Ultrasound and microbubble mediated Doxil delivery in a murine breast cancer model: Therapeutic efficacy dependence on tumor growth rate

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Abstract—The effect of tumor growth rate and treatment repeats are examined as parameters in pressure-mediated ultrasound treatments with microbubbles and Doxil in a murine breast cancer model. For this purpose, mice with a tumor doubling time of 8 and 13 days respectively received either a single or two ultrasound treatments (at 1 MHz/1 MPa) in conjunction with Definity microbubbles (1:1 dilution) and Doxil (3 mg/Kg dose) after the tumor size reached 150 mm³. The tumor model was generated using MDA-MB-231-luc cells implanted into the lower mammary fat pad of SCID beige mice. At 15 days post-treatment, tumor size was reduced by 3±18%, 8±14%, and 20±10% as compared to control for the Doxil only, ultrasound + microbubbles + Doxil (single treatment), and ultrasound + microbubbles + Doxil (2 treatments) groups, respectively, in the mice with the slower growing tumors. The mice with the faster growing tumor yielded tumor size reductions of 46±27%, 71±10%, and 61±26%, respectively, for the same groups. We hypothesize that treatment efficacy is dependent on the dynamics of the tumor itself, even within the same cell line.

Keywords-ultrasound mediated drug delivery; non-invasive therapy; targeted delivery

I. INTRODUCTION

Localized ultrasound mediated drug delivery could improve the therapeutic efficacy for the treatment of malignant tumors and reduce toxic exposure to healthy organs and tissues. The long term objective of this study is to investigate the usage of ultrasound (via pressure-mediated effects, i.e. non-thermal effects), microbubbles, and a co-injected (systemic) anti-neoplastic small molecule drug in a liposomal formulation (Doxil) in a murine breast cancer model to test this hypothesis, as this technique has shown promise for a safe and effective method of drug delivery [1]. The transient increase of vascular and cellular permeability, caused by the interaction of the ultrasound wave with the contrast-agent microbubble (sonoporation) is believed to be the mechanism of increased delivery for the drug [4][5]. Many factors influence the overall efficacy of such procedures, including ultrasound exposure frequency, sonication time, duty cycle, focal pressure [6], microbubble composition, concentration, stability, and injection site [7], microvascular pressure [8], treatment repeats, etc. Here we report on the effect of ultrasound mediated drug delivery using microbubbles on tumors growing at different rates, as well as the effect of treatment repeats on tumor growth. This study became possible due to the opportunistic observation that the tumor growth rate of two otherwise identical animal groups was different, likely caused by small variations in tumor cell conditions prior to injection.

II. METHODS

In order to investigate the efficacy of ultrasound mediated small-molecule drug delivery on tumor growth rate, two separate studies were performed under a Pfizer IACUC approved protocol. In the first study (“Study 1”), MDA-MB-231-luc cells were implanted into the lower mammary fat pad of SCID beige mice that yielded a tumor doubling time of approximately 8 days. In the second study (“Study 2”), the same cells were implanted similarly, but yielded a tumor doubling time of approximately 13 days. In all other aspects, both studies were identical for the control and single-treatment groups. To investigate the efficacy of ultrasound mediated small-molecule drug delivery on treatment repeats, both Study 1 and Study 2 included groups that were either treated a single time, or two times, separated by a pre-set number of days. Doxil, the liposomal formulation of doxorubicin, was chosen for these experiments due to the wide body of literature available for this small-molecule drug. Thus, the experimental groups of both studies included: vehicle-treated control, single Doxil treatment only, single ultrasound + microbubbles + Doxil treatment, and two ultrasound + microbubbles + Doxil treatments, as shown below in TABLE I.

<table>
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<th>Study</th>
<th>Group</th>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>1</td>
<td>3 mg/Kg Doxil only</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>3 mg/Kg Doxil + Definity µB + US at day=0</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>3 mg/Kg Doxil + Definity µB + US at day=0, repeat treatment at day=5</td>
<td>4</td>
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<tr>
<td>2</td>
<td>Control (Saline)</td>
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<tr>
<td>2</td>
<td>3 mg/Kg Doxil only</td>
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</tr>
<tr>
<td>2</td>
<td>3 mg/Kg Doxil + Definity µB + US at day=0</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>3 mg/Kg Doxil + Definity µB + US at day=0, repeat treatment at day=10</td>
<td>4</td>
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</table>

TABLE I. EXPERIMENTAL STUDIES AND SUB-GROUPS
Treatments for all groups were performed when the tumor size reached 150 mm$^3$ on average, using a 3 mg/kg Doxil dose (bolus), Definity (Lantheus Medical Imaging) microbubbles (1:1 dilution, constant infusion via tail vein), and focused ultrasound (TIPS, Philips Research North America [9]), at 1 MHz and 1 MPa. A 1x1 cm$^2$ region was targeted with the focused ultrasound transducer, which covered the entire tumor. As the focal zone of the focused transducer (approximately 1x1x6 mm$^3$) is smaller than the target region (approximately 5x5x5 mm$^3$), the focal zone was mechanically scanned across this region, following a back-and-forth scanpath, as shown in Figure 1. This scanpath was traversed 6 times, for a total sonication time of 6 minutes/tumor.

At the beginning of the experiment, microbubble wash-in to the tumor site was confirmed by low-MI (0.1) ultrasound imaging prior to starting with the focused ultrasound exposure, as shown in Figure 1. This step is critical, as it ensures that microbubbles have arrived at the target area. Microbubble infusion continues during the entire sonication time of the experiment.

During the procedure, the animal is kept under anesthesia using isofluorane and on a heated warming pad to maintain its body temperature as close as possible to 37°C. Degassed ultrasound gel, pre-warmed to 37-40°C is used to couple the ultrasound transducer to the target region. The overall animal experimental setup is shown in Figure 3. The animals were monitored for 15 days after treatment, during which time the animal weight and tumor size (using calipers) was measured, typically at two-day intervals. At 15 days post-treatment, the animals were euthanized and the results analyzed.

III. RESULTS

First, the growth rate between both study groups was determined. The control group animals for both studies were used for this purpose, as they did not receive any treatment. In order to determine the tumor doubling time for the animals of both studies, the tumor size data was modeled as an exponential, after it had been normalized to size=1 at day=0, the day of the treatment, and is shown in Figure 4. An average tumor doubling time of 8.1 days was measured for the mice of study 1, and an average tumor doubling time of 13.3 days was measured for the mice of study 2, respectively.

Second, at day 15, the overall tumor size reduction for all 6 groups was also determined, the effect of Doxil alone on tumor growth, and the effect of Doxil + microbubbles + ultrasound was calculated, and the results are shown in Figure 5.

The tumor size was reduced by 46±27%, 71±10%, and 61±26% as compared to control (i.e. no tumor size reduction=1) for the Doxil only, ultrasound + microbubbles + Doxil (1 treatment), and ultrasound + microbubbles + Doxil (2 treatments) groups in study 1. This cohort of animals had the smaller (faster) tumor doubling time, or the faster growing tumor. The tumor size was reduced by 3±18%, 8±14%, and 26±9% as compared to control for the Doxil only, ultrasound + microbubbles + Doxil (1 treatment), and ultrasound + microbubbles + Doxil (2 treatments) groups in study 2, respectively.
20±10% as compared to control for the same groups in the study 2, respectively. This cohort of animals had the larger (slower) tumor doubling time, or the slower growing tumor.

Figure 4. Tumor growth of the untreated control group mice.

Figure 5. Relative tumor size reduction (as compared to the control group), measured at day 15 for the various groups of the 2 studies.

IV. DISCUSSION

The faster growing tumors responded significantly better to both the administration of Doxil by itself, as well as the administration of Doxil + ultrasound (with microbubbles). The added effect of ultrasound and microbubbles was able to enhance the tumor size reduction by 5% (in absolute terms) for the slow growing tumors, and by 25% (in absolute terms) for the fast growing tumors. In relative terms, the added effect of the ultrasound (as compared to Doxil alone) was approximately 5% for the slow growing tumors, and 46% (i.e. almost by a factor of 2 additional size reduction) for the fast growing tumors. These results indicate that tumor growth rate does also seem to be an important factor in the efficacy outcome of ultrasound mediated delivery of small-molecule drugs to tumor targets. While this increase in therapeutic index (TI) is noticeable and similar increases due to ultrasound have been reported by other researchers in the field (see, for example [10]), conversations with industry experts have indicated that TI increases on the order of 3 to 10 need to be achieved in pre-clinical experiments for new delivery and treatment modalities to be seriously considered as new clinical candidates. By this measure, while encouraging, the results achieved in this work via the systemic (venous) administration of both the drug and microbubble still need to be improved upon.

The tumor size reduction results as a function of retreatment are more difficult to interpret. Repeated treatments did show on average an additional tumor size reduction effect on the slower tumor growth cohort. This effect, however, was not statistically significant, and the faster tumor growth cohort actually showed an overall tumor size increase. The experimental results remain inconclusive for this subgroup, suggesting additional studies need to be performed with a larger number of animals (and potentially different repeat treatment scenarios) to quantify this parameter. None of the animals in the study showed complete tumor regression.

Many factors have been shown to influence the ability of ultrasound and microbubbles to effectively deliver small-molecule drugs to the intended target region. A key factor is the microbubble concentration in the target region during sonication [11]. Over the ranges investigated, larger microbubble concentrations typically lead to better agent delivery. We have observed similar microbubble concentration dependence on Evans Blue extravasation studies, as well as in pDNA delivery and expression studies. Higher microbubble concentration tend to lead to better extravasation (in the case of Evans Blue) and expression (in the case of pDNA), but also tends to lead to increased tissue toxicity and damage (data not shown). Such balance of requirements typically results in undertaking organ-dependent microbubble concentration/characterization studies prior to commencing with the main research activities. Differences in tumor vasculature between fast and slow growing tumors may lead to more or less microbubbles being present in the tumor region during treatment, possibly being partially responsible for the observed results. Differences in tumor vasculature also influence the formation of tumor necrotic cores, whose size could again influence the outcome of the treatment. Ultrasound Doppler data would be required to test these hypotheses, which was not collected in this study.

V. CONCLUSIONS

Results suggest that the ultrasound mediated treatment efficacy is not only dependent on the drug and microbubble dosages, ultrasound sonication parameters, microbubble composition, and tumor model (as previously reported in the literature), but we hypothesize that it is also dependent on the dynamics of the tumor model itself, even within the same cell line. Additional experiments are needed to validate this hypothesis.
REFERENCES


