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Europium(III)-doped yttrium vanadate nanoparticles reduce the toxicity of cisplatin

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ABSTRACT

The aim of this study was to evaluate the antitumor efficiency of chemotherapy with cisplatin alone and incorporated into europium(III)-doped yttrium vanadate nanoparticles functionalized with 3-chloropropyltrimethoxysilane with folic acid and without folic acid in a syngeneic mouse melanoma model. Histopathological, biochemical and genotoxic analyses of treated animals were performed to assess the toxicity of treatments. The treatment of the animals with cisplatin alone and the nanoparticles functionalized with cisplatin at a dose of 5 mg/kg b.w. for 5 days reduced tumor weight about 86% and 65%, respectively. Histopathological analysis showed lower mean frequency of mitoses in tumor tissue of the groups receiving cisplatin alone (90% reduction) and the nanoparticles functionalized with cisplatin (70% reduction) compared to the tumor control group. A reduction in body and liver weight and an increase in serum creatinine and urea levels were observed in animals treated with CDDP, but not in those receiving the nanoparticles functionalized with cisplatin. Genotoxicity assessment by the comet assay revealed lower frequencies of DNA damage in animals treated with the nanoparticles functionalized with cisplatin (mean score = 140.80) compared to those treated with cisplatin alone (mean score = 231.80). Marked toxic effects were observed in animals treated with cisplatin alone, while treatment with the nanoparticles functionalized with cisplatin showed no toxicity. Moreover, folic acid in the inorganic nanoparticles reduced the genotoxicity of cisplatin in the bone marrow micronucleus test (10 ± 1.4 and 40 ± 0.0 micronucleus, respectively). These results demonstrate the antitumor efficiency and significantly reduced systemic toxicity of the nanoparticles compared to CDDP.

1. Introduction

The incidence of non-melanoma and melanoma skin cancer has increased in recent decades. Between 2 and 3 million cases of non-melanoma skin cancer and 132,000 cases of melanoma are diagnosed worldwide each year [1]. The infusion of cytotoxic drugs continues to be the first-line treatment of metastatic melanoma. The most widely used drugs are dacarbazine, cisplatin (CDDP), nitrosoureas (carmustine and lomustine), vinca alkaloids (vincristine and vinblastine), and taxanes (etoposide, teniposide and taxol).

CDDP, *cis*-[$PtCl_2(NH_3)_2$], is an inorganic compound with a square-planar geometry that contains a central platinum atom surrounded by two chlorine atoms and two ammonia groups. This compound is widely used for cancer treatment and mediates its antitumor activity *via* several cytotoxic mechanisms. The best known is for DNA damage, even though CDDP also causes cytoplasmic organelle dysfunction particularly with the endoplasmic reticulum and mitochondria. The general mechanism of action of CDDP is that it forms crosslinks with the purine bases of the DNA and this adducts interferes with the DNA replication and repair mechanisms, with subsequently induces apoptosis [2]. It also activates apoptotic pathways and inflicts cellular damage *via* oxidative stress and inflammation. Despite its effectiveness, treatment with CDDP is associated with different side effects, particularly on the central nervous system and kidneys, as well as ototoxicity. Consequently, the

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continuation of treatment with CDDP is often difficult due to its systemic toxicity [3]. Another limitation is the acquisition of resistance to the drug after a period of administration. Factors attributed to acquired CDDP resistance include its binding to plasma proteins and the consequent inactivation of a large number of CDDP molecules [4].

In an attempt to identify drugs that are more effective against cancer and less toxic to the organism, different substances are being tested to reduce these effects without a decrease in their antitumor activity. In this respect, contemporary methods for cancer treatment, particularly drug delivery systems to specific targets, have led to advances compared to traditional therapies by targeting specific sites and being less toxic to normal cells. In this sense, one of the distinguishing features between normal cells and cancer cells, which can be used as preferred targets for treatment, is a combination of folic acid (FA) with nanocarriers. This is because folate receptors (FR) are found overexpressed in different types of cancer cells and normal cells not expressing or are located on the apical surface of polarized epithelial, where drugs cannot reach [5]. Over the past decade, a great effort has been made to develop a system that is able to transport a therapeutic compound to a specific target. The objective of a controlled release system is to maintain a therapeutic concentration of a drug in the body for an extended time by controlling its rate of delivery and, therefore, the concentration of the drug between toxic and effective levels [6]. Directed or targeted therapy involves the development of drugs that will be carried by nanomaterials such as nanoparticles, nanospheres or nanotubes, called nanodrugs.

Considering the need for more effective therapeutic methods against cancer and less toxic to the organism, the present study investigated the antitumor efficiency of CDDP incorporated into functionalized yttrium vanadate nanoparticles doped with europium(III) ($Y^{III}V^{V}O_4:Eu^{III}$) coated with 3-chloropropyltrimethoxysilane (CPTES), and folic acid (FA) ($Y^{III}V^{V}O_4:Eu^{III}$:CPTES:FA:CDDP) or without FA ($Y^{III}V^{V}O_4:Eu^{III}$:CPTES:CDDP), as well as the toxicity of these treatments in melanoma-bearing mice.

2. Materials and methods

2.1. Synthesis of nanoparticles and physicochemical characterization

The $Y^{III}V^{V}O_4$ nanoparticles were synthesized by the sol-gel method as described in the literature [7]. An ethanol solution of yttrium chloride (YCl_3) was mixed with vanadium isopropoxide at a molar ratio of 1:1 and 1% europium (III) chloride was added to the solution as luminescent probe. The reaction was shaken for 48 h and the solid obtained after removal of the solvent was washed and treated at 800 °C. This sample was designated $Y^{III}V^{V}O_4:Eu^{III}$.

The $Y^{III}V^{V}O_4:Eu^{III}$ nanoparticles underwent surface modifications by functionalization in a solution containing the alkoxide CPTES, ethanol, and water. For this purpose, 300 mg of $Y^{III}V^{V}O_4:Eu^{III}$ nanoparticles were added under constant shaking. After 24 h, the solid was washed and dried and received the designation $Y^{III}V^{V}O_4:Eu^{III}$:CPTES. The chemotherapeutic agent and FA were incorporated into the nanoparticles by shaking the functionalized nanoparticles ($Y^{III}V^{V}O_4:Eu^{III}$:CPTES) in a solution of CDDP and FA. This preparation is referred to as $Y^{III}V^{V}O_4:Eu^{III}$:CPTES:CDDP and $Y^{III}V^{V}O_4:Eu^{III}$:CPTES:FA:CDDP. The physicochemical characterization of these nanoparticles is described in the literature [8].

2.2. Animals and tumor cell line

Male C57BL/6 mice, with a body weight (b.w.) of approximately 25 g, obtained from the Animal House of the University of São Paulo, Ribeirão Preto Campus, were used in the experiments. The animals were kept in plastic boxes in an experimental room under controlled conditions of temperature (23 ± 2 °C) and humidity (50 ± 10%) on a 12-h light-dark cycle, with *ad libitum* access to ration and water. The treatment protocols were approved by the Ethics Committee on Animal Use of the University of Franca (007/13).

The B16F10 cell line (murine melanoma) was a courtesy of the Department of Biochemistry, School of Medicine, University of São Paulo, Ribeirão Preto Campus, São Paulo, Brazil. The cells were maintained in HAM-F10 culture medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Nutricell), 1.2 g/mL sodium bicarbonate (Sigma-Aldrich), 0.1 g/mL streptomycin (Sigma-Aldrich), and 0.06 g/mL penicillin (Sigma-Aldrich) in a 5% CO_2/air atmosphere at 37 °C. The medium was routinely changed every 3 days and the cells were separated by trypsinization before reaching confluency.

Cell viability was assessed by the Trypan blue exclusion method [9]. Only cell dilutions with ≥95% viable cells were used.

2.3. Antitumor activity

Male C57BL/6 mice (n = 5 per group) were injected subcutaneously into the right dorsolateral region with 1×10^6 viable B16F10 cells in 100 µL culture medium (HAM-F10). When the tumor had reached approximately 100 mm^3, which occurred 18 days after cell implantation, the animals received once a day, for 5 consecutive days, 15 mg/kg b.w. of $Y^{III}V^{V}O_4:Eu^{III}$:CPTES:CDDP and $Y^{III}V^{V}O_4:Eu^{III}$:CPTES:FA:CDDP, being 5 mg/kg b.w. of CDDP (diluted in 1% of dimethyl sulfoxide [DMSO]/normal saline) in a volume of 100 µL/animal, at a distance of 1.0 cm from the tumor. According to Van Moorsel et al. [10], the maximum tolerated dose (that causes a weight loss of 15%) of CDDP in C57BL/6 mice is 6 mg/kg b.w. (intraperitoneal). Based on these data, the maximum dose of CDDP was 5 mg/kg b.w. for the groups treated with CDDP alone or incorporated into $Y^{III}V^{V}O_4:Eu^{III}$:CPTES nanoparticles with or without FA. The solutions were administered to the animals immediately after preparation. Negative (no tumor; no treatment), solvent (with tumor; 1% DMSO/normal saline), $Y^{III}V^{V}O_4:Eu^{III}$ (with tumor; 15 mg/kg b.w.), CPTES (with tumor; 7.3 mg/kg b.w.) and $Y^{III}V^{V}O_4:Eu^{III}$:CPTES (with tumor; 15 mg/kg b.w.) controls were included. The body weight of the animals, water intake and tumor size were assessed daily throughout the experiment. The tumor volume was calculated using the following equation [11]:

$$V\ (mm^3) = [length\ (mm) \times width\ (mm)^2]/2$$

The animals were euthanized with thiopental sodium (0.84 g/kg b.w.) 24 h after the last treatment. The blood, liver, kidneys, spleen, heart, lungs, brain and tumor of each animal were collected and properly stored for subsequent analysis.

2.4. Histopathological analysis

The tumor and organs removed from the animals after euthanasia were fixed in buffered formalin. Five sections (5.0 mm thick) were cut from different parts of each tumor tissue and five random fields were analyzed at 40× magnification, for a total of 25 areas per group. Necrotic, pleomorphic and hyperchromatic cells, binucleation and cellular anisokaryosis, necrotic areas, and coagulation were observed in the tissues analyzed. The kidneys, heart, lungs, spleen, liver and brain were analyzed to identify metastatic foci.

2.5. Biochemical analysis

The levels of creatinine and urea were measured to determine alterations in renal function. The analysis was carried out in an automated equipment (Mindray®) using enzymatic colorimetric kits. The method of the Mindray equipment is based on the principle of absorption.