Delivery of Oseltamivir Phosphate and Gemcitabine from Poly (D, L-lactic-co-glycolic acid) for the Treatment of Pancreatic Cancer

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INTRODUCTION AND OBJECIVE

Pancreatic cancer often has a poor prognosis, in large part because it frequently goes unnoticed until reaching an advanced stage. When detected, pancreatic tumours are often too large to be surgically removed and must be treated with chemotherapy, radiation, or both. Gemcitabine (GEM), a nucleoside analogue (Burris III 1997), has been the international standard of care for advanced pancreatic cancer for the last decade. GEM by itself cannot cure pancreatic cancer (Wang 2009).

Several growth factors in the receptor tyrosine kinase (RTK) family are overexpressed during the progression of pancreatic cancer including EGF, VEGF, and insulin. When a ligand binds to its RTK, such as Trk (Jayanth 2010), EGFR (Gilmour 2013), and insulin (Alghamdi 2013), the receptor undergoes a conformational change to activate matrix metalloproteinase-9 (MMP-9) which induces Neu1 sialidase (Neu1). Both MMP-9 and Neu1 form a complex with the RTKs at the ectodomain. Activated Neu1 hydrolyzes α-2,3-sialyl residues on the receptor, enabling removal of steric hindrance to receptor association and cellular signalling. Oseltamivir phosphate (OP) inhibits Neu1 sialidase activity associated with ligand-induced receptor activation.

Production of an encapsulation vehicle allows for the highest concentration of the drug to be administered in a sustained manner at the affected area. A capsule containing OP was developed using Poly (D, L-lactic-co-glycolic acid) (PLGA) as an encapsulation polymer. PLGA is degraded through bulk erosion, releasing OP over an extended period of time.

Success with a single-layered cylinder led to the development of a double-layered cylinder, containing OP in the outer layer and GEM in the inner layer. It is proposed that OP will sensitize the tumor to GEM, which will destroy the tumor.

The aim of the study was to develop a method of delivering OP and GEM over an extended period of time, and to test the efficacy of the released drug(s) in impeding tumor growth and spread in heterotopic xenografts of human pancreatic tumors growing in RAGxCγ double mutant mice.

MATERIALS AND METHODS

Rolling film cylinder production

OP crystals, SPAN 80, PLGA and acetone were mixed together. The solution was drawn into a 1 mL syringe and ejected onto a Teflon sheet, then cured at 5°C overnight. The polymer film was scraped off the Teflon sheet and rolled around an 18 gauge needle tip. Once rolled and sealed, the hollow polymer cylinder was removed from the needle tip. When including both OP and GEM, a double-layered cylinder was formed by producing two separate films, one containing OP and the other GEM. The GEM film was rolled first, and the OP film was rolled around the GEM cylinder.

OP release kinetics

Kinetic trials were conducted with single-layered cylinders containing 0, 10 or 20 mg of OP, and the double-layered cylinders containing both OP and GEM. PLGA cylinders were suspended in sodium phosphate buffer (pH 7.4), mixed, and stored at 37 ˚C. Supernatants were extracted periodically and kept at -20 ˚C. For the single-layered cylinders, OP in the supernatant was measured by a colorimetric assay developed by the Centre for Disease Control. OP and GEM in the double-layered cylinders were measured as a percent of theoretical amount of drug in the capsule assuming full incorporation.

Animal trials

RAG2/Cγ double mutant xenograft mouse model of human pancreatic cancer was prepared by implantation of $5x10^7$ human Panc-1 pancreatic cells on the back right flank. Tumor volumes were monitored over time. Drug loaded cylinder was inserted under the skin through a small incision on the back right flank when tumor reached 100-200mm^3. Four mice were implanted with a blank PLGA cylinder that contained no OP, while four were implanted with a cylinder containing 20 mg OP.

RESULTS AND DISCUSSION

Kinetic trials for PLGA single-layered cylinders containing 10 and 20 mg OP demonstrated sustained release of the OP throughout a 30 day period as shown in Figure 1. Nearly 100% of the loaded OP was released from both 10 mg and 20 mg OP cylinders.

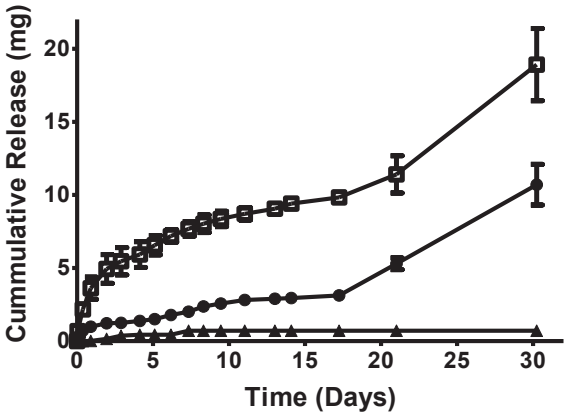

Figure 1. Cumulative release of OP from PLGA cylinders containing 0 mg (▲), 10 mg(●), and 20 mg (■) OP.

Double-layered cylinders also demonstrated sustained release, with OP and GEM being released over 26 days as seen in Figure 2. A higher percent of OP compared to GEM, was released in the first 10 days which was the objective. It was intended that OP halt tumor growth before subsequent release of GEM, intended to kill the cancerous cells.

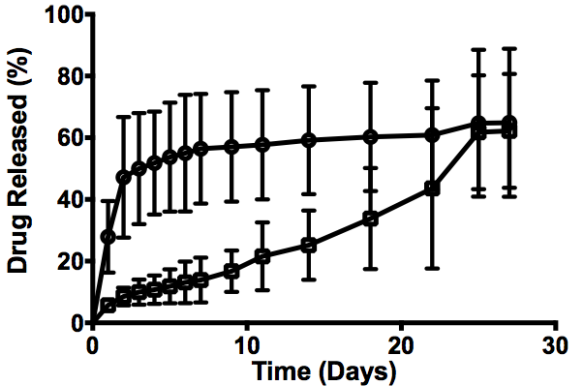

Figure 2. Double-layered cylinder release kinetic profile of OP (●) and GEM (■) in sodium phosphate buffer of pH 7.4.

Implanted OP cylinders effectively inhibited tumor growth in mice as shown in Figure 3. A significant difference in tumor size between the mice implanted with the 20 mg OP cylinders and those implanted with the blank cylinder can be observed between days 40 and 71. It is only at day 75, 40 days after implantation and approximately 10 days after OP was last released, that all mice show similar tumor volumes.

Post termination autopsies revealed neovascularization was greatly reduced in mice implanted with the 20 mg OP cylinder in comparison to the blank cylinder. Limiting angiogenesis decreases the risk of metastasis,

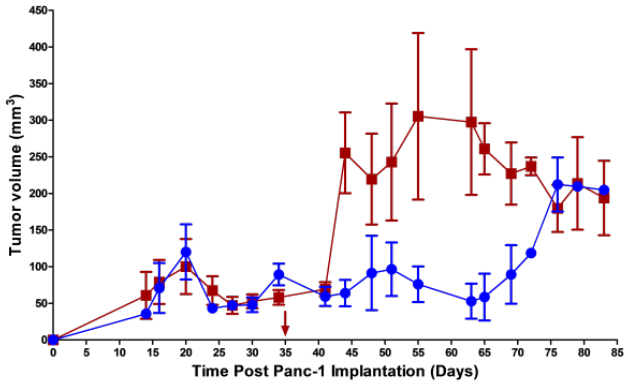

Figure 3. Change in tumor volume following implantation of Panc-1 cells. PLGA cylinders were implanted on day 35 with 20mg OP " ● ", and are compared to blank cylinders, " ■ " .

since the easiest method of transport for cancer cells is through the blood stream (Jayanth, 2010).

CONCLUSIONS

Both single-layered and double-layered cylinders were shown to release OP and OP/GEM respectively over 30 days. Implanted single-layered 20 mg OP cylinders arrested tumor growth for one month. A marked decrease in angiogenesis was also observed. The next stage in the research will be to implant double-layered cylinders to both arrest tumor growth, and subsequently destroy the tumor.

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