PBR
Pharmaceutical & Biomedical Research

December 2019. Volume 5. Number 4

Review Article: Applying Nanoparticles in the Treatment of Viral Infections and Toxicological Considerations

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Article info:
Received: 08 Jun 2019
Accepted: 03 Oct 2019

Keywords:
Nanoparticles, Drug delivery, Nanomedicine, Toxicity, Antiviral

ABSTRACT

Background: Despite the tremendous progress in human medicine, viral infections can cause death in them. Due to the high prevalence of viruses, it is required to develop novel treatment strategies and provide the site-specific delivery of antiviral agents at the viral reservoirs.

Objectives: Particle size is a vital physical characteristic that can affect the bioavailability and circulation time of nanoparticles. The ratio of large surface area and volume of nanoparticle could enhance the solubility of poorly soluble drug compounds.

Methods: Therefore, the optimum surface charge of the nanoparticle with the possibility of encapsulation and large drug cargo, are some of the nanoformulation advantages of these agents.

Results: This study reviewed various nanocarriers and modern smart delivery technologies, such as liposomes and immunoliposomes, polymeric nanoparticles, nanoemulsions, nanosuspensions, dendrimers, solid lipid nanoparticles, and so on.

Conclusion: We aimed to overcome the problems of traditional formulations and resistance to currently available therapies in various infections. Moreover, the concerns about the potential toxicities of nanoparticles to humans have been raised; therefore, the present study has reviewed the toxicity effects of nanoparticles used in controlling viral infections.

Introduction

Nanomedicine, as a field of nanotechnology, has potential applications for tissue targeting, controlled release, increasing permeability, and drug solubility with higher efficacy, improved safety and decreased toxicity [1]. The biopharmaceutical properties contain Absorption, Distribution, Metabolism, and Excretion (ADME), which are strongly associated with the physicochemical characteristics of drug formulation [2]. The use of nanomedicine offers some advantages that can improve the fate of drug molecules. Nanocarriers act as the transporters of therapeutic drug moiety to the target tissue.

Citation Fasili Z, Mehri F, Ebrahimi HA, Jamali Zh, Mohammad Khanlou E, Kahrizi F, Salimi A. Applying Nanoparticles in the Treatment of Viral Infections and Toxicological Considerations. Pharmaceutical and Biomedical Research. 2019; 5(4):1-20. DOI: http://dx.doi.org/10.18502/pbr.v5i4.2392

Interestingly, nanoparticle formulation can change the biochemical properties of drug molecules, causing sustained/controlled release, modified pharmacokinetics, and targeting specific sites of action in the cells. This can increase the effectiveness of treatment and decrease its complications [3]. Significant advancements are used in developing nanomedicine in the treatment of many diseases. Treating viral infections is among global challenges in a healthy society. Furthermore, it significantly impacts the health and economy status of millions of people and may cause diseases and death worldwide. Treating viral infections reduces drug's efficacy with the development of drug resistance. This phenomenon is a threat to public health and increases the mortality and morbidity rates and medical costs [4].

When infectious virus particles (virions) attached to susceptible cells and interned them, viral infections are generated. Accordingly, the virions can spread locally or over long distances through blood flow, lymph, or neural routes. The spread of infectious viruses from cell to cell often involves the direct transfer of infectious particles into the extracellular environment. To spread the infection, it is necessary to transport viral particles within the neurons and spread to epithelial cells; however, the number of viruses transmitted between the cells during these events is unclear [5].

The most important advantages of nanocarriers that make them good candidates for antiviral drug delivery are their biochemical properties, such as small particle size [6], the ratio of large surface area to volume [7], and tunable surface charge; which facilitates the penetration of particles through the negative charge of cell membrane [8]. Furthermore, nanocarriers can increase the bioavailability of encapsulated drug contents, improve the solubility and stability of the drug compounds in physiological conditions, facilitate take-up by phagocytic cells as viral reservoirs, deliver the drug compound intracellular, reduce antiviral drug compound toxicities, delay the drug resistance, overcome the biological barriers [e.g. Blood Brain Barrier (BBB) and blood test barrier] [9], improve cellular uptake, and increase drug delivery to the target sites [10]. This review study will discuss various recent investigations (past 10 years) on nanocarriers regarding antiviral drugs and vaccine delivery and their toxicities and other adverse effects.

Nano drug delivery systems for antiviral drugs

Liposomes

Liposomes are efficient delivery systems for drug compounds or biological entities. They are closed spherical vesicles, containing phospholipids and cholesterol. Liposomes can encapsulate hydrophilic, amphipathic, and lipophilic drug molecules into inner water phase or within lipid leafleted. Liposomes, as carriers, could release their cargo (drug or antigen) in specific target sites. This nanocarrier can provide a focused antimicrobial innate and immune response against pathogens.

Moreover, it could be used as a novel agent for prophylaxis or therapy against microbial infections [11]. Small and single-lipid bilayer liposomes (20-100 nm diameter), enclose a large aqueous core; they are ideally suited for the encapsulation of hydrophilic drug compounds/antigens. In return, Multi Lamellar Vesicles (MLV) are characterized by the presence of two or more concentric lipid bilayers (500-5000 nm diameter) that preferentially entrap hydrophobic drug molecules. Liposomes structure forms a protective barrier, which can typically protect the cargo from degradation. It can also help them to release at the target cell or organ.

Liposomes are biocompatible and biodegradable and have low toxicity [12]. Despite many advantages, liposome formulations have several significant issues. The first one is the elimination of liposomes from systemic blood circulation by the Reticuloendothelial System (RES) which phagocyte the se nanoparticles and causes the short plasma half-life times of these nanoformulations [13]. Some studies suggested that more substantial size and negative charge at the liposomal surface will decrease the half-life of the liposomes [14]. Removing liposomes from the circulation by phagocytosis is directly associated with their diameter. MLV with diameters from 500 to 5000 nm are quickly eliminated by phagocytes; whereas, Unilamellar Vesicles (ULV) with diameters between 20 and 50 nm are less internalized by macrophages [14].

Croci et al. argued that ivermectin cytotoxicity, when delivered via liposomes, was reduced up to 5 times, in comparison to free drugs. They reported a significant increase in the antiviral activity of the liposomal forms of ivermectin. This nanocarrier can effectively inhibit the replication of dengue virus with half-maximal Effective Concentration (EC50) values. They found that the administration of ivermectin by a liposomal formulation yielded higher Cmax and significantly faster absorption time. This finding suggested that applying liposomes could improve the in vivo efficacy of ivermectin. This technology can also improve the spectrum of ivermectin therapeutic activities [15]; it can be a promising starting point for the future development of liposomal nanocarriers against pathogenic viruses.

Local antiviral formulations have been produced with nano-liposomes technology. Liposomal gel, as the topical formulation of idoxuridine, was prepared by Seth et al. using reverse phase evaporation method [16]. The antiviral efficacy of topical liposomal gel was tested; the relevant result revealed that this new formulation had improved therapeutic efficacy in treating herpes simplex virus type 1 and 2 infections [17]. Ramana et al. developed a liposomal nano delivery system for nevirapine. Nevirapine, a newly licensed drug, was the first member of non-nucleoside reverse transcriptase inhibitors. It has demonstrated potent activity against Human Immunodeficiency Virus type 1 (HIV-1).

The obtained results demonstrated that the encapsulation efficiency was elevated. Another advantage of nevirapine-loaded liposomal nano delivery was improving the targeted delivery of antiretroviral drugs with fewer adverse effects [18]. Coating the external surface of liposomes with Polyethylene Glycol (PEG) reduces immunogenicity and the percentage of uptake by macrophages and leads to a prolonged blood circulation half-life. Therefore, pegilation provides enough time for them to leakage through endothelium towards the tissues [19]. In addition to paginated liposomes, stimuli responsive liposome and targeted liposomes are also being developed.

Immunoliposomes

Immunoliposomes are examples of targeted delivery technologies and have been extensively investigated for their potential in targeted drug delivery methods [20]. The aim of developing targeted liposomes is to selectively deliver the cargo to target cells. Targeted liposomes are therefore designed to counterbalance the broad body distribution of stealth liposomes. The membranes of targeted liposomes are functionalized with glycoproteins, polysaccharides, or ligands for specific receptors that cause the accumulation of liposomes in particular tissues; thus, the liposome cargo can be preferentially released in target sites [20].

Another strategy to deliver the drug or antigen transported by liposomes in the desired site is represented by immunoliposomes. Antibodies and antibody fragments, as targeting moieties for liposome, are highly specific for the target antigens and combined antibody [20]. Antibodies can be attached to the surface of the liposomes and form valid specific binding to the specific site of target cells in vitro; however, their in vivo performance is often inefficient due to enhanced uptake through RES [21]. Examples of stimuli-responsive liposomes are the pH-responsive, redox-responsive, enzyme–responsive, and light-responsive liposomes, which keep their cargo encapsulated at body condition, but specifically discharge it in the target site [22].

Many liposome formulations are available for treating viral infections in clinical use. Liposome encapsulated antivirals (e.g. ribavirin, azidothymidine, or acyclovir) have been formulated and reported to reduce toxicity; today, these formulations are evaluated through more precise tests which are related to their efficacy [23]. Liposomal capability to induce immune responses to antigens bound to it was first reported by Gregoriadis and Allison [24]. Liposomes and virosomes have become such important carrier systems that motivation for liposome-based vaccines significantly has increased in vaccine delivery. Virosomes are liposomes prepared by the fusion of natural or synthetic phospholipids and virus phospholipids, viral glycoproteins, or other viral proteins [25].

Epaxal is the first liposomal vaccine, used for hepatitis A prevention. Its formulation has been developed and patented by CrucellBerna Biotech, in Switzerland. This formulation is free of aluminum and thiomersal; therefore, has high tolerability and fewer adverse local effects compared with conventional aluminum-adsorbed vaccines. Epaxal is highly effective after the first dose administration, but can protect immunity for a limited time. Following the second dose, the immune system will be boosted up for the next 20 years [26]. Inflexal V is composed of haemagglutinin surface molecules of the influenza viruses. It was developed and patented by the CrucellBerna Biotech, in Switzerland.

These virosomes contain globular and unilamellar vesicles with an approximate diameter of 150nm. The immunogenicity and tolerability of Inflexal V vs. conventional flu vaccines have indicated statistically significant improvements [26].

Polymeric nanoparticles

Polymeric nanoparticles are designed in two ways. In the first form, the drug molecule is entered into a cavity and surrounded by a polymeric membrane, known as nanocapsule. In the second form, the drug is uniformly mixed with polymeric matrix, which is known as nanosphere (Table 1) [27]. The most common polymer, which is extensively used as a carrier for polymeric nanoparticles, is Poly Lactic Acid Co Glycolic Acid (PLGA). PLGA, because of its biocompatibility and biodegradability, is a Food and Drug Administration (FDA) approved polymer. It provides an excellent delivery carrier

for the controlled administration of drugs, peptides, and proteins. PLGA, as a copolymer, consists of Poly Lactic Acid (PLA) and Poly Glycolic Acid (PGA) [28].

Polymeric nanoparticles immigrate into the cell through the endocytosis process. Phagocytosis, pinocytosis, receptor-mediated endocytosis, and clathrin-mediated endocytosis are endocytosis pathways by which these systems can be taken up. Polymeric nanoparticles, like liposomes, are rapidly taken up by the RES in the parent or opsonized form; this results in limited contact time with their target organs in systemic circulation [29]. To prevent their uptake, various coating agents or shielding are used. This goal can also be achieved by some other solutions, such as modifying surface, decreasing these nanoparticles' size, and exerting hydrophilic molecules, like PEG [30].

Polymeric nanoparticles are useful systems for drug delivery and drug targeting. They can deliver drugs to specific sites, and help to improve the solubility of poorly soluble drug molecules. Compared to traditional formulations, polymeric nanoparticles have better applications and more effective drug delivery. This would ultimately improve treatment outcomes and patients' compliance [31]. These specific properties prevent the rapid clearance of drug compounds and increase their stability. In turn, that makes the administration of drugs at lower doses possible, i.e. essential in reducing drugs' toxicity [10].

A significant number of scientific researchers reported using polymeric nanoparticles to deliver antiviral medicines. Shibata et al. studied PLGA nanoparticles that contain a combination of three Antiretroviral drugs (cART NPs) for the inhibition of HIV-1 replication. PLGA, as a biocompatible and biodegradable polymer, Encloses defavirenz (EFV) and boosts lopinavir (lopinavir/ritonavir; LPV/r). The average size of cART NPs was equal to 55.4-138.3 nm and its average surface charge was calculated as -13.7–4.5 mV. These results suggested that all the three antiretroviral drugs are efficiently encapsulated (>79%) into the cART NPs. The lack of cytotoxicity in the cell lines within 28 days in vitro with IC50 values in the nM range was investigated.

The relevant results revealed that the cART NP formulation has more advantages with the equal amounts of the drugs orally administered to patients with HIV-1 infection. Compared to the soluble antiretroviral drugs, this study demonstrated significant increase in the uptake of the cARTNPs and higher levels of antiretroviral drugs in the nuclear, cytoskeleton, and membrane fractions of cells. This experiment demonstrated that the higher intracellular antiretroviral drug delivery by PLGA NPs formulation significantly reduces the HIV-1 infectivity by inhibiting HIV replication at lower doses [32]. In a study, Guedj et al. applied the PLGA-based nano polymer as an efficient and safe carrier to increase the transport of active molecules into human monocyte-derived macrophages.

They developed PLGA NPs containing bovine serum albumin (size =126 nm, zeta potential =−5.61 mV) which were taken up rapidly and efficiently by macrophages for the prevention and elimination of intracellular pathogens, like HIV. They concluded that PLGA NPs are swallowed by macrophages within 30 minutes. Furthermore, electron microscopy revealed they are found into the cytoplasm of human monocyte-derived macrophages after 45 minutes following administration. Several studies have documented that the use of polymeric-based nanocarriers is a promising strategy to deliver drug compounds in human monocyte-derived macrophages. Moreover, it could be beneficial in targeting pathogens that amplify inside macrophages such as HIV, malaria, visceral leishmaniasis, and tuberculosis [33].

Machado et al. tested PLGANPs contained Tenofovir (PLGA-TFV-NPs) embedded into a thin polymeric film base to allow the vaginal administration. PLGA-TFV-NPs were prepared by a double emulsion solvent evaporation method, and the encapsulation efficiency of TFV was high. The mean diameter value of nanoparticles was equal to 127nm, and the drug encapsulation efficiency was above 50%. The films containing TFV-loaded NPs; however, demonstrated two release steps, as follows: at first 15 min, 30% of TFV was detected in media because of an initial mild burst effect, which was followed by approximately linear sustained release.

In particular, both in vitro drug release profiles for TFV-loaded NPs films are favorable to potentially allow immediately; they also sustained TFV levels in the vagina. The described mouse model was used for the first time in the evaluation of vaginal films and corroborated the safety of PLGA- TFV/Stearyl amine NPs in films. This new formulation can be considered as an interesting substitute to currently used TFV gels in clinical sttings, if in vivo testing such as pharmacokinetics, safety and efficacy is justified. This film could be an appropriate alternative to rings; the only difference is that the film has the advantage of being suitable for women in need of only occasional protection [34].

Nanosuspensions

Nanosuspensions are the highly fine colloidal dispersions of nanoparticles stabilized by surfactants and polymers. They are also recognized as a biphasic system, consisting of drug nanoparticles with the mean diameter values of <1 µm, dispersed in an aqueous vehicle [35]. Nanosuspensions consist of the poorly water-soluble drug molecules without any matrix material suspended in dispersion [36]. This nanotechnology can enhance the solubility of poorly water-soluble drug molecules. This approach is not only useful for molecules with poor solubility but also poorly permeable drug compounds; this is a significant challenge for the formulators [37].

Nanosuspensions containing antiviral agents have been taken up by macrophages and showed time-dependent kinetics. Because of their nanosized form, they can quickly enter the cell. For increased uptake, their surface can be modified; that results in their specific receptor-mediated cellular endocytosis, which in turn leads to increased drug concentration inside cellular compartments. The suspensions can also be lyophilized and converted into a solid matrix [10]. Dash et al. studied the nanosuspensions of atazanavir, ritonavir, and poloxamer 188 as stabilizers obtained by high-pressure homogenization for neuroprotection in a humanized HIV infected animal model (nonobese diabetic/severe combined immunodeficiency-γcnull mice).

This formulation was administered intravenously and weekly, leading to the development of neuroprotective reaction (diagnosed by reduced neuronal, synaptic, and astrocyte damage). In addition, it decreased the viral loads and remained CD4+ cells in peripheral blood [38]. Roy et al. applied the same in vivo model and authenticated these findings; they correlated their relation with the tissue and blood serum levels of atazanavir, efavirenz, and ritonavir when these drugs were administered as nanosuspensions. Their data indicated that the nanoformulations of protease inhibitors show high efficacy in cell-line.

This model can be successfully used in the animal model of HIV-1 disease to stop CD4 + T cells loss and reduce viral replication [39]. These studies endorsed the role of monocytes/macrophages in the enhanced CNS delivery of the antiretroviral drug compounds. Shegokar et al. prepared nevirapine nanosuspensions by cold high-pressure homogenization technique. Their surface properties were modified by the addition of surface modifiers, such as serum albumin, PEG 1000, and dextran 60.

Their trial resulted in the production of nanoparticles with mean particle sizes of 515.8 nm, 520 nm, and 520.3 nm, respectively. The nanosuspensions were easily taken up by primary macrophages, and indicated time-dependent uptake kinetics. Nevirapine-loaded surface modified albumin nanosuspensions could pass through the blood brain barrier and accumulate in the brain for >24 h. This is one of the major problems in treating Acquired Immune Deficiency Syndrome (AIDS). Thus, this finding can generate a new perspective for treatment of HIV-1 infection. Nanosization of nevirapine can significantly improve its in vivo behavior; additionally, it can potentially target the infected cells [40].

Nanoemulsions

Nanoemulsions are colloidal particulate systems with 10 to 1000 nm sizes, transporting drug molecules. An emulsion is a water and oil biphasic system where one phase is dispersed in the other. Macroemulsions are thermodynamically unstable systems; in these cases, they can be used as an emulsifying agent for its stabilization. In contrast, nanoemulsions, due to the presence of surfactants and co-surfactants, are more stable. Because of their small size, nanoemulsions are transparent. Nanoemulsions are divided into three categories, as follows: first, oil is dispersed in the continuous aqueous phase and forms oil in water nanoemulsion; (b) second, water droplets are dispersed in oil phase and make water in oil nanoemulsion, and the third form is bi-continuous nanoemulsions [41].

Hobson et al. described the controlled radical polymerization synthesis of an amphiphilic branched copolymer, enable to stabilize nanoemulsions and dissolve efavirenz, and lopinavir as anti-HIV drug compounds. This process leads to increased drug absorption and a subsequent decrease in the required oral dose for treating HIV patients. To evaluate the antiviral activity of nanoemulsions, MT4 cells are infected with the Human Immunodeficiency Virus 1, strain IIIB (HIV-1) virus.

The experiment is designed in the presence of the cells in which the virus replicates. Therefore, to show the effect of efavirenz, the drug should enter the cell and harness more proliferation of the virus. The MTT assay is performed to evaluate the cell viability of all cells in culture against HIV, leading to cell death. Unloaded nanoemulsion suggested no inherent antiviral activity against HIV-1 IIIB expected [42]. However, the drug-loaded nanoemulsion samples efficiently inhibited the HIV-1 IIIB cells.

Dendrimers

Dendrimers are nanoscale carriers with symmetric molecules, homogeneous, and monodisperse structures, which have branches like a tree [43]. Dendrimers are monodisperse macromolecules containing a small molecule or a linear polymer in core and symmetrical branches around itself [44]. Polyconicdendrimers have no persistent shape and may change in size, shape, and flexibility as a function of increasing generations [45]. Dendrimers are hyperbranched macromolecules which their end-groups can be functionalized. As a result, their biochemical activity can be modified [46].

Dendrimers have unique features, allowing them to be variously applied. Dendrimers are defined as artificial macromolecules, containing several functional groups and a highly-branched structure [43]. Dendrimers are essential in molecular chemistry because of their high functional groups. This ability is named as host-guest property, i.e. based on the reaction of functional group of substrate molecule (guest) to a receptor molecule (host) [47]. Dendritic polymers have advantages in biomedical applications. They are analogous to proteins, enzymes, and viruses; therefore, their application is convenient. Dendrimers and other molecules can either be attached to the periphery or encapsulated in their interior voids [48].

Vacas-Cordoba et al. explained the mechanism of polyanionic carbosilanedendrimers G3-S16 and G2-NF16 inhibiting HIV-1 infection. They demonstrated that the formulation prevented the infection of the cells by the HIV-1 at the first step. Carbosilanedendrimers can prevent the binding of virus particles to target site. This process occurs through the occlusion of the gp120-CD4 interaction. G3-S16 and G2-NF16 are polyanionic molecules, and their mechanism relates to the electrostatic interactions of their functional groups and proteins of the HIV-1envelope, like gp120.

That finally blocks target points and prevents virus binding to the target sites. They argued that dendrimers can also bind to some structures, like proteins at the cell surface; i.e. related to the HIV-1 infection, including CD4 or CCR5 coreceptors. In addition, they reported dendrimers inhibit cell-to-cell HIV-1 transmission and complicate the formation of infectious synapse. In case of donor dendritic cell treatment, most dendritic cells containing HIV-1 diffused into the cytoplasm; whilst an increase of dendritic cells containing the HIV-1 in the intracellular sac-like compartment was observed when receptor cells were treated.

They hypothesized that it may be related to a different inhibitory mechanism of dendrimers in each case. They assumed that dendrimers would be preventing the specific protein interactions at the cell-to-cell contact area; thus, they block the correct signaling pathways for synapse formation. Dendrimers can importantly affect the primary steps of HIV-1 infection in a multifunctional manner; therefore, their combination with other anti-HIV-1 drug compounds could be interesting to generate new potent combinatorial microbicides [49].

Sepúlveda-Crespo et al. reviewed important role in preventing HIV/HSV-2 (herpes simplex viruses) co-infection. Accordingly, they recommended new therapeutic options to manage individuals harboring multidrug-resistant viruses. To improve the microbicide pharmacokinetics, better targeting, and innovative approaches, with a decreased chance for the development of resistance, nanotechnology-based microbicides, and especially dendrimers, seem to be efficient strategies. The polyanionic dendrimers have shown exciting features for clinical use as antivirals against HIV/HSV-2 co-infection and other sexually transmitted infections. Polyanionic dendrimers have biocompatible, biodegradable, and monodispersed structures. This nanostructure can entrap several drug compounds by encapsulation or electrostatic interactions.

Furthermore, it can target specific sites and deliver drug compounds (e.g. inside the cell). Polyanionicdendrimers have proper biological stability and can mimic biological receptors or cofactors by surface modifications. Most of the development of resistance occurs spontaneously because of the high mutation rate of the virus. However, if viruses overcome the inhibitory effects of dendrimers through mutations, dendrimers can interact with other specific sites of gp120, and therefore inactivate the viruses. The majority of dendrimers can target the variability of HIV/HSV-2 envelope proteins.

This process would explain the susceptibility of different HIV and HSV-2 variants to these inhibitory nanoparticles. Molecular modeling systems can develop optimized anti-HIV/HSV-2 dendrimers. A dendrimer with dual action against viral infections will reduce the cost of therapy. This is due to its main advantage that can prevent both HIV and HSV-2 virus infections. There are some other dendrimers with dual antiviral activity, including SB105-A10, SPL7013, and even several carbosilanedendrimers [50].

Solid Lipid Nanoparticles

Solid Lipid Nanoparticles (SLNs) were developed since 1990 as a substitute carrier system to liposomes, emulsions, and polymeric nanoparticles. SLNs are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle-matrix being solid at room and body temperatures [51].They have an average size of 40-1000 nm and a spherical morphology [52]. Gupta et al. revealed the solid lipid nanoparticles of efavirenz. They prepared those by high-pressure homogenization and solvent evaporation methods. In this study, the sizes of the average nanoparticles were <110 nm.

Such findings illustrated this formulation is suitable for administration through different routes. This is because it increases the permeability and bioavailability of the poorly soluble drug efavirenz. The intranasal formulation of efavirenz demonstrated 150 times more efficient to reach the brain targeting and 70 times better absorbed, in comparison to the traditional formulation (capsule). Therefore, this novel formulation has higher potency for reducing the viral levels in plasma with a low dose of efavirenz; it has less toxicity as well as significant potential to target the brain. Thus, this new formulation has a high potential to eradicate HIV reservoir and improve AIDS according to the clinical trials [53].

Penumarthi et al. developed a new SLNs formulation as a non-viral Deoxyribonucleic Acid (DNA) vaccine delivery system. They prepared SLNs by solvent emulsification method and DNA–SLN complexes were prepared at different mass ratios. The synthesized SLNs indicated an average 110 nm hydrodynamic size with a Polydispersity Index (PDI) of 0.5. They concluded this system might be an excellent model for the non-viral delivery of DNA. This result could be a basis for future research in DNA vaccine delivery [54].

Niosomes

Niosomes are structurally similar to liposomes. They are composed of microscopic lamellar bilayer vesicles. Their difference with liposomes lied on their compositions, which non-ionic surfactants are using for preparing niosomes. Therefore, niosomes are more stable and cost-effective, and their maintenance is more accessible. Some energy is required for heat and physical agitation to form this structure. In the bilayer structure, the hydrophilic heads are in contact with the aqueous solvent, whereas hydrophobic parts are oriented away from the aqueous solvent [55, 56]. Niosomes might be unilamellar or multilamellar, relying upon the technique used to set them up [57].

Sherry et al. studied the new formulation-niosome gel, containing acyclovir for enhancing dermal deposition. The highest percentage entrapment efficiency was in the vesicle size of 28 nm. The observed data suggested a greater permeation of niosomes across the skin from this formulation. They assumed the best ratio as span 60 and tween 60 as well as between cholesterol and lecithin as 1:3 and 1:2, respectively in the niosome gel formulation. Therefore, different ratios of surfactants, cholesterol, and lecithin have been formulated and evaluated. Their optimum ratios majorly impacts the control of permeation and skin penetration from the vesicles.

Localization of drug inside the derm or epiderm can provide effective concentrations in the skin while avoiding the systemic exposure. Drug delivery from the skin depot site in a sustained and controlled rate can be bioavailability improved; therefore, they could control the disease for a prolonged period, compared to conventional dosage forms [58]. All mentioned above nanocarriers for drug delivery of antiviral agents are presented in Table 1.

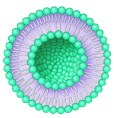
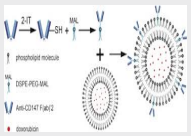
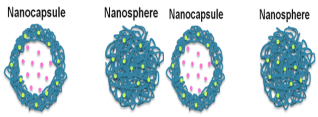
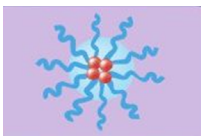
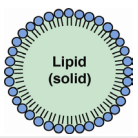
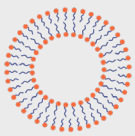
Toxicological consideration

Role of nanoparticle characterization in toxicity

Considering the importance of nanoparticles in estimating toxicological endpoints, accurate identification, and the characterization of nanoparticles are essential. Otherwise, the obtained toxic effects cannot easily be attributed to a particular property of the nanoparticles [59]. Internationally suitable protocols and standards for identifying the property of nanoparticles have not been implemented. Therefore, due to the lack of toxicity databases and proper characterization techniques, the toxicity effect evaluation of nanoparticles is very hard. Different toxicity of nanoparticles relates to the function of their chemical structure or deposition site in human body [60].

Regarding the chemical structure, nanoparticles classify into two hard and soft types. The instances of hard nanoparticles are metals, and non-metal compounds and soft materials include dendrimer-, latex-, polymer- and protein-based nanoparticles [61]. To demonstrate the toxicity description of nanoparticles, different techniques have been applied. Electron microscopy is a precise method and is frequently applied to determine the different size, shape, and structure of particles. Bourdon et al. used animated light scattering and transition electron microscopy methods to assess the dynamic size of nanoparticles [62].

Table 1. Possible nanocarriers used in viral infections with structure and loaded drugs

Nanocarriers	Cargo	Structure
Liposomes	Ivermectin Nevirapine	
Immunoliposomes	Epaxal® Inflexal® V	
Polymeric nanoparticles	Defavirenz Lopinavir Tenofovir	
Nanosuspensions	Nevirapine Atazanavir Ritonavir Poloxamer 188	
Nanoemulsions	Efavirenz Lopinavir	
Dendrimers	G3-S16 G2-NF16	
Solid lipid nanoparticles	Efavirenz Non-viral DNA vaccine	
Niosomes	Acyclovir	

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Other identification procedures are centrifuge sedimentation and ultra-high light microscopy. These approaches have been applied in several studies for evaluating extensive collections of nanoparticles, including carbon-based materials, metal oxides, metals, in cell culture and water media. Murdock et al. used these methods in many of their research studies [63]. In many articles, for determining the Au content in HeLa and A594 cells when exposed to oligonucleotide-modified Au nanoparticles from inductively coupled plasma mass spectrometry (ICP-MAS) has been used as an identification probe [64]. Another highly applicable technique is fluorescence spectroscopy. This method was used to identify the dynamic tracking of nanoparticles in cells [65]. In conclusion, considering the different methods and characteristics of nanoparticles, a precise evaluation about the side effects of particles is only possible through conducting various studies about the nanoparticles' properties [66].

Dose and dose criterions in toxicity

The dose is explained as the quantity of a substance that will reach a biological system and affect the target organ. Dose determination is among the main factors in toxicology studies. In nanotoxicology surveys, the dose

is directly calculated by the concentration of substance in different mediums (e.g. air, food, & water) multiplied by the contract duration. The dose determination is vital to describe conclusions obtained from in vitro and in vivo studies for assessing health risks [67]. Although this definition may seem easy and straightforward, dose determination has many complexities. This is because various nanoparticle has different properties, such as size, surface chemistry, and diverse structure, which can change particle-particle (e.g. aggregation) or particle-cell (e.g. uptake rates) interactions [68].

Toxicity analysis, to achieve a biological response, should be performed by considering doses in real conditions. In nanotoxicology studies, dosing is expressed in two levels; high doses and low doses. High doses are useful in short contact for explaining mechanisms. Although this parameter can be improper to predict the process of human pathology from environmental contacts [69]. Moreover, low doses can be precise and sensitive in prolonged contact for inhalation experiments and are more likely to be predictive of human dangers [70].

Surface reactivity of nanoparticles and toxicity potential

Regarding the surface properties of particles, toxicity studies about particles with similar physicochemical structures have demonstrated that severe toxicity occurs from smaller particles compared to larger ones [71]. Increased surface area is proportional to increased chemical reactivity. Therefore, immediately after receiving similar mass doses, including billions of nanoparticles to human body, it will respond very differently to contact with the biological systems [72]. In addition, when certain particles move into biological or environmental contexts, smaller structures, such as atoms, molecules, or macromolecules adjoin to the particle's surface to form strong or weak bonds.

Some studies used titanium dioxide particles in mice; their obtained results suggested that nanoparticles with diameters of about 20nm create much higher inflammatory responses, compared with larger nanoparticles with mean diameters of 250nm for a similar mass dose [73]. It has extensively been reported that nanoparticles in biological or environmental mediums never consist of "bare" particles [74]. There are comprehensive research studies available on the particles' surface; however, because of their different properties, it seems necessary to perform more studies about physical and chemical characteristics of nanoparticles when determining their biological activities [75].

Comment mechanisms in the toxicity of nanoparticles

Data are scarce about the metabolism of nanoparticles. Due to the existence of different breakdowns of nanoparticles and unpredictable molecular responses, their degradation processes in the body when contacting with biological systems are critical and different. Various protocols have suggested measuring particle uptake proportion and intracellular distribution in different cells [76]. The activity mechanisms of nanoparticles when entering the cell and interacting with subcellular structures divide into chemical or physical types. The primary chemical mechanism is the formation of Reactive Oxygen Species (ROS) [77].

In vivo and in vitro studies have reported that ROS production is a crucial factor in primary and secondary processes that can cause cell damage that function (by the peroxidizing of lipids, altering in proteins, disrupting to DNA, interfering with signaling functions, and modulating gene transcription) and even cell death [78]. The other cases of chemical mechanisms are as follows: the activity and release of toxic ions [79], the disturbance of electron chain /ion cell membrane transport activity [80], catalysis damage [81], lipid peroxidation [82], and surfactant properties [83]. Physical mechanisms include damages membranes [83], changed membrane function [72], modified transport processes [84], and protein conformation/folding [85].

Chemical and physical mechanisms constitute several responses. These responses may occur before or after the contact of particle with organ targets. Wang et al., in an in vivo study, indicated once the nanoparticles enter the human body via different pathways, such as ingestion, dermal, and inhalation, nanoparticles' metabolism occurs in blood circulation; then, their profiles clear in organs, such as the lung, liver, and kidney [86]. Therefore, in addition to identifying mechanisms, patterns clearance of nanoparticles in organs are important for understanding their fate in the body.

Cell toxicity of nanoparticle

Describing the cytotoxicity of nanoparticles is beneficial for the accurate interpretation of their biological activities. However, identifying the proper molecular mechanisms underlying cell toxicity plays an essential role in the cytotoxicity of nanoparticles [87]. Several studies have reported the cytotoxicity of particles may be affected by their small size and surface in terms of cellular processes and numerous disease developments

[88]. Bahadar et al. evaluated the cytotoxicity effects of different nanoparticles using tetrazolium-based techniques, such as MTT and MTS.

They exposed different cells, like cancer cell lines to nanoparticles with different compositions and sizes to determine cell viability. They concluded that these parameters (size and composition) primarily affect the determination of intracellular responses, the degree of cytotoxicity, and the potential mechanisms of toxicity [89]. Lanone et al. investigated the cytotoxicity effects of 24 nanoparticles with similar spherical diameter, and various elemental compositions and structure on 2 important pulmonary cell lines; THP-and 1A549.

They found that copper- and zinc-based nanoparticles appeared to be the most toxic ones, compared with other samples. Titania-, alumina-, ceria-, and zirconia-based nanoparticles revealed moderate toxicity, and no toxicity was observed for tungsten carbide [90]. Uboldi et al. surveyed the different cytotoxicity effects of SiO_2 nanoparticles using MTT assay and fluorescence microscopy on Balb/3T3 mouse fibroblasts. They observed nanoparticles that were exclusively located in the cytoplasmic district of cell had no cytotoxicity effect [91]. Results obtained from different investigations on nanoparticles indicated these components might be toxic in experimental conditions; however, further studies are required to access fresh internationally agreed of bias toxicological models. Therefore, it is difficult to interpret these data.

Liver toxicity of nanoparticle

The liver has been documented as a vulnerable and main organ involved in the metabolism and expulsion of xenobiotic compounds of the body. All compounds entering the body are sorted by the liver tissues before being allocated to various body parts. The liver has many advantages, such as its high blood flow speed, its exposure with different compounds, and its high metabolic activity. Many investigations have suggested nanoparticles are trapped by the reticuloendothelial system in the liver; therefore, hepatotoxicity testing is a famous experiment for the safety assessment of nanoparticle toxicity. Studies are limited to the hepatotoxicity of nanoparticles in the liver.

Teodoro et al. used the liver cells line as an in vitro study model to determine the toxicity of AgNP particles in BRL3A rats. They observed remarkable reductions in mitochondrial function, increment in lactate dehydrogenase (LDH leakage speed from cells, the elimination of antioxidants and a rise in ROS production [92]. Gaiseret al. surveyed the level of inflammation and oxidative stress produced in the liver of female Wistar rats by Ag NPs. They found that Ag NPs were very toxic for hepatocytes and created hepatocyte homeostasis by decreasing albumin release levels [93].

Yang et al. evaluated hepatic cell toxicity and its important mechanism by exposing cells to SiO_2 NPs in ICR mice. They indicated SiO_2 NPs have a possible distribution into different liver cells, including Kupffer and hepatic stellate cells; finally, the process may lead to hepatics dysfunction as well as granuloma production in the liver [94]. Various studies exploring the hepatotoxicity of nanoparticles demonstrated that these components might be talented hepatotoxins for human exposure. Nevertheless, these studies, because of using different models and methods for toxicity measurement are complex for data interpretation. In addition, considering the limited studies in this area, further investigations are required (Figure 1).

Kidney toxicity of nanoparticle

The kidney, similar to the liver, is among the vital target organs for nanoparticle toxicity and a primary organ for clearing them from the body. Rana et al. investigated the effects of exposure to CdSNPs (cadmium sulfide nanoparticles) by some mechanism (lipid peroxidation generation and H_2O_2 production) in the rats' kidneys. Their observations highlighted impairments in proximal tubules, mitochondrial dysfunction, disorders in nuclear and ER (endoplasmic reticulum), and eventually reductions in alkaline phosphatase level from the brush border of proximal tubules [95]. Moisan, Brochard et al. evaluated the toxicity of Carbon Black (CB) and titanium dioxide nanoparticles using immunofluorescence assay in glomerular mesangial cell (IP15) and the epithelial proximal tubular of renal.

They demonstrated that immunofluorescence microscopy assay using latex beads revealed NP materials considering size, the cells internalized particles, and accumulation in the cell cytoplasm, significantly increased ROS production in IP15 and LLC-PK1 cells [96]. Lu Xiao et al. conducted studied the toxic effect of zinc oxide on podocytes and rats. The in vitro experiment revealed podocytes exposed to ZnO NPs, compared to controls demonstrated an intracellular increase of Reactive Oxygen Species (ROS), apoptosis formation, decreased SOD value, and increased MDA level. Results obtained from in vivo investigations on adult male Wistar rats decreased the activity of catalase and SOD in kidney cortex [97].

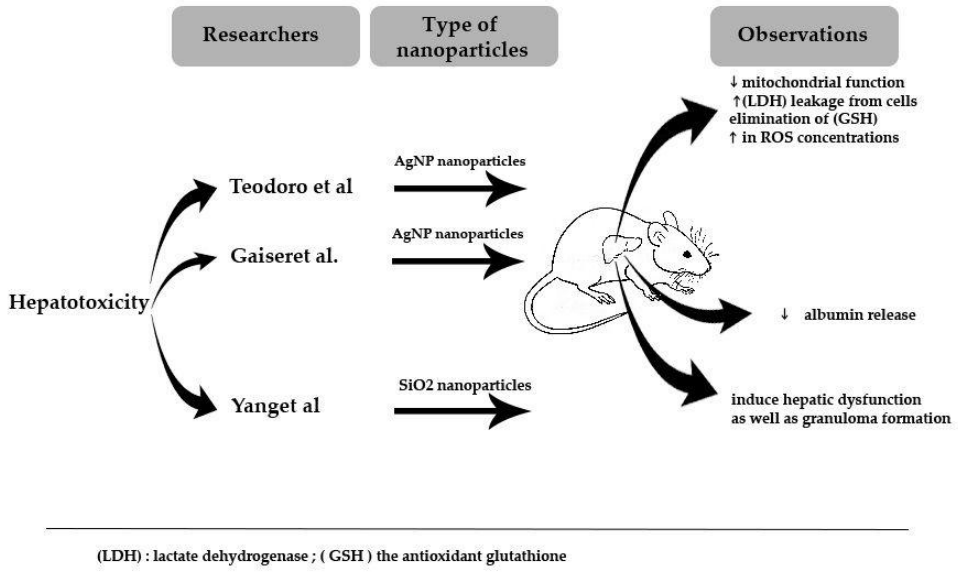

Figure 1. Hepatotoxicity induced by nanoparticles according published studies

Previous studies performed about particles' toxic effects on kidney are partial and different. The outcome of these studies speculated that many nanoparticles, due to translocation and accumulation in the kidney, could induce nephrotoxicity in these organs.

Dermal toxicity of nanoparticles

Skin is the largest and the widest organ and the possible path for entrance of many nanoparticles of environmental conditions and occupational exposure to the body. Therefore, assessing health risks and dermal toxicity of them is important. Various parameters may influence the dermal absorption of nanoparticles. The metal-based nanoparticles, such as nanosilver, nanogold, nanocapsules, nanocrystals, liposomes, and solid lipid nanoparticles, due to certain advantages, including enhance solubility, affect the transparency and color of cosmetic products; thus, they have been extensively used by the cosmetic, pharmaceutical, paint, and paper industries [98].

Additionally, owing to the minimal size of nanoparticles, assessing their toxic effects following long-term dermal exposure forms an essential discussion in nanotechnology studies. Jianhong et al. investigated the penetration rate and toxicity of titanium dioxide (TiO_2) nanoparticles on dermal tissues using in vitro and in vivo experiments. Their in vitro model trial revealed titanium dioxide cannot penetrate through the stratum corneum of dermal; however, the in vivo model provided different results. The latter trial indicated TiO_2 particles could enter the body through the skin, reach different tissues, and induce several pathological lesions in the central organs [99].

Jebali et al. studied the skin toxicity of different nanoparticles, including zinc dioxide, titanium oxide, magnesium oxide, silver, and gold. They applied Lactate Dehydrogenase (LDH) and Reactive Oxygen species (ROS) generation assays on Triglyceride (TG) of the skin. They found an increase in LDH release and ROS generation. Such decreases occurred with pristine metal nanoparticles that are associated with many health risks for humans [100]. Publications on the dermal toxicity of nanoparticles are scarce and complex. These studies documented that a certain nanoparticle may have different effects on the skin. However, these studies have used different models, tests and experimental conditions; thus, their achieved data are challenging to interpret.

Pulmonary toxicity of nanoparticles

Inhalation is the only route of human exposure to airborne nanoparticles. After inhalation, the deposition of nanoparticles available in the air in different sections of the respiratory tract occurs by diffusion starting from the nose and pharynx, down to the lungs [101]. The deposition process causes larger particles achieve biological tissues that would not frequently be accessed. For example, TiO_2 nanoparticles, by inhalation, translate into the lung texture of rats and mice and form inflammation [102]. Limited studies on the toxicity of nanoparticles

in pulmonary tissue reported its nontoxicity. Hong et al. investigated the influence of Silica Nanoparticles (SNs) on the lungs of rats to evaluate the toxicity and possible injury of SNs. Their results suggested SNs can make pulmonary fibrosis by increasing lipid peroxidation and high expression of cytokines [103].

Morimoto et al. performed studies about intratracheal instillation and inhalation of Zinc Oxide (ZnO) nanoparticles to assay pulmonary toxicity. In the inhalation study, they found high concentration may increase total cell and neutrophil counts. In the intratracheal instillation study, in addition to the observations as mentioned above, their results revealed an increase in the expression of Cytokine-Induced Neutrophil Chemoattractant (CINC)-1, CINC-2, chemokine for neutrophil, and Heme Oxygenase-1 (HO-1), an oxidative stress marker in the BALF [104]. Loret et al. evaluated biological responses (pro-inflammatory effects and quantitative comparisons) caused by poorly soluble and toxic TiO_2 and CeO_2 nanoparticles through in vivo and in vitro methods, using compatible dose metrics.

They found that more advanced detection techniques could enhance prediction ability about the pulmonary toxicity of toxic TiO_2 and CeO_2 nanoparticles [105]. Different studies have been conducted on the inhalation toxicity of nanoparticles. The results of these studies indicated that specific nanoparticles might be prone to induce harm in respiratory tract. However, the findings of these studies, because of using different methods for nanoparticles identification, are difficult to interpret.

Gastrointestinal toxicity of nanoparticles

A possible route of nanoparticle entry to the body is the gastrointestinal tract. These components, in gastrointestinal tract, operate directly through intentional ingestion and indirectly via nanoparticle dissolution from food containers. Evaluating nanoparticles in gastrointestinal tract is required not only due to absorption and accumulation of them in this organ but also because of their potential role in altering gut microbes and the effects of this perturbation on the host. Waldman et al. explored different toxicities of nanoparticles (e.g. zinc oxide, Silica, and titanium dioxide) to assay changes, such as necrosis, apoptosis, membrane damage, and mitochondrial activity on intestinal epithelial C2BBe1 cells.

They concluded that silica and titanium dioxide nanoparticles were nontoxic, although all nanoparticles were internalized by cells. Mild acute toxicity of zinc oxide nanoparticles was observed after 24-hour treatment of intestinal epithelial. Thus, silica, titanium dioxide, and zinc oxide nanoparticles induced slight toxicity in intestinal epithelial cells [106]. The literature provides limited information on the gastrointestinal toxicity of nanoparticles. This information is controversial and inconsistent. Such controversy and inconsistency may be due to the lack of proper characterization of the nanoparticles, including their source and configuration. Therefore, the data obtained from these studies are difficult to interpret.

Cardiotoxicity toxicity of nanoparticles

Our information about the toxicity effects of particulate available in air pollution as well as a gastrointestinal tract on cardiovascular health is limited. Furthermore, this problem has involved numerous studies in toxicology. Miller et al. investigated the cardiovascular toxicity of gold nanoparticles in healthy volunteers using robust and accurate detectors like mass spectrometry and Raman microscopy. They evaluated different hematologic parameters, inflammatory factors, oxidative stress reaction, endothelial disorders, and myocardial enzyme dysfunction in serum samples. They expressed gold nanoparticles provided vascular inflammation in the studied sample.

Their results suggested that the cardiovascular toxicity of gold nanoparticles had a direct relationship with particle size and dosage [107]. Beltrán et al. evaluated cardiomyocyte shortening and intracellular Ca+2 and disorder contractility and intracellular Ca+2 transient amplitude during adrenergic stimulation in exposure with SiO_2 nanoparticles. Their results indicated SiO_2 leads to the depolarization of the mitochondrial membrane, decreases ATP production, glutathione depletion, H_2O_2 generation, and increases oxidative stress and mitochondrial dysfunction. They concluded that exposure to SiO_2 nanoparticle is a potential risk factor for the cardiovascular system [108].

Hussainy et al. evaluated the toxicity effects of aluminum oxide (Al_2O_3) nanoparticles on the different parameters of myocardial as electrical activities, morphology structure, inflammatory factors, and the myocardial expression of connexin 43 in rats. Their results suggested disorder in ECG, significant increase in Creatine Phosphokinase (CPK), Triglycerides (TGs), Cholesterol (LDL), and significant decreases in serum HDL and myocardial GSH, and Catalase (CAT). They concluded that aluminum oxide nanoparticles cause myocardial dysfunctions [109].

There is limited study on the cardiotoxicity of nanoparticles. Previous studies performed many investigations about nanoparticle properties, like their mechanism, which is vital for the cardiotoxicity identification of these particles. Additionally, because there are different experimental conditions for nanoparticle characterization, the information obtained from these studies are hard to interpret. The effects of nanoparticles on humans and animals are illustrated in Figure 2.

Neurotoxicity of nanoparticles

Researchers have extensively indicated evidence that airborne NPs available in environmental or occupational exposure can penetrate to Blood-Brain Barrier (BBB), access to the brain through the olfactory nerve pathway, and finally cause injury by the induction of oxidative stress, inflammatory responses, and cytotoxicity. Studies on the CNS toxicity of nanoparticles are limited. Tian et al. investigated the neurotoxicity effects obtained by exposure to zinc oxide nanoparticle in different-aged mice

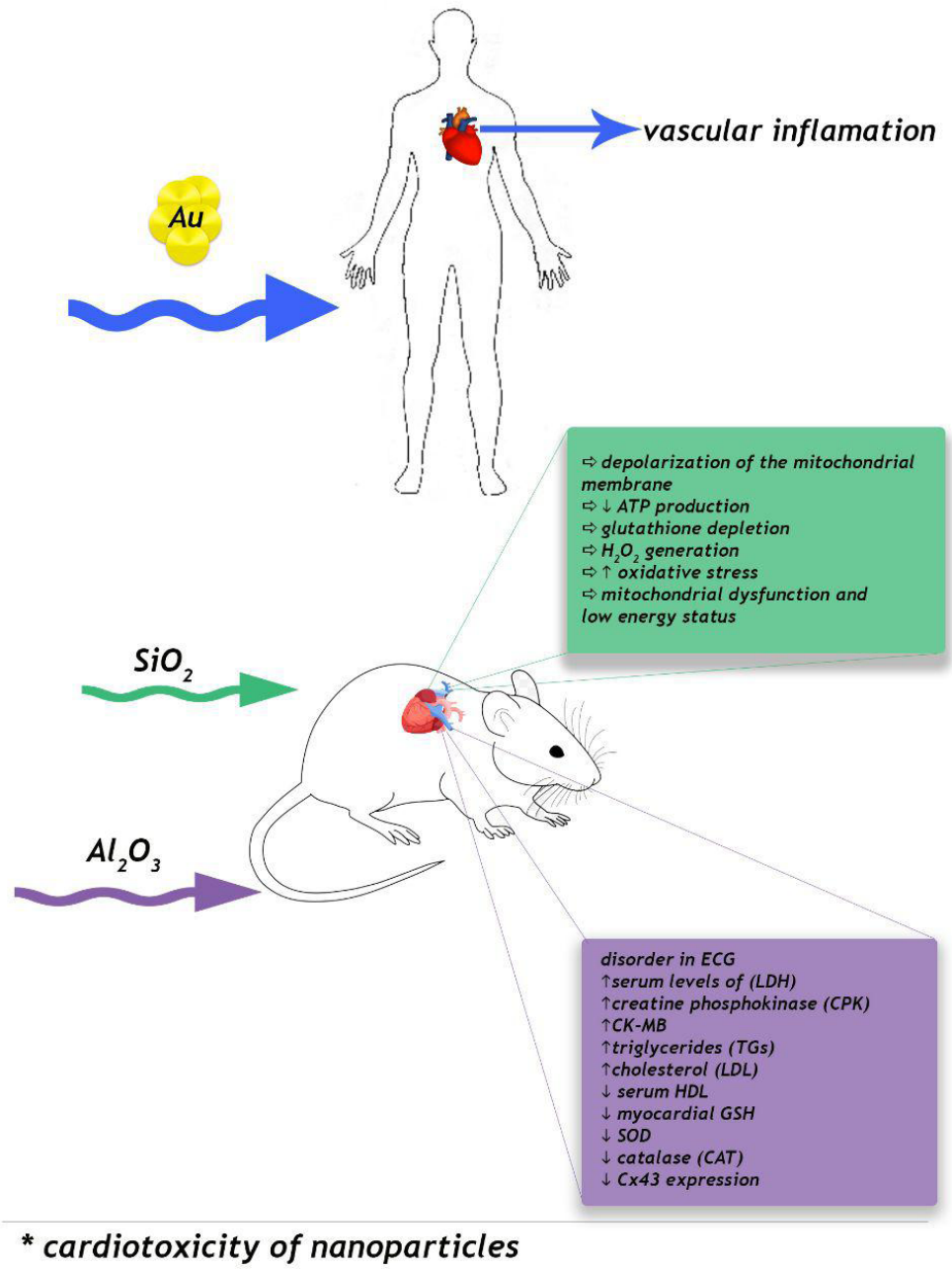

Figure 2. The cardiovascular toxicity effects of nanoparticles on humans and animals

using field emission scanning electron microscope. They concluded in old mice, subsequent exposure to ZnO NP increases oxidative stress values, impair learning and memory, and change hippocampal pathological.

Therefore, their findings demonstrated zinc oxide nanoparticles could induce neurotoxicity [110]. Song et al. evaluated the neurotoxicity of Titanium Dioxide Nanoparticles (TiO_2NPs) in different-rodents using in vivo and in vitro experiments. They investigated the morphology structure and function of glial cells. Their results indicated TiO_2NPs might induce necrosis in cells, significant impair mitochondrial, lysosome, and cytoskeleton, and change the recognition ability, spatial memory, and learning ability in TiO_2NPs-treated rodents. Therefore, their findings demonstrated titanium dioxide nanoparticle could stimulate neurotoxicity and neurodegeneration diseases [111]. Literature about the neurotoxicity of nanoparticle is insufficient; therefore, to increase knowledge about the potential risks of nanoparticle on brain health, extensive studies are required (Figure 3).

Immunotoxicity of nanoparticles

Immunotoxicity is a new field emerging in nanotoxicology. Reports on the immunotoxicity of nanoparticles are inadequate and inaccurate. The compatibility of nanoparticles with the immune system mainly depends on the physicochemical properties(e.g. size, form, congestion state, chemical compound, surface area, & charge) that can stimulate or suppress the immune response [112]. The reaction between nanoparticles and biological tissues or immune system can be via the generation of active oxygen species (oxidative burst) and the release of pro-inflammatory cytokines. This process leads to the production of responses, including the suppression of immune system, increased sensitivity, immunogenicity, and autoimmunity in the human body [113].

Studies have reported that TiO_2 nanoparticle, via change in the signaling pathway of p38-Nrf-2, can significantly increase ROS accumulation in splenic and subsequent lipid peroxidation or HO-1 expression in cell [114]. Exposure to silver nanoparticles with diverse sizes form ROS production, glutathione depletion, and inhibits superoxide dismutase enzyme [115]. Investigations suggested silica nanoparticle not only activates the MAPKs signaling pathway employing increased expression of phosphorylated JNK and p38 MAPK but also inactivates the Extracellular Signal-Regulated Kinases (ERKs). This process leads to ROS production and oxidative stress that regulate apoptosis in cell. Data are scarce on the immunotoxicity of nanoparticles. Available investigations on the immune system toxicity of nanoparticle are limited. Therefore, it is necessary to conduct numerous studies for understanding the mechanisms associated with the side effects of particles on immune system (Figure 4).

Conclusion

Nanotechnology in drug delivery systems is a new method for confronting problems associated with conventional

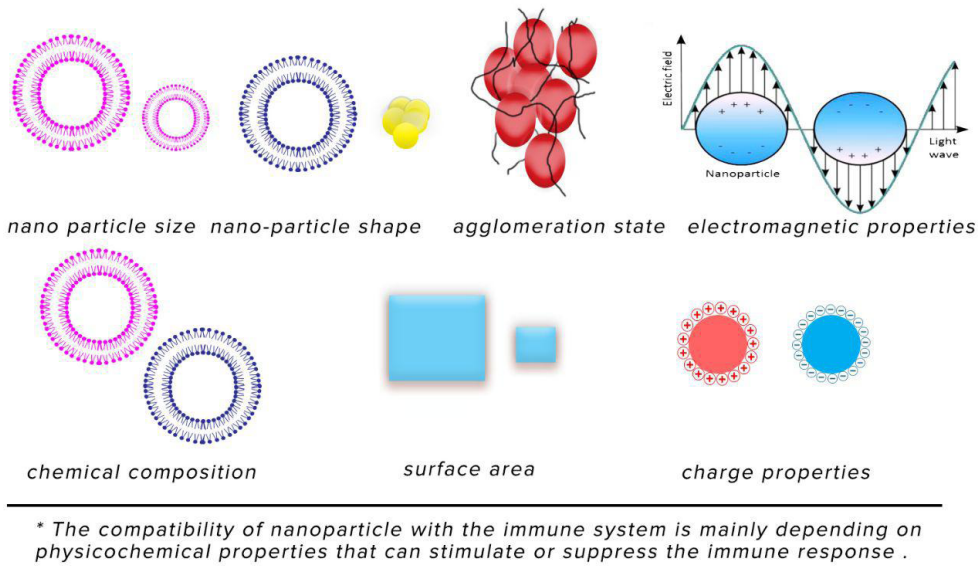

Figure 3. Neurotoxicity effects of nanoparticles

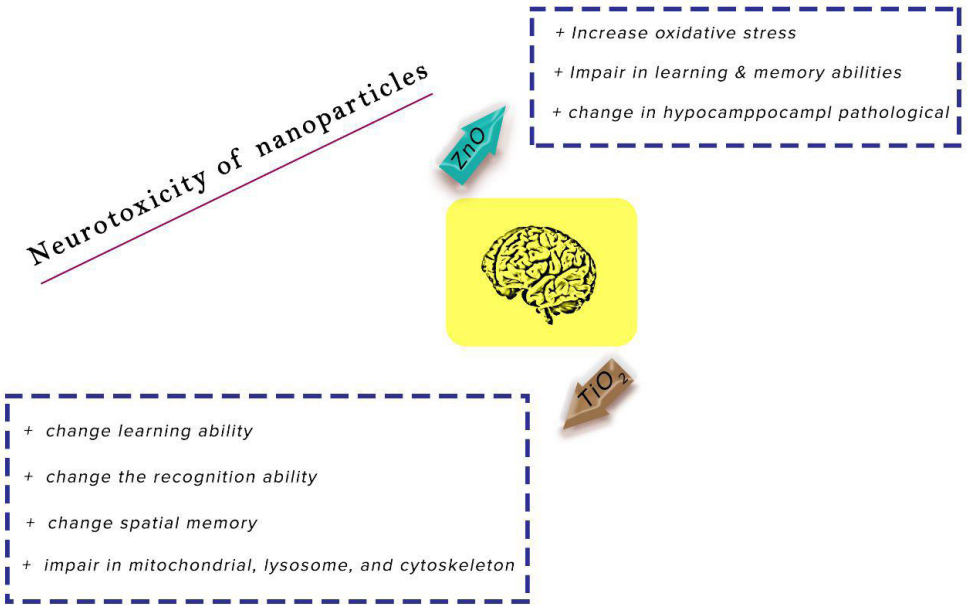

Figure 4. Interaction of nanoparticles with immune system and subsequent effects

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drug therapies, especially in antiviral drugs. Nanomaterials can improve pharmaceutical efficacy by optimizing the physical properties of antiviral drug compounds, as well as improving their bioavailability. Advancements in this field have led to targeted drug delivery, controlled release, or slow-release, reduced dosage, and drug toxicity, compared with conventional approaches. Nanotechnology, especially in antiviral therapeutic agents, increased the permeability of poorly water-soluble drug compounds and the stability of unstable drugs.

Despite many advantages, these nanoparticles, compared to conventional therapies, require more investigation regarding their toxic effects. Identifying the toxicity profiling and biological interaction of nanomaterials due to their natural present in the ecosystem environmental as well as the therapeutic use of their antiviral drug compounds are essential. They may generate toxic effects on human exposure by many mechanisms, such as reactive oxygen species generation, protein misfolding, membrane perturbation, and direct physical damage. Our knowledge about the safety of nanomaterials is limited.

Different studies have used in vitro and in vivo test models, different sources, and techniques in this regard. Therefore, their data are hard to interpret. However, the applications of nanomaterials in the nano-formulations of drugs require a different structure that balances their disadvantages versus their advantages and therapeutic benefits. Due to the use of a wide range of nanoparticles, like nanomedicine and their undesirable effects on healthy human and environment caused many researchers to assess the toxicity of nanoparticles. The physicochemical properties of nanoparticles, including different size, form, morphology, and chemical structure are essential components, contributing to their side effects [73].

There are no appropriate toxicity tests for the safety evaluation of nanoparticles. National Center for Toxicological Research and National Institute of Standards and Technology are the leading centers for studying nanoparticles; they have not reported a positive standard protocol for the toxicity testing of nanoparticles [67]. To increase performance, developing standard protocols for nanoparticles is necessary for expert investigators, appropriate laboratory conditions, proper experimental observations, and the accurate identification of nanoparticles. Therefore, extensive studies are required for increasing our general knowledge of toxicity tests and the effects of nanoparticles on biological systems and human health.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by Ardabil of Medical Sciences, Deputy of Research.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed in preparing this paper. Zohreh Fasili shared co-first leadership with Freshteh Mehri.

Conflict of interest

The authors declared no conflicts of interest.

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