Nanomedicine

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Journal:	*Nanomedicine*
Manuscript ID	NNM-2023-0323.R2
Manuscript Type:	Editorial
Keywords:	Nanoparticles, Animal Models, Clinical Translation

Can animal models help to explore functions of nanoparticles in health and diseases?

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Over the decades, nanotechnology has rapidly bloomed in a variety of fields and generated a wide range of nanomaterials. Nanoparticles (NPs), approximately 1-100 nm in diameter, make up a particularly innovative symbol of this increasingly growing field. A multitude of NP formulations are still under investigation in the laboratory, such as lipid nanoparticles (LNP), dendrimers, metal-based NPs and extracellular vesicles. NPs exhibit distinct properties, attributed to their increased surface area-to-volume ratio [1]. Currently, NPs are widely adopted in nanomedicine, including early detection, diagnosis, treatment and follow-up of different diseases [2]. For diagnosis, NP-based contrast agents offer high-resolution imaging, and other NP-based approaches enable the identification of specific biomolecules, which substantially improves the sensitivity and specificity of disease detection. In therapeutics, utilizing targeted and stimuli-responsive NPs as delivery vehicles enhances drug efficacy and reduces adverse effects [1]. Despite these excellent advantages that NPs offer, an extremely limited number of NPs were approved by the U.S. Food and Drug Administration (FDA). These authorized materials largely favor formulations that are polymeric, liposomal and nanocrystal, and are mainly employed in mRNA vaccination and cancer treatment [3]. Eligard® (Tolmar) for prostate cancer and Onivyde® (Merrimack) for pancreatic cancer are two examples of FDA-approved LNPs [4]. There are also several NPs under Phase II or III investigation in clinical trials. For example, MRT5005, a codon-optimized CFTR mRNA, delivered by aerosol in LNP, has been proven to be safe and well tolerated in a randomized, double-blind, placebo-controlled

Phase 1/2 study performed in the US. There is a trend in recent clinical studies toward developing more sophisticated materials containing micelles and protein-based NPs, and various inorganic and metallic particles [4].

In light of the success of these NPs, the demand for therapies using NPs and *in vivo* animal experiments that offer more reliable extrapolation to human biology has increased significantly. Animal models have contributed tremendously to understanding disease pathophysiology, identifying novel targets, optimizing dose regimens, assessing pharmacokinetic/pharmacodynamic relationships and, importantly, measuring the therapeutic index of novel drugs [5]. In response to the 3R principle (replacement, reduction, refinement), which attempts to limit the use and potential suffering of animals used in scientific research, a variety of animal alternatives have been developed to reduce the use of living animals, including the use of *in vitro* alternatives to explore NPs. However, at the moment, animal-alternative methods are far from answering all the necessary questions in biological and medical research. A study by Braakhuis *et al.* compared the translocation of NPs between in vitro models and in vivo models [6]. They observed that, while the *in vitro* translocation of certain NPs is comparable to their *in vivo* translocation, significant disparities appear to arise between the *in vitro* and *in vivo* translocation rates for the majority of NPs [6]. This suggests that these *in vitro* approaches to NP testing are insufficient in certain contexts. Currently, small animals (e.g., rodents) are the most popular experimental animals in NP research due to their low cost, easy operability, and high reproducibility. Moreover, the availability of transgenic and knockout strains makes rodents, particularly mice, one of the most attractive models for research. For example, Tang *et al.* demonstrated that in contrast to healthy mice, hypercholesterolemic mice had a lower probability of NP delivery to the lungs and a higher probability of NP delivery to the liver, spleen, and brain. This finding suggests that the metabolome profile of hypercholesterolemic mice is an untapped factor that influences the target efficacy and safety of NPs [7]. Guo *et al* found that solitary administration of a hydrogel co-delivering Gemini-like NPs in mouse models of first-line resistant malignancies (breast, liver, pancreatic, and colorectal), could induce immune activation, reinstate chemosensitivity, and ultimately

lead to tumor regression [8]. Rodents are different from humans in critical biological processes such as immunological responses and have exhibited constraints when it comes to producing data that is clinically applicable to human diseases, such as cancer, cardiovascular disease, neurodegenerative diseases, and diabetes [5]. Large animals, including non-human primates (NHPs), mustelids, pigs *et cetera*, may assist in bridging the gap between rodent models and clinical studies since they more closely resemble humans at the organ and cellular levels. For instance, ferrets are widely accepted for investigating respiratory diseases, as their anatomy and behavior are physiologically close to humans, and they have been used extensively to simulate disease caused by many respiratory viruses including influenza, respiratory syncytial virus and SARS-CoV [9]. A recent example is that during the COVID pandemic, NHPs took an important role in displaying the pathogen entry, replication and transmission, immune responses, and pathology, which helped extensively to prevent disease transmission and advance vaccine therapy [10]. A crucial prerequisite for the achievement of successful clinical development is the preclinical translation to higher species. This is especially true with respect to NPs. Historically, rodents have been the subject of most animal research considering cost, availability and ethical concerns. As it is challenging to attain therapeutically significant effects at tolerable dose levels in larger species, it has been documented that the efficacy of NPs may not consistently persist when progressing from rodents to NHPs [11]. For example, a tenfold reduction in protein expression was observed when Lam *et al*. tested an mRNA-LNP that had been previously optimized in rodents but failed to perform as expected in NHPs [12]. The successful delivery of NHPs was observed with a particle size reduction to ≈50–60 nm and a PEG concentration increase to ≈2.2–2.8%; neither of these aspects were predicted by murine data [12].

Furthermore, it has been observed that the toxicity findings derived solely from small animals lack consistency. Large animals have been used to study the systemic toxicity of several organic and heavy metal nanoparticles, including graphene oxide, detonation nanodiamonds, silica quantum dots, and CdSe/CdS/ZnS quantum dots [13]. However, in one recent study, anaphylactic death of NHPs occurred with graphene oxide but not

with single-walled carbon nanotubes and nanodiamonds, which were not reported in murine studies [14]. These studies underline the great significance of evaluating nanotoxicological effects on large species.

In summary, the clinical translation of NPs continues to face a multitude of obstacles that must be surmounted. These encompass the reduction of toxicity, enhancement of targeting specificity, and comprehension of the interactions that occur between NPs and the human body. Animal experimentation, particularly using NHP models is indispensable to help to explore the function of NPs in health and achieve the transition from bench to bed.

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