Pluronic Triblock Copolymers Enhance Low Grade Hyperthermic Tumor Cell Injury

Tianyi M. Krupka[1,2], Ilya R. Bederman[3], David Dremann[1], Ann-Marie Broome[1,2], Agata A. Exner[1,2].
Departments of Biomedical Engineering[1], Radiology[2] and Pediatrics[3], Case Western Reserve University, Cleveland, OH 44106.

Statement of Purpose: Sublethal temperature at the tumor – normal tissue interface has been shown to be a major cause of tumor recurrence after radiofrequency (RF) ablation treatment. One means to overcome this shortcoming is to pharmacologically enhance low grade hyperthermic (LGH) cytotoxicity in cancer cells. Previous studies in our group have shown that Pluronic, triblock copolymer of poly (ethylene oxide, EO) and poly (propylene oxide, PO), namely Pluronic P85 (EO_{26}-PO_{40}-EO_{26}) and L61 (EO_2-PO_{30}-EO_2) were effective in sensitizing tumor cells to LGH in vitro; P85 and L61 also increased the efficacy of RF ablation in vivo. In an attempt to understand the nature of their thermal sensitizing effects, we report the time and dose dependence of Pluronic and their role in modulating palmitic acid turnover rate and heat shock protein expression.

Keywords: Hyperthermia, Pluronic, thermosensitizer

Methods: DHD/K12/TRb rat colorectal cells originating from dimethylhydrazine induced colon adenocarcinoma were exposed to P85 or L61, at 0-70mg/ml at 37°C for 0-360 min with the addition of 15min of exposure at 43ºC for 0 or 15min. *Dose and time dependence* were determined by assessing levels of intracellular ATP via cellTiter Glo® luminescent assay. *Cell survival* was determined via mitochondrial enzyme activity (Rapid Cell Proliferation assay, EMD Biosciences) at 0, 1, 2, 3 days after treatments. *Palmitic acid turnover rate* was determined by deuterium incorporation. Cells treated with Pluronic and/or heat were exposed to 10% (MPE) ^2H_2O for various time periods (0-240 min). Label incorporation data were determined using GC/MS of TMS esters of palmitate. Fractional synthesis rates were determined immediately after treatment or after 24 hours recovery. *Heat shock protein (hsp70) expression* levels were determined on cell lysate at 0-24 hrs using a BioRad protein assay (antibodies: primary, hsp70 monoclonal; secondary, sheep anti mouse). Exposure was affected using enhanced chemiluminescence. Optical density scans were obtained with Versadoc (BioRad). *Statistical analysis:* two-tailed unpaired Student's t-test was conducted; Bonferroni correction was performed for multiple comparisons. Data are reported as mean ± standard error of mean.

Results: *Cell viability* data showed that once combined with heat the IC_{50} (Pluronic concentration required to reduce 50% of cell viability) and PT_{50} (Pluronic pre-exposure time required to decrease cell viability to 50%) were decreased compared to cells exposed to Pluronic alone (IC_{50} of L61 from 0.81 to 0.16mg/ml and PT_{50} from 248 to 0min; IC_{50} of P85 from 166 to 22mg/ml and PT_{50} from 498 to 419min). *Cell survival:* Under the optimal conditions (defined as the concentration and pre-exposure time at which Pluronic alone did not lead to altered cell viability but significantly increased the cytotoxic effects of LGH), cells not only lost their viability but their ability to proliferate (**Figure 1**).

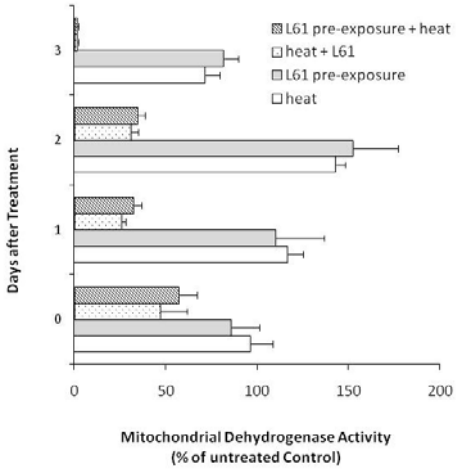

Figure 1: Cell survival results on L61 treated cells (n = 4).

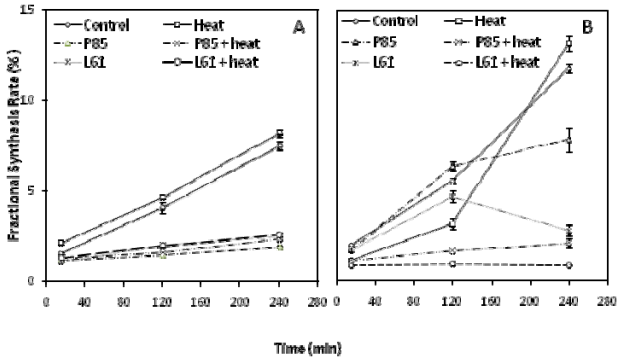

Figure 2: both P85 and L61 have significantly inhibited rate of palmitic acid turnover. When combined with heat, rate of palmitic acid turnover failed to recover; while 15min heat alone showed no inhibition effects.

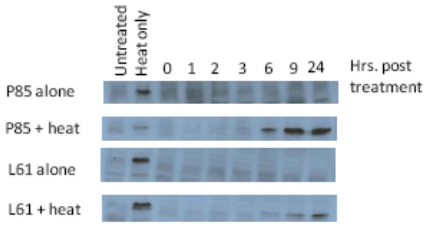

Figure 3: Heat exposure, for 30min at 43°C, elevated hsp70 expression compared to untreated control. Temporary P85 or L61 exposure was effective in inhibiting the cellular heat shock response up to 6 hrs after removal of these Pluronics.

Conclusions: Both P85 and L61 in synergy with sublethal heat were able to cause permanent tumor cell injury in a dose and pre-exposure time dependent manner; L61 appears more potent than P85. In addition either L61 or P85 is able to inhibit the palmitic acid turnover rate and hsp70 expression. These findings may lead to the discovery of an unprecedented Pluronic thermal sensitizing pathway.