Topical 2: Discovery, Development and Delivery of Medicines (*T2007 Advances in Drug Delivery)*

In vivo study for the controlled delivery of Paclitaxel from Electro-hydrodynamic Atomized Microparticles for the post-surgical treatment of Glioma Blastoma Multiforme

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Introduction

Glioblastoma multifome is the most common and most aggressive primary malignant brain tumor. The highly invasive nature of glioblastoma, as well as the fact that few cells are actively replicating at any given point in time, mean that therapies need to act in areas of the brain distant from the site of the tumor origin and for long periods of time after their introduction. This criterion negates established treatments such as surgery radiation and chemotherapy which have not altered the median survival of glioblastoma significantly [1, 2].

The use of controlled chemotherapeutic release from implants as an adjunct treatment to mandatory tumor debulking surgery continues to look promising as it allows high drug localization besides ensuring a sustained release over long periods of time. Implantation in the cavity following a tumor debunking procedure, has been shown to improve drug distribution over the site of action due to high pressure build up in the cavity from edema effects and the higher interstial tumor pressure present.

Paclitaxel, a chemotherapeutic drug originating from the pacific yew Taxus brevifolia, and other members of the Taxaceae family [3] is commonly used as a chemotherapeutic agent of for ovarian and breast cancer. Paclitaxel functions through promotion of the assembly and stabilization of microtubules inhibiting cellular division. It also prevents de-polymerization of the assembled microtubules and thereby halts mitosis or cell division and binds to Bcl-2 [4, 5] which normally blocks the process of apoptosis, allowing apoptosis to proceed. Physically, Paclitaxel is highly hydrophobic and studies have indicated that it penetrates the BBB poorly [6, 7]

Fabrication by Electro-Hydrodynamic Atomization

Microparticles were fabricated by subjecting a solution of Poly (DL-lactic-co-glycolic acid 50:50) (Mol. Wt. 40,000 – 75,000) and Paclitaxel through Electro-Hydrodynamic Atomization (EHDA) to yield particles at 15.0 μm in diameter within a narrow size distribution of 1.7 μm at 20% w/w drug loading. The particles are shown to be able to provide a linear release of 1.27-1.38% of the encapsulated Paclitaxel/day, after an initial burst, for more than 30 days.

In this method, a solution of organic solvent and polymeric solute is sprayed using EHDA. An electrical potential is applied to the nozzle which is countered by a grounded copper plate placed below. This leads to the formation of a liquid cone at the tip of the nozzle, which is termed the Taylor Cone. A very fine jet is formed at the tip of the Taylor Cone. This jet would then break up to produce a cloud of mono-disperse droplets. The organic solvent will then evaporate from the surface of the droplets, leaving behind solid polymeric particles. Details of the Fabrication by EHDA can found in the studies by Ding el al. [8] and Xie el al. [9]

In Vivo Release Profiles Experiment

In order to evaluate the release profile for 20% EHDA Microparticles (F1) within an in vivo environment and to assess the safety against failure, the formulations (F1) were surgically implanted subcutaneously in the flank of male Wistar Rats (wt ~ 250 g, n = 5 animals, total paclitaxel implanted = 4.5 mg) and blood was drawn at regular intervals from the lateral tail vein for analysis of Paclitaxel concentration in the plasma analyzed by LcMSMS.

These results were compared to samples collected from a control group (C1, n = 5) which received a bolus injection of 4.5 mg Paclitaxel in the form of the commercial Taxol®. (See Figure 1)

Plasma Paclitaxel levels in the Control group reached a peak of 23.6 ± 6.5ng/ml plasma within 2 days of injection and trace levels were detectable after 14 days. However, this was marked by large weight drops of the animals indicative of the toxic response over the first 7 days.

In contrast, F1 (EHDA microparticles) showed a constant stable release with an initial burst captured by the peak of 2.38 ng/ml on day 7 at which gradually tapers off with sustained release up till 28 days.

No weight loss was observed in F1 group at any time in the experiment as opposed to that observed in the control C1 groups. Hence a level of sustained release is achievable in an in vivo environment by the EHDA microparticles over the commercial product and no sign of failure of the formulations were observed.

In Vivo Tumor Response

To evaluate tumor growth reduction of the EHDA microparticles, the particles were injected suspended in PBS into C6 glioma tumors pre-implanted subcutaneously in Balb/c nude mice models injected to a total 1 mg paclitaxel (F1, n = 5) 6 days after tumor implantation (See Figure 2).

These were compared with two control groups. The first control group is received blank microparticles without Paclitaxel (C1, n = 5) and the second control group received a bolus injection of commercial Taxol® of 1 mg Paclitaxel 6 days after tumor implantation (C2, n = 5). At regular intervals, the tumor volume was measured under the skin and the weight of the animal taken.

In the study, the experimental group (F1) saw tumor volume suppression of 79% over the control group's volume (C1) by day 23. When compared with commercial taxol (C2) group, F1 had a tumor volume suppression of 43.3% on day 28. The study shows that sustained release effect from the 20% EHDA microparticles increased the tumor suppression over the commercial Taxol®.

Conclusions

In this study, we've established the release behavior of 20% Paclitaxel loaded microparticles fabricated by Electro-hydrodynamic Atomization in an in vivo environment. The results showed that there is no sign of bulk failure and presented the tumor suppression response of the microparticles over the commercial Taxol®.

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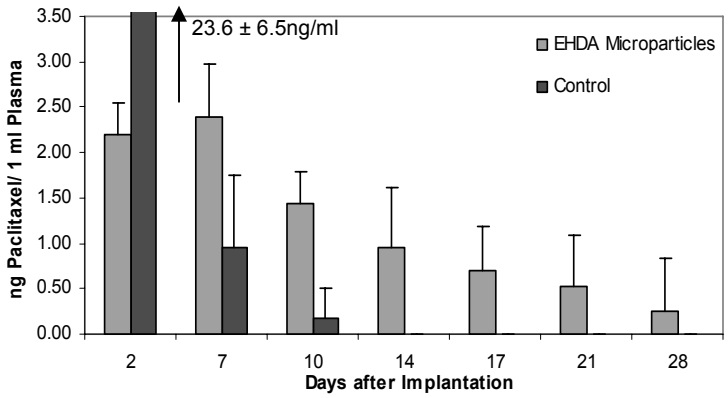

Figure 1: In vivo Paclitaxel Release Profile from 20% EHDA Microparticles in comparison with Control (bolus injections of commercial Taxol®) both at a total Paclitaxel concentration of 4.5 mg. (n = 5 rats)

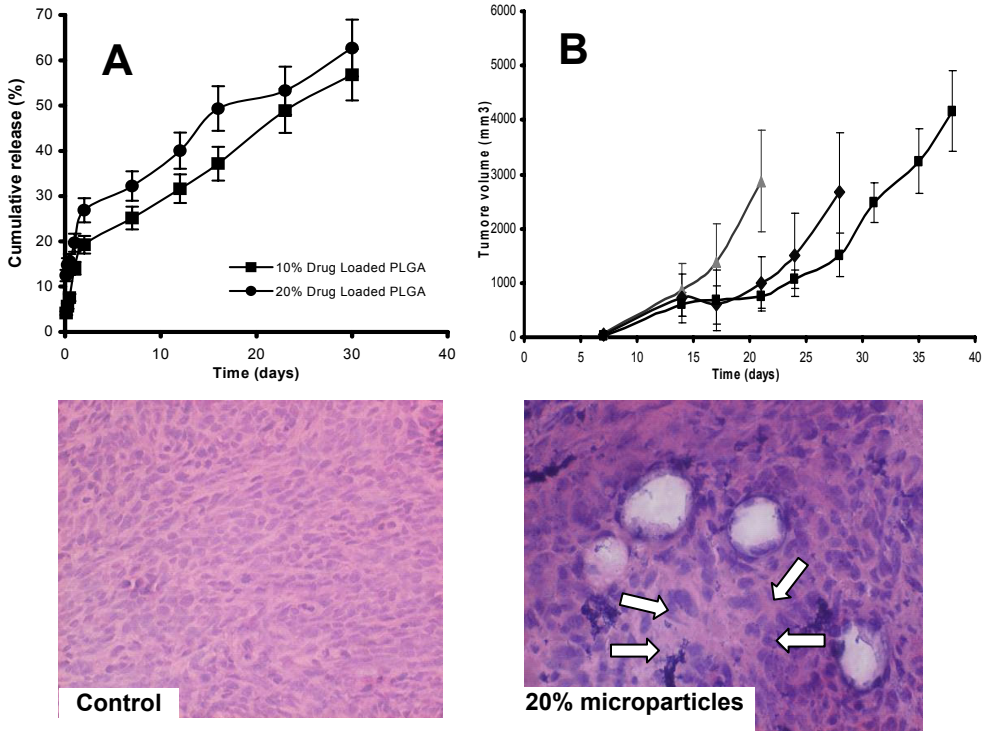

Figure 2: In vitro release comparison with tumor volume suppression effects of Paclitaxel loaded EHDA microparticles. Figure A: In vitro release profile in PBS for EHDA microparticles at 10% and 20% Paclitaxel loading. Figure B: Tumor volume response to EHDA microparticles in Balb/c nude mice. (▲) Blank EHDA microparticles control group C1, (◆) Commercial Taxol control group C2 injected at 1.0 mg Paclitaxel on day 6, (▲) 20% drug loaded microparticles injected at 1.0 mg Paclitaxel on day 6. Figure 2C: H and E stains of tumor tissue taken at 40 x magnifications from EHDA microparticles groups. 20% Microparticles observable with regions of dead tumor cells (white arrows).