release. The antitumor activity of PTXGLC225 in a DU145 human

BOX 3

Doxil is not a targeted drug.

Encapsulation of doxorubicin (DOX) in pegylated liposomes alters the drug's pharmacokinetic data and leads to a marked improvement in the toxicity profile, compared with nonliposomal DOX [41]. However, the apparent increase in the therapeutic efficacy of this preparation has little to do with targeting to the tumor site. Gabizon *et al.* [42] showed that the clinical pharmacokinetic data of Doxil follow a dose-dependent clearance saturation phenomenon. It has also been observed that liposome-encapsulated DOX has a toxic effect on liver macrophages because it impairs their phagocytic function, reducing the ability of colloid particle clearance. In studies with tumor-bearing mice, in which the dose of Doxil was elevated from 2.5 mg/kg to 20 mg/kg, Gabizon *et al.* [42] demonstrate that the dose increase results in the saturation of Doxil clearance, facilitating an increase of the liposomal drug accumulating in tumors. A dose-dependent liposomal DOX blockade of the reticuloendothelial system appears to prolong liposome circulation time and, therefore, significantly enhance drug delivery to tumors.

implanted prostate-tumor mouse model was higher than the antitumor activity of PTXSXC225.

A bifunctional linker, AZ-CINN Linker, was used to combine a targeting agent, the monoclonal antibody trastuzumab (Herceptin®), with paclitaxel (via ester bonds), creating a targeted prodrug, AZ-CINN 310 [21]. The effectiveness of a single treatment of AZ-CINN 310 in decreasing tumor volume and tumor cell density (of human HER-2-positive BT474 mammary tumor cells implanted in SCID mice) was shown and compared with treatments with simultaneously administered trastuzumab and paclitaxel, as well as with a saline control. The results suggest that local release of paclitaxel from AZ-CINN 310 can take place at the tumor site.

Liposomes

Liposomes, when first described 30 years ago, generated great expectations as pharmaceutical carriers [22–25]. However, these lipid structures are phagocytozed rapidly by macrophages (mainly in the liver) and thereby removed from the circulation. Liposomes that can stay in the circulation (so-called 'sterically stabilized' or 'stealth' liposomes) can, at least in principle, accumulate passively at a selected site via the enhanced permeation and retention (EPR) effect or they can be targeted by attaching them to a suitable receptor-interacting ligand [26]. The encapsulation of doxorubicin (DOX) in pegylated liposomes is one example of liposome use, explained in greater detail in Box 3.

Targeting

A drug-loaded carrier with optimal targeting properties should contain structural features that specifically interact with the designated target, for example a particular receptor (in this way it utilizes ligand–receptor interactions [26]). Ideally, such a receptor should only be associated with a diseased organ or tissue.

DOX-loaded long-circulating liposomes were linked to a monoclonal nucleosome (NS)-specific antibody (mAb 2C5) that recognizes a variety of tumors via their surface-bound NSs [27]. This concept is based on observations in humans where NS-specific, antinuclear monoclonal autoantibodies recognize the surface of various tumor cells but not the surface of normal cells. Surface-bound NSs have previously proved to be accessible targets on the outer membrane of tumor cells [28]. They were shown to kill various tumor cells *in vitro* with higher efficiency than nontargeted DOX-loaded liposomes. However, almost a decade since this observation, the efficacy of these NSs in the clinic is yet to be established.

Two different monoclonal-antibody-targeted hydroxypropyl methacrylamide (HPMA)–copolymer–DOX conjugates (classic and star-like) were synthesized and tested for site-specific cancer therapy [29]. The anti-mouse monoclonal antibody, Thy-1.2 (IgG3), and two anti-human monoclonal antibodies, CD71/A (IgG1) and CD71/B (IgG2a), were used as targeting structures. Biodistribution studies showed that the star-like conjugate remained in a relatively high concentration in the blood, whereas the classic conjugate was found at a concentration 6.5-times lower. Compared with low antitumor activity of free DOX and nontargeted HPMA–copolymer–DOX conjugate, both of the anti-Thy-1.2-targeted conjugates (classic and star-like) successfully treated all mice bearing T cell lymphoma EL4. Results from xenografts show that star-like conjugates, containing anti-CD71/A or anti-CD71/B monoclonal antibodies as their targeting structures, were also more effective against human colorectal cancer SW 620 than the classic conjugates.

By chemically coupling a single-chain variable fragment (scFv), scFv A5, to the surface of liposomes, the fragment being directed against human endoglin, Volkel *et al.* [30] generated immunoliposomes that target proliferating endothelial cells. In clinical trials these liposomes, containing angiogenesis inhibitor TNP-470 (TAP Pharmaceutical Products, Deerfield, IL, USA), slowed tumor growth in patients with metastatic cancer. However, at higher doses (necessary for tumor regression) many patients experienced neurotoxicity [30], suggesting that drug targeting failed. Satchi-Fainaro *et al.* [31] synthesized a water-soluble conjugate of the HPMA copolymer, Gly-Phe-Leu-Gly linked to TNP-470, that accumulated selectively in tumor vessels and that substantially enhanced and prolonged the activity of TNP-470 *in vivo* (in murine tumor and hepatectomy models). The same synthetic polymer was used to develop a polymer-directed enzyme prodrug therapy (PDEPT) [32], a novel two-step antitumor approach that uses a combination of a polymeric prodrug and a polymer–enzyme conjugate to generate a cytotoxic drug rapidly and selectively at the tumor site, presumably via the EPR effect. This PDEPT combination caused a significant decrease in tumor growth (T/C = 132%), whereas neither free DOX nor HPMA-co-MA-GG-C-DOX alone displayed

activity. It would be exciting to see this approach developed into an effective cancer therapy; however, fresh mouse tumors do not always exhibit the same EPR effect as established human tumors.

Kukowska-Latallo *et al.* [33] employed modified PAMAM dendritic polymers, <5 nm in diameter, as carriers. Acetylated dendrimers were conjugated to a folic-acid-targeting agent and then coupled to methotrexate. These conjugates were injected intravenously into immunodeficient mice with human KB tumors that overexpress the folic acid receptor. By contrast to nontargeted polymer, folate-conjugated nanoparticles accumulated in the tumor and the liver tissue over four days following administration. Targeting methotrexate increased its antitumor activity and markedly decreased its toxicity, allowing therapeutic responses that are not possible with a free drug. It is hoped that similar results will be obtained in human clinical studies.

Targeting to disease sites is not generally based on the unique ligand–receptor interaction but more on the enhanced expression of receptors at the disease site, or on the existence of a physical difference (e.g. pH or enzyme concentration) between the disease site and the normal tissue. It is, therefore, important to consider the overall 'mass balance' and evaluate, for example, the amount of the receptor expressed and accessible at the disease site, compared with the total amount of the receptor expressed and accessible in the rest of the body. It needs to be recognized that the efficacy of a site-selective delivery system designed to utilize this interaction will reflect the relative distribution ratio for the given receptor (e.g. transferrin).

Nakase *et al.* [34] examined the efficacy of p53 gene therapy in human osteosarcoma (HOSM-1) cells derived from the oral cavity using a cationic liposome supplemented with transferrin. *In vivo*, the HOSM-1 tumor transplanted into nude mice grew ~5–6 mm in diameter. Transferrin–liposome–p53 was locally applied to these peripheral tumors (on day zero) and then applied once every five days, making a total of six applications. During the administration period, tumor growth did not occur and the mean tumor volume on the final day of administration (day 25) was 10% of that seen in the saline control group. Because additional controls using liposomes without transferrin (e.g. free DNA plasmid) were not used, it is not possible to say whether any enhancing effect of targeting via transferrin is actually involved.

Antibody

Antibodies, produced by the adaptive immune system, exquisitely recognize their antigen. Antibodies are increasingly used as drugs, as drug carriers and as targeting moieties for other drug carriers [26,27,29]. Addressing the issue of targeting efficiency, Wu and Ojima [35] showed that delivery of a drug to a site of action might not result in sufficient benefit. A series of paclitaxel–monoclonal antibody conjugates (conjugated via C-2′ ester linkage) were tested but did not show improved paclitaxel efficacy. In a separate study [36], the same group designed and synthesized new taxoids bearing methyldisulfanyl (alkanoyl) groups and tested their antitumor activities. A highly cytotoxic C-10 methyldisulfanyl-propanoyl taxoid was, this time, conjugated to monoclonal antibodies recognizing the epidermal growth factor receptor (EGFR). These conjugates possessed remarkable target-specific antitumor activity *in vivo* against EGFR-expressing A431 tumor xenografts in SCID mice, resulting in complete inhibition of tumor growth in 100% of treated mice without detectable toxicity. Similarly, Grifiths *et al.* [37] conjugated DOX to murine and humanized anti-B-cell antibody LL1 (targeting CD74). When SCID mice were challenged with a dose of 2.5 million Raji lymphoma cells, the mice died of disseminated disease within 15–25 days. A single dose (117–350 μg) of DOX–LL1 conjugate (8–10 drug molecules per antibody), given 5–14 days (advanced disease) after injection of the cells resulted in successful treatment (as documented by 100% survival) of most animals up to 180 days after injection of the cells [37]. However, as Judah Folkman, a cancer researcher at the Children's Hospital in Boston, USA, has been reported to say, 'If you have cancer and you are a mouse, we can take good care of you' (www.ishipress.com/cancer-c.htm).

Conclusions

Apart from the case of exquisitely specific antibody-based drugs, such as Rituxan®, the development of macromolecular, target-specific drug–carrier delivery systems has not yet been broadly successful at the clinical level. It can be argued that drugs generated using the conventional means of drug development [i.e. relying on facile biodistribution and activity after (preferably) oral administration] are not suitable for a target-specific delivery and would not benefit from such delivery even when a seemingly perfect delivery system is available. Therefore, successful development of site-selective drug delivery systems will need to include not only the development of suitable carriers, but also the development of drug entities that meet the required pharmacokinetic profile. Future efforts will need to be directed to solve, in practical terms, the following fundamental issues:

- The drug–carrier system (including the drug to be delivered) must avoid nonspecific interactions in the vascular compartment.
- The system should retain its ability to accumulate at the target site(s) (defined in terms of unique anatomical, physiological or disease conditions) and be in a form capable of acting on its pharmacological activity target.
- Drugs need to be selected, or rather designed, to have the pharmacokinetic properties compatible with the demands of target-selective drug delivery (especially drug retention at the site of delivery and its ability to access its site of molecular action).

Application of antibodies currently offers the most exciting prospect for site-specific drug delivery.

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