IN VITRO RELEASE OF CISPLATIN FROM BIODEGRADABLE POLY(DL-LACTIC-CO/GLYCOLIC ACID) MICROSPHERE VEHICLES

INTRODUCTION: Cisplatin, alone or in combination with other chemotherapeutic drugs, is currently in clinical use for patients with osteosarcoma. However, the frequent occurrence of systemic toxicity, especially nephro- and neuro-toxicity, limits its dose in these patients. Localized controlled delivery of the drug to the tumor would decrease systemic cisplatin levels and possibly result in higher local doses. Additionally, the development of a biodegradable vehicle containing a depot dose of cisplatin would improve local treatment options that otherwise require drug loaded non-degradable beads and repeat surgery for their removal. The goal of this study is to investigate the in vitro release of cisplatin from biodegradable microsphere vehicles and evaluate their efficacy against an osteosarcoma cell line.

METHODS: Cisplatin loaded poly(dl-lactic-co-glycolic acid) (PLGA) microspheres were synthesized using a double emulsion (water-in-oil)-in-water solvent extraction method. Briefly, 250 mg PLGA (Medisorb, Alkermes, Cambridge, MA) having a 75:25 lactic acid to glycolic acid ratio and a molecular weight of 62 kDa was dissolved in 1 ml dichloromethane (Fisher). While vortexing, 125 µl of cisplatin (Sigma) dissolved in phosphate buffered saline (PBS) was injected, followed by injection of 2 ml poly(vinyl alcohol) (Aldrich). The formed microspheres were placed in 100 ml isopropyl alcohol (Aldrich) and allowed to mix for 1 hour to assure complete extraction of dichloromethane. The microspheres were then collected via centrifugation (2000 rpm for 5 minutes) and washed three times with distilled deionized water. Subsequently, the microspheres were frozen overnight at -20°C, vacuum dried, and stored in a -20°C freezer.

A total weight of 25 mg of each microsphere formulation was placed in a 1.5 ml microvial and bathed in 1 ml of phosphate buffered saline (PBS, pH 7.4). This weight of microspheres corresponded to 0, 0.05, 0.1, and 0.2 mg cisplatin per mg of microspheres, respectively.

A cisplatin dose response curve was generated using an MG63 osteosarcoma cell line with 3×10^4 cells incubated with four drug concentrations over 24 hours. Results are reported as the percentage of viable cells compared to untreated controls. The results are expressed as the percentage of viable cells compared to untreated controls. The results are expressed as the average for n=6 ± s.d.

RESULTS: Release of cisplatin from all the loaded microsphere formulations demonstrated a biphasic profile over the 8 week experimental period (Figure 1). During the first 24 hours the 5, 10, and 20 percent formulations released 55%, 89%, and 70% of their theoretical release after 8 weeks. The burst release was followed by a trough period with minimal release between week 1 and 2. A second phase of drug release began during week 3 and peaked between weeks 5 and 6. As expected, the higher loading doses released more cisplatin. However, the release profiles showed that all three formulations released approximately 30% of their theoretical loading dose by week 8 (Figure 2). This indicates that the entrapment efficiency of cisplatin within the microspheres is significantly less than the intended loading.

The MG63 cell response to cisplatin dose indicates that a 3×10^{-7} ng of cisplatin is necessary to achieve a potent cytotoxic effect (Figure 3). The maximal concentration that was attained during the release period was in the 3×10^{-6} ng/ml range by the 20% cisplatin microspheres.

DISCUSSION: Optimization of the loading efficiency and subsequent release of cisplatin is essential to attain and maintain a locally cytotoxic concentration of drug. This in vitro study demonstrates that a higher cisplatin loading dose decreased the number of MG63 cells by more than half their initial value over 24 hours. These results support the feasibility of a PLGA sustained release system for local cisplatin delivery to osteosarcoma cells, and suggest that such a system may be an appropriate candidate for in vivo animal model evaluation.