pH-sensitive nanoparticle for delivery of lonidamine to triple-negative breast cancer: A preliminary 31P MRS study

Kavindra Nath[1], Hoon Choi[1], David S Nelson[1], Daniel F Heitjan[1], Dennis B Leeper[2], Jerry D Glickson[1], I-Wei Chen[1], and Rong Zhou[1]

[1]*University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Thomas Jefferson University, Philadelphia, Pennsylvania, United States*

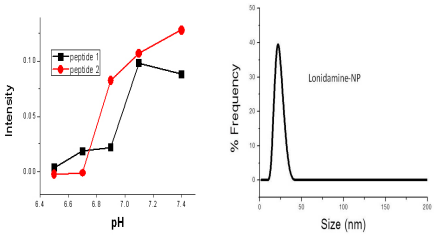

Fig. 1. Turbidity of pH-responsive peptides solution over a range of pH (left). Particle size distribution (right).

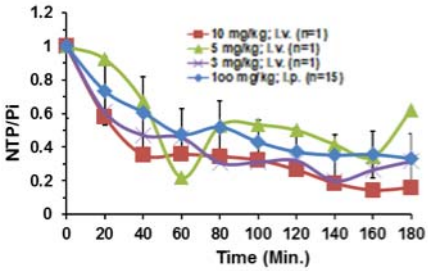

Fig. 3. Bioenergetics (βNTP/Pi; ratio of peak area) normalized to baseline as a function of time in human HCC1806 xenografts in response to i.v. injection of LND-nanoparticles at 10 (n=1), 5 (n=1) and 3 (n=1) mg/kg in comparison with i.p. injection of LND/tris-glycine at 100 mg/kg (n=15, **Ref. 1**) at time zero.

Introduction: A primary challenge in cancer therapy is to transport a drug to a tumor without affecting normal tissues. We previously reported that lonidamine (LND), a small molecule, was able to sensitize human melanoma to chemotherapeutic drugs such as melphalan by reducing the intracellular pH (pHi) and de-energizing the cancer cells (1). Due to its poor water solubility, LND was dissolved in heated tris/glycine buffer (pH =8.3) and administered i.p. at a relatively large dose (100 mg/kg); such formulation, however, may affect the bioavailability of LND, as it could precipitate at blood pH of 7.4 after injection. To exploit the slightly acidic extracellular pH (pHe) environment of cancer in comparison with normal tissue, we have made ultra pH-responsive nanoparticles to encapsulate LND for intravenous administration. Once at the tumor site (pHe = 6.9), the nanoparticles will melt away, releasing LND molecules that diffuse to surrounding cancer cells; in normal tissues (pHe $\geq$ 7.2), however, LND will not be released as the nanoparticles remain stable. We aim to test whether LND can sensitize triple-negative breast cancer (TNBC) to chemo-, radio- and thermo-therapy, since, owing to the absence of estrogen & progesterone receptors and HER2/neu receptor (2, 3), TNBC is insensitive to the most successful treatments of breast cancer, namely, endocrine therapy (tamoxifen) and HER2/neu-targeted therapy. Here we test the hypothesis that formulation of LND in pH-sensitive nanoparticles allows i.v. injection and therefore the dose of LND can be dramatically reduced for achieving similar extent of intracellular acidification and energy depletion in TNBC xenografts.

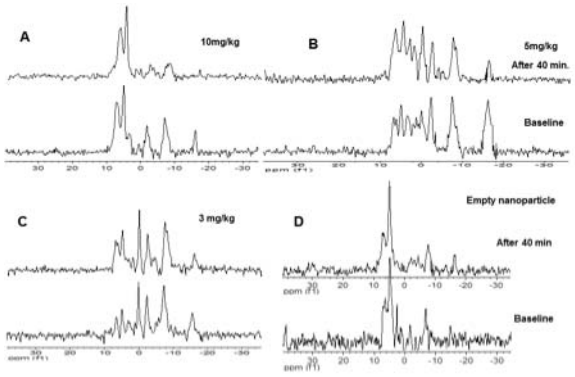

Fig. 2. *In vivo* localized (Image Selected In vivo Spectroscopy - ISIS) ^{31}Phosphorus MR spectra of HCC1806 TNBC xenografts at baseline and 40 min after i.v. injection of LND-nanoparticles at 10 (A), 5 (B) and 3 mg/kg (C) or empty nanoparticles (D). In each set lower spectrum denotes (Baseline) and upper after 40 min. after administration.

Material and Methods: LND was encapsulated in nanoparticles that are made of pH-responsive short peptides (15 amino acids) that self-assemble into nanoparticles of 20-50 nm in size at neutral to slightly basic pH (*e.g.*, pH 7.4, as in serum). As these nanoparticles enter a neutral or slightly acidic environment (*e.g.*, pH 6.8-7.0), they melt away as the result of peptide protonation, releasing individual water-soluble peptides (**Fig. 1 Left**). Importantly, at pH >7.0, nanoparticles contain both slightly positive and hydrophobic moieties, thus giving them the ability to non-covalently incorporate both water-insoluble drugs (*e.g.*, LND) and water-soluble anionic molecules. Inclusion of polyethylene glycol (PEG) molecules further stabilizes nanoparticles, resulting in a narrow size distribution (**Fig. 1 Right**). A human, basal-like TNBC line, HCC1806, was maintained in DMEM culture media supplemented with 10% fetal bovine serum and 0.5% penicillin/ streptomycin at 37°C in 5% CO_2. Cells in exponential growth phase were harvested; 10^6 HCC1806 cells suspended in 100 µL culture medium were inoculated in the flanks of female nude mice (NCI Production). When the tumor reached 7-8 mm in diameter, MR studies were performed on a 9.4 T/31 cm horizontal-bore Varian system using a homemade 10 mm inner diameter double-turn solenoidal coil. Mice were anesthetized by 1% isoflurane in oxygen at a flow rate of 1 L/min delivered through a custom-built nose cone. A rectal thermistor and respiration pillow were placed for monitoring core temperature and respiration rate of the mouse, respectively. The animal's core temperature was maintained at 37(±1) ^{0}C during the scan. A 26 gage catheter was placed for intravenous delivery of LND-nanoparticle doses of 10 mg/kg, 5 mg/kg and 3 mg/kg while the mouse remained in the magnet. Changes of pH and energetic status of the tumor were monitored continuously before and after injection. Estimation of pH and area under NTP and Pi peaks was performed as described elsewhere (1). **Results:** Localized ^{31}P MR spectra of HCC1806 xenografts at baseline and at 40 min after i.v. injection of LND- nanoparticles at doses of 10 (n=1), 5 (n=1) and 3 mg/kg (n=1) mg/kg are shown in **Fig. 2A-C**. Empty nanoparticles (not containing LND) were also injected as controls (**Fig. 2D**). LND-nanoparticles at 10 mg/kg appear especially effective in diminishing tumor bioenergetics status for a prolonged period of time: βNTP/Pi was reduced by 65% at 40 min relative to the baseline level, and by 85% at 160 min post injection; the extent of energy status decrease exceeds that achieved with 100 mg/kg LND-tris/glycine i.p. injection (**Fig. 3**). LND-nanoparticles also led to a decrease in pHi from 6.90 to 6.62 at 10 mg/kg, 6.90 to 6.78 at 5 mg/kg and 6.92 to 6.70 at 3 mg/kg (**Fig. 4**). To assess the toxicity of LND-nanoparticles, mice were injected i.v. at doses of 10 mg/kg (n=3) and 5 mg/kg (n=3); all animals survived with weight loss < 20% (data not shown). **Discussion:** The preliminary data strongly suggest that pH-responsive nanoparticles can efficiently solubilize LND for i.v. injections. 10 mg/kg of LND-nanoparticles is safe but able to reduce pHi and de-energize TNBC tumors to a similar or greater degree compared to i.p. injection of 100 mg/kg LND/tris-glycine, suggesting that the nanoparticles formulation following i.v. injection concentrates the bioavailability of LND in the tumor. Since the monocarboxylate transporter-1 (MCT-1) is overexpressed in about 50% of TNBC, MCT-1 is likely to play an important role in the export of cellular lactic acid and maintenance of high glycolytic flux. Intracellular acidification resulting from MCT-1 inhibition by LND is expected to sensitize the TNBC to chemo-, radio- and thermotherapy (1, 4 and 5).

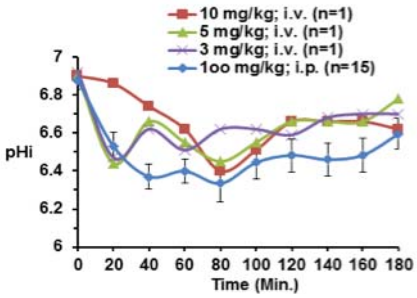

Fig. 4. pHi profile as a function of time of human HCC1806 xenografts in response to i.v. injection of LND- nanoparticle at 10 (n=1), 5 (n=1) and 3 (n=1) mg/kg in comparison with i.p. injection of 100 mg/kg LND/tris-glycine (n=15, **Ref. 1**) at time zero.

Acknowledgements: NIH grants 5R01CA129544-04, 1RO1CA172820-01A1, DoD: W81XWH-10-1-0320 and W81XWH-10-1-0604.

References: 1). Nath K et. al. NMR Biomed 26(1); 98-105, 2013. 2). Chu QD et. al. Int J Breast Cancer 764570; May 8, 2012. 3). Amos KD et al. Int J Breast Cancer 385978; Jan 24, 2012. 4). Chu G L et. al. Radiat. Res. 115(3): 576–585, 1988. 5). Jahde E et al. Cancer Res 49(11): 2965–2972, 1989.