Ranpirnase-induced changes in blood flow, lactate, and ATP levels in A549 human NSCLC measured by noninvasive near infra-red spectroscopy and magnetic resonance spectroscopy

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Introduction: The novel cytotoxic RNase, ranpirnase (ONCONASE®: ONC), isolated from the eggs and early embryos of the leopard frog *Rana pipiens*, is known to significantly improve the blood flow, interstitial fluid pressure, and oxygenation of MCaIV tumors (1). We recently observed that ONC enhanced radiation response in A549 human non-small cell lung carcinoma (NSCLC) cell lines *in vitro* and *in vivo* (2). Using dynamic contrast-enhanced (DCE) MRI, we observed that ONC transiently increased tumor perfusion in A549 tumors (2). This led us to consider the therapeutic effectiveness of ONC ± cisplatin on A549 tumors. In cell culture studies, we studied whether apoptosis was induced by ONC. Using a growth delay assay, we observed the change in tumor volume after treatment with ONC ± cisplatin in A549 tumor xenografts of athymic nude mice. To confirm ONC-induced changes in tumor blood flow (TBF) observed in our prior results, using a DCE-MRI method (2), we noninvasively monitored TBF using a Near Infrared Spectroscopy (NIRS) method. We tested whether a transiently improved ONC-induced TBF could efficiently remove tumor acidic metabolites such as lactate in tumors. We also tested whether ATP levels were decreased in tumors, based on prior observations of reduced O_2 consumption by treatment with ONC.

Methods: *Cell culture*: A549 cells were cultured in RPMI1640 (15% FBS in 5% CO_2 in air at 37 °C). *Apoptosis analysis*: A549 cells ($2.5x10^4$/chamber) were plated on either 2- or 4-chamber slides in RMPI 1640 containing no phenol and incubated for 72 h prior to ONC treatment. ONC was added to each chamber well, and incubated for 72 h. Apoptotic cells were detected by TUNEL assay and visualized using a Nikon Eclipse E800 fluorescence microscope. *In-vivo growth delay assay*: Eight- to ten-week-old, female athymic NCR-nu/nu nude mice bearing A549 tumor xenografts were utilized. 2 x 10^6 viable cells suspended in 50 µl of RPMI 1640 medium were injected subcutaneously into the right thighs of mice. Experiments were carried out when the tumor volume was between 200 and 400 mm^3. *NMR spectroscopy*: Lactate level in the tumor tissue was measured by MRS 0-2 hrs post-treatment with ONC using a 9.4 T 30 cm vertical bore spectrometer (Varian INOVA) equipped with 55 mm, 55 G/cm gradients, and a slotted tube resonator. ONC-induced changes in ATP levels were monitored by non-localized ^{31}P NMR spectroscopy. The animal body temperature was maintained at 37 °C during MRS experiment by blowing warm air through the magnet bore. Non-localized ^1H *in vivo* spectra were acquired using a SelMQC pulse sequence (3,4). *NIRS perfusion studies*: Non-invasive blood flow was assessed by diffuse correlation spectroscopy. Since the electric field temporal autocorrelation function is explicitly related to the motion of the red blood cells, information about tissue blood flow far below the tissue surface was derived from measurements of temporal fluctuations impressed upon light diffusing through tissue.

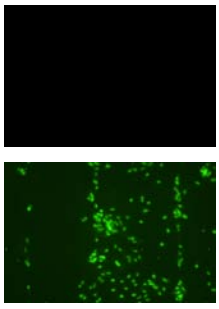

Figure 1: Apoptosis was induced by ONC (Upper: control, Lower: ONC-10 µg/mg)

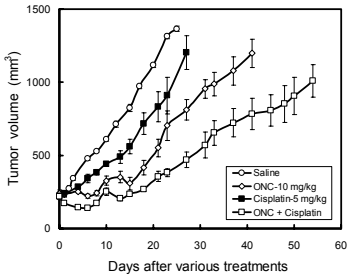

Figure 2: Growth delay assay after treatment with ONC and cisplatin (N= 5 mice per group)

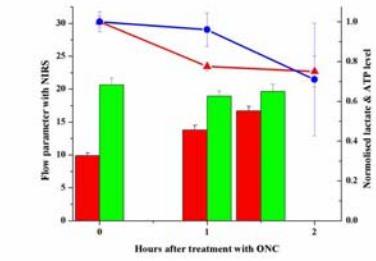

Figure 3: Time dependence of tumor perfusion (red bars), normal skeletal muscle perfusion (green bars), lactate (red line) and ATP (blue line) level after an i.p. administration of 10 mg/kg of ONC

Results and Discussion: Figure 1 shows the apoptosis analysis of untreated A549 tumor cells and cells treated with ONC. No apoptosis is observed in untreated A549 cells (Fig. 1-Upper). However, ONC-treated cells show considerable induction of apoptosis (Fig. 1-Lower). Using a growth delay assay, ONC alone or ONC + cisplatin delayed tumor growth by 15 days and 25 days, respectively (Fig. 2). ONC alone was found to produce an anti-tumoral effect on A549 tumors ($EFGR^{WT}$ and gefitinib resistant). Furthermore, ONC enhanced the cisplatin-induced tumoricidal effect on A549 tumors. Previously, we observed ~25% increase in perfusion in A549 tumors using a DCE-MRI method at 90 min post-treatment of ONC (2). In Figure 3, ONC increased tumor blood flow by ~40% (at 60 min) and ~70% (at 90 min), measured by a NIRS method (N=8). However, perfusion of the corresponding muscle tissues on non tumor-bearing leg of mice was not altered by ONC. We observed ONC-induced increases in tumor perfusion during the first 120 minutes using a NIRS method. In contrast, ~20% decrease of lactate level was observed using non-localized MR spectroscopy (N=3), caused by the removal of tumor acidic metabolites. ATP levels were also decreased (N=3), probably due to ONC-induced inhibition of QO_2 in A549 tumor cell lines (2). In conclusion, ONC may be a new and promising drug for the treatment NSCLC.

References: 1. Lee, I. et al., J. Surgical Oncology 73: 164-171, 2000. 2. Kim, D. et al., "Possible mechanisms of improved radiation response by cytotoxic RNase, Onconase, on A549 human lung tumor xenografts of nude mice", submitted for publication in Adv. Exp. Med. & Biol., September, 2005. 3. He, Q. et al., JMR 106: 203-211, 1995. 4. He, Q. et al., JMR 112: 18-25, 1996.