Nanotechnology and nanomaterials in the strategy for the treatment of neoplastic diseases

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SUMMARY

Nanoparticles (NPs) have nanometric dimensions, a large surface-volume ratio, physical and chemical stability and individual optical electronic properties. These characteristics have allowed the use of nanostructured materials for the prevention and treatment of various diseases, such as cancer. NPs have been designed and modified to improve the pharmacokinetics and pharmacodynamics of drugs, and to target drugs to cancer cells only. Various nanomaterials can be used for nanomedical applications. In particular, inorganic NPs, such as zinc oxide (ZnO-NPs), gold (Au-NPs) and silver (Ag-NPs) NPs, have been used to improve anticancer therapies. Biosynthesized inorganic NPs were loaded with chemotherapeutic drugs and subsequently functionalized to selectively target cancer cells. Many studies have identified the cellular mechanisms involved after cell-NP interaction: oxidative stress, mitochondrial alterations, lysosomal dysfunction, apoptosis or alternatively autophagy. To improve knowledge of the interaction between drugs loaded on NPs and cells and optimize their use by reducing toxic effects, transmission electron microscopy (TEM) techniques proved to be a good investigation tool. TEM observations have shown, for example, that ZnO-NPs enter the cells by passive diffusion or endocytosis. Ultrastructural analysis showed that Au-NPs enter the cells by invagination of the plasma membrane and are subsequently internalized in the autophagosome. This brief review shows that each new NP needs to be evaluated individually considering all its properties.

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Introduction

Nanotechnology is a science that combines the study of biological principles with chemistry and physics to obtain nanometer-sized particles with specific possibilities and functions. Due to their size, nanoparticles (NPs) have been considered in recent years for applications in biomedicine. As it is known, NPs have a large surface-volume ratio, which provides them with mechanical, physical and chemical stability, and peculiar optical and electronic properties (Nabil *et al.,* 2019). The development of different nanostructured materials has opened up new paths for the prevention and treatment of different diseases. In many cases, specific NPs have been designed and superficially treated in order to provide compatible drugs for targeting regions in the human body, and to improve their pharmacokinetics and pharmacodynamics. Nanotechnology has made it possible to use many tools to attempt new therapeutic strategies in many diseases, including cancer. In particular, it was possible to study and implement personalized therapies to overcome obstacles that are very frequently encountered using conventional drugs (Khan *et al.,* 2020). Therefore, nanotechnology investigates nanomaterials that can behave as cytotoxic substances or increase the effectiveness of radiotherapy and chemotherapy, reducing their side effects. The use of nanomaterials in the treatment of neoplasms must be preceded by careful cytotoxicity studies of the materials. In fact, sometimes these materials can damage healthy cells, the immune system or determine the possibility of developing malignancies in other sites.

In the choice of a nanomaterial to be used for the delivery of the drug in the tumor mass, there are several parameters that must be considered, some of which are: size, biocompatibility, biodegradability of the nanocomposite, toxicity and antigenicity of the drug to be encapsulated, stability, solubility in water of the drug and properties suitable for release by the material (Jindal, 2017) (Table 1). Recent studies have shown that less than 1% of the injected NPs reach the site in the solid tumor; this also depends on NPs once injected into the bloodstream, if the nanomaterial is covered with proteins (opsonins) that can be recognized from the mononuclear phagocytic system (Abbina *et al.,* 2020). The interaction between proteins and nanomaterials strongly depends both on NP properties and on factors, such as the gradient of the plasma, the constants of the kinetic equilibrium, the circulation time and temperature (Gao and He, 2014; Ban *et al.,* 2020). The adsorbed proteins are divided into opsonins and dysopsonins (Liu *et al.,* 2010) that have different functions: in fact opsonins induce a rapid clearance of the blood of the NPs whereas dysopsonins are able to promote a prolonged circulation of NPs in the blood. In a cellular system, processes such as endocytosis, biodistribution, characteristics of NPs are influenced by the adsorption of proteins (Vu *et al.,* 2019). Very often the NPs are functionalized with specific surface ligands to ensure that the nanomaterial reaches the set target. The surface state of the nanomaterial leads to a different degree of opsonization, which greatly influences targeting, regardless of whether or not it favors reaching the drug in the tumor. From here, we understand how important is to study these phenomena for each nanomaterial to be used, to improve the targeting effect and reduce the adsorption of proteins that can lead to target failure (Srivastav *et al.,* 2019).

Nanomaterials can be of various nature, diversifying itself in size and chemical properties such as biopolymer NPs, carbon nanotubes, dendrimers, inorganic particles, liposomes, polymer micelles, protein NPs, and quantum dots (QDs).

In this brief review, however, we will only talk about inorganic

Table 1. Advantages and disadvantages of NPs.

Nanoparticles	Advantages	Disadvantages
Biopolymeric nanoparticles	High specific surface area Biodegradable and biocompatible	Hydrophobic materials Poor encapsulation efficiency of drugs
Carbon nanotubes	Easy surface functionalization Cell membrane penetrability Efficient loading	Low biodegradability Toxicity
Dendrimers	Synthesis of well-defined structures High chemical and biological stability High surface area, loading capacity Biodegradable and biocompatible	Complex synthetic route
Inorganic nanoparticles	Easy surface functionalization Good stability	Non-biodegradable Toxicity
Liposomes	Easy surface functionalization Biocompatibility	Instability Insufficient drug loading Short circulation times in blood
Polymeric micelles	Efficient carrier system for hydrophilic drugs Biodegradable and biocompatible	Short circulation times in blood
Protein nanoparticles	Low toxicity Biodegradability	Low drug loading efficiency
Quantum dots	Strong fluorescence intensity	Cytotoxicity to lung cells Induction of stress oxidative

NPs, such as zinc oxide (ZnO-NPs), gold (Au-NPs) and silver (Ag-NPs) NPs.

Inorganic nanomaterials

As mentioned above, inorganic NPs have been studied in recent years precisely for their particular optical, magnetic and chemical characteristics such as stability, inertia and functionalization simplicity (Wang *et al.,* 2020a).

ZnO-NPs

The zinc ions (Zn^{2+}) content in the human body is about 15 mg per day, it is the most abundant trace element in tissues and biological fluids; its absorption occurs through the small intestine and excretion through the kidneys and skin. It has an important role in the immune system, and it stabilizes the molecular components of the cell and the membranes. It also has a regulatory role for many enzymes involved in the synthesis and degradation of lipid carbohydrates, proteins and nucleic acids, but also has an important role in cellular homeostasis. A Zn^{2+} deficiency can lead to the onset and progression of a neoplastic disease (Ho, 2004), while controversial researches have shown that Zn^{2+} accumulation can be important for tumor progression (Leslie *et al.,* 2016; 2017). It can certainly be concluded that cancer cells are characterized by a low or altered concentration of Zn^{2+}. Many research studies have shown that the release of Zn^{2+} by the ZnO-NPs can cause a cascade of events that are responsible for cytotoxic effects in human cancer cells (De Berardis *et al.,* 2010). ZnO-NPs have shown preferential cytotoxicity for cancer cells both in *in vitro* and *in vivo* models. The main advantages for the use of ZnO-NPs in cancer research are summarized below. The concentration of chemical groups (-$ZnOH_2$, -ZnOH, -ZnO-) on the surface of ZnO-NPs is pH dependent. The availability of chemical reactive groups allows functionalizing the ZnO-NPs with antibody and proteins that can improve the selectivity for cancer cells compared to normal cells. Indeed, under physiological conditions, ZnO-NPs have a strong positive charge. It is known that cancer cells have high concentration of anionic phospholipids on their outer membrane so the electrostatic interaction with ZnO-NPs may effectively promote cellular uptake, phagocytosis and cytotoxicity on cancer cells.

Observations at transmission electron microscopy (TEM) showed that ZnO-NPs enter into the cancer cells by passive diffusion or endocytosis (Figure 1A). Hence, some ZnO-NPs release Zn^{2+} after interaction with the acidic environment of lysosomes. The simultaneous presence of Zn^{2+} and ZnO-NPs within the cells induces the formation of reactive oxygen species, mitochondrial alterations (Figure 2B) and severe nuclear modifications, leading to apoptosis (Condello *et al.,* 2016).

The specific selectivity of ZnO-NPs towards cancer cells compared to healthy cells and the intracellular retention of NPs showed that ZnO-NPs are suitable candidates for antitumor activity as single or synergistic agents in combination with chemotherapeutic drugs (Mishra *et al.,* 2017; Jin and Jin, 2019). ZnO-NPs alone were effective against different human cancer cell lines from hepatocarcinoma, leukemia, and colon, breast, lung, ovarian, cervical, gastric, and epidermal tumors (Jiang *et al.,* 2018). ZnO-NPs, as single agents, have been tested on *in vitro* and *in vivo* models of small cell lung cancer (Tanino *et al.,* 2020). They induced oxidative stress and DNA damage on tumor cells, but they were not toxic against normal lung cells. Recent work has suggested that ZnO-NPs inhibited the progression of melanoma, the drug resistance and metastasis, associated with the ERK-AKT signaling pathway. These data were promising for further applications on melanoma animal models and other types of cancer (DeLong *et al.,* 2019). ZnO-NPs, synthesized from the plant *Cardiospermum halicacabum*, induced toxicity on human melanoma (A375 cells) through the modulation of apoptosis pathway (Duan *et al.,* 2020). Finally, in other tumor models, as human tongue cancer (CAL 27 cells), ZnO-NPs increased the intracellular reactive oxygen species levels, decreased the mitochondrial membrane potential in a time-dependent manner, and activated the PINK1/Parkin-mediated mitophagy (Wang *et al.,* 2018).

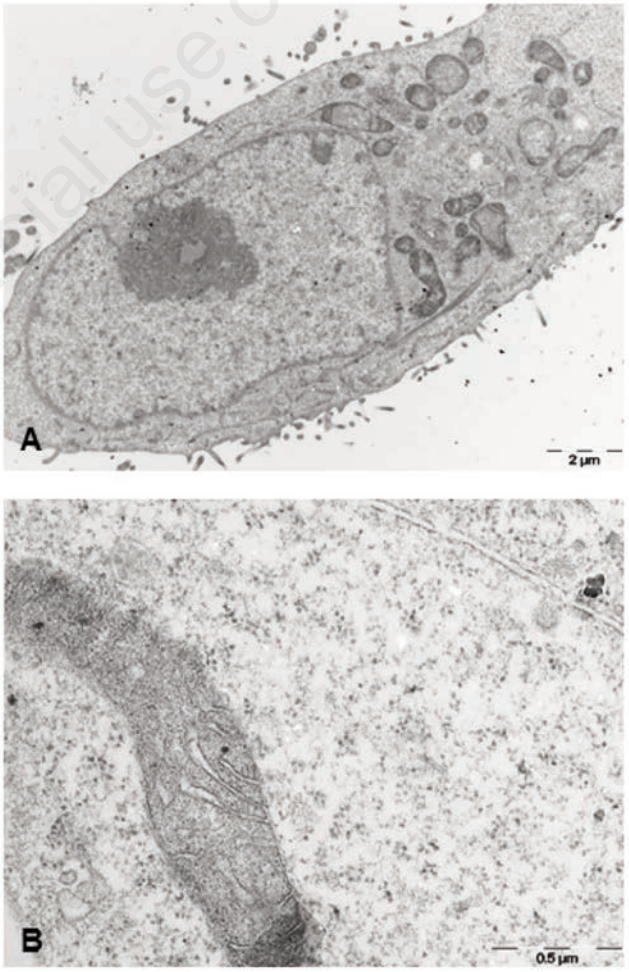

Figure 1. TEM micrographs of colon carcinoma cells treated with ZnO-NPs. Ultrastructural analysis shows that ZnO-NPs enters the cell by passive diffusion (A), and induces mitochondrial alteration (B).

Taking advantage from the peculiar characteristics of ZnO-NPs, it was possible to load them with drugs and modify their structure to increase the absorption and interaction specificity with cancer cells. For example, the ZnO-NPs engineered with glycol, conjugated with the arginine-glycine-aspartate (RGD) peptide and loaded with doxorubicin, have been shown to have specific interaction with U87MG glioblastoma cells demonstrating strong cytotoxic effect compared to cervical cancer cells (HeLa) (Yang *et al.,* 2019). This therapeutic combination of NPs is considered an efficient system for target antitumor therapy of glioblastoma.

In addition, other researchers have studied ZnO-NPs loaded *via* a mesoporous silica nanowire and conjugated with the anti-Frizzled-7 antibody (FZD-7), a receptor that is very often upregulated in different breast cancer cells. This study has shown that ZnO-NPs modified by exploiting the potential clinical utility of FZD-7 receptors are promising tools for the treatment of triple-negative and drug-resistant breast tumors (Ruenraroengsak *et al.,* 2019).

Au-NPs

Other inorganic NPs used for the drug release system in biomedicine are Au-NPs. They have particular optical and electronic properties (Kohout *et al.,* 2018), are easily synthesized, and their surface can be modified (Li *et al.,* 2019). The inert nature of Au-NPs makes them relatively biocompatible but in general, as previously described, the cytotoxicity of NPs also depends on their shape, size, surface properties and chemical composition. Another of the problems that can lead to cytotoxicity is that the uncoated Au-NPs, which exhibit high surface binding energy, can bind human plasma proteins, increasing the NP-cell interaction (Długosz et al., 2020). When NPs interact with the cell, as previously described, a coating of proteins from the physiological environment called the crown complex is formed on their surface (Aggarwal *et al.,* 2009). Thus, the Au-NPs contain on their surface, in the crown complex, the opsonins, which are recognized by the immune cells. This complex mechanism is crucial for the distribution of NPs in the organs of the human body. To partially avoid immune recognition, limit the toxic effect and increase their biocompatibility, Au-NPs can be coated with biopolymers, chitosan, proteins, glucan or polyethylene glycol (PEG) (Spinelli *et al.,* 2019).

As previously described, Au-NPs have unique optical and plasmon resonance properties that make them very interesting in the biomedical field in general and especially in ultra-sensitive detection as imaging-based therapeutic techniques in neoplastic diseases. Furthermore, due to the presence of a negative surface charge, they can be functionalized by means of ligands or drugs.

Here we summarize some applications of Au-NPs in anticancer therapy.

Recent studies have shown the antitumor apoptotic activity of newly synthesized Au-NPs on lung cancer (Wang *et al.,* 2020b). Au-NPs also induced apoptosis on A498 renal carcinoma cells (Liu *et al.,* 2019). They inhibited the proliferation of human gastric adenocarcinoma and exerted anticancer effects on hepatocellular carcinoma, on HCT116 colon cancer cells, on MCF-7 breast adenocarcinoma cells, on T24 bladder cancer cells, on HeLa cervical carcinoma and K-562 leukemic cells (Raghunandan *et al.,* 2011; Ismail *et al.,* 2018; Patil *et al.* 2018; Wu *et al.,* 2019, Ji *et al.,* 2019).

To overcome the side effects of the main chemotherapeutic drugs such as 5-fluorouracil (5-FU), doxorubicin (DOX) and paclitaxel (PTX), functionalized and non-functionalized Au-NPs have been used as a drug delivery system on many types of cancer (Jahangirian *et al.,* 2019). For example, 5-FU has been loaded onto Au-NPs using ligands, such as thiol and GSH. These formulations were tested on patient-derived colorectal cancer tissue: this study showed that the release of 5-FU was pH-dependent and that Au-NPs loaded with 5-FU were more effective than the free 5-FU (Safwat *et al.,* 2016). Another study showed that DOX-loaded AuNPs limited DOX-induced dose-dependent cardiotoxicity (Du *et al.,* 2018). Finally, Sun and coauthors synthesized Au-NPs coated with pluronic-b-poly (L-lysine) and loaded with PTX, and used them for chemo-photothermal therapy. An *in vitro* and *in vivo* study of breast cancer showed that PTX-loaded NPs were more cytotoxic than PTX alone (Sun *et al.,* 2017).

The correct evaluation of the interaction between Au-NPs and cells and the potential adverse effects compared to the beneficial ones are important for the biomedical application of these NPs. An example is the evaluation of the effect of Au-NP size on their absorption and cytotoxicity by normal and cancerous cells (Gioria *et al.,* 2014; Xia *et al.,* 2019). As shown in Figure 2, when NPs interact with cells different endo- and exo-cytotic mechanisms are involved. In our study, TEM analysis showed that Au-NPs enter the

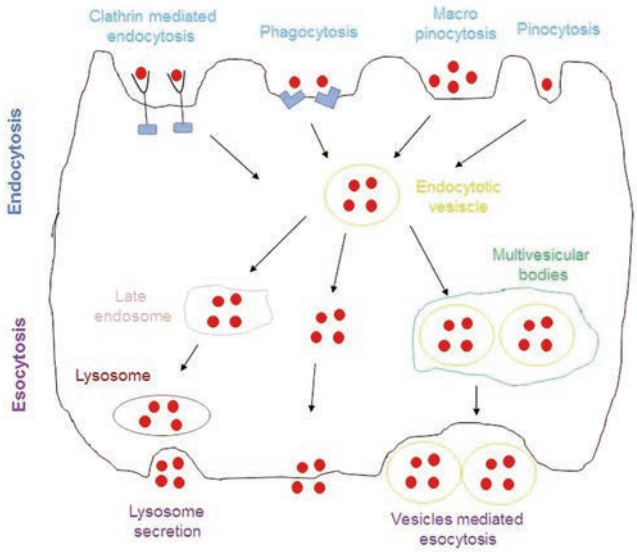

Figure 2. Schematic representation of cell-NPs interactions. NPs enter the cells by multiple endocytic mechanisms, as clathrin mediated endocytosis, phagocytosis, micropinocytosis or pinocytosis. Then, NPs are transferred by late endosomes, or lysosomes or multivesicular bodies. Exocytosis may occur by passive diffusion or be mediated by lysosome secretion or by vesicles.

cells through the invagination of the cell membrane (Figure 3A), they are mainly internalized in the autophagosomes (Figure 3B), and subsequently induce alteration in the endoplasmic reticulum and at the mitochondrial level (Gioria *et al.,* 2014).

Ag-NPs

Ag is another bioinorganic element with promising chemical characteristics for the production of NPs and their application in nanomedicine (Hecel *et al.,* 2019). Ag-NPs synthesized from medicinal plants have anticancer properties, as demonstrated by *in vitro* studies on prostate and human lung cancer cells (Dadashpour *et al.,* 2018; Valsalam *et al.,* 2019; Zhang *et al.,* 2019). The anticancer activity of Ag-NPs can be due to several mechanisms, such as dynamic interaction with DNA and proteins, oxidative stress, lysosomal destruction leading to apoptotic or necrotic cell death (Jadhav *et al.,* 2018). Like other NPs, the anticancer effect of Ag-NPs depends on various chemical properties, such as particle size, surface charge, bonding properties, aggregation potential (Jadhav *et al.,* 2018). Some anticancer drugs have been administered *via* Ag-NPs, to increase efficacy and reduce the side effects. Epirubicin-coated Ag-NPs were synthesized and analyzed on human HepG2 liver carcinoma cells, showing good anti-tumor properties at low drug concentration (Ding *et al.,* 2019). Ag-NPs coated with a camptothecin-based polymeric prodrug were developed; the study on HeLa cell showed that Ag-NPs increased drug release based on pH sensitivity (Qiu *et al.,* 2017). Ag-NPs have also been used to overcome resistance to drugs by simultaneously administering two drugs such as trichosanthin protein and albendazole; this Ag-NPs co-delivery system also inhibited the proliferation of drug-resistant cells of lung and colon cancer (Tang *et al.,* 2017).

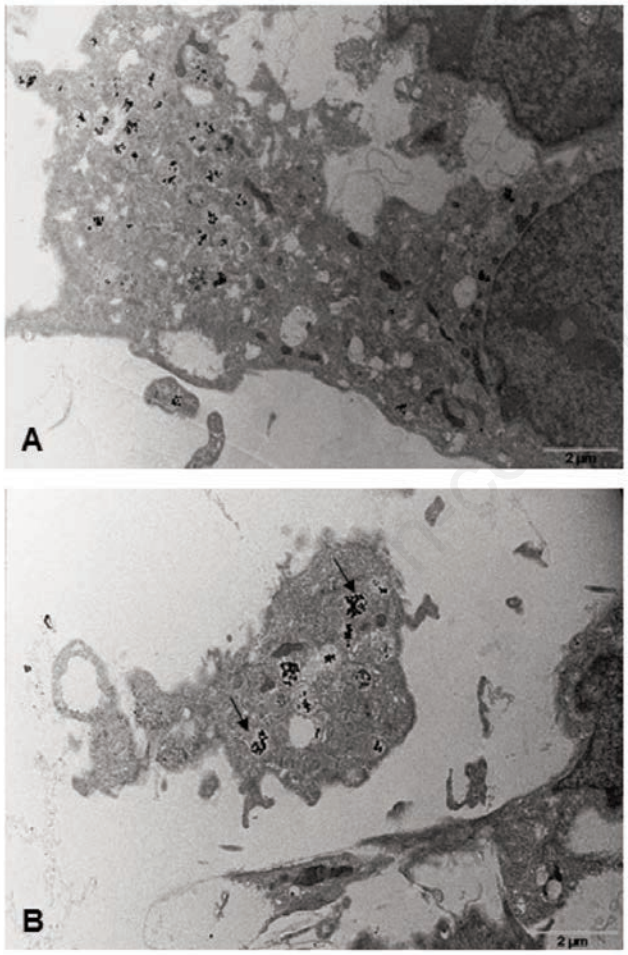

Figure 3. TEM micrographs of Balb/3T3 mouse fibroblast cells treated with Au-NPs (arrows). Ultrastructural analysis shows that Au-NPs enter the cells by invagination of the cell membrane (A), and they are mainly internalized in the autophagosomes (B).

Conclusions

NPs are fundamental tools for biomedical research. However, the development of NPs with therapeutic efficacy requires a thorough knowledge of their interactions with cells in order to improve their use and reduce any toxic effects. To improve the understanding of the interaction of a composite structure such as the NP-drug complex with the cell, TEM is the perfect investigation tool. Due to the high number of variables that influence the potential toxicity of nanomaterials, it is not possible to make general conclusions on the effects associated with the exposure to nanomaterials: each new NPs must be evaluated individually, considering all its properties to make it biocompatible to become an efficient carrier for use in drug delivery. This review showed some recent experimental results that reported the application of inorganic ZnO-, Au- and Ag-NPs in biomedicine, in view of an increasingly safe and responsible use of nanomaterials.

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