Botulinum toxin increases tumor uptake of gemcitabine chemotherapy as measured with fluorine spectroscopy

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Introduction ^{19}F MR spectroscopy (FMRS) may be used to study non-invasively the kinetics of fluorinated chemotherapy agents in tumor (1). Most such FMRS studies have focused on how the kinetics of 5-fluorouracil correlate with treatment outcome and/or administration of co-treatments (2). Few FMRS studies, however, have investigated the relatively new fluorinated chemotherapy drug gemcitabine (Gemzar®, Eli Lilly) (3). Additionally, to our knowledge no FMRS study has been performed where a co-drug that acts directly on tumor vasculature was used to increase chemotherapy perfusion and thereby enhance chemotherapy efficacy.

We have shown previously that intra-tumoral injections of Botulinum neurotoxin type A (BoNT-A, commonly known as the cosmetic drug Botox®, Allergan, Inc.) inhibit neurogenic tone in tumor vasculature, thereby causing a vasodilation effect. BoNT-A increased perfusion of P792 (a Gd contrast agent) and enhanced the efficacy of cyclophosphamide (a non-fluorinated chemotherapy drug) (4). The goal of the present work was to extend these results to FMRS with gemcitabine. We hypothesized that BoNT-A would increase the perfusion of gemcitabine in tumor and enhance the efficacy of gemcitabine chemotherapy.

Methods TLT tumors were implanted in 16 ♂NMRI mice (35-40 g). BoNT-A 29 U kg^{-1} (N=8) or saline solution (control, N=8) was injected when tumors reached 8 ± 1 mm diameter. 4.7T FMRS (gemcitabine i.p. 800 mg/kg) and proton MRS were done 2 days after BoNT-A or saline treatment. A 25 mm diameter surface coil which could be tuned separately to either ^{1}H or ^{19}F was placed directly over the tumor in such a way as to maximize the NMR signal received from the tumor and minimize the signal from the upper leg and paw. Non-localized proton (full spectrum, no water suppression) and fluorine spectroscopy were performed with the following parameters: α=90° (20-80 μs block RF pulse), spectral width=25 kHz, acq. size = 8k, TR=6 s, Navg = 4 for proton, 150 for fluorine (total acq. time for the latter = 15 min). One fluorine spectrum was acquired approximately 45 minutes after the administration of gemcitabine in order to characterize initial gemcitabine uptake. For 6 mice (N=3 BoNT-A, N=3 control), 15 further fluorine spectra were acquired (total time ~4 hours) to characterize gemcitabine elimination. FIDs were Fourier transformed (line broadening 25 Hz for fluorine spectra), phased, baseline corrected, and integrated (real part only, integration width =20 ppm for proton, 12 ppm for gemcitabine peak) to obtain the gemcitabine and proton signals. The ratio of gemcitabine signal to proton signal provided the relative concentration of gemcitabine ([gemc.]) with a precision of ~7%.

Results and Discussion The initial uptake of gemcitabine in tumors treated with BoNT-A was ~40% higher than in controls ($p<0.001$) (Fig 1 shows mean and SEM of each group). No statistically significant difference in elimination time constant was observed (Fig 2 shows the mean of each group, SEM=~0.015 for each group). The total quantity of gemcitabine to which the tumor is exposed (AUC) should therefore be ~40% higher in tumors treated with BoNT-A. This supports our second hypothesis that BoNT-A should chemosensitize tumors to gemcitabine. Definitive experiments to test this latter hypothesis are under way.

Refs 1 Martino et al, *J Pharm Biomed Anal* 2005; **38**:871. 2 McSheehy et al, *Cancer Chemother Pharmacol* 2005; **55**:117.
3 Blackstock et al, *Clin Cancer Res*. 2001; **7**:3263. 4 Ansiaux et al, submitted.

Fig. 1 Initial uptake (@ 45 min)

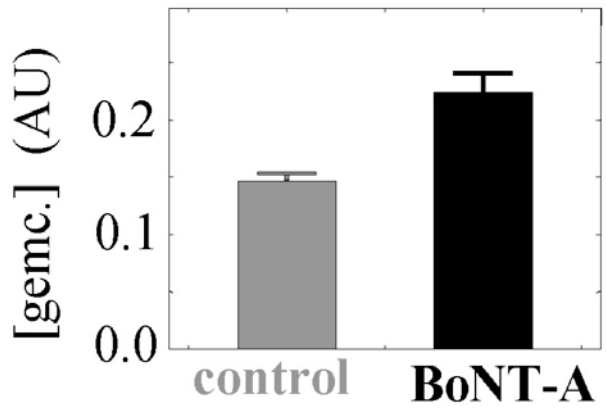

Fig. 2 Elimination kinetics