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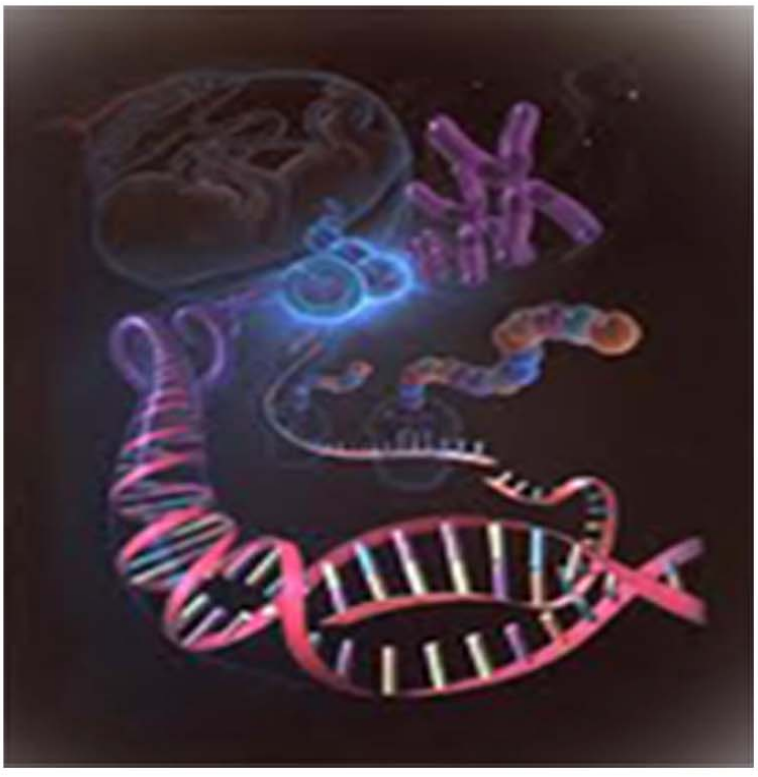

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Research Paper

EFFECTS OF CISPLATIN CONJUGATE NANOPARTICLES ON BREAST CANCER *IN VITRO* AND *IN VIVO*

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In the present study, we synthesized the magnetic nanoparticles (NPs) loaded with cisplatin. Then PEG-COOH/Fe3O4 nanoparticles were loaded by cisplatin. Its cell toxicity evaluated by the MTT and in vivo assay. In vitro breast cancer studies, we compared the effect of cisplatin and cisplatin nanoparticles in MCF-7 breast cancer cell line. Finally, *in vivo* part of this study, the breast adenocarcinoma allograft in Balb/c mice was investigated. Different doses of cisplatin and cisplatin loaded nanoparticles were administrated to mice. Tumor size was evaluated by calculation of tumor volume. We found that PEG-COOH/Fe_3O_4 nanoparticles are effective anticancer agents. We also found that cisplatin nanoparticles induce apoptosis in human breast cancer cell line. In the present study, we have shown strongly increased in vitro cytotoxicity of cisplatin nanoparticles compared with the free drug in MCF-7 cell line. In summary, our results indicate that cisplatin loaded nanoparticles are effective anticancer agent.

Keywords: Nanoparticles, Pegylation, Cisplatin, MCF-7, Breast, Adenocarcinoma

INTRODUCTION

Chemotherapy is the only option for treating the malignant breast cancer and condition for increases the life span of the patient. Successful chemotherapy of cancer depends on the delivery of sufficient concentrations of an effective drug to tumor cells without causing intolerable toxicity to the patient (Decatris *et al*., 2004). Cisplatin is used widely for the treatment of lung, breast, bladder, ovarian, cervical, prostate, testicular and head and Neck malignancies (Galea and Murray, 2008). One of the disadvantages is inactivation of cisplatin secondary to complex formation with plasma and tissue proteins and resistance to cisplatin (Burger *et al*., 2002). One promising approach for overcoming the side effects is using nanoparticles for carrying the drugs specifically to the breast cancer cells. Nanoparticles are defined as submicroscopic particles between 1 to 100 nm. Nanoparticulate drug delivery systems are being developed to deliver smaller doses of chemotherapeutic agents in an effective form and control drug distribution within the body

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(Praetorius and Mandal, 2007). Magnetic nanoparticles (MNPs) have demonstrated great promise for diagnostic and therapeutic applications (Sheng and Huang, 2011). Magnetite directly conjugated with specific drug has some limitations, drug release control and drug loading capacity (Yang *et al*., 2006). To solve these problems, many researchers have used specific organic polymers. Pegylation was shown to prolong the lifetime of cisplatin loaded with nanoparticles in serum to almost 1 h (Staffhorst *et al*., 2008). Our aims of this study were to synthesis cisplatin loaded with Fe_3O_4 nanoparticles and comparison the anticancer potential of cisplatin nanoparticles *in vitro* and *in vivo*.

MATERIALS AND METHODS

Synthesis of Cisplatin-Loaded with PEG-COOH/Fe_3O_4

At first PEGylated magnetic nanoparticles of $FeCl_2$ and $FeCl_3$ were synthesized using co-precipitation method with ammonia reducer, followed by pegylation of MNPs. Cisplatin solution (0.5 mg/ml, 1340 µM) and sonicated MNPs (0.2 mg/ml) were mixed in the same ratio and stirred at 600 rpm for 48 h at RT. The solution was then ultracentrifuge for 5 min. The supernatants and cisplatin solution (3440 µM) were used to determine cisplatin continent and loading efficacy using -Atomic Absorption Spectroscopy (AAS). Drug-loading Efficiency was determined by this equation:

$$\text{Loading (\%)} = 1-\left(\frac{\text{Quantity of Pt in the supernatant fluid}}{\text{Quantity of Pt in the cisplatin solution}}\right)\times 100$$

Characterization of MNPs and Pegylated Cisplatin MNPs

Particle size (diameter, nm) and morphology was examined using SEM. Surface charge (zeta potential, mV) were measured. MNPs were quantitatively assessed by FTIR spectroscopy. The FTIR spectra of MNPs loaded with cisplatin is shown in Figure 5.

Cell Culture

Cells were cultured in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mM glutamine, penicillin (100 IU/ml) and streptomycin (100 mg/ml). Cells were allowed to grow in plastic tissue culture flasks and were kept in CO_2 incubator at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air (Mortazavi *et al*., 2011).

Cell Treatment with Cisplatin and PEG-COOH/Fe_3O_4 Loaded with Cisplatin

In each experiment, six MCF-7 cultured wells with no sample were used as negative controls. Furthermore cells cultured on 96-well plates were incubated with different concentrations (0, 3.125, 6.25, 12.5, 25, 50 and 100 µM) of cisplatin and MNPs loaded with cisplatin for 48 and 72 h. Each concentration of cisplatin was tested on three wells of the 96-well plates containing 1×10^4 MCF-7 cell lines.

MTT Assay and IC_{50} Determination

One hundred µl of MTT solution (0.5 mg/mL in PBS) was added to cell monolayer in each 96-well plate. Cells were incubated in the humidified incubator at 37ºC for 3 h. In soluble formazan dye was dissolved in solution containing 100 µl isopropanol and its Optical Density (OD) was read against blank reagent with an ELISA reader at a wavelength of 570 nm (Mortazavi *et al*., 2011).

Breast cancer cell viability treated with cisplatin and MNPs loaded with cisplatin was calculated with these equations:

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

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

IC_{50} was determined by probit analysis using the Pharm/ Pharmacologic Calculation System (PCS) statistical package (Springer Verlag, USA).

In Vivo Studies

Breast adenocarcinoma allograft in Balb/c mice was investigated. After 2 weeks, allograft of tumor start IP injection of cisplatin loaded nanoparticles (2 and 5 mg/kg) weekly and size mass of tumor twice a week with this equation (Chen *et al*., 2009):

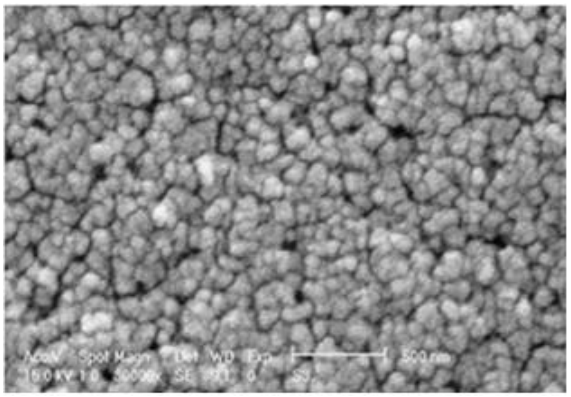
$V = W^2 \times L/2$

RESULTS AND DISCUSSION

Particle Size and Morphology

The particles size of MNPs-cisplatin fluids were measured by SEM. The results observed with the SEM show that, the particles in MNPs-loaded cisplatin fluid are spheroid (Figure 1).

Figure 1: Scaninng Electron Microscopy (SEM) Images of (A) MNPs (26 nm) and (B) Cisplatin-Loaded MNPs (48 nm)



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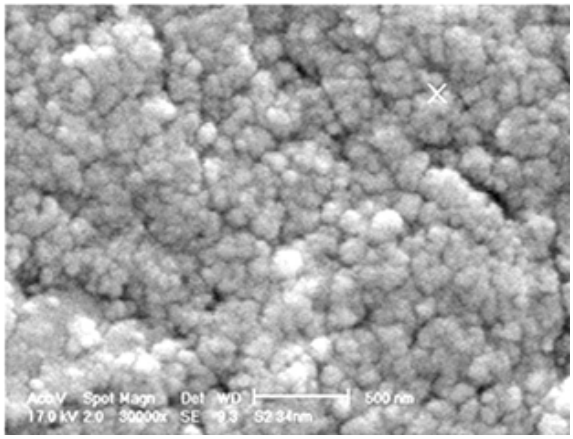
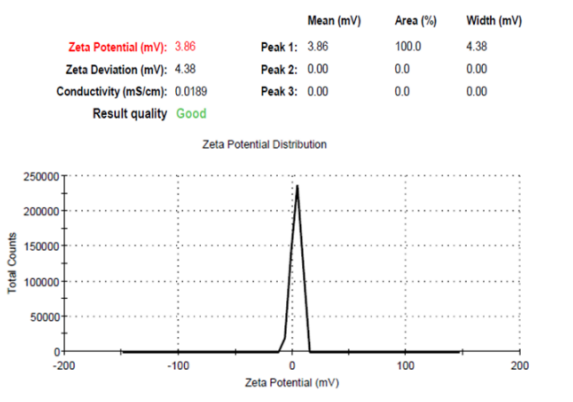


Storage Stability of Magnetic Nanoparticles

The dispersions of Fe_3O_4 nanoparticles are easy to aggregate due to their high specific surface areas. It was expected that the storage stability of Fe_3O_4 nanoparticles could be improved after coating with PEG-COOH. The zeta potential results suggest that the net surface charge on the MNPs are positive (3.86 mV) but the negative zeta potential values (–29.3 mV) indicate the pegylated NPs because of negative charge of carboxyl groups of PEG (Figure 2).

Drug Loading Efficiency

Figure 2: Figure 2: Zeta Potential of (A) Fe_3O_4 Nanoparticles (B) PEG-COOH/ Fe_3O_4 Nanoparticles



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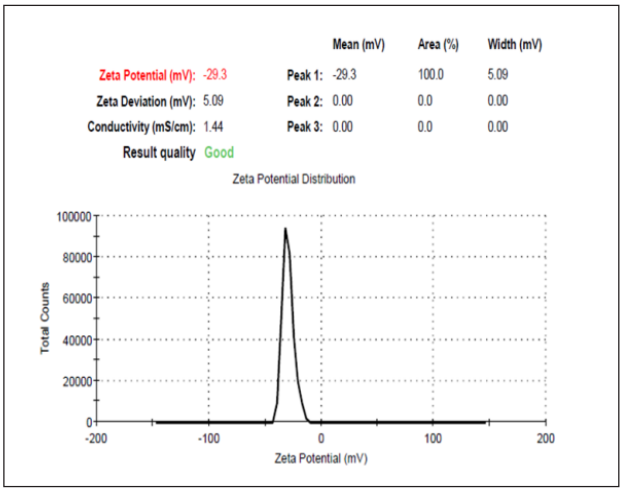
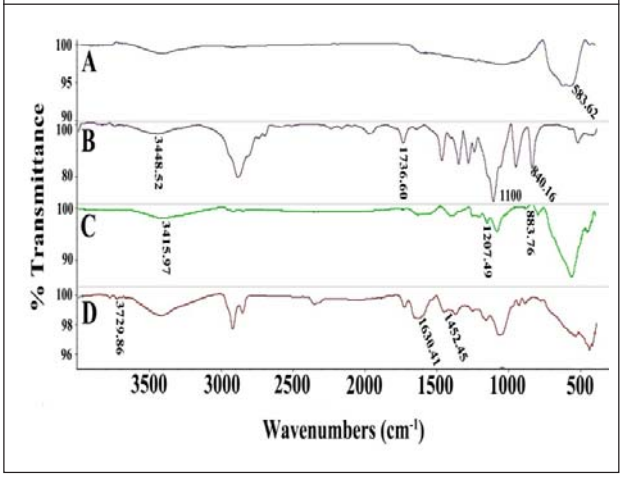


The cisplatin content of the nanoparticles was assessed by FAAS using H_2PtCl_6 (Sigma) as a standard. The average loading efficiency was 14%.

FTIR Spectra

Fourier Transform Infrared Spectroscopy of Fe_3O_4, PEG-COOH, PEG-COOH /Fe_3O_4, PEG-COOH /Fe_3O_4 loaded with cisplatin are shown in Figure 3. Specific band of Fe_3O_4 NPs is shown at 583 cm^{-1} (Figure 3, plot A). Absorbance picks located at 1110, 840, 3448 cm^{-1} indicate C-O-C bands, CH_2CH_2O, C=O bands, O-H in PEG-COOH, respectively (Figure 3, Plot B). Absorbance picks at 1630, 3729, 1452 cm^{-1} related to respectively C=O, N-H, C=C bands of NPs loaded with cisplatin (Figure 3, Plot D), these pikes indicate the amide bands between carboxyl group of NPs and amine group of cisplatin.

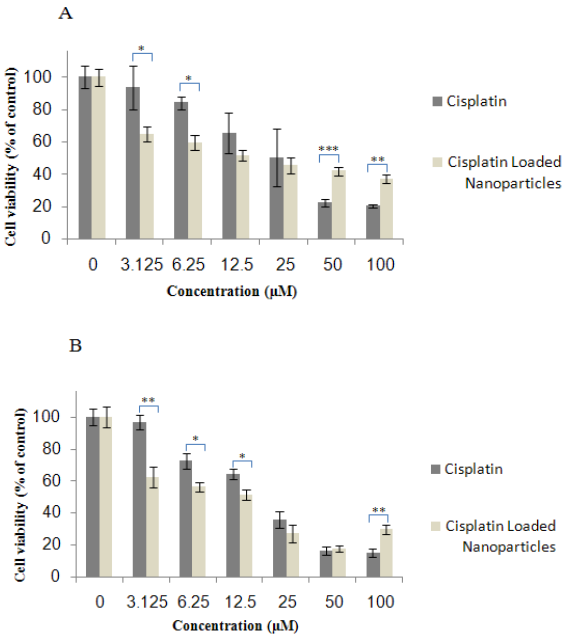
Figure 3: FTIR Spectra of (A) Fe_3O_4, (B) PEG-COOH, (C) PEG-COOH /Fe_3O_4, (D) PEG-COOH /Fe_3O_4 Loaded with Cisplatin



Cytotoxicity of Cisplatin-Loaded Fe_3O_4 Nanoparticles *in Vitro* and Calculated IC_{50}

The effect of cisplatin and MNPs-loaded cisplatin with indicated concentrations on MCF-7 viability are compared in Figure 4(A) and (B) after 48 and 72 h treatment, respectively. The IC_{50} of cisplatin-loaded MNPs was significantly lower than

Figure 4: Viability of Cisplatin-Treated Cells with Different Concentrations of Cisplatin (0, 3.125, 6.25, 12.5, 25, 50 and 100 µM) Were Determined for 48 (A) and (B) 72 Hours Treatments. Error Bars Represent the Standard Deviation of the Mean. The Result Is Indicated in Form of Percentage of Viability Compared to Control and Presented as Mean ± SD (*P<0.05; **P<0.01; ***P<0.001 Student's T-test)

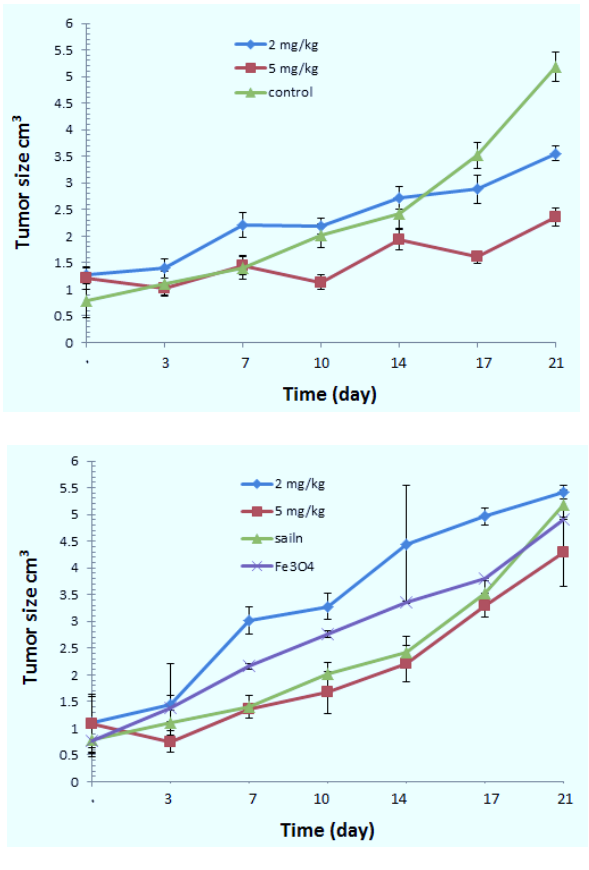


cisplatin. IC_{50} of cisplatin and MNPs-cisplatin were 43.694 and 24.721 µM, respectively.

Treatment Efficacy *In Vivo* Studies

Drug administration was performed twice a week and size of tumors was calculated according to the equation. Calculated amounts for each group mentioned as the average volume of the tumors. Maximum reduction of tumor volume in cisplatin-loaded nanoparticles treated group is seen in concentration of 5 mg/ml per body weight significantly ($p<0.05$) but no reduction was seen in 2 mg/ml per body weight (Figure 5).

Figure 5: Tumor Size of Mice Treated with Two Different Doses of (A) Cisplatin (B) Cisplatin-MNPs for 21 Days



DISCUSSION

Several studies demonstrated that nanocarriers could passively extravagate through the leaky vasculature, which is characteristic of solid tumors (Yu *et al*., 2008). The advantage of the magnetic targeted drug delivery systems over other drug targeting techniques is their ability to minimize the uptake by reticuloendothelial system (Chomoucka *et al*., 2010). Some investigators have reported successful tumor remission in animal experiments upon the use of magnetically responsive anticancer drug carriers under magnetic fields (Chertok *et al*., 2008). The findings of previous studies suggest that Fe_3O_4 nanoparticles can increase cisplatin concentration in SKOV3 cells and enhance the effective accumulation of anticancer agents in resistant cancer cells (Jiang *et al*., 2009). In laboratory tests, the gold-iron oxide nanoparticle combination successfully targeted the cancer cells and released the cisplatin into the malignant cells, killing the cells in up to 80% of cases (Lee *et al*., 2010).

CONCLUSION

We showed that the cytotoxic effect of cisplatin and cisplatin loaded nanoparticles increased in a dose and time dependent manner. Furthermore, we have shown that the strongly increased *in vitro* cytotoxicity of cisplatin-loaded nanoparticles compared with the free drug in MCF-7 cell line. Antitumor activity *in vitro* was determined by MTT test. *In vitro* cell proliferation assay showed that administration of cisplatinloaded with MNPs significantly reduced the IC_{50} of cisplatin from 43.694 µM to 24.721 µM. Furthermore we determined the efficacy of cisplatin loaded with nanoparticles (NPs) on tumor inhibition. *In vivo*

studies in Balb/c mice showed significantly decreased tumor growth and increased survival in treatment groups using nanoformulation.

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