PhD THESIS

Action of Gold and Silver nanoparticles. Toxicological and therapeutic considerations

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INTRODUCTION

ANanotechnology is a branch of science that deals with the study of materials smaller than 100 nm. It is a major area of research interest, with a focus on the functional mechanism of nanoparticles, especially metallic ones. Nanotechnology is used to develop nanoparticles for various purposes: drug delivery, pathogen detection, biomolecular sensors, anti-tumour therapy (Nanotechnology and Medicine 656, n.d.).
Nanoparticles (NPs) are clusters of atoms of variable size 1-100 nm that possess highly promising chemical, optical and metallic properties. Nanoparticles have attracted attention due to their unique physical and chemical properties that derive from the large amount of surface atoms, but also from the high surface/volume ratio. As their diameter decreases, the available surface area of the particle increases dramatically, hence an increase in the original properties of the corresponding bulk materials occurs (Dos Santos şi colab., 2014).

Nanoparticles are promising candidates for various applications, however, their potential toxicity raises significant concerns. The toxicity of metal NPs is attributed to their small size, large surface-to-volume ratio and unique physicochemical properties, which can lead to interactions with biological systems and induce adverse effects. Studies have shown that metal NPs can induce oxidative stress, disrupt cell membranes, interfere with cell signalling pathways and induce inflammation and genotoxicity. In addition, the release of toxic metal ions from NPs can further exacerbate their adverse effects. The toxicity of metal NPs varies depending on factors such as composition, size, shape, surface chemistry and surface charge. Therefore, a comprehensive understanding and careful evaluation of these parameters is essential for the safe and sustainable development of metallic NPs for various applications.
Currently, nanoparticles are the core area of current research, with the main aim of replacing antibiotics, antiviral and antitumour substances in the future, thus eliminating the risk of antibiotic resistance and viral resistance (Nanotechnology and Medicine 656, n.d.).

THE STRUCTURE OF THE THESIS

The paper entitled "The Action of Gold and Silver Nanoparticles. Toxicological and Therapeutic Considerations" contains 161 pages and is written according to the rules in force, being structured in two parts.

The first part, the bibliographical part, is structured in 3 chapters and comprises 35 pages. In this part of the thesis we have summarized the current general framework of knowledge regarding the antibacterial, antitumor and toxicity action of metallic Gold and Silver nanoparticles respectively.

In the second part, extended over 100 pages and structured in 5 chapters, I detailed my personal research carried out in the period 2020-2024. Each chapter is divided into sub-chapters presenting the aim and objectives, the materials and methods used, the results obtained with discussion of their novelty and the partial conclusions drawn from carrying out each individual study. The research results have been illustrated in 76 figures and summarised in 8 tables. The paper concludes with the bibliography cited (185 titles).

RESULTS OF RESEARCH

In the second part of the present work, we studied the toxicological and antitumor potential of metallic Gold and Silver nanoparticles. Our main objectives were:

- Descrierea and characterize the nanoparticles used in the studies and the potential macroscopic and histopathological lesions encountered following nanoparticle administration.
- Analizarea distribution of metal nanoparticles using dark-field hyperspectral microscopy (CytoViva).
- Evaluarea some biochemical and haematological parameters.

Chapter 5, entitled "Evaluation of sub-acute systemic toxicity induced by oral administration of an aqueous solution of GNP-CAE in the mouse" aimed to assess the sub-acute systemic toxicity occurring following oral administration of a solution of gold nanoparticles functionalised with carcinoembryonic antigen GNP-CAE in the mouse.A number of objectives have been set to achieve this goal: To analyse the systemic biodistribution of GNP-CAE using "dark field" hyperspectral microscopy (Cytoviva), to analyse potential adverse effects after long-term (30 days) exposure to GNP-CAE by histopathological examination of organs to characterise the type and severity of GNP-CAE-induced damage and to analyse markers of systemic inflammatory response (IFN-gamma, Il 1 beta, Il 4, Il 10, Il 12, TGF-beta). In the sub-acute administration version of the prototype, it induces hepatic mononuclear and

polymorphonuclear infiltrate, (control, n=0; 5mg GNP-CAE/kg group, n=5; 10mg GNP-CAE/kg group n=4; 20mg GNP-CAE/kg group , n=4; and group 50mg GNP-CAE/kg , n=4), hepatic amyloidosis, (control, n=0; group 5mg GNP-CAE/kg, n=0; group 10mg GNP-CAE/kg n=0; group 20mg GNP-CAE/kg , n=3; and group 50mg GNP-CAE/kg , n=1). At the splenic level, the lesions encountered were splenic amyloidosis, (control, n=0; 5mg GNP-CAE/kg group, n=0; 10mg GNP-CAE/kg group n=2; 20mg GNP-CAE/kg group , n=3; and group 50mg GNP-CAE/kg , n=3), white pulp hyperplasia, (control, n=0; group 5mg GNP-CAE/kg, n=2; group 10mg GNP-CAE/kg n=1; group 20mg GNP-CAE/kg , n=0; and group 50mg GNP-CAE/kg , n=0). At the renal level, the lesions encountered are represented by: chronic progressive nephropathy, (control, n=0; 5mg GNP-CAE/kg group, n=1; 10mg GNP-CAE/kg group n=2; 20mg GNP-CAE/kg group, n=3; and 50mg GNP-CAE/kg group, n=2). At the gastric level, the lesions encountered were yeast, (control, n=0; group 5mg GNP-CAE/kg, n=1; group 10mg GNP-CAE/kg n=1; group 20mg GNP-CAE/kg , n=2; and group 50mg GNP-CAE/kg , n=1), bacterial overgrowth, (control, n=0; group 5mg GNP-CAE/kg, n=3; group 10mg GNP-CAE/kg n=1; group 20mg GNP-CAE/kg , n=4; and group 50mg GNP-CAE/kg , n=1), hyperkeratosis (control, n=0; group 5mg GNP-CAE/kg, n=0; group 10mg GNP-CAE/kg n=0; group 20mg GNP-CAE/kg , n=1; and group 50mg GNP-CAE/kg , n=1), gastric amyloidosis (control, n=0; group 5mg GNP-CAE/kg, n=0; group 10mg GNP-CAE/kg n=0; group 20mg GNP-CAE/kg , n=2; and group 50mg GNP-CAE/kg , n=0). Cardiac, intestinal and brain lesions were not observed in any group
studied. Particles persist in small (minimal) numbers at tissue level for all
all 4 experimental groups. From a biochemical point of view, an immune activation with predominance of cellular immunity is observed.

Chapter 6, entitled "Evaluation of acute systemic toxicity induced by oral administration of an aqueous solution of GNP-CAE in the mouse" was primarily aimed at evaluating acute systemic toxicity,
following oral administration of a solution of gold nanoparticles
carcinoembryonic antigen (GNP-CAE) nanoparticles in the mouse. To achieve this aim, we set the following objectives: To analyse the systemic biodistribution of GNP-CAE using "dark-field" hyperspectral microscopy, to analyse potential adverse effects after short-term exposure to GNP-CAE by histopathological examination of organs (liver, spleen, kidneys, lungs, heart, stomach, and intestine) to characterize the type and severity of GNP-CAE-induced lesions, analysis of systemic inflammatory response markers (IFN-gamma, Il 1, Il 4, Il 10, Il 12, TGF-beta). In the acute administration version of the prototype, it induced hepatic amyloidosis, (control, n=0; 10mg GNP-CAE/kg group n=0; 20mg GNP-CAE/kg group , n=0; and 50mg GNP-CAE/kg group , n=3), increased mitosis, (control, n=0; 10mg GNP-CAE/kg group n=0; 20mg GNP-CAE/kg group , n=0; and 50mg GNP-CAE/kg group , n=6). At the splenic level, the lesions encountered are represented by: splenic amyloidosis, (control, n=0; group 10mg GNP-CAE/kg n=1; group 20mg GNP-CAE/kg , n=0; and group 50mg GNP-CAE/kg , n=1).At the pulmonary level, the lesions encountered are represented by: interstitial bronchopneumonia, (control, n=0; group 10mg GNP-CAE/kg , n=1).

CAE/kg n=0; group 20mg GNP-CAE/kg , n=1; and group 50mg GNP-CAE/kg , n=0). At the gastric level, the lesions encountered were bacterial overgrowth, (control, n=0; group 10mg GNP-CAE/kg n=0; group 20mg GNP-CAE/kg , n=2; and group 50mg GNP-CAE/kg , n=2), hyperkeratosis (control, n=0;; group 10mg GNP-CAE/kg n=0; group 20mg GNP-CAE/kg , n=0; and group 50mg GNP-CAE/kg , n=2). Renal, cardiac, intestinal and brain lesions were not observed in any of the groups studied. Particles persist in small (minimal) numbers at tissue level for all 3 experimental groups. From a biochemical point of view, an immune activation with predominance of cellular immunity is observed.

Chapter 7, entitled "Induction of hepatic carcinogenesis by intraperitoneal administration of DEN in mice and evaluation of the effects of AuNP-Cad-1-PEI-FA at the tumour level" aimed mainly at the stepwise induction of hepatic carcinogenesis, and evaluation of the local effects of the nanoparticles used. To achieve this aim, we proposed the following objectives:Investigation of histopathological changes in the liver induced by intraperitoneal administration of diethylnitrosamine (DEN) using standard stains (H&E) and special stains (PAS, and Reticulin). Immunohistochemical evaluation of E-cadherin. Evaluation of progression of liver carcinogenesis in the mouse after intraperitoneal injection of DEN. Evaluation of the role of Concanavalin-A in the diagnosis of hepatocellular carcinoma (HCC). Evaluation of eosin fluorescence intensity in preneoplastic, basophilic liver nodules. Analysis of tumor-level changes following intratumoral administration of AuNP-Cad-1-PEI and detection of nanoparticles using hyperspectral microscopy. Intraperitoneal administration (single dose) of diethylnitrosamine (DEN) induces in Swiss line mice progressive pretumor lesions (foci of hepatocellular alteration) at 3 months, hepatocellular adenomas at 5 months after administration and hepatocellular adenocarcinomas at 8 months. In DEN-induced hepatocellular carcinogenesis, tumour-initiated and neoplastically transformed cells show significant alterations in carbohydrate metabolism, manifested by accumulation of glycogen and carbohydrates, the latter demonstrated by increased cellular affinity for Con-A. In HCC, there is an increase in Con-A expression and a shift in cytoplasmic location from perimembrane (predominantly seen in normal cells) to perinuclear-cytoplasmic location. E-cadherin expression decreases in HCC tumour foci, demonstrating alterations in intercellular junctions. From this study, we were able to demonstrate the presence of changes in the fluorescence spectrum of eosin when bound to proteins in both normal and tumor liver tissues. This highlights how fluorescence spectroscopy can complement histopathological observations and reveal information that aligns with classical H&E staining methods. Intratumoral administration of cadherin-1-PEI-FA-functionalized gold nanoparticles is associated with intratumoral persistence for more than 24 hours without damage to tumor structure or toxic effects in the peritumoral liver parenchyma.

Chapter 8, entitled "Evaluation of sub-acute pulmonary toxicity by intratracheal administration of AuAg nanoparticles" was initiated to evaluate the sub-acute (28 days) toxicity of gold-silver alloy nanoparticles (Au-AgNp) in the lung following aerogenic

(intratracheal) exposure. To achieve this aim, we proposed the following objectives: physicochemical characterization of the Gold-Silver nanoparticles used, respecting the recommended requirements for toxicity studies involving nanomaterials; investigation of clinical (hematological and BAL) and anatomopathological (macroscopic and histopathological) changes induced by intratracheal administration of Gold-Silver nanoparticles of different sizes; identification of a possible effect related to variable amounts of Au-Ag alloy on this toxicity; investigation of pulmonary distribution and persistence of nanoparticles at tissue level using hyperspectral microscopy (Cyto-Viva). At the lung level, the lesions encountered were pigmented/inert material, (control, n=0, group 1, n=2; group 2 n=1 and group 3, n=1), inflammation, chronic, interstitial (control, n=0, group 1, n=2; group 2 n=3 and group 3, n=1). Liver, splenic, renal, cardiac, gastrointestinal and brain lesions were not observed in any of the groups studied. Particles persist in a small (minimal) number at the lung level for all 3 experimental groups.

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