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Original Article

Evaluation of cytotoxic activity of platinum nanoparticles against normal and cancer cells and its anticancer potential through induction of apoptosis

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ABSTRACT

Background: Plant mediated green synthesis of nanoparticles is an eco-friendly and efficacious approach which finds immense application in the field of medicine. This study aimed to evaluate the cytotoxicity of platinum nanoparticles (ptNPs) synthesized through green technology against normal and different cancer cell lines.

Methods: Platinum nanoparticles were synthesized by green technology and characterized earlier. In this study we examined the cytotoxic effect of platinum nanoparticles (ptNPs) on human lung adenocarcinoma (A549), ovarian teratocarcinoma (PA-1), pancreatic cancer (Mia-Pa-Ca-2) cells and normal peripheral blood mononucleocyte (PBMC) cells and evaluate anticancer potential through induction of apoptosis on PA-1 cells if any. Cytotoxicity was evaluated using MTT assay, trypan blue dye exclusion assay and anticancer potential assessed through clonogenic assay, apoptosis assay, cell cycle analysis.

Results: We found that ptNPs exerted cytotoxic effect on cancer cell lines, whereas no cytotoxic effect was observed at highest dose on normal cells. The results showed that ptNPs had potent anticancer activities against PA-1 cell line via induction of apoptosis and cell cycle arrest.

Conclusion: Overall, these findings have proved that biosynthesized ptNPs could be potent anti-ovarian cancer drugs. Further studies are required to elucidate the molecular mechanism of ptNPs induced anti-tumor effect *in vivo*.

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Introduction

In recent years, the interest in the synthesis and properties of metal nanoparticles has been increasing because of their unique properties and promising applications as catalysts, ferrofluids, and semiconductors.[1,2] Nanotechnology is the most promising field for generating new applications in medicine. However, only few nano-products are currently in use for medical purposes.[3]

For many years, platinum-based molecules have received considerable attention because of their electro-catalytic properties.[4,5] For instance, platinum-based therapeutic drugs, notably cisplatin and carboplatin, have been exploited in chemotherapy to kill cancer cells.[5] However, these drugs are not selective for cancer cells, because normal cells are also affected, leading to substantial dose-limiting acute and chronic toxicities. Since toxic side effects (particularly nephrotoxicity and gastrointestinal) and frequent development of drug resistance represent the major challenges in the clinical outcome of these patients, it was conceivable to search for cisplatin analogs or other metal complexes able to offer a more acceptable level of toxicity and improved antitumor activity.[6,7]

Nanoparticles are making significant contributions to the development of new approaches of drug delivery in cancer and can provide a platform for combined therapeutics with subsequent monitoring of response.[8] Increasing evidence suggests that the special physicochemical properties of nanomaterials pose potential risks to human health.[9] Therefore it is necessary to understand how cells respond to nanomaterials and through what mechanisms. Green nanotechnology is generating attention of researchers toward eco-friendly biosynthesis of nanoparticles. With a view toward developing nano-therapeutics, we have performed experiments using eco-friendly platinum nanoparticles. Platinum nanoparticles were synthesized by green technology, characterized its zeta potential and size by dynamic light scattering (DLS) as well as scanning electron microscopy (SEM) earlier.[10]

The present study is the continuation of the earlier work and is carried out to assess the cytotoxicity of ptNPs on normal and three different types of cancer cells. Based on highest cytotoxicity results, anticancer activities of platinum nanoparticles against ovarian terotocarcinoma cells were evaluated.

Methods

Cell culture medium reagents were purchased from Himedia laboratories. Fetal bovine serum (FBS) was purchased from Invitrogen (US). An Annexin V-FITC apoptosis detection kit was purchased from BD-Bioscience (Catalogue no. 556547). Cisplatin, used as a positive control was purchased from Cipla (India). Platinum nanoparticles were synthesized through green technology and characterized by particle size, zeta potential and surface morphology.[10] The recovered ptNP sample was used for cytotoxicity and anticancer studies.

Cell culture and exposure of drug

A human cancer cells, A549, PA-1, Mia-Pa-Ca-2 were obtained from National Center for Cell Science (NCCS), sub-cultured and then used to determine cell cytotoxicity after exposure to the drug. The cells were cultured in minimum essential medium (MEM), Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS at 5% CO_2 and 37 °C. At 85% confluence, the cells were harvested using 0.25% trypsin and seeded in 25 cm^2 flasks, 96 well plates, 6-well plates, according to the experiment being performed. The cells were allowed to 70% attach to the surface prior to treatment. A stock solution of ptNPs 10 mg/ml was made in vehicle and diluted to appropriate concentrations for treatment. Suspensions were vortexes and aspirated 10 times before treatment. Cells treated with vehicle control were taken as control.

MTT cell proliferation assay

The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay measures the cell proliferation rate and conversely, the reduction in cell viability when metabolic events lead to apoptosis or necrosis. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water insoluble blue formazan compound, depending on the viability of the cells.[11]

A549, PA-1, Mia-Pa-Ca-2 cells (2×10^4 cells/ml) were seeded in 96-well plates and exposed to different concentrations (50, 100 and 200 μg/ml) of ptNPs and 10 μg/ml of cisplatin for a period of 48 hours. After the treatment period, the cells were allowed to react with MTT for a period of 3–4 hours in dark at 37 °C. At the end of the incubation period, dark purple formazan crystals were formed. These crystals were solubilized with an organic solvent (e.g. isopropanol) and absorbance at 595 nm was measured spectrophotometrically. The experiment was repeated at least three times. Cisplatin was used as positive control for this experiment. To determine the cell viability, we calculated percent viability as % viability = [(Optical density {OD} of treated cell − OD of blank)/(OD of vehicle control − OD of blank) × 100].

Preparation of PBMC and assessment of cytotoxicity using trypan blue assay

Peripheral blood mononuclear cells (PBMC) were isolated from healthy human volunteer by Ficoll-Paque (Histopaque 1077, Himedia laboratories) density gradient centrifugation as per standard procedure.[12] PBMC (2×10^5 cells/well) were cultured in complete RPMI-1640 media as usual and incubated with ptNPs (200 μg/ml), cisplatin (10 μg/ml) to evaluate cytotoxicity for 48 hours using trypan blue exclusion assay. This methods yields approximately more than 95% viable PBMC.

Clonogenic survival assay

Clonogenic assay or colony formation assay is an *in vitro* cell survival assay based on the ability of a single cell to grow into a colony. The colony is defined to consist of at least 50 cells. The assay essentially tests every cell in the population for its ability to undergo "unlimited" division.[13] Clonogenic assay is the method of choice to determine cell reproductive death after treatment with ptNPs. After harvesting with 0.05% trypsin, 200 (depending on the treatment) cells were plated 24 hours before treatment in MEM at 37 °C. Cultured cells were treated with

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