



Does doxorubicin survive thermal ablation? Results of an *ex vivo* bench top study

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PURPOSE

We aimed to test the hypothesis that doxorubicin (DOX) survives thermal ablative heating in an *ex vivo* model of combined transarterial chemoembolization (TACE) and thermal ablation.

METHODS

Fresh porcine psoas major muscle (3 samples, 15×10×3 cm) was submerged in aqueous DOX solution (60 µg/mL, 0.1 M) for 24 hours to passively saturate tissue. DOX-infused tissue was then dried and treated with microwave ablation (MWA) using a 2.45 GHz antenna at 65 W for 2, 5, and 10 minutes. Ablations were repeated in triplicate (9 total). Tissue was then sampled at both ablated and unablated control sites, and DOX concentration was quantified via ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), with samples analyzed in triplicate. Tissue DOX levels in ablation and control groups were compared using one-way ANOVA.

RESULTS

Homogeneous DOX uptake into porcine tissue was evident in all three samples. Mean DOX concentration in unablated tissue was 8.0±2.2 µg/mL. MWA was technically successful in all 9 procedures (100%), with tissue heating to 95–100°C. Mean tissue DOX concentration showed progressive reduction with increasing ablation time, measuring 6.7±1.3, 4.9±0.9, and 4.8±1.3 µg/mL in MWA-treated tissue after 2, 5, and 10 minutes, respectively. Differences in tissue DOX levels between unablated tissue and MWA groups were statistically significant ($P < 0.001$).

CONCLUSION

Contrary to the initial hypothesis, tissue DOX concentration progressively decreased after MWA of longer ablation times. These results suggest that TACE followed by ablation may result in lower intratumoral DOX than would otherwise be anticipated for TACE alone.

Combined transarterial chemoembolization (TACE) and percutaneous thermal ablation has shown clinical efficacy in the treatment of hepatocellular carcinoma (1). Not only do the embolic effects of TACE potentiate ablative heating by reducing vascular heat sink effects, but it is purported that TACE-mediated local chemotherapy delivery facilitates cytotoxic killing of any tumor cells that survive thermal destruction. When TACE precedes thermal ablation, such cytotoxic effects are contingent on the survival of chemotherapeutic drugs through the ablative heating period. However, the thermal effects of ablation on the structural integrity of locally delivered chemotherapeutic drugs are unknown. This bench top study was undertaken to test the hypothesis that doxorubicin (DOX) chemotherapy survives thermal ablative heating in a simple *ex vivo* model of combined TACE and thermal ablation.

Methods

This *ex vivo* bench top study did not require institutional review board approval.

Sample preparation and treatment

Three fresh porcine psoas major muscle specimens (15 cm length × 10 cm width × 3 cm thickness) were submerged in aqueous DOX solution (empirically selected concentra-

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tion of 60 µg/mL or approximately 100 M) for 24 hours to passively saturate the tissue with chemotherapeutic agent. After 24 hours, the tissue specimens were removed and dried with a disposable towel (Fig.). Each specimen was then treated with microwave ablation (MWA) using a 2.45 GHz antenna (Certus 140, Neuwave Medical) at 65 W for 2, 5, and 10 minutes (Fig.) with generator monitoring of ablation temperatures. Ablations were repeated in triplicate for each ablative time interval, resulting in 9 ablations in total (3 per tissue specimen).

Sample harvest and analysis

Immediately following MWA, tissue samples were harvested from the psoas muscle specimens at both unablated control sites (n=2) and ablation sites (n=1 per ablation site, total n=9) using an 8 mm punch biopsy device (Sklar Instruments) to obtain 8 mm diameter × 10 mm depth samples (Fig.). Samples were stored at -80°C until the time of drug quantification.

Ultra-high performance liquid chromatography tandem mass spectrometry

DOX concentration within tissue samples was quantified using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The UHPLC-MS/MS method has been previously described (2). Each analysis was performed in triplicate. Briefly, tissue samples were homogenized in phosphate buffer (0.05 M, pH 7.4) to produce a homogenate containing 0.2 g tissue per mL buffer. Homogenized tissue was extracted with ice-cold acetonitrile and then centrifuged at 5000 g for 10 minutes. The supernatant was removed and evaporated

Main points

- In an *ex vivo* ablation system, tissue doxorubicin (DOX) concentration progressively decreased after longer microwave ablation times.
- The basis for reduction in DOX concentration is uncertain, but may be related to thermal decomposition or thermolysis.
- The findings herein mirror previously reported DOX degradation after prolonged heating in solution.
- The results of this study suggest that transarterial chemoembolization (TACE) followed by ablation may result in lower intratumoral DOX than would otherwise be anticipated for TACE alone.

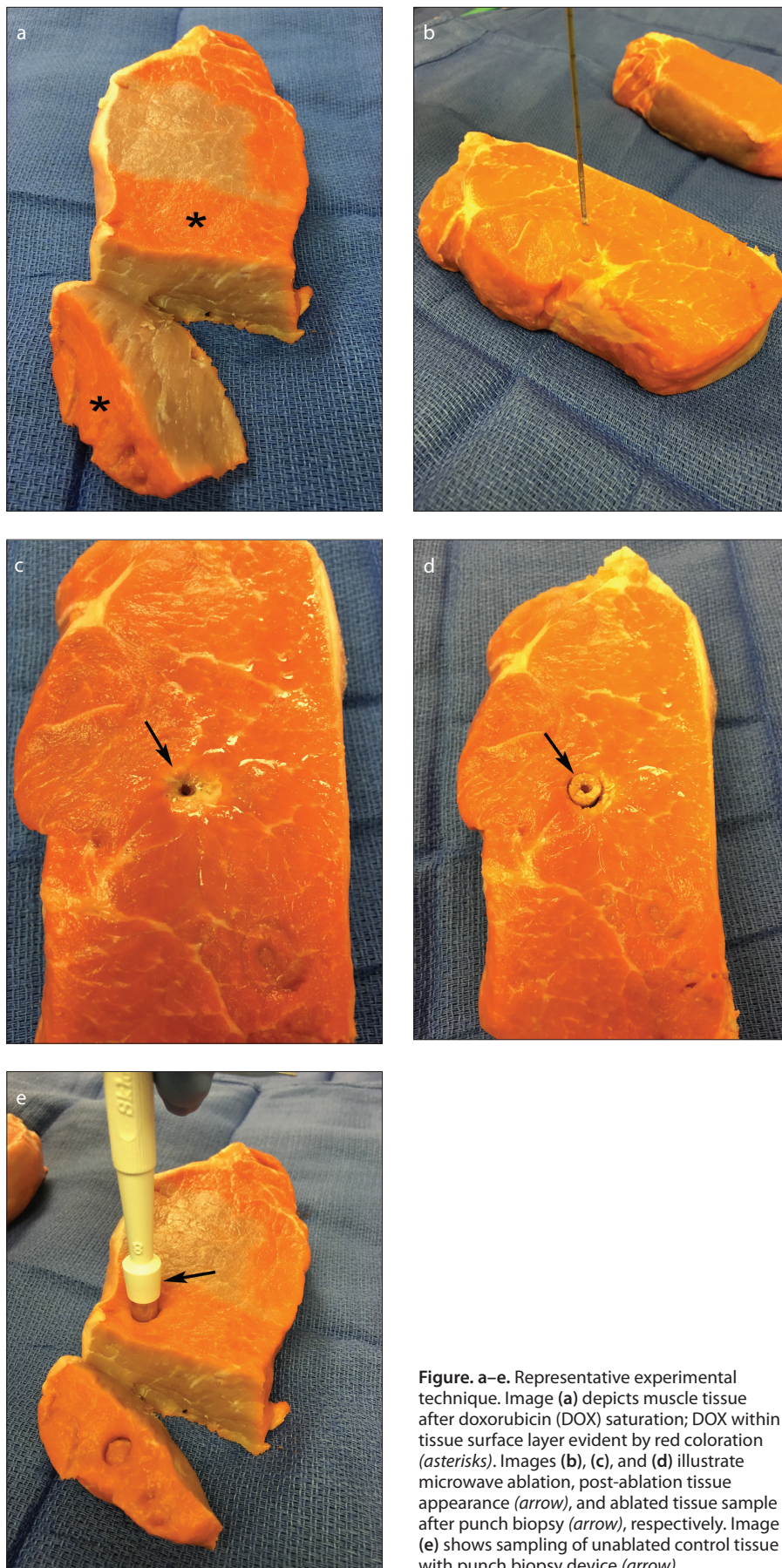


Figure. a–e. Representative experimental technique. Image (a) depicts muscle tissue after doxorubicin (DOX) saturation; DOX within tissue surface layer evident by red coloration (asterisks). Images (b), (c), and (d) illustrate microwave ablation, post-ablation tissue appearance (arrow), and ablated tissue sample after punch biopsy (arrow), respectively. Image (e) shows sampling of unablated control tissue with punch biopsy device (arrow).

to dryness. The extract was reconstituted in UHPLC mobile phase, and daunorubicin was added as an internal standard (50 ng/mL). Extracts were analyzed on a Nexera X2 UHPLC system (Shimadzu) interfaced to a Shimadzu LCMS-8060 triple quadrupole mass spectrometer. UHPLC separations were carried out using a Cortecs C18 column (2.0 × 50 mm, 1.6 μm). DOX and daunorubicin were ionized using positive ion electrospray. Quantitative analysis was carried out using the selected reaction monitoring mass transitions of m/z 544 to 397 and m/z 528 to 361 for DOX and daunorubicin. Standard curves were prepared using blank tissue, with DOX added immediately before extraction at a range of concentrations spanning 250–7500 ng/mL. Data was analyzed using LabSolutions software v5.8 (Shimadzu).

Measured outcomes and statistical analysis

Technical success of MWA was defined as application and heating of the MWA antenna without the need for interruption or repositioning. The primary outcome measure of the study was DOX concentration in porcine psoas tissue samples after exposure to thermal ablation. Tissue DOX concentrations were compared between specimens subject to varying thermal ablation exposure time. Mean tissue DOX levels were compared between ablation and control group using the one-way ANOVA test and the unpaired Student's t-test. Statistical analyses were performed using Microsoft Excel 2010.

Results

After 24 hours of aqueous DOX saturation, DOX uptake in the surface layer of the tissue specimens was observed in all three cases (100%). MWA was technically successful in all 9 procedures performed (100%), with tissue heating to 95–100°C in all cases.

DOX eluted at 1.095 minutes and daunorubicin eluted at 1.38 minutes. The UHPLC-MS/MS standard curve was linear from 250–7500 ng/mL with a coefficient of determination $R^2=0.997$. Quality control samples were determined to be within 15% error.

The mean DOX concentration in unablated control tissue was 8.0 ± 2.2 μg/mL ($n=6$ UHPLC-MS/MS analyses). Ablated tissue samples showed a progressive reduction in tissue DOX concentration with increasing ablation time. Tissue ablated for 2, 5, and 10 minutes demonstrated mean DOX concentrations of 6.7 ± 1.3 , 4.9 ± 0.9 , and 4.8 ± 1.3 μg/mL, respectively ($n=9$ UHPLC-MS/MS analyses each), corresponding to 16%, 39%, and 40% decrease

from baseline concentration. The difference between unablated control tissue DOX concentration and ablated tissue DOX concentrations was statistically significant ($P < 0.001$) across all groups. DOX concentrations in tissues ablated for 5 minutes ($P = 0.004$) and 10 minutes ($P = 0.006$) were significantly different than unablated control tissue, while DOX concentration in tissue ablated for 2 minutes was not significantly different from unablated control tissue ($P = 0.185$).

Discussion

The results of this study revealed a decrease in tissue levels of DOX after MWA in a simple *ex vivo* model of combined TACE and thermal ablation. The outcomes obtained herein opposed the initial hypothesis by showing incremental reduction in tissue DOX concentration with increasingly longer MWA duration. The basis for reduction in DOX concentration is uncertain, but may be related to thermal decomposition or thermolysis. Thermal decomposition refers to loss of molecular structural integrity due to heating, and is known to occur for DOX at temperatures as low as 50°C (3). Despite the decrease in tissue chemotherapy concentration, it is notable that DOX did remain present within ablated tissues, albeit in lesser quantity. In all, the study results suggest that a clinical locoregional therapy strategy of TACE followed by thermal ablation may result in lower tissue DOX than would otherwise be anticipated for TACE alone. If local DOX concentration is reduced below known cytotoxicity thresholds—DOX half maximal inhibitory concentration ranges from approximately 0.4–8.0 mM (4)—this may have implications for clinical therapeutic efficacy. On the other hand, hyperthermia is known to enhance DOX cytotoxicity (5), which may counteract this effect.

Previously, DOX degradation has been reported after prolonged heating in solution. In 2004, Ahrar et al. (6) studied the cytotoxic activity of DOX after systematic heating of chemotherapy solution to higher temperatures (60–120°C) over longer periods of time (15–120 minutes). The authors noted a decrease in DOX cytotoxicity towards adenocarcinoma cells after heating, and noted that a 2-hour exposure to 120°C resulted in 95% DOX degradation (6). Our study demonstrated congruent effects in an *ex vivo* tissue model after exposure to therapeutic temperatures (95–100°C) using MWA across various time points (2, 5, 10 minutes). Further investigations may be warranted in an *in vivo* animal model system to assess

whether these effects are retained in living systems, and whether intraparenchymal DOX concentrations remain at cytotoxic levels following thermal ablation after delivery at standard therapeutic dosing.

This study has limitations. First, a simple *ex vivo* system, which employed muscle rather than liver tissue, was used. Such a system cannot model the complexities of viable, *in vivo* tissue, including heat sink effects. Second, this investigation spanned a small sample size. Third, this study utilized passive saturation of tissue with DOX rather than active injection as is performed during TACE. While this approach was used to ensure homogeneous DOX distribution within tissue, the DOX diffusion method does not faithfully mimic TACE drug delivery. Fourth, MWA was employed herein, and extrapolation of results to other thermal ablation modalities, such as radiofrequency ablation, may not be feasible. Fifth, the study protocol did not include any cytotoxicity assays to assess cellular killing by heated DOX.

In conclusion, contrary to the initial hypothesis, tissue DOX concentration progressively decreased after MWA of longer ablation times. These results suggest that TACE followed by ablation may result in lower intratumoral DOX levels than it would otherwise be anticipated for TACE alone.

Conflict of interest disclosure

The authors declared no conflicts of interest.

References

1. Chen QW, Ying HF, Gao S, et al. Radiofrequency ablation plus chemoembolization versus radiofrequency ablation alone for hepatocellular carcinoma: A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2016; 40:309–314. [CrossRef]
2. Baumgarten S, Gaba RC, van Breemen RB. Confirmation of drug delivery after liver chemoembolization: direct tissue doxorubicin measurement by UHPLC-MS-MS. *Biomed Chromatogr* 2012; 26:1529–1533. [CrossRef]
3. Kaushik D, Bansal G. Four new degradation products of doxorubicin: An application of forced degradation study and hyphenated chromatographic techniques. *J Pharm Anal* 2015; 5:285–295. [CrossRef]
4. Namur J, Citron SJ, Sellers MT, et al. Embolization of hepatocellular carcinoma with drug-eluting beads: doxorubicin tissue concentration and distribution in patient liver explants. *J Hepatol* 2011; 55:1332–1338. [CrossRef]
5. Hahn GM. Potential for therapy of drugs and hyperthermia. *Cancer Res* 1979; 39:2264–2268.
6. Ahrar K, Newman RA, Pang J, Vijjeswarapu MK, Wallace MJ, Wright KC. 2004 Dr. Gary J. Becker Young Investigator Award: Relative thermosensitivity of cytotoxic drugs used in transcatheter arterial chemoembolization. *J Vasc Interv Radiol* 2004; 15:901–905. [CrossRef]