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Synthesis and Cytotoxicity Assessment of Novel Nano Copper Naphtoquinone Derivative on PC12 Cancer Cells

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With respect to the unique properties of nanoparticles' small size and efficient penetration into the cell membrane and considerable drug delivery currently put an special emphasis on design, synthesis and biological evaluation of nanoparticles(NPs) in cancer treatments. The present investigation was carried out to design, synthesis and bioevaluation of the new copper NPs complex of heterocyclic naphtoquinone derivatives in the PC12 cancer cell lines. Cytotoxic effect was evaluated from cell viability, LDH release assay and lipid peroxidation measurement. Cell viability was found to decrease as Cu(II)NQ14 NPs content increased from 0.675 to 80 µM but when compared with Cu(II)NQ14 microparticles cell mortality observed from 2.5 to 80 µM for 24 h. Likewise, LDH leakage induced by NPs inclined with dose more significantly compared to MPs (p<0.001). Moreover, lipid peroxidation occurred markedly worse in NPs- than MPs-treated PC12 cells in a dose- dependent manner. Therefore, Cu(II)NQ14 NPs can effectively kill PC12 cancer cells, enabling potential application as anticancer agent.

Keywords: Nano copper naphtoquinone; Cytotoxicit; PC12

Introduction

Recent advances in nanopharmacy open a new horizon in the design and development of novel anticancer agents. Due to drastic physicochemical changes of nanoparticles(such as size, shape, surface area, phase and composition) compared to micro counterparts, nano anti-cancer drugs can achieve improved therapeutic efficacy through enhanced cytotoxic activity, prolonged blood circulation time and intracellular delivery capacity over traditional one[3].

Among the diverse natural and synthetic compounds noticed by antitumor potential, compound with quinone containing structures are of great importance Quinones, particularly 1,4-naphtoquinones are reported to exhibit various pharmacological properties like antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic and anticancer activities. These quinones with the ability to induce oxidative stress are responsible for initiation of tissue damage selectively in tumor cells and this seems to be a promising approach for targeting cancer cells.

Preparation of copper 1,4 naphtoquinone micro and nanoparticles:

On the other hand, combining a reactive nano metal moieties to quinone can potentiate anticancer activity of parent ligand. Thus, the present investigation was carried out to synthesize and compare cytotoxic effects of novel micro and nano Cu(II) complexes derived from 1,4-naphtoquinone[5]..

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Material and methods

Based on Michael's reaction, naphtoquinone derived from heterocycle was boiled for 1 hour with 2-

methyl-amino-pyridine (amine group) to get 1 -4-naphthoquinone brown precepitate with molecular mass of 264.28 per mole. To prepare the copper nanocomplex derived from the heterocyclic 1 and 4-naphtoquinone, 10 milliliters of 0.5M copper salt solution are exposed to ultrasonic probes at a frequency of 20 kHz and a power output of 600 watts for 15 minutes. Then, 20 ml of 1M 1,4-naphtoquinone ligand added to salt solution as droplets and exposed to an ultrasound probe for 1 hour. The resulting precipitate was filtered, washed with methanol and then dried to air. Nanoparticles with molecular weight of 660.99 gram/mole were obtained.

Structural characterization:

The size distribution of dispersed particles was measured using a Zetasizer Nano ZS90(Malvern Instruments Ltd, Malvern, UK). Further characterization of changes in the surface and surface composition was performed by Fourier transformed infrared spectroscopy (PerkinElmer Spectroscopy GX, PerkinElmer Inc., Waltham , MA, USA). Transmission electron microscopy (TEM), using a Philips CM-120 was performed to determine the size and morphology of CuNPs(Fig-1).

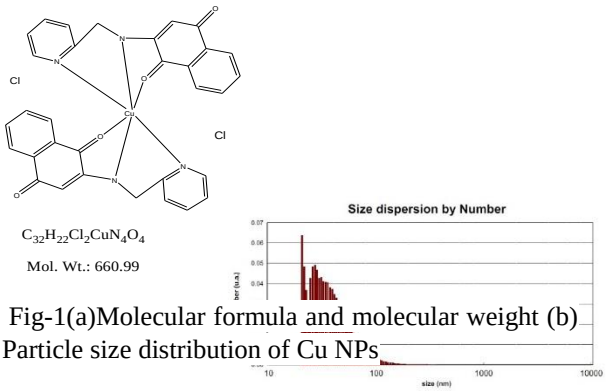

Fig-1(a)Molecular formula and molecular weight (b) Particle size distribution of Cu NPs

Cell culture:

Pheochromocytoma(PC12) cancer cells were obtained from Pasteur Institute of Iran. Cells were cultured in Dullbecco's modified Eagels's medium (DMEM, Sigma Alderich, MO, USA) supplemented with 5% fetal calf serum and 0.1% penicillin-streptomycin-amphotericin B (Sigma Alderich). Cells were maintained at 37ºC in a humidified 5% CO_2 air incubator for 3 days to achieve 80% cell confluence. Cells were then trypsinized with trypsin and resuspended in DMEM medium.

Cytotoxicity assessment

The cytotoxic effect of micro and nano Cu(II)NQ14 on PC12 cells was measured by MTT assay. Prior to using in the experiments, cells were assessed for viability (more than 95% viability). Cells (5×10^3) were allowed to adhere for 24 h in 96-well culture plates. The next day, cells were treated with increasing concentrations of micro and nano Cu(II)NQ14 (0.675,1.25, 2.5, 5, 10,20, 40 and 80 µM) for 24h. After the respective exposures, MTT was added and plates were incubated for 4 h. Next, the solution was discarded and cells were lysed with DMSO to obtain purple solution. The plates were kept for 10 min at room temperature and then read at 595 nm using multiwell microplate Reader (Awareness Technology, USA).

Lactate dehydrogenase (LDH) release assay

LDH release assay is a method to measure the membrane integrity as a function of the amount of cytoplasmic LDH released from damaged cells to the medium. LDH assay was carried out using LDH assay kit, BioVision, California, USA). Supernatant of each well was transferred to a fresh bottom 96-well culture plate and further processed for enzymatic analysis as per the instructions given in the kit.

Lipid peroxidation measurment

The measurement of Thiobarbituric Acid Reactive Substances(TBARS) is a well-established method for screening and monitoring of lipid peroxidation. MDA-TBA adduct formed in cells treated with Cu(II)NQ14 micro and nanoparticles at varying concentrations for 24 was measured colorimetrically at 540nm using commercially available kit (Cayman, Item No. 10009055) following manufacturer's instruction.

Statistical analysis

Results were expressed as means of at least three replicates ± standard error. Statistical analysis was performed by one-way analysis of variances (ANOVA) and post hoc Tukey-Kramer test to comparethe control versus treated group by SPSS Version 12. In all the cases, $p<0.05$ was considered as significant.

Results and discussion

Effect of Cu NQ14 micro and nanoparticles following 24h exposure to PC12 cells was studied

using MTT, LDH and MDA assays(Fig-2). Both the micron and nanoparticles exhibited significant dose dependent toxicity on PC12 cells. Size was shown to be an important parameter affecting the biological activities so that nanoparticles compared to microparticles demonstrated more effective dose response cytotoxcicity. Findings from several studies have pointed out that ROS generation and oxidative stress occur as an early event leading to NP-induced injury[1,3]. Oxidative stress corresponds with the physicochemical reactivity of NP. Various metal oxide NP including Zn, Cu, Ti and Zn accelerate the ROS response via mechanisms such as Haber-Weiss and Fenton-type reactions[2]. NP-mediated ROS responses have been reported to orchestrate a series of pathological cellular events such as necrosis and LDH leakage[4].

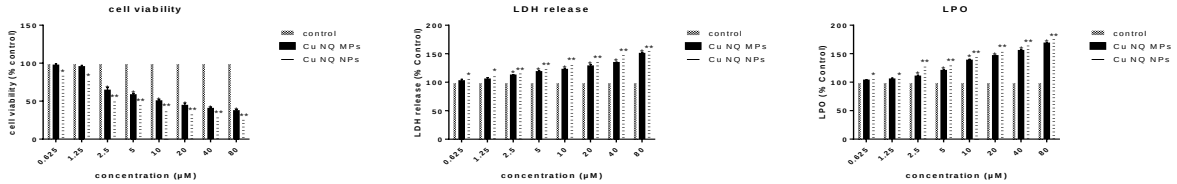

Fig-2. Identification of cell viability of Cu NQ MPs and NPs in PC 12 cells assessed by standard endpoints such as (a) MTT assay (b) LDH release and (c) LPO assay. Data are represented as mean ± SE of replicate.* P<0.05 indicates significance.

Conclusion

This work describes an approach toward preparation and biological evaluation of new class of nano antitumor agent. We observed that the synthesized Cu NQ14 exhibits excellent cytotoxic activity against PC12 cancer cells. This NP can be examined for complementary in vitro and in vivo tests.

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