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Nanotoxicity: Can We Use Traditionary Methods?

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Having regard to the rapid growth of nanomaterials in the human environment, both in assortment range and in absolute weight, it is necessary to determine the issues of their harmful effects on humans and the environment. This is especially important, when traditional nanosize elements are used in medicine and pharmacology. As known, depending on the size and technological conditions of fabrication of these materials, they acquire new distinctive properties. This one requires changes in traditional algorithms used in pharmacotoxicology. This article reviews the methods and offers a rejuvenation of algorithms for the nanotoxicological studies.

Враховуючи швидке зростання наноматеріялів в оточенні людини як за асортиментом, так і в абсолютному ваговому вимірі, необхідно визначитись з питаннями шкідливого впливу їх на людину та навколишнє середовище. Зокрема, це важливо, коли традиційні елементи у нанорозмірах використовуються у медицині та фармакології. Відомо, що, в залежності від розмірів, а також технологічних умов виготовлення цих матеріялів, вони набувають нових відмінних властивостей. Це потребує змін традиційних алґоритмів, що використовують їх у фармакотоксикології. В статті проводиться огляд методик і пропонується перегляд алґоритмів нанотоксикологічних досліджень.

Key words: nanomaterials, toxicity, nanotoxicity.

Ключові слова: наноматеріяли, токсичність, нанотоксичність.

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1. INTRODUCTION

The last decades of the development of human civilization have been marked by multiple rapid technological breakthroughs. One of such

areas of scientific thought was the desire to know the properties of matter at micro- and nanolevels. Physics, chemistry, biology, medicine, industry, agriculture, and a few other areas are actively developing nanomaterials. Nanomaterials are intensively developed and researched for the food industry, agriculture, electronics, *etc*. Very promising studies are involving the use of nanoparticles (NPs) in medicine and pharmaceuticals for diagnostic or therapeutic purposes. To date, a big quantity of nano-based drugs has been designed to treat various diseases such as neurological disorders, diabetes, cancer, infectious diseases, and allergy [1, 2].

2. THEORETICAL DETAILS

The specific properties of traditional elements at the nanoscale not only significantly affect many biological processes, but also make it possible to use them as carriers, significantly changing the bioavailability and kinetics of drugs. The known already and alleged technological capabilities of nanomaterials are causing the rapid growth of their development and production.

However, the hasty introduction of substances with new, not always predictable unique properties into the biosphere should be combined with the confidence of their safety for both the man and his environment. The historical experience of the technological revolutions had demonstrated us not only the significant advantages of industrial growth, chemical synthesis, the development of chemotherapy, radio energy, but also the ongoing and accumulating pressure of the negative consequences of the technological boom.

Given the wide range of areas of human contact, as a biological system, with nanomaterials and requiring various methods for assessing human impact, we plan to discuss only assessment methods in pharmacology.

To solve surveys of safe use of new materials in medicine for health in medicine, it is necessary, from our point of view, to determine some important aspects. First, is it justified to copy simply traditional methods for assessing the drug toxicity of new materials for use as a medicine or an agent for changing pharmacokinetics?

Secondly, how diverse should the studies of each sample of nanomaterials be, given the difference in their properties depending on the synthesis conditions? Besides, it is very difficult to correlate, probably, only structured nanotoxicity. It is suggested that additional information should be considered, such as (*1*) synthesis conditions, (*2*) technological characteristics, (*3*) nanoparticle size, (*4*) concentration, and (*5*) attributes associated with cell membranes. Molecular descriptors cannot be defined for very complex substances such as NPs and engineered nanomaterials (ENMs), since there is

usually no clear idea of their molecular structure [3].

It was established that the study of the mechanisms underlying the kinetics of NPs in biological media and their physiological and toxic effects strongly depends on their physical properties such as size, shape, structure, surface charge, and surface area, hydrophilicity, agglomerate and aggregate formation. Together with solubility, chemical, and geometric properties of new materials, this may be a prerequisite to obtaining reliable data on their toxicity [4].

Today, we have an ever-increasing database of increasing toxicity of nanomaterials in comparison with those elements of standard size and structure. Given the same mass, smaller nanoparticles have a larger specific surface area (SSA) and, thus, more available surface area to interact with cellular components such as nucleic acids, proteins, fatty acids, and carbohydrates. The smaller size makes also likely it possible to enter better the cell, causing cellular damage. For example, gold nanoparticles with a diameter of 1.4 nm were found to be toxic, while the same particles with a diameter of 15 nm did not display toxicity [5]. Several studies have revealed cytotoxic effects of silver nanoparticles [5, 6]. Moreover, iron oxide particles have also been found to exhibit harmful characteristics both *in vitro* and *in vivo* [7–9], mainly due to the generation of reactive oxygen species [10, 11].

Particle surface charge may affect the cellular uptake of particles as well as how the particles interact with organelles and biomolecules. Consequently, particle surface charge influences cytotoxicity. According to mathematical probability and assuming, particles are toxic, high particle uptake (*i.e.*, higher bioavailability) correlates with higher toxicity [12].

The form also may influence levels of toxicity. These authors found that: (*1*) as the atomic number of the element increases, cytotoxicity increases; (*2*) alteration of cell viability is a function of particle surface charge, available binding site on a particle surface, and particle metal dissolution, but not of band-gap energy.

In addition, we cannot exclude the possibility that the crystal structure after interaction with water or other liquids or biological structures can be modified and significantly differ from the original one. In addition to changes in nanoparticle characteristics, endogenous biomolecules, which are exposed to the nanoparticle interface, may also undergo structural and functional alterations. Such changes can have important implications for the safety of nanoparticles [13–15].

New materials require new methods of analysis. Traditionally, the assessment of chemicals, including pharmaceuticals, relied on data from animal testing; however, there are many motivations to

move to a situation, which is free of such testing. In part, the new paradigm for safety assessment embraces the ethos of twenty-first century Toxicology, whereby every effort is made to maximize the information that may be obtained without animal testing [16].

The toxicity of certain nanoparticles can be manifested at the molecular, cellular, and tissue levels [17]. It has been demonstrated that NPs can cause neurotoxicity through different mechanisms, such as lipid membrane damage, which serves to compartmentalize cellular components [17], cell cycle interference, reactive oxygen species (ROS) formation, and accumulation of autophagosomes, depending on their physicochemical properties and stability in physiological media. Low, *in vivo*, achievable concentrations of NPs induced only minor or no changes *in vitro*; however, prolonged exposure and accumulation *in vivo* could negatively affect the cells. This was also shown in case of autophagy dysfunction for the TiO_2 P25 NPs and decrease of cell viability for the TiO_2 FG NPs, which were only evident after 72 h of incubation [18].

3. DISCUSSION

Toxicity mechanisms of selected engineered NPs on human.

Available data indicate that the protective barriers of the brain against the movement of nanoparticles into the brain are incomplete. This raises concerns about the potential effects of manufactured nanoparticles on brain function, given that the ability of nanoparticles to cause oxidative stress, inflammation, death from apoptosis, or changes in the expression level of certain neurotransmitters [19].

Recently, information has been accumulating on studies, in which machine learning (ML) methods are used in the field of nanotoxicology to identify, assess and classify potential risks, taking into account costs and time with very encouraging results. This area has proven to be very useful in this area to get a preliminary idea of the features that affect toxicity, predict possible adverse effects as part of a proactive risk analysis and report on a safe design [20–22].

The introduction of ML into nanotoxicology is quite promising, although it is still in its infancy towards scientific consensus and subsequent guidelines and rules. Decision-making, machine-learning applications are transforming, according to some authors [23], our ability to predict toxicity based on nanofunctions and experimental conditions. Research is underway on integrating and curating fragmented data in compliance within the silico methods, which will allow for method testing and intercomparing and will help come to the standardization of methods.

The use of a prospective assessment of the potential toxic threat methods requires the use of the so-called big date, extensive databases of accumulated information [24, 25].

Today, we are in the process of accumulating data on the positive and negative effects of nanoparticles on living objects and humans especially. Without setting ourselves the task of describing the entire variety of the identified effects on biological structures, we focus only on some harmful systemic influences. More often, scientists are discussing influencing effects on the immune system [26], pulmonary system [27], and we should understand and analyses the most common features of these impacts.

One of the principal aspects of the toxic action of NPs on biological systems is their potential property for penetration through histological barriers. This can be very dangerous, since, it is specifically NPs and their actions for changing the protective properties of the placental and brain barriers against the penetration of other substances [28].

On the other hand, nanoparticles can cause comparatively fewer side effects in comparison with macrodrugs, improving their accumulation in the affected tissue, thereby, reducing the dose needed to achieve therapeutic efficacy [29].

Nanoparticles can decrease the toxicity of drugs by improving the biodistribution profile or by eliminating the need for harmful solubilizing agents [13, 30].

Nanoparticles, acting as a drug conductor across cell membranes, can serve as an alternative to toxic solubilizing agents, which are widely used to improve the delivery of water-insoluble drugs. In conclusion, nanoparticles can reduce drug toxicity by improving the distribution profile or by eliminating the need for harmful solubilizing agents.

It is necessary to take into account the features of kinetics. Creating a protein corona upon entering the body can drastically change nanoparticle properties, such as shape, size, and charge. For example, protein interactions can increase or decrease the size of nanoparticles, and typically cause the zeta-potential to become more anionic [13, 17, 30].

In addition, according to Maocai Shen and colleagues [31], nanomaterials, for example, micro(nano)plastics, can: 1) accelerate the diffusion of organisms in the environment, which can lead to biological penetration; 2) increase the exchange of genes between attached biofilm communities, causing the transfer of pathogenic and antibiotic resistance genes; 3) increase the flow rate of energy, materials, and information in the environment. This will increase the level of harmful effects on healthy organisms and, possibly, change the virulence of pathogens and the traditional picture of the devel-

opment of diseases. Unfortunately, plastics and their constituents are produced at a faster rate than their toxicities can be evaluated [32].

We can conclude the most general results of many different investigations in this field, for example, predictions or generalizations on how different characteristics of the nanoparticles affect their ultimate toxicity [33] and how those properties can be used to create guidelines and rules for the application of safer materials in NPs design [34].

Decreasing the particle size generally increases the toxicity and the amount of cellular uptake.

Positively charged nanomaterials are more toxic due to their increased interactions with primarily negatively charged biological surfaces and entities.

From a composition perspective, ionic dissolution correlates with the toxicity index.

Anisotropic morphology or rod-shaped NPs are taken up less efficiently; but once internalized, they exhibit significant damage to near-infrared plasmonic criterions.

The division of research methods in traditional toxicology into *in vivo* and *in vitro* groups is also used for nanotoxicological studies. *In vivo* studies can inform the choice of relevant model system for further *in vitro* studies as well as provide toxicity information not available through *in vitro* studies. The most commonly used *in vitro* assessment methods generally assess viability (live/dead ratio) or mechanism of toxicity. The main methods of analysis of viability, in

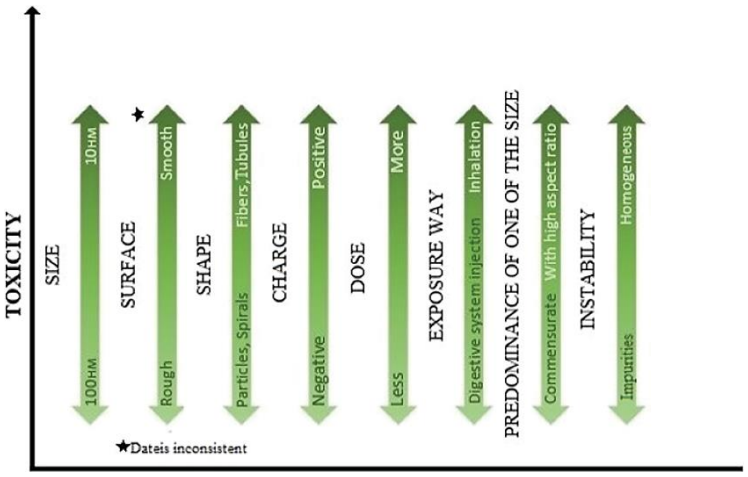

Fig. General trends in the influence of some parameters of nanomaterials on their toxicity.

turn, can be divided into the categories of proliferation, necrosis, or apoptosis, as well as analysis. The main mechanisms of toxicity are classified as oxidative stress or methods for detecting DNA damage. Recently, many new approaches have been applied to characterizing *in vitro* nanotoxicity. Gene expression analysis has been recently applied to the study of nanotoxicity. This technique compares labelled RNA collected from nanoparticle exposed and control cells through a competitive binding assay, using commercially available microarrays of human *c*DNA libraries [35].

In vivo nanoparticle toxicity studies typically focus on one or more of three major areas: changes in blood serum chemistry and cell formulas, changes in morphology of different tissues, examined using morphopathologycal investigations, or the overall nanoparticle biodistribution and clearance.

Novel *in Vitro* Toxicological Techniques. To obtain material for bioanalytical research, dynamic control methods are widely used, such as microfluidics and microelectrochemistry. In this case, samples are taken directly from freely moving animals through an implanted probe. This allows us to apply *in vivo* dynamic measurements limited to sample and detection rates of probes and detectors, respectively. An automated blood collection system for use with free-roaming animals allows overcome some of the limitations commonly used in *in vivo*, when measurement usually gives a static picture, which can be distorted by artefacts caused by sample preparation and handling.

Some authors [36] propose to use not only animal or fish models, but also to investigate phytotoxicity in plants [37]. Plant metabolomics is a simple and effective tool for solving the above problems, as it includes a comprehensive study of changes in metabolic profiles. Since the dominant metabolites and metabolic pathways are similar in different plants, they suggest universal applicability of

TABLE. A summary of traditional and innovative nanomaterial toxicity testing

Traditionary toxicity test	Innovative tests for new materials
Methods for determining the values of toxic and average lethal doses, maximum permissible concentrations (morphological microscopy, biochemical tests, functional studies) *in vivo* and *in vitro*.	Various cell tests for viability or increase/decrease in a designated inherent biological pathway. Genetic studies. Tests *in vivo*-like on 3*D* human organs. Machine Learning.

metabolomics analysis.

4. CONCLUSIONS

1. At present, the stage of active accumulation of data on the effect of new nanomaterials on living organisms, both positive and negative, continues.
2. In most cases, traditional methods of assessing the toxic effect of nanomaterials on the body of animals and humans are used with the use of modern laboratory and instrumental research technologies.
3. It is necessary to continue the development of appropriate algorithms and standardized methods of nanotoxicology, taking into account the specific properties of nanomaterials, which depend on the size of nanoparticles and their structure, production method, *etc*.

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