Evaluation of Controlled Delivery of Carboplatin from a Poly-Ether-Ester Urethane System in a Murine Model

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Statement of Purpose: As cancer is a large concern in our society, multiple efforts are being made to fight this disease more effectively. While surgery, radiation, chemotherapy, or a combination of these approaches are available, chemotherapy is often employed for metastized or inoperable tumors.

Chemotherapy employs cytotoxic drugs, which usually work by hindering a tumor cell's ability to undergo mitotic division and replicate. This results in selective targeting of cancerous tissue, due to the fact that tumor cell replication rates tend to be greater than that of a healthy cell[1]. Of particular interest in the field of oncology is the platinum family of drugs, characterized by excellent mitotic disruption abilities, but also relatively high systemic toxicity (Low LD_{50} values)[2]. One such member of this family is carboplatin, a second generation drug often employed in cases of ovarian cancer.

For these reasons, it is desirable to develop methods to deliver these powerful anti-neoplastics locally. As divulged in previous communications, Poly-Med, Inc. (PMI) has developed a polymeric drug delivery system comprising a blend of PEG 400 and one of PMI's patented family of polyether-ester urethane copolymers, the Viscoprene^{TM} polymer system. This communication summarizes the delivery of carboplatin in such a system within a murine model with the goal of exhibiting a higher LD_{50} value than freely administered drug.

Methods: Delivery system was dry-heat sterilized at 130°C for one hour. Subsequently, these polymers were mixed with 5 w/w% carboplatin (Sigma Aldrich, St. Louis MO) by mortar and pestle in a sterile field.

Athymic Nude-Foxn1^{nu} mice were purchased from Harlan Laboratories (Indianapolis, IN). These mice (20 in total), were split into 5 equal groups: untreated (control), polymer mixture only, 10 mg dose, 8 mg dose, and 6 mg dose. For all treated groups except for "polymer only", carboplatin was injected in a 5 wt. % polymer solution to deliver the targeted amount. "Polymer only" injections delivered no drug.

All mice were housed in pairs at Godley-Snell Research Facility (Clemson University, Clemson, SC). Mice were monitored for healthy appearance and weight daily. Once deceased, liver and kidneys were excised and fixated using immersion techniques in 10% formalin. Mice were exsanguinated at euthanasia and blood was provided to AnMed Medical Center (Seneca, SC) for a complete blood count (CBC).

Results and Discussion: CBC data indicated no increased white blood cell or decreased red blood cell levels beyond those provided by Harlan Laboratories, indicating no severe systemic immune response to implants or anemic conditions. This was found in all carboplatin treated, control and placebo mice.

Mouse	Dose	Mouse Body Weight by Days, g			
		0	7*	14	21
1	None	23	24	24	24
2		22	21	22	21
3		24	24	26	24
4		22	23	25	25
5	175µL Polymer Only	21	22	23	22
6		22	22	23	23
7		22	23	22	23
8		22	23	24	24
9	175µL Conjugate (10 mg Carboplatin) ~LD_{50} X 3.3	21	21	22	21
10		23	24	26	26
11		24	14	X	X
12		24	16	X	X
13	150µL Conjugate (8 mg Carboplatin) ~LD50 X 2.5	21	22	23	22
14		21	21	21	21
15		19	13	X	X
16		23	16	X	X
17	100 µL Conjugate (6 mg Carboplatin) ~LD_{50} X 2.0	20	21	23	23
18		23	24	25	24
19		21	15	X	X
20		22	13	X	X

Table I. Daily Weights Note: 'X' indicates deceased

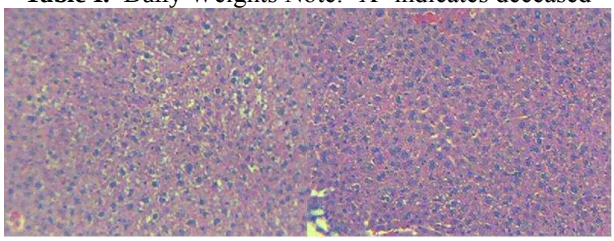

Figure 1: Liver histology images (H&E staining) of mice 1 and 20 at 10x objective magnification

Survival rates were at least 50% for all groups, including the group where injected drug amounts were as high as 3.3 times the published LD^{50} (See Table I. Intraperitoneal LD^{50} for mice, LD^{50} = 150mg/kg[3]).

Examination by a licensed pathologist of H&E stained slides from all mice for both kidney and livers exhibited no significant cytotoxic effect, indicating liver and kidney health, even in deceased mice. See Figure 1 for sample histological images showing no liver damage.

Conclusions: The Viscoprene^{TM} drug delivery system does not appear to adversely affect health in a murine model over a period of 21 days. Carboplatin was delivered in doses 2-3.3 times that of the LD^{50}, with a 50% survival rate, indicating a potential increase in the deliverable amount of drug in a small sample size.

References:

[1] Verweij J and de Jonge M. "Achievements and future of chemotherapy" European Journal of Cancer 36 (2000) 1479-1487.

[2] St Germain C, Niknejad N, et al. "Cisplatin Induces Cytotoxicity through the Mitogen-Activated Protein Kinase Pathways and Activating Transcription Factor" 12:7 (2010) Neoplasia 527-538.

[3] Product Information Sheet for TEVA parenteral medicines Carboplatin injection (November 20, 2003).