

The Role of Molecular Imaging in Drug Delivery

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Abstract

The parallel development of molecular imaging and drug delivery allows the combination of therapeutic agents with imaging moieties, which facilitates visualisation of the drug delivery process and provides a realtime readout on the *in vivo* efficacy of a therapeutic agent. Although challenging, it is feasible to construct a highly versatile, multifunctional single 'theranostic' probe for quantitative molecular imaging, targeted drug delivery and controlled drug release to obtain an effective therapeutic response. Compared with conventional methods for the evaluation of pharmacokinetics/pharmacodynamics, molecular imaging has advantages such as substantially decreasing the workload and increasing the volume of more precise data with statistical relevance. More importantly, molecular imaging techniques bridge the gap between pre-clinical and clinical research to develop candidate drugs that have the optimal target specificity, pharmacodynamics and efficacy. With the advancement and integration of technology in various fields, diverse types of targeted imaging probe coupled with drug delivery potential have been developed. Preliminary data have demonstrated that it is feasible and promising to use these targeted carriers for simultaneous target imaging and drug delivery.

Keywords

Drug delivery, molecular imaging, positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasound, magnetic resonance imaging (MRI)

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The main goal of drug delivery, especially targeted drug delivery, is to optimise a drug's therapeutic index by strictly localising its pharmacological activity to the site or organ of action, i.e. to achieve site specificity. By doing so, targeted drug delivery will reduce the drug toxicity and dose required and increase treatment efficacy. In addition, delivery of poorly water-soluble drugs, transcytosis of drugs across tight epithelial and endothelial barriers, delivery of large macromolecule drugs to intracellular sites of action and co-delivery of two or more drugs or therapeutic modalities for combination therapy are hotspots in the field of drug delivery. However, there are several challenges for the effective evaluation of drug delivery in pre-clinical and clinical studies. These challenges include identifying the 'correct' biologically active concentration and dose schedule, selecting the patients likely to benefit from treatment, monitoring inhibition of the target protein or pathway and assessing the therapy response.

The parallel development of molecular imaging and targeted drug delivery may offer great potential for a single multifunctional system containing both therapeutic and imaging components for imaging-guided drug delivery. By combining therapeutic agents with imaging moieties, the drug delivery process can be visualised¹ to provide a real-time read-out on the *in vivo* efficacy of a therapeutic agent.² *Figure 1* shows two typical complexes based on liposomes and iron oxide particles, respectively, for targeted drug delivery and multiple

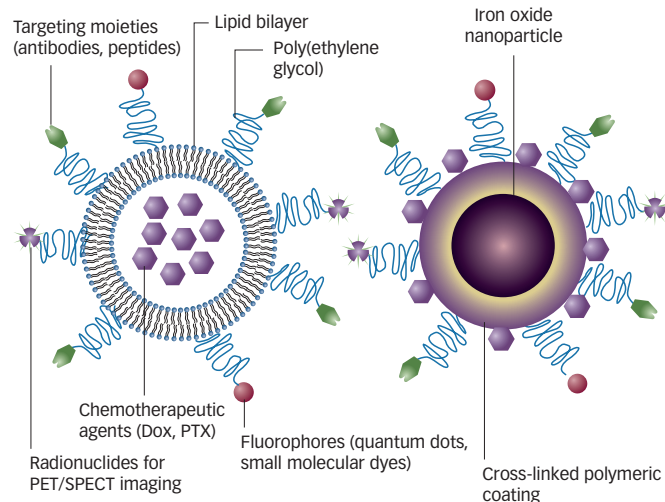
modality imaging. In this short review article, the recent development of such multifunctional systems and imaging performed with corresponding imaging modalities will be focused on.

Molecular Imaging

Molecular imaging exploits specific molecular probes as well as intrinsic tissue characteristics as the source of image contrast. It provides the potential for the understanding of integrative biology, earlier detection and characterisation of disease and evaluation of treatment.³ Imaging technologies can yield tremendous amounts of high-quality experimental data per protocol by increasing the number of times that quantitative data can be collected and guiding tissue sampling for subsequent biochemical or histological analyses, resulting in a rapid and powerful combination of analyses.

By imaging the whole animal at multiple time-points, researchers can better understand disease pathology, pharmacokinetics and other contextual aspects of the biomolecular processes taking place in the living animal. The images can provide both the structural and functional information under physiological conditions, mimicking the situation observed in the clinic. In addition, non-invasive and repetitive study of the same living subject at different time-points decreases statistical variance and reduces the number of animals required and thus cost.⁴ Pharmacokinetic knowledge obtained from imaging

Figure 1: Schematics of Multifunctional Drug Delivery Systems Based on Liposome (A) and Iron Oxide Nanoparticles (B)



PET/SPECT = single photon emission computed tomography/positron emission tomography.

enables continuous monitoring of the disposition of the drug candidate, not just snapshots of the plasma concentration of the unmetabolised component, which may have little relevance to the concentration of the drug candidate at the intended site of action.⁵

Technologies encompassed within molecular imaging include single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), X-ray CT, ultrasound, optical bioluminescence imaging and optical fluorescence imaging.³ Currently, PET and optical imaging are the most prevalent molecular imaging technologies. Each modality has its own pros and cons. For example, PET and SPECT are the most commonly used molecular imaging modalities in drug delivery, offering the potential to detect the molecular and cellular changes of diseases, but they suffer from relatively poor spatial resolution with the currently available technology. Optical imaging is cost-effective and highly sensitive, but quantification is difficult and there is a high background signal due to tissue autofluorescence. Limited tissue penetration and scattering are two other problems optical imaging encounters. MRI and ultrasonography are characterised by high spatial resolution, but they are unable to detect diseases until structural or functional changes in tissue are large enough to be detected.

Radionuclide Imaging

Drug delivery systems work mainly by altering the pharmacokinetic and pharmacodynamic properties of therapeutic agents. For example, paclitaxel (Taxol), a complex taxane diterpene, is a potent antitumour agent that has been commonly used in the treatment of various types of cancer.⁶ However, the use of paclitaxel is limited by the drug's toxicity (i.e. acute myelosuppression and peripheral neurotoxicity) and its extremely low aqueous solubility. To increase the therapeutic index, various drug delivery systems are being developed that include the use of liposomes, microspheres, micelles, prodrugs and polymer-drug conjugates.⁷⁻⁹ Liposome was one of the first drug delivery systems to be described (in the 1960s). A series of studies including pH-triggered drug release¹⁰ and pegylation of liposome¹¹ paved the way for the development and subsequent approval of doxorubicin (Doxil) in 1995 for the treatment of AIDS-associated Kaposi's sarcoma.¹²

In order to optimise the formulation of a drug delivery system, it is critical to evaluate the pharmacokinetics and pharmacodynamics of free drugs and their deliverable formulations. Due to its high sensitivity, radionuclide imaging can be used to assess the drug delivery vehicle's pharmacokinetic/pharmacodynamic properties. Kleiter et al.¹³ investigated doxorubicin administered concomitantly with ^{99m}Tc-labelled liposomes to estimate the effect of hyperthermia on intratumoural accumulation of doxorubicin in rat fibrosarcomas using scintigraphy. They found that co-administration of radiolabelled liposomes did not negatively influence the amount of drug delivered with doxorubicin and there was a significant positive correlation of intratumoural doxorubicin concentration and intratumoural uptake of the radiolabelled tracer. The results indicated that ^{99m}Tc-labelled liposomes could be used to monitor the drug delivery of doxorubicin and estimate the effect of an intervention aimed at increasing liposomal accumulation, such as local hyperthermia.¹³

For relatively large-sized biomolecular therapeutic agents, effective drug delivery is usually achieved by linking or fusing a targeting moiety onto the therapeutic to be used. For instance, an integrin $\alpha\beta3$ -binding RGD (Arg-Gly-Asp) peptide has been used to realise tumour-targeted delivery of therapeutics^{14,15} as integrin $\alpha\beta3$ is known to be upregulated in proliferating endothelial cells and a variety of cancer cells.

The delivery of these therapeutic molecules can be evaluated by molecular imaging after labelling them with radionuclides. After being conjugated with DOTA and labelled with ⁶⁴Cu, the ⁶⁴Cu-DOTA-RGD tumour necrosis factor (TNF) showed similar binding affinity and cytotoxicity to the RGD-TNF protein. The tumour uptake of ⁶⁴Cu-DOTA-RGD-TNF correlated very well with integrin $\alpha\beta3$ expression levels (U87MG [glioblastoma astrocytoma] > MDA-MB-435 [breast or melanoma] > C6 [glioma]). In addition, RGD-TNF showed a more potent antitumour effect in the integrin $\alpha\beta3$ -positive MDA-MB-435 tumour model than TNF protein.¹⁴

Paclitaxel has also been conjugated with a dimeric RGD peptide [C(RGDYK)]₂ to improve tumour selectivity of the anticancer drugs.¹⁶ When RGD₂-paclitaxel was labelled with ¹²⁵I through the tyrosine residue on the RGD peptide, integrin-specific accumulation of ¹²⁵I-RGD₂-paclitaxel in the MDA-MB-435-expressing tumour was observed.¹⁶

Optical Imaging

Poor penetration and difficulty with quantification have in general limited the application of optical imaging in drug delivery. However, recent investigations have revealed several interesting drug delivery systems endowed with optical imaging capacity. One example is semiconducting quantum dots (QDs), which have emerged as a promising alternative to organic dyes as fluorescent biomarkers for both *in vitro* and *in vivo* imaging. Their superior brightness and photostability make them excellent candidates in the development of traceable multifunctional agents.^{17,18} QDs can be incorporated into a single nanoscale structure with tumour-targeting, imaging and drug delivery functions. Bagalkot et al.¹⁹ reported a ternary novel system QD-aptamer (doxorubicin) composed of a QD, A10 RNA aptamer (Apt) targeted to prostate-specific membrane antigen and the small molecular anticancer drug doxorubicin for *in vitro* targeted imaging, therapy and sensing of drug release. The QD-Apt (doxorubicin) was able to deliver doxorubicin to the targeted prostate cancer cells and sense the delivery of doxorubicin by activating the fluorescence of QDs, which concurrently highlights the cancer cells. Recently, Weng et al.²⁰ conjugated QD605/

QD800 to doxorubicin-loaded HER-2-targeted immunoliposomes. The doxorubicin-loaded QD-immunoliposomes showed selective and efficient internalisation and anticancer activity in HER-2 over-expressing tumour cells *in vitro*. Serial *in vivo* optical imaging showed that systematically administrated QD-immunoliposomes localised in tumours that were over-expressing HER-2.

Another interesting aspect is the synergistic combination of near-infrared fluorescence imaging and photodynamic therapy. It is well-known that most photosensitisers can both emit fluorescence for near-infrared fluorescence imaging and produce cytotoxic reactive singlet oxygen (1O_2) for photodynamic therapy when activated by light.^{21,22} Coupling photosensitisers with near-infrared dyes will therefore facilitate fluorescence image-guided photodynamic therapy. For example, a 'bifunctional agent' consisting of a highly effective photosensitiser, HPPH (Photochlor) and a cyanine dye exhibiting long-wavelength absorption at 660 and 836nm, respectively, has been designed and synthesised.²³ The resulting conjugate was found to localise in the mitochondria, the most sensitive intracellular target for photodynamic therapy. Whole-body fluorescence imaging indicated a significant tumour-imaging capability of the conjugate, even at a dose of 0.3 μ mol/kg, which was 10-fold less than the most effective therapeutic dose.

Photodynamic molecular beacons have also been developed by combination of fluorescence resonance energy transfer and photodynamic therapy.²⁴ Photodynamic molecular beacons comprise an enzyme-specific linker, a photosensitiser and an 1O_2 quencher so that the photosensitiser's photoactivity is silenced until the linker substrate interacts with a target molecule, usually a tumour-associated protease. As proof-of-principle, an MMP7-triggered photodynamic molecular beacon (PP_{MMP7}B) was synthesised by linking pyro as photosensitiser and black-hole quencher 3 with a MMP7-cleavable short peptide sequence, GPLGLARK. In KB tumour-bearing mice, the fluorescence signal started to increase in tumours 20 minutes after the PP_{MMP7}B probe injection and reached its highest level at the three-hour time-point.

The recently developed Raman spectroscopy is also within the scope of optical imaging. Raman active molecules are more photostable compared with regular fluorophores and the narrow spectral features are easily separated from the broadband autofluorescence.²⁵ The single-walled nanotube is inherently Raman active,²⁶ which allows one to track, detect and image single-walled nanotubes to understand the *in vivo* behaviour and drug delivery efficacy. In addition, single-walled nanotubes provide a very high surface area per unit weight for high drug loading. Consequently, various biological molecules including drugs^{27,28} have been incorporated onto single-walled nanotubes by either covalent coupling or non-covalent adsorption methods. Liu et al.²⁸ conjugated paclitaxel to branched polyethylene glycol chains on single-walled nanotubes via a cleavable ester bond to obtain a water-soluble single-walled nanotube-paclitaxel conjugate. The intravenous (IV) injection of single-walled nanotube-paclitaxel into tumour-bearing mice resulted in a tumour growth inhibition of 59.4%, which was significantly more effective than the control formulations. In addition, the intrinsic Raman scattering properties of single-walled nanotubes were used to determine the blood circulation half-life and biodistribution of single-walled nanotube paclitaxel by using Raman spectroscopy without relying on a radiolabel or fluorescent label.

Micro-Raman imaging of single-walled nanotubes in tumour slices confirmed tumour uptake of single-walled nanotubes with a spatial resolution of $\sim 1\mu$ m. Furthermore, single-walled nanotubes exhibit photoluminescence in the near-infrared region.²⁹ Near-infrared imaging of the photoluminescent single-walled nanotubes has been shown both *in vitro* and *in vivo*.^{28,30}

Contrast-enhanced Ultrasound

Ultrasonography is by far one of the most commonly used clinical imaging modalities because it is safe and cost-effective. Ultrasonic contrast agents, such as microbubbles, have been the subject of active research, especially in recent years. There has been increased interest in developing site-directed ultrasonic contrast agents. Microbubbles (typically 1–4 μ m in diameter) vibrate particularly strongly at the high frequencies used for diagnostic ultrasound imaging, which makes them several thousand times more reflective than normal body tissues and consequently enhances both greyscale images and flow-mediated Doppler signals.³¹

There are several strategies to facilitate drug delivery with microbubbles. First, microbubbles and the drug are injected simultaneously and circulate freely in the small vessels. Once a sufficiently strong ultrasound pulse is applied to the area, the microbubbles expand and rupture the endothelial lining. The drug is then able to extravasate.^{5,32} Second, the microbubbles can be loaded with drugs and the drug-laden microbubbles freely circulate throughout the vasculature.³³ A pulse of ultrasound applied locally can rupture the microbubbles to liberate the drug for local delivery.

More desirably, microbubbles can be modified with a targeted motif and also loaded with drugs for enhanced targeted drug delivery. Microbubbles with a surface ligand when drug-laden preferentially bind to the endothelial target, resulting in an accumulation of microbubbles in the target region. An ultrasound pulse is then applied to liberate the drug. However, as yet there is no report on using microbubbles equipped with both a targeting ligand and therapeutic agents.

Magnetic Resonance Imaging

MRI is a versatile and powerful imaging modality available in both clinical and research settings for visualising soft tissues with high spatial resolution. Nasongkla et al.³⁴ first described the development of multifunctional polymeric micelles composed of three key components:

- a chemotherapeutic agent, doxorubicin, that is released from micelles through a pH-dependent mechanism;
- a cyclic RGD ligand that can target integrin $\alpha_v\beta_3$ on tumour endothelial cells; and
- a cluster of superparamagnetic iron oxide (SPIO) nanoparticles loaded inside the hydrophobic core of each micelle for ultrasensitive MRI detection.

Nanomolar concentration of SPIO-doxorubicin micelles was MRI-detectable due to the high loading density of SPIO (up to 50w/w%). Hanessian et al.³⁵ covalently attached two well-known antitumour agents, 5-fluorouracil and doxorubicin, to a mixed polymer of polyvinylalcohol (PVA) and poly(vinylalcohol/vinylamine) (aminoPVA) through appropriate bifunctional linkers. Upon enzyme cleavage the drugs were released at the target site, then the ferrofluid consisting of iron oxide nanoparticles (IONPs) was added to obtain the drug-SPION conjugates. The drug-SPION demonstrated highly synergistic

antiproliferative activity *in vitro*, superior T_2 relaxivity and elongated circulation half-life *in vivo*.

Although the application of these drug-nanoparticles is limited to *in vitro* assays, this integrated nanomedicine platform opens many exciting opportunities for the targeted delivery of therapeutic agents as well as the use of MRI as a non-invasive strategy to monitor treatment efficacy to improve the therapeutic outcome of drug therapy. Recently, Yu et al.³⁶ synthesised doxorubicin-loaded thermally cross-linked SPIOs (TCL-SPIOs). Doxorubicin-TCL-SPIOs showed superparamagnetic behaviour and the doxorubicin was released faster under the mildly acidic environment as a consequence of weakened binding between doxorubicin and the partially neutralised carboxyl groups in TCL-SPIO. MRI imaging showed noticeable darkening in the tumour area at 4.5 hours after doxorubicin-TCL-SPIOs injection, with a relative signal enhancement value of about 58%. This indicates that a large amount of doxorubicin-TCL-SPIOs accumulated within the tumour. Consequently, doxorubicin-TCL-SPIOs (12.5mgFe/kg and doxorubicin 0.64mg/kg) showed a superior antitumour effect compared to 5mg/kg free doxorubicin without showing any toxicity to major organs. Thus, it is reasonable to anticipate that TCL-SPIO may be used to develop combined therapeutic and diagnostic modalities.

Multimodality Imaging

As no single technique possesses all the required capabilities for comprehensive imaging, multimodality imaging using a combination of CT, MRI, PET, SPECT or optical imaging may provide more accurate and reliable data of the probes than single modality imaging alone. The development of imaging agents for multimodality imaging is more challenging than that for single-modality agents, requiring more complex design, multistep synthesis and careful selection of nuclear and optical tracers to avoid physical-chemical interference between molecular components. By providing the imaging agents on the same carrier, differences in distribution of the agents would be minimised if not eliminated. For example, the development of dual-function PET/near-infrared fluorescence probes allow for accurate assessment of the pharmacokinetics and tumour-targeting efficacy of the probes, and additionally aid the direct observation of the probe location microscopically.

With a chelator CHX-A', NIR dye Cy5.5 and a radiometal were conjugated to the HER-2-targeting antibody trastuzumab.³⁷ The resulting trastuzumab-¹¹¹In-Cy5.5 showed comparable immunoreactivity with native trastuzumab and was used as a multimodality imaging probe for HER-2-expressing tumours. The trastuzumab itself is a therapeutic agent used in the clinical setting and furthermore ¹¹¹In can be replaced by therapeutic radioisotopes, such as ⁹⁰Y and ¹⁷⁷Lu for radioimmunotherapy.

A few other examples of PET/near-infrared fluorescence dual-modality probes have also been carried forward for investigation and evaluation *in vivo*, such as a QD-based nanoprobe for dual PET and near-infrared fluorescence imaging of tumour vascular endothelial growth factor receptor expression³⁸ and integrin $\alpha_v\beta_3$ expression.³⁹ MRI and optical techniques are also highly complementary imaging methods. MRI/optical dual-modal probes provide a macroscopic image with a ~50 μ m spatial resolution by MRI. *In vitro* fluorescent imaging can exhibit detailed microscopic information at the subcellular level-an MRI contrast agent can be directly labelled with fluorescent dyes.⁴⁰ Magnetic resonance contrast agents can be coupled with radionuclide

labels for dual-modal MRI/radionuclide imaging using a gamma camera, SPECT or PET. Results of the combination of high-resolution MRI and high-sensitivity radionuclide imaging included better spatial and anatomical information and also improved signal sensitivity.^{41,42}

Conclusions and Perspectives

An ideal drug delivery system would have the following features:⁴³

- a high payload of drugs to deliver effective dose;
- strong signals for easy detection;
- *in vivo* stability;
- surface modification to improve pharmacokinetic/pharmacodynamic properties by attaching targeting moieties; and
- little to no toxicity to normal tissues.

So far, most of the drug delivery systems developed can be categorised as nanomedicine. Indeed, the development of nanotechnology has had a revolutionary impact on all areas of biomedicine, from research to diagnostics and therapeutics. There are more than 100 nanosized particles containing anticancer agents in various stages of pre-clinical and clinical development. The biophysicochemical properties of the nanoparticles – such as size, charge, surface hydrophilicity and the nature and density of the ligands on their surface – can all affect the circulating half-life of the particles as well as their biodistribution.⁴⁴⁻⁴⁶ To develop and optimise a nanosized drug delivery system, strong collaborative efforts between imaging experts, pharmaceutical and biomedical scientists and physicians are required.

Compared with conventional methods for the evaluation of pharmacokinetics and pharmacodynamics, molecular imaging definitely has advantages, such as substantially decreasing the workload and enabling the acquisition of more precise data with statistical relevance. More importantly, molecular imaging techniques bridge the gap between pre-clinical and clinical research, aiding the development of candidate drugs that have the optimal target specificity, pharmacodynamics and efficacy. With the advancement and integration of technology in various fields, diverse types of targeted imaging probes coupled with drug delivery potential have been developed. Preliminary data have demonstrated that it is feasible and promising to use these targeted carriers for simultaneous target imaging and drug delivery. It can be anticipated that molecular imaging moieties in these drug delivery systems will accelerate the progress of clinical translation to achieve better disease control. ■



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