Toxicity assessment of nanoparticles in various systems and organs

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Abstract: In the past decades, much attention has been paid to toxicity assessment of nanoparticles prior to clinical and biological applications. While in vitro studies have been increasing constantly, in vivo studies of nanoparticles have not established a unified system until now. Predictive models and validated standard methods are imperative. This review summarizes the current progress in approaches assessing nanotoxicity in main systems, including the hepatic and renal, gastrointestinal, pulmonary, cardiovascular, nervous, and immune systems. Histopathological studies and specific functional examinations in each system are elucidated. Related injury mechanisms are also discussed.

Keywords: function; histopathology; in vivo; systematic assessment; nanoparticle; toxicity.

1 Introduction

Nanoparticles (NPs) are particles with the size of 1–100 nm. Current investigation progresses have rendered NP applications in biomedical, bioengineering, and optical fields. Much attention has been drawn to NPs’ toxicology, which poses possible threat both medically and environmentally. From a biomedical perspective, NP toxicology reveals an interaction between the physicochemical characteristics of NPs and their biological effects. Assessments of NP toxicity need a set of design rules. During the past decades, in vitro toxic characterizations of NPs have been well summarized and compared. Marquis et al. [1] reviewed the analytical methods in respect to proliferation, necrosis, apoptosis, DNA damage, and oxidative stress, which are related to the main mechanisms of cytotoxicity. While in vitro studies have been performed extensively [2], the significance of nanotoxicity in vivo studies is being emphasized [3]. Relatively long-term, complicated, and animal-sacrificed in vivo studies come after in vitro assessments and are the inevitable progress before wide-range application. Although many reviews have summarized the methods in both in vitro and in vivo studies in certain nanostructures in different model systems (Table 1), systemic evaluation remains indefinite. Fischer [3] emphasized the importance of developing predictive models of NP toxicity assessment, whereas many researchers have focused on histological changes [25] and pharmacokinetic parameters like exposing [26], biodistribution [27, 28], biochemistry metabolism, and clearance. However, exploration of nanotoxicity remains superficial in sacrificed animals. Based on the in vivo results of NP pharmacokinetics, including homeostasis regulation, systematically evaluating the impact of NPs on the major systems, including the hepatic, renal, digestive, pulmonary, hematological, cardiovascular, nervous, immune systems, may provide profound insight into this field. This review summarizes the recent progress of toxic assessments of NPs from the perspective of single systems.

2 Systemic assessment

On a physiological basis, nanotoxicity can be tested from pathological changes, including morphologic changes (gross, microscopic, and ultrastructural changes) and functional damage. The mechanisms underlying NPs’ physiological interactions range from cells to organs and were discussed in the latest review [29]. Additionally, in terms of
in vivo studies, the selection of animal models is the initial step. Although many animal models have been built, a standard and predictive model is still underdetermined. Besides the most common experimental mice and rats, zebrafish [30, 31], rabbits [32], and Caenorhabditis elegans [33] were also used in nanotoxicology studies. As the explicitly known genome and developed application in toxicity investigation, mice and rats would be more appropriate models in nanotoxicology studies, which is addressed below (Figure 1).

In vivo assessments are divided into two main categories. One involves tissue structure changes [34, 35], apoptosis [36, 37], and inflammation infiltration in main organs (kidney, spleen, lung, brain, and heart). Another one targets certain systems, whose structural specificities are liable to concentrate NPs. For instance, hepatic sinusoid and kupffer cells are the fundamental structures for liver function in metabolism and detoxication, in which NPs are liable to be deposited, as well as in renal filtration

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**Table 1: Assessments of some NPs in medical use.**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Application</th>
<th>Assessments</th>
<th>In vitro</th>
<th>In vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe3O4</td>
<td>Contrast agent (MRI) labeling and tracking</td>
<td>Cytotoxicity</td>
<td>Distribution</td>
<td>[4, 5]</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>Antimicrobial agent</td>
<td>Genotoxicity, cytotoxicity, cellular uptake</td>
<td>Pulmonary toxicity, hepatotoxicity, immunotoxicity</td>
<td>[6–8]</td>
<td></td>
</tr>
<tr>
<td>Au</td>
<td>Biolabel, biosensor, drug carriers</td>
<td>Cytotoxicity</td>
<td>Hepatotoxicity, spleen/lung toxicity</td>
<td>[9–11]</td>
<td></td>
</tr>
<tr>
<td>TiO2</td>
<td>Biomedical ceramic implanted biomaterial sterilization</td>
<td>Cytotoxicity (lung, nervous, hematopoietic, etc.), genotoxicity, microvascular and mitochondrial dysfunction</td>
<td>Skin toxicity</td>
<td>[12, 13]</td>
<td></td>
</tr>
<tr>
<td>PEG</td>
<td>Drug carriers</td>
<td>Cytotoxicity</td>
<td>Immuno- toxicity</td>
<td>[14, 15]</td>
<td></td>
</tr>
<tr>
<td>CNT</td>
<td>Building blocks</td>
<td>Toxicokinetics</td>
<td>Hepatotoxicity, pulmonary toxicity</td>
<td>[16–19]</td>
<td></td>
</tr>
<tr>
<td>PLGA</td>
<td>Drug carriers</td>
<td>Macrophage uptake, phototoxicity</td>
<td>Nephrotoxicity</td>
<td>[20–22]</td>
<td></td>
</tr>
<tr>
<td>SLN</td>
<td>Drug carriers</td>
<td>LD50, Cytotoxicity, tissue injury</td>
<td>Lung toxicity</td>
<td>[23, 24]</td>
<td></td>
</tr>
</tbody>
</table>

Fe3O4, Iron oxide; TiO2, Titanium dioxide; PEG, Poly(ethylene glycol); CNT, Carbon nanotube; PLGA, Poly(D,l-lactide-co-glycolide); SLN, solid lipid nanoparticles; LD50, Median lethal dose.

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**Figure 1:** In vitro toxic characterization has been tested in respect to proliferation, necrosis, apoptosis, DNA damage, and oxidative stress. Mice and rats would be more appropriate models due to their explicit genome for toxicity tests of organs including hepatic and renal, gastrointestinal, pulmonary, hematological, cardiovascular, nervous, reproductive, and immune systems in terms of histopathological changes and functions.

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membrane. Consequently, combined with the exposing ways (ingestion, injection, transdermal delivery, and inhalation) and applications, appropriate assessment models could be established, especially for the drug delivery of NPs [38].

2.1 Biodistribution

Any single physicochemical property of NPs can influence the distribution, which at a certain extent determines the material toxicity. Size [27, 39, 40], surface charge, constituent [41, 42], and chemistries of the coating could affect NP aggregation and excretion. Elucidating the biodistribution of NPs in organs is useful to guide the adjustment and modification of NPs.

The most common way of exploring the distribution is to remove the main organs [27] or tissues (lung, liver, kidney, heart, spleen, pancreas, brain, fat, and muscle) after animal sacrifice, then track the NPs according to their respective characteristics using physical detection methods. For some metal NPs, their intrinsic properties could be probed by specific instruments. Indeed, gold composite nanodevices in mouse tumor tissues could be explored by instrumental neutron activation analysis [27]. Si and Cd [34] concentrations could be determined by inductively coupled plasma optical emission spectrometry. For other varieties of NPs, some radiolabeled and fluorescent-labeled skills have been extensively applied. [3H]-PLA [42] has served as a marker to show blood concentration and organ distributions of poly(ethylene glycol)-grafted nanocapsules quantitatively. Some fluorescent-labeled particles [43] in tissue homogenates were analyzed by plate reader. In some other materials [44], like quantum dots with heavy metal cores, it was more appropriate to apply multiplexing and multicolor imaging through single-particle Förster resonance energy transfer assays [45]. However, qualitative analysis with light microscope (LM) and electron microscope (EM) failed to show sufficient and significant evidence in current studies. Other imaging techniques, including typical imaging instruments like magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography, optical imaging, and ultrasound, were introduced in the latest reviews [46, 47]. Furthermore, multiscale, real-time, and quantitative technologies were reported lately [48]. As some imaging contrast agents have potential unintentional toxicity, these tracing methods may aggravate the toxicity of NPs. Moreover, the combination of contrast agents may alter the characteristics and consequently change the biodistribution of NPs.

2.2 Hepatic and renal system

Hepatic sinusoid with Kupffer cells and renal glomerular basement membrane, as main metabolism and clearance organs, are fragile to toxic stimuli. Hepatic and renal toxicities are basic biosafety evaluation for drugs, as well as NPs. Oxidative stress can be determined by the index of superoxide dismutase (SOD) and glutathione peroxidase (GPx) [49]. Ultrastructural alterations of the liver and kidney are recommended primarily for morphologic changes [42]. Cationic nanobubbles [50] were visualized in histologic tests by section staining with haptoglobin and H&E. Afterward, tissue apoptosis was observed with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

2.2.1 Hepatic assessment

Immunohistochemistry was used to detect liver fibrosis and inflammation [50]. Serum enzymology analysis was used for hepatic function evaluation, mainly including alanine aminotransferase, aspartate aminotransferase [51], c-glutamyl transferase, and alkaline phosphatase [52], which indirectly and quantitatively reflect functional changes. Multiple automated hematology analyzer and automated chemistry analyzer are advantageous for the comprehensive analysis if conditions are permitted [53, 54].

2.2.2 Renal assessment

Histopathological study is useful to indicate renal glomerulus degeneration. The different pathologic changes, like glomerulosclerosis and collagenous tubulointerstitial matrix, can be confirmed by immunohistochemistry by detecting fibrotic and mesenchymal markers transforming growth factor-β1, interferon-6, type I collagen, fibronectin, and vimentin [55]. Cell proliferation can be assessed by measuring proliferating cell nuclear antigen [37]. Some kidney special dyeing, including PAS, PASM, and Masson, can be observed under LM [56]. The pathological diagnosis can be also determined by EM. From a functional aspect, kidney indices [57] are commonly measured. Gandhi et al. [58] tested renal variables including total proteins, albumin, and glomerular filtration rate. Urine protein, hematuria, urine albumin, and creatinine ratio [59] were also examined to evaluate the injury degree of the glomerular filtration membrane.
2.3 Gastrointestinal system

Compared with injection, oral administration of drugs or others is considered favorable due to convenience and compliance for patients. Drug bioavailability through oral administration, however, is limited because of physiological barriers of the gastrointestinal tract (GIT). With the improvement of nanocapsule transportation, some new drug delivery systems are gradually being developed, e.g., polymeric NP [60], which prevents biologicals from inactivation and degradation by acid and enzymatic barriers of the GIT. There are a majority of researches that report the benefits of NPs, such as the reversal of nonsteroidal anti-inflammatory drug-induced gastrointestinal injury [61] and radioprotection [62] from cancer radiotherapy. Cerium oxide NPs [63] were also reported to protect gastrointestinal epithelium from reactive oxygen damage. Nevertheless, the hazardous impact from NPs cannot be ignored. Exposure to TiO₂ NPs interfered in nutrient absorption of normal epithelium from reactive oxygen damage. Nevertheless, the hazardous impact from NPs cannot be ignored. Exposure to TiO₂ NPs interfered in nutrient absorption of metal contents [64]. Histological assay should be the initial step in which GIT microvilli and epithelial atrophy [65, 66] are observed under EM, and the number of mast cells [64] in the stomach should be counted. Besides, functional tests of GIT were also necessary. Absorption function could be reflected indirectly by the evaluation of metal content and electrolyte. Acid-base unbalance like metabolic alkalosis occurred after the overstock of HCO₃⁻, when certain mental NPs reacted drastically. A novel method quantitatively monitored the digestion of intramolecular-quenched protein under fluorescence spectroscopy in zebrafish [65] and showed digestive malfunction and developmental abnormalities.

2.4 Pulmonary system

Considering environmental issues, the initial researches of pulmonary toxicity were concentrated on inhalable particles [67, 68]. NP, as a new material with targeting and noninvasive characteristics in drug delivery system, is another category related to pulmonary impact. To detect pulmonary accumulation of NPs, MRI [69] was used to visualize antibody-conjugated superparamagnetic iron oxide (SPIO) NPs in a lipopolysaccharide-induced chronic obstructive pulmonary disease mice model. The most common method is intratracheal instillation for long-term [70, 71] and short-term [72] toxicity assessments, in which the bronchoalveolar lavage (BAL) fluid [73] is collected for biochemical analysis, including lactate dehydrogenase (LDH) assay. LDH index is useful to indicate pneumonocyte injury. As a crucial indicator of inflammatory reaction, the number of BAL-recovered neutrophils [74] is counted to indicate the extent of the inflammation. Although tissue studies are less commonly applied, pathology and apoptosis research was occasionally used in these systems. Additionally, lipid peroxidation (LPO) and glutathione production were used to reveal oxidative stress [75].

2.5 Cardiovascular system

For each of the NPs whose exposure is by way of intravenous injection, impact on the cardiovascular system is definite. The biocompatibility of hematology and serum drew lots of attention. Phlebitis is arranged at the first place of the common risk in clinical observation. Venous tolerance was explored in the auricular vein [76], where eye wetness, brachychronic breath, purple lump, blood clot, and edema were recorded. Typical symptoms of phlebitis [77] can be seen through pathological sections. Hemolysis and thrombosis are other common risks. While hemolysis was often tested in vitro [78], the vascular thrombosis model [79] of the carotid artery was applied by platelet aggregation and ATP release in rats. The routine study involving circulation was to count each variation of complete blood, including erythrocytes, total leukocytes, hemoglobin, and hematocrit [36]. As for cardiac injury, serum markers, like troponin-T, creatine kinase-MB, and myoglobin, should be analyzed [80]. Cardiac calcium concentration associated with contraction function and DNA damage also needs to be determined. Although energy-related function damage is concerned, ATP/ADP ratio and concentration of myoglobin in the cardiac cells were calculated less in vivo. Oxidative stress [81] biomarkers should be emphasized, including LPO, reactive oxygen species, and antioxidant enzymes like SOD, GPx, and catalase. Cardiac uptake of NPs was revealed through radioisotope [125I] of iron oxide NPs [82]. An energetics study involving nanocarriers, which needs specific instrumentations, was performed by Vlasova et al. [83] by monitoring the cardiovascular parameters of arterial pressure and heart rate telemetrically, along with an ex vivo study on isolated aorta rings.

2.6 Nervous system

In the nervous system, assessments were focused on the drug delivery of solid lipid NPs (SLNs) in the brain. Especially, brain visualization was improved and drug permeability across the blood-brain barrier was assessed with
radiotracers like PET and PET/CT system [84]. Magnetite-labeled NPs can act as a contrast agent in rat brain MRI [85]. Transportation was further assayed by noninvasive in vivo imaging and ex vivo optical imaging after injection via the carotid artery [86]. The uptake of drug into the brains was determined by the ratio of concentration in the brains (Cbr) to that in the plasma (Cpl) after intravenous injection. The impairment can be reflected by glutamate uptake dysfunction when NPs were translocated to the olfactory bulb [87]. Confocal fluorescence [86] studies were also applied to evaluate the uptake of brain endothelial cells. Meanwhile, acute toxicity was visualized by brain histology examination (LM & TEM) [88] and fluorescence imaging [85].

Nervous injury is mainly assayed by behavioral and electrophysiological studies. Behavioral studies were performed in a well-established animal model [89] with a series of clinical signs of toxicity, including tremors, convulsions, salivation, nausea, vomiting, diarrhea, lethargy, etc. Moreover, spatial learning ability and memory function associated with the hippocampus can be studied by examining the expression of related genes [90]. Electrophysiological investigation with electroencephalogram bands [91] is convenient and reliable. Peripheral electrical stimulus is one common method used in neurophysiology study, in which caudal sensory nerve action potentials [92] (caudal SNAPs) are recorded centrally from the tail. As the high oxidative metabolism in the brain, the function of brain mitochondria, which is easily isolated by differential centrifugation, is a good index to evaluate brain nerve injury [93, 94]. The respiratory chain [95] of the mitochondria provides opportunities for potential mechanism study.

2.7 Immune and reproductive system

The interactions between all new chemical and biological entities of NPs and the immune system need to be clarified prior to application in medicine and biology. Superficial modification of NPs determines their immunogenicity, which may cause immunotoxicity by interacting with the immune system [96]. Due to the complexity of the immune system, the toxicity assessment of NPs relies on overall changes of immune cytokines and molecules, immune organs, hematological system, etc. For instance, the generation and release of ILs and tumor necrosis factors are usually examined upon the use of NPs at high aspect ratio, some metal or certain cationic [97, 98]. Histological changes of major immune organs (e.g. spleen) are preferably observed by H&E dyeing [97, 99].

The toxicity assessment of the reproductive system is a relatively long-term study. Only a few researches [100–104] have focused on this kind of chronic study. However, it is a necessary step for the clinical approval of drug products. So far, various in vitro and in vivo models have been developed to study NP-associated toxicity in relative organs of this system (as reviewed in [105]). Different from other systems, the reproductive system is sexually divided into male female and male. Routinely for the male, the testicular tissue structure, the epididymal sperm parameters, the serous sexual hormones (e.g. testosterone), and the concentration of NPs in serum and testis need to be tested [106–108]. As for the female, sexual hormone (e.g. follicle stimulating hormone, luteinizing hormone, and estradiol) levels in serum need to be measured [105, 109]. Functions of major organs (ovary, uterus, and vaginal tract) also need to be evaluated [110]. The histopathological analysis is supposed to be done. Specifically, organs (testes, uteri, placenta, etc., of parental or offspring generation) with potential resorption of NPs are suggested to be inspected [111–114]. And last but not the least, reproductive index and offspring development need to be explored as some NPs were reported to penetrate the placenta-blood barrier in rats [115, 116]. Mostly, the teratogenicity of NPs on the fetus is of great concern [115, 117]. Also, the survival, growth, development, and reproduction of the offspring need to be mainly assessed upon prenatal exposure [101, 113, 118].

3 Summary

The application of NPs is increasing along with the development of nanotechnology, including drug delivery system [119], contrast agent of imaging, and engineering. Apart from the undesirable environmental NPs such as nanosilver nano-TiO₂ and carbon nanotubes [120], more attention needs to paid to medically applied nanoscale materials in systematical biosafety assessments. While in vitro studies indicate a promising direction, in vivo evaluation, as a closer step to the clinical application, more directly reflects the adaptation and injury of the organism. The present study elaborated the major systems and organ assessments from the aspects of general histopathology and specific function (Table 2). The ultrastructural changes and the relationships between tissues and NPs were clearly revealed through electric microscope. Original and specific pathologic changes were visualized by specific dyeing on paraffin sections. Functional injuries were detected by diverse biomarkers and high-tech...
equipment. However, in further studies and applications, more attention should be paid to the correlations between internal environment affecting in vivo studies and some single factors controlled in the in vitro studies, although some investigations have indicated that no correlations existed between them [121, 125].

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