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Nanoparticulate Systems for Bioactive Agent Delivery: What Is the Missing Link in Research for Real Applications?

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Diverse nanoparticulate systems, ranging from polymeric nanoparticles to liposomes, have been exploited as carriers of bioactive agents in recent years; however, the use of these systems has been confined largely to the laboratory context till now. Systems that can successfully be adopted for bioactive agent delivery in practice are few. Herein, such low efficiency in clinical translation is partly due to the lack of awareness of the similar nature between a carrier and a real drug. To rectify this situation, it is important to treat a carrier as an ordinary drug despite its absence of therapeutic effects. The current situation in prevalent bioactive agent delivery research, as well as those routine research practices that should be changed to enhance clinical translation, will be discussed here.

1. Introduction

Diverse polymeric, liposomal and metallic nanoparticulate systems have been developed over the years for bioactive agent delivery. Examples of these systems include the nanoparticles formed between polycations (e.g., poly(L-lysine),[1] chitosan,[2] and poly(ethylenimine) [PEI][3]) and therapeutic nucleic acids, as well as the metallic nanoconstructs (e.g., upconversion nanoparticles[4] and metal nanoclusters[5]) surface-modified for agent loading. Apart from synthetic carriers, biological entities have been exploited for bioactive agent delivery. One example is viral vectors (e.g., lentiviruses, adenoviruses, and adeno-associated viruses [AAVs]), which can deliver exogenous nucleic acids into target cells for transcription and translation. Other examples of biological carriers include exosomes,[6] erythrocyte ghosts,[7] and virus-like particles.[8] Despite the plethora of carriers reported in the literature, carriers that can successfully be translated into real applications are few. Clearly, there are barriers between preclinical trials and practical use. In fact, upon careful examination of most of the existing studies in delivery research, it is not difficult to discern that the performance of the reported carriers, at the moment, is elevated predominately based on the toxicity and therapeutic outcomes. But, are these two factors sufficient to indicate the transferability of the reported carriers to real applications? This is exactly the question we would like to and should consider.

As far as the use of nanoparticulate systems as carriers is concerned, multiple purposes are generally desired to be achieved. Conventionally, nanoparticulate systems are adopted to enhance the aqueous solubility and bioavailability of bioactive agents. One example is Abraxane, which is a formulation fabricated by binding paclitaxel to albumin nanoparticles to improve the aqueous solubility of paclitaxel.[9] Another example lies in the case of sirolimus, which is an immunosuppressant that shows poor aqueous solubility. Upon the use of NanoCrystal technology, the drug has been formulated into a solid dosage form which displays enhanced palatability and storage stability, facilitating the clinical application of the drug.[10] Apart from enhancing the solubility and bioavailability, nanoparticulate systems may help enhance the cellular uptake of the loaded agent, and may prolong the blood circulation time by protecting the agent from recognition by the immune system. Meanwhile, some nanoparticulate systems are designed to enable the loaded agent to be deposited preferentially in specific tissues in a body, and to allow for sustained and controlled agent release. Because of this, it is understandable if a study mainly focuses on the evaluation of the carriers in those specific aspects when the performance of the carriers is assessed. The human body, however, is a complex system. Because of the differences between the permeability of the vasculature (and many other factors including the rate and volume of blood flow) between humans and animal models, this leads to discrepancies between the performance of a carrier in a model organism and that in a human body, rendering translation of research works into real applications challenging and ineffective.

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2. Missing Links for Real Applications

In drug development, preclinical studies on the absorption, distribution, metabolism, and excretion (ADME) profiles are often conducted. These studies relate to the excretion balance, metabolic profile, and toxicology of drug candidates. Related data are not only reported in research articles and required for journal publications, but are also needed for regular dossier submission. In research on bioactive agent delivery, the situation, however, is totally different. This is partly because many of the articles on newly developed carriers are disseminated in materials science journals, which require more thorough investigations on the physical and chemical aspects of the reported carriers rather than detailed preclinical and clinical data. This shapes the research practice in carrier development and characterization, and largely impedes successful translation of reported carriers from the laboratory to real applications.

Indeed, similar to an ordinary drug candidate, upon the administration of a carrier, it will undergo ADME processes. We, therefore, hold that to facilitate the translation process, carriers should be regarded as ordinary "drug candidates" when they are characterized and optimized. This is particularly true for those carriers that are designed to show intrinsic bioactivity. One example of such carriers is the potentially immune-modulating copolymer generated by conjugating polysaccharopeptides from *Coriolus versicolor* with PEI.[11] The copolymer has been demonstrated to form nanoparticles upon complexation with therapeutic nucleic acids. Another example is the antitumorigenic agent generated from structural modification of calixarenes for micelle formation and doxorubicin encapsulation.[12] These carriers are indeed no difference from real drugs in practice. For this, the metabolic pathway of such carriers should be monitored as what research on an ordinary drug candidate does. In addition, the guideline issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) requires the safety of all metabolites that represent 10% or more of drug-related exposure to be properly determined.[13] This should also be applied to future carriers, which are supposed to be for human use. High-resolution mass spectrometers, as well as triple quadrupole or ion trap mass spectrometers, are some of the emerging tools applicable to determine and characterize metabolites of a carrier in the future.[14]

3. Factors Affecting Ultimate Performance

As far as the development and characterization of carrier are concerned, the concentration of the carrier, upon administration, in major tissues (ranging from liver to heart) has to be determined so that the success of manipulation of the pharmacokinetic profile of the loaded agent can be evaluated. Several parameters (such as half-life [$t_{1/2}$], maximum concentration [C_{max}], clearance [Cl], mean resident time [MRT], and the area under the curve [AUC]) can also be derived based on the pharmacokinetic profile. If the enhancement of a carrier for blood retention is accomplished, an increase in AUC (as well as MRT and $t_{1/2}$) and a decrease in Cl are expected to be observed. In fact, many properties of a carrier can be adjusted to manipulate the pharmacokinetic profile achieved. The particle size is one of the foremost factors. Considering poly(ethylene glycol) (PEG) hydrogel particles fabricated using the mesoporous silica (MS) templating method as an example, by increasing the PEG molecular weight or decreasing the particle size, a decline in the association of the particles with phagocytic blood cells has been observed.[15] This observation is in accordance with the findings in vivo, in which smaller particles (150 nm) have been reported to be more efficacious in blood retention than the larger counterparts (>400 nm).[15]

The surface property of a carrier is another factor determining the pharmacokinetic profile. This has been revealed by an earlier study,[16] which has generated four types of gold nanoparticles possessing various surface charges (neutral, positive, negative, and zwitterionic). Upon intravenous and intraperitoneal injection, neutral and zwitterionic nanoparticles have been found to lead to not only a longer blood circulation time but also more effective tumor uptake. On the other hand, shorter half-lives have been noted in those gold nanoparticles possessing negative or positive surface charges. The route of administration affects the pharmacokinetic profile, too. Intravenously administered gold nanoparticles have been found to accumulate largely in the liver and spleen, with little deposition having been observed in the brain, kidneys, and lung.[16] For intraperitoneally administered nanoparticles, they have accumulated largely in the pancreas.[16] Using different routes to administer the same carrier can cause the carrier to undergo different processes of blood circulation and lymphatic clearance, leading to a change in the biodistribution profile of the carrier.

To optimize the biodistribution profile, the shape of the carrier should be considered as well. This is shown by the case of fluorescent mesoporous silica nanoparticles (MSNs).[17] While intravenously administrated short-rod MSNs accumulate mainly in the liver in mice, the spleen is the place that long-rod MSNs accumulate.[17] Comparing with long-rod MSNs, short-rod MSNs are more effective to be removed by renal and fecal excretion.[17] Furthermore, as revealed by the case of magnetic mesoporous silica nanoconstructs,[18] rod-shaped cylinders with dimensions of 400–450/120–150 nm are the most effective one to get accumulated in the spleen and tumor after intravenous administration to tumor-bearing mice;[18] on the other hand, spherical nanoparticles with a diameter of 200 nm exhibit a high level of accumulation in the liver.[18] Last but not least, the pharmacokinetic profile is affected by the number of times of carrier administration. This has partly been revealed by Dams et al.,[19] who have researched into changes in the pharmacokinetic profile of radiolabeled PEGylated liposomes after multiple injections. A more significant decline in the blood content of injected liposomes, as well as a dramatic increase in the uptake of liposomes in the liver and spleen, is observed upon repeated liposome administration.[19] This suggests that the previously injected dose of a carrier may cause changes in the pharmacokinetic behavior of subsequently injected doses in a time- and frequency-dependent manner.

4. Technical Challenges and Perspectives

Nanoparticulate systems may show inherent polydispersity due to the technical difficulty of precisely controlling the size of the

system and the molecular weight of constituents, particularly when the constituents are polymeric in nature. This causes batch-to-batch variations in the biodistribution profile of the nanoparticulate system. In addition, while the use of nanoparticulate systems is often confined largely to the enhancement of the efficiency in delivering bioactive agents, more and more efforts have been reported in the literature to merge multiple functionalities in to one system. For instance, in an earlier study β-cyclodextrin (β-CD)-terminated polyfluorene (CD-PF-CD) has first been generated from polyfluorene (PF-OH), followed by dissolution in pyridine and injection into water to induce self-assembly for the formation of water-dispersible conjugated polymer nanoparticles (CPNs).[20] Due to the presence of β-CD, inclusion complexation with the guest molecule adamantane (ADA) for surface functionalization is possible,[20] providing a path for the incorporation of cell-specific fluorescent tags and other functional moieties. More recently, PEG-coated and doxorubicin-loaded multimodal gadolinium oxide nanoparticles have been made to simultaneously mediate drug delivery and multimodal imaging.[21] Such multifunctional carriers can enhance the performance in, and controllability of, bioactive agent delivery.

Despite such promising potential, it is worth noting that when carriers consisting of multiple functional yet detachable moieties are applied to a body, the pharmacokinetic profile of each of the detachable moieties should be characterized separately so that comprehensive understanding of the fate of the multifunctional nanoconstruct can be attained. This can be done by first radiolabeling each of the moieties and then monitoring the fate of each of them; however, whether the carrier will behave differently after the radiolabeling process is an issue that should be considered. Yet, right now effective strategies to track the fate of a carrier while completely avoiding changes in carrier properties (e.g., size, shape, and surface properties) are lacking. In addition, at the moment animal experimentation is the only approved strategy to evaluate the pharmacokinetic profile of an entity before clinical trials. Unfortunately, because the total blood volume in model organisms is different from that in human bodies, this creates an unavoidable barrier for effective translation into human use as blood per se is a tissue that has significant effects on the pharmacokinetic profile of the administered carrier.

In addition, while carriers having extended blood circulation time is desirable for purposes such as systemic drug delivery, these carriers may have a higher chance to stimulate the coagulation cascade, leading to the formation of blood clots and hence the occlusion of the blood vessel by the thrombus. This problem can be ameliorated by manipulating the surface properties of the carrier. Such feasibility has been demonstrated by an earlier study,[22] in which the extent of platelet aggregation and activation induced by cetyl alcohol/polysorbate-based nanoparticles has been found to be reduced upon PEGylation of the particle surface. An alternative approach to prevent blood coagulation induced by a carrier is to coadminister the carrier with an anticoagulant. However, as revealed by an earlier study examining the thrombogenicity of various forms of carbon-based nanomaterials (including multiple-wall and single-wall nanotubes, C60 fullerenes and mixed carbon nanoparticles),[23] the form of the nanomaterial per se may affect its interactions with blood components. Illuminating the exact mechanism underlying platelet aggregation induced by nanoparticulate systems is in dire need to enhance translation of carriers from the laboratory to human use. Yet, solving this takes time. It is anticipated that this will still be a barrier to clinical translation of research on bioactive agent delivery in the next 5–10 years unless unexpectedly significant progress in solving the problem has been achieved.

5. Conclusion

Diverse types of nanoparticulate systems, ranging from polymeric nanoparticles to liposomes, have been developed and reported in the literature since the turn of the past century; however, systems that finally get to real applications are few. This is partially attributed to the routine research practice, which largely focuses on physiochemical and structural characterization of a carrier, without paying much attention to the fate of the carrier upon administration to a living body. As discussed in this perspective piece, treating a carrier as a real drug will be the way a change should be made in the field. Here, it is worth mentioning that changing a research practice is not an individual issue but is a matter that requires collaborative efforts. Such efforts should be established to ensure areas (particularly the efficiency, safety, and pharmacokinetic profiles) specific to nanoparticulate systems are properly characterized in routine research so that the potential of a carrier to be translated into real applications can be better predicted and assessed. Apart from the technical factors as discussed in this article, industrial considerations (such as scalability, manufacturing costs, and ease of application) may affect the practical potential of a nanoparticulate system. Addressing these concerns, however, is often beyond the scope of a scientist's work. An open dialogue between various stakeholders (including the regulatory bodies, funding agencies, research laboratories, and industry) should be made to come up with a more application-oriented research culture for future efforts in the development and use of nanoparticulate systems for bioactive agent delivery. Achieving this is easier said than done, but is necessary in order to make a change in the status quo.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

bioactive agent delivery, carriers, nanoparticles, pharmacokinetics, sustained release

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