Encapsulation in nanoparticles improves anti-cancer efficacy of carboplatin
Tanmoy Sadhukha¹ and Swayam Prabha¹,²

¹Department of Pharmaceutics, University of Minnesota, Minneapolis, MN 55455, USA; ²Center for Translational Drug Delivery, University of Minnesota, Minneapolis, MN 55455, USA
sadhu001@umn.edu

ABSTRACT SUMMARY
Poor intracellular uptake of carboplatin contributes to its limited therapeutic efficacy. We investigated encapsulation in poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles as a means to improve intracellular delivery and therapeutic efficacy of carboplatin. Nanoparticle-encapsulation resulted in enhanced cellular uptake and a remarkable reduction in the IC₅₀ of carboplatin in different tumor cell lines. Encapsulation in PLGA nanoparticles can thus reduce the dose of the drug required and potentially reduce drug toxicity commonly observed at high carboplatin doses.

INTRODUCTION
Platinum-based drugs like carboplatin are used in the treatment of a number of malignancies including ovarian, lung, head and neck cancers.¹ However, low uptake of carboplatin by tumor cells is considered a key reason for its limited therapeutic efficacy.² Several attempts have been made to increase the effectiveness of platinum compounds and thereby reduce the number of required dosing cycles.³ We investigated PLGA nanoparticles as a safe and effective drug carrier for improved intracellular delivery of carboplatin. Previous studies have shown that nanoparticles are effectively endocytosed by tumor cells. We hypothesized that encapsulation in PLGA nanoparticles will significantly increase the delivery of carboplatin into tumor cells, and thereby result in improved anticancer efficacy. To test this hypothesis, carboplatin loaded PLGA nanoparticles were formulated and their effect on cell survival was evaluated in a panel of cancer cell lines.

EXPERIMENTAL METHODS
PLGA nanoparticles containing carboplatin and fluorescent dye, coumarin-6, were formulated by a modified double emulsion-solvent evaporation technique.⁴ Aqueous solution of carboplatin (3 mg/300 µl of 1% BSA) was emulsified into an organic phase consisting of 32 mg PLGA and 250 µg coumarin-6 in 1 ml chloroform by sonication and further emulsified into a 2.5% w/v aqueous PVA solution. Chloroform was evaporated using a rotary evaporator. The resulting nanoparticle dispersion was washed three times by ultracentrifugation, suspended in purified water and lyophilized. Control nanoparticles containing coumarin-6 but no carboplatin were also prepared in similar manner. Nanoparticles were characterized for size, zeta potential, carboplatin loading and release. Drug loading was determined using methanol extraction and HPLC analysis. Cumulative carboplatin release was determined by incubating nanoparticles in phosphate buffer at 37 °C. Carboplatin concentration was determined at different time points using HPLC.

Cytotoxicity studies were performed in MA148 (ovarian cancer), A549 (lung adenocarcinoma), NCI-ADR/RES (ovarian cancer) and MDA-MB-231 (mammary adenocarcinoma) cells. Effect of carboplatin-loaded nanoparticles and free carboplatin dissolved in growth medium on cell viability was determined using MTS assay. Untreated cells and cells treated with control nanoparticles served as controls. The cell survival profile was fit to a sigmoidal dose response curve using GraphPad Prism to determine the IC₅₀ of the drug treatments.

Intracellular accumulation of carboplatin was determined after incubation of A549 and MA148 cells with 2 µg/ml carboplatin solution or 300 µg/ml carboplatin nanoparticles for 6 hrs. The cells were washed, lyophilized, dissolved in 70% nitric acid at 85 °C and analyzed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

RESULTS AND DISCUSSION
Carboplatin loaded PLGA nanoparticles were 325.8 ± 6.7 nm in size with a zeta potential of -9.6 ± 0.4 mV. Carboplatin loading in the nanoparticles was 3.4 µg/mg of nanoparticles, with an encapsulation efficiency of 3.9 %. Carboplatin release from nanoparticles followed a relatively constant release rate of ~1% per hour for the first 3 days with essentially no initial burst. After about 70% of the drug was released, the rate decreased and complete drug release was achieved in 7 days. Nanoparticles demonstrated time dependent uptake in MA148 cells. Confocal microscopy confirmed the uniform intracellular distribution of the nanoparticles.
The percent cells surviving for select cell types after optimized exposure time to carboplatin solution and carboplatin nanoparticles are shown in Figure 1. In all the cases, encapsulation in nanoparticles dramatically improved the cytotoxicity and reduced the IC$_{50}$ of carboplatin. Control nanoparticles were not cytotoxic to the cells at the concentrations tested.

To explore the mechanism of enhanced cytotoxic efficacy of carboplatin nanoparticles, the cellular accumulation of carboplatin with nanoparticles and solution exposures was determined. ICP-MS analysis revealed that the intracellular platinum concentrations were 6 and 15-fold higher in A549 cells and MA148 cells, respectively, in nanoparticle treatment groups relative to the solution treatment groups. Based on the analysis of the intracellular concentration and added dose, less than 5% of the carboplatin accumulated within the cells when delivered as solution, whereas more than 20% of the drug was recovered in the cells when introduced in the form of nanoparticles.

**CONCLUSION**

Despite its hydrophilic nature, carboplatin was successfully loaded into PLGA nanoparticles. Significantly lower IC$_{50}$ values in four different cancer cell lines were observed with carboplatin nanoparticles in comparison to carboplatin solution. This greater cytotoxicity was associated with higher intracellular accumulation of nanoparticle-encapsulated drug. Our studies suggest that encapsulation in PLGA nanoparticles can reduce the total dose of the drug required for achieving effective cell kill and can thus potentially reduce drug toxicity commonly observed at high carboplatin doses.

**REFERENCES**


**ACKNOWLEDGMENTS**

We thank Dr. Rick Knurr, Geochemical Lab in the Department of Earth Sciences at the University of Minnesota for ICP-MS analysis.