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Full Length Research Paper

In vitro evaluation of the effects of acetone, on the potency of cisplatin: Is it a good candidate for cisplatin carrier preparation?

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One of the major problems for cisplatin nanocarrier production has originated in the low water solubility of this anticancer drug. The solubility power of acetone as an organic solvent and its effect on antitumor activity of cisplatin in order to produce a drug carrier constituents was therefore evaluate. Powder of cisplatin (secondary cisplatin) was obtained after evaporation of acetone. Its potency was determined by MTT and MIC tests using Hep-G2 cell line and *E. coli* respectively. A further insight into the similarity between two chemical compounds has also performed using Fourier Transform Infrared Spectroscopy technique. Compared with control groups, there was no significant difference between primary and secondary cisplatin cytotoxicity by MTT assay. MIC results also did not show significant discrepancy (MIC for primary and secondary cisplatin were 9.5 and 9.7 micro molar respectively). The similar spectra obtained of primary and secondary cisplatin at FTIR indicated these compound were approximately identical. Based on the results of current study it can be concluded that under proper condition, acetone as an organic solvent can be considered for cisplatin carrier preparation.

Key words: Cisplatin, Acetone, Hep-G2 cell line, Drug delivery.

INTRODUCTION

Nowadays cisplatin is one of the most powerful anticancer drugs used widely to treatment of many malignancies including ovarian, breast, lung and stomach carcinomas (Galsky *et al*., 2011; Voss *et al*., 2011). However, use of cisplatin is along with side effects that restricted administration dose of the drug. Some of the cisplatin side effects are acute nephrotoxicity, lesion of homopoetic system and loss of hearing (Hamdi *et al*., 2010; Lamerie *et al*., 2011; Sanchez-Gonzalez *et al*., 2011). However selective or targeted administration of cisplatin against cancer cells leads to reduction of the side effects with improvement of the drug efficiency (Gladieff *et al*., 2009). Passive targeting of drugs against tumor is achieved by their conjugation or entrapment to the soluble and stable particle carriers (Galsky *et al*., 2011). Nevertheless, efforts have not succeeded in developing a suitable cisplatin carrier to deliver this drug effectively to tumor cells yet. One of the main reasons is the low water solubility of cisplatin that results in disability to manufacture a correct carrier and as a result, deliver of drug to tumors (El-Gendy, and Berkland 2009; Meerum Terwogt *et al*., 2002). We here take advantage of dissolving capability of acetone as an organic solvent and evaluate the effects of this solvent on the cisplatin potency. The results show that a milliliter of acetone is competent to dissolve 25 mg of cisplatin. Acetone is a simple example of ketone compounds with high water

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Table 1. Array of different materials used for MIC assay

Primary cisplatin	Secondary cisplatin	bacterial suspension	Culture medium	Compound row
–	+	+	+	1
–	+	–	+	2
+	–	+	+	3
+	–	–	+	4
–	–	–	+	5

miscibility and because of low boiling point (56/53 ˚C) it has been demonstrated that this substance is quickly evaporated even at the water and soil (El-Gendy and Berkland 2009). Acetone, as a member of ketone bodies, is produced during normal metabolic processes in human body (Patakova *et al*., 2012). Because of higher demand for energy consumption, increased production rates of acetone is observed in the pregnancy, breast feeding and children (Kulin *et al*., 1979). Acetone is also used as food additives and cosmetic materials (Kang *et al*., 2006). Hence, this solvent can be considered as a safe substance for human usage (Kang *et al*., 2006). Here in this study, the results show that anti-tumor activity of cisplatin after solvent evaporation was persevered that was confirmed with 3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) and minimal inhibitory concentration (MIC) tests (Hussain *et al*., 1993). Fourier transform infrared spectroscopy (FTIR) is a forceful technique for investigation of the chemical compounds (Wong *et al*., 1987). In this study it has also shown that spectra achieved with applied FTIR technique from both primary and secondary forms of cisplatin were approximately the same.

MATERIALS AND METHODS

Cisplatin, acetone and MTT were purchased from sigma chemicals, USA. HEP-G2 cell line was obtained from National Cell Bank of Iran (NCBI) and *E. coli* strain was obtained from microbial collection of pasture institute of Iran. Analytical grade miscellaneous chemical reagents and solvents were also purchased from Sigma (USA) and Merck (Darmstadt, Germany) companies. Firstly, 25 mg cisplatin in powder form (primary cisplatin) was dissolved in one milliliter of acetone, after 24 hours acetone evaporated and secondary cisplatin in the powder form was once again obtained. Primary and secondary cisplatin then undertook MTT and MIC assays using Hep-G2 cell line and *E. coli* strain, respectively. MIC defines as a minimal concentration of interest substance that causes bacteria growth inhibition (Andrews *et al*., 2001). Since bacteria reproduction generate turbidity, it is possible to compare the sample's turbidity using spectroscopy instruments or optically. MIC assay was performed according to a method described previously (Panghal *et al*., 2011). Briefly, correspond to half McFarland Scale, 100 µl of LB (Luria Broth) bacteria culture medium dispensed to 96 well plates and then bacterial suspension, Secondary cisplatin, and Primary cisplatin were added according to Table 1. Total final volume existing to each well was equal to 200 µl. Finally, plate was incubated at 37 °C for 24 hours. This assay was performed at duplicate plates. Additionally, in order to determine cytotoxicity effects of these cisplatins, HEP-G2 cell line was cultured in DMEM containing 10% FBS, 2 mM glutamine, antibiotics (penicillin G, 60 mg/L; streptomycin, 100 mg/L; amphotericin B, 50 µL/L) under a humid atmosphere (37 ℃, 5% CO2, 95% air). Then MTT assay was included. Briefly, 10^4 cells/well were treated with various concentrations of cisplatin (0, 20, 40, 80, and 160 µM). After 24-hour incubation, MTT (0.5 mg/mL PBS) was added to each well and incubated at 37 ℃ for 3 hours. The formazan crystals that formed were dissolved by adding 100 µL/well of 100% isopropanol and the absorbance was read at 570 nm using a microplate scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands). Toxicity levels were calculated with these equations (Mokhtari et al., 2008):

$$\%\ \text{cytotoxicity} = 1 - \frac{\text{mean absorbance of toxicant treated cells}}{\text{mean absorbance of negative control}} \times 100$$

$$\%\ \text{Viability} = 100 - \%\ \text{cytotoxicity}$$

Furthermore, samples for FTIR analysis were prepared by pressing powdered KBr pellets combined thoroughly with about 5% – 10% of finely ground powder of the each sample, and after that settled on a Nicolet 740SX FTIR spectrophotometer with a MCT-B detector (USA). The spectra were recorded in the range 4000 ~ 450 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans.

RESULTS

Our results demonstrated that MIC tests for two samples were almost the same in which these values were 9.5 and 9.7 µM for primary and secondary cisplatin, respectively. At concentrations of 20, 40, 80, and 160 µM of primary cisplatin in MTT assay, HEP-G2 cell viability reduced to 64.41 ± 0.52% ($P < 0.01$), 58.00 ± 2.00% ($P < 0.01$), 54.00 ± 1.00% ($P < 0.01$), and 34.85 ± 2.81% ($P < 0.01$), respectively. For secondary cisplatin, at concentrations of 20, 40, 80, and 160 µM, HEP-G2 cell viability reduced to 66.17 ± 0.97% ($P < 0.01$), 61.37 ± 2.00% ($P < 0.01$), 56.40 ± 3.13% ($P < 0.01$), and 40.44 ± 0.88% ($P < 0.01$), respectively, (Figure. 1). In addition, the results of MTT assay associated to primary and secondary cisplatin on the cell viability showed no significance difference between cytotoxicity effects of

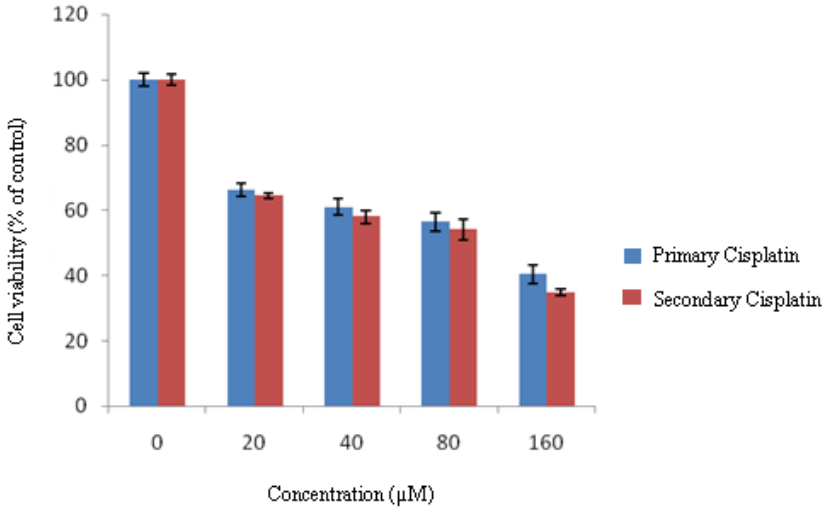

Figure 1. Cytotoxicity effects of primary and secondary cisplatin on HEP-G2 cells for 24 hours incobation. Results are expressed as a percentage of viability compared to control and are presented as mean ± SD from at least three independent experiments.

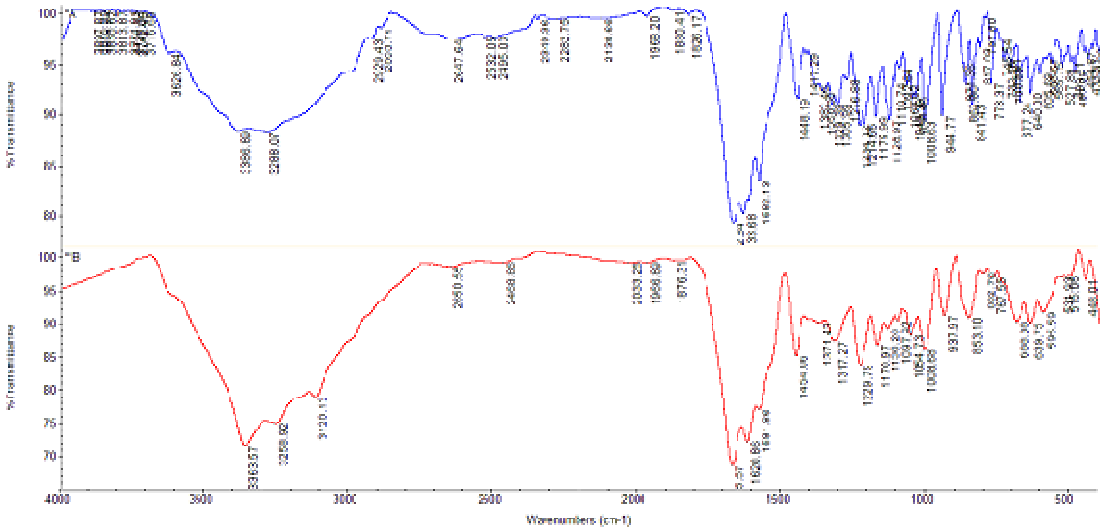

Figure 2. FTIR spectra of primary and secondary cisplatin (beneath and upper graphs respectively). As picture shows marked similarity occurs between two graphs.

these cisplatins. Evaluation of spectra came from the FTIR technique also had shown similar results. It has been demonstrated that bond between platinum and nitrogen of ammoniac ligands appears at 463 cm^{-1}. These values for bonds between chloride and platinum elements of cisplatin come to 338 cm^{-1} and 362 cm^{-1}. In addition tensional trembles associated to ammoniac groups became visible at 1550-1680 cm^{-1} and 3300-3500 cm^{-1} region. According to the results obtained of spectra of two samples, it could be concluded that they have approximately the same configurations (Figure. 2).

DISCUSSION

In this study, we have succeeded to produce a form of

cisplatin drug usable for drug carrier construction. One of the main methods for preparation of poorly-water soluble drugs such as cisplatin is solvent evaporation. Polymer is dissolved in a volatile organic solvent at this process. Various organic solvents were used for this method (Mattheolabakis *et al*., 2009; Hiraia *et al*., 2010). Cisplatin carrier is also obtained with some other protocols that utilize of organic solvents too (Nishiyama *et al*., 2003). At the all-aforementioned methods, the solvents used have some disadvantages compared to acetone including side effects and the power of solubility. In our study, cisplatin dissolved in acetone had crystallized appearance and was more colorful than the original one. It was also clarified that acetone is a competent solvent for cisplatin regarding that 25 mg of cisplatin had dissolved in just one milliliter of acetone. Because of the low boiling point of this solvent (56/53 ºC), preparation of secondary cisplatin was almost easy. Acetone is classified as a nearly safe material as well, so, it can be used in pharmaceutical preparations (Kang, 2006; Tanaka *et al*., 1996). Cisplatin is one of the most common anti-cancer drugs used worldwide (Voss *et al*., 2011). From the statistical point of view, we here observed that differences between values achieved in MIC and MTT assays at primary and secondary cisplatin were not meaningful. Even these slight differences may be originated in solubility troubles associated to the secondary cisplatin. Because secondary cisplatin had approximately crystalline appearance and unlike primary one, it was more difficult to obtain dissolved form of that at cell line and bacteria culture medium. Therefore, obtained homogeneity from secondary cisplatin was not perfect as much as primary one. Findings of FTIR spectra also confirmed the results of MTT and MIC assays in that there was no markedly difference between two spectrums of samples. As shown here, acetone as a solvent had no detrimental effects on the cisplatin potency, thus use of this solvent is acceptable for cisplatin carrier's preparation. Evaluation of methods for cisplatin carrier construction and selection of compatible method may be considered as a future work.

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