Investigating the relationship between interstitial fluid pressure, perfusion, and liposome accumulation in solid tumors.

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ABSTRACT SUMMARY
The relationship between elevated tumor IFP, tumor perfusion, and liposome accumulation is investigated. A strong negative correlation was found between central tumor IFP and total tumor liposome accumulation in an orthotopic breast mouse xenograft model. No strong correlation was found between tumor blood flow, vascular permeability, or plasma volume fraction and liposome accumulation. These results suggest that IFP plays a significant greater role in mediating the total tumor accumulation of CT-liposomes compared to tumor perfusion.

INTRODUCTION
Liposome-based drug delivery systems have been developed to improve the efficacy and toxicity associated with routinely used chemotherapeutics. Encapsulating chemotherapeutics in liposomes increases circulation time and improves tumor accumulation through the enhanced permeability and retention (EPR) effect (1). However, heterogeneity in the total tumor accumulation of liposomes between subjects has been observed both pre-clinically and clinically (2-4).

The heterogeneity in the total tumor accumulation of liposomes has been attributed to variations in the morphology and physiology of tumor blood vessels (5). However, it has also been suggested that interstitial fluid pressure (IFP) may play a significant role in mediating liposome accumulation (6). There is a paucity of studies that are able to untangle the relative contributions that tumor perfusion and IFP have on the accumulation of liposomes.

In this study we investigate the relationship between tumor perfusion, IFP, and total tumor liposome accumulation. Dynamic contrast enhanced computer tomography (DCE-CT), wick-in-needle measurements, and CT imaging of a CT-liposome contrast agent are used to measure tumor perfusion, tumor IFP, and total tumor accumulation of liposomes respectively.

EXPERIMENTAL METHODS
A CT-liposome contrast agent was prepared according to the methods described in (4). The CT-liposomes included iohexol (Omnipaque, 300 mg/mL of iodine, GE Healthcare) encapsulated within a lipid bilayer, which is composed of phosphatidylcholine, cholesterol for stability, and brushed with a polyethylene glycol (PEG) coating to reduce immunogenicity. The native concentration of iohexol was 40 mg of iodine mL⁻¹ of solution. The liposomes had diameters between 75 and 80 nm. Measurements of CT-liposome accumulation kinetics were made in 6 female SCID mice bearing orthotopic breast tumors (MDA-MB231) using micro-CT (80 kV, 70 mA, eXplore Ultra, GE Healthcare, London, Canada). Volumetric anatomical images were acquired pre-administration of the CT-liposome formulation and at 10 min, 24 hrs, and 48 hrs post-administration. The DCE-CT measurements of tumor perfusion were performed 10 minutes post CT-liposome injection and accomplished by injecting approximately 90 µL of free iohexol (Omnipaque® 300, GE Healthcare, New Jersey, USA) mixed with 10 µL of saline through the tail vein catheter using the same injection pump settings. Images were acquired continuously every second for the first 30 seconds and then intermittently every 10 seconds for 4.5 minutes to capture the rapid wash-in and slow wash-out kinetics typical of free-ioxhol. IFP measurements were made using the wick-in-needle technique at the 48hr time point (7). Approximately five IFP measurements were made at multiple locations within each tumor. The IFP needle placement was performed using robotic system (CT Sabre, Parallax Innovation, Canada) that operated under CT image guidance such that the exact location of the IFP measurement within the tumor volume could be assessed.

The CT-Liposome and DCE-CT data sets were contoured and three-dimensional volumes of interest (VOI) were generated of each tumor at each time point and averaged to get the total tumor concentration of iohexol and CT-liposome as a function of time. A novel quantification method (8) was used to estimate tumor blood flow (F), permeability surface area product (PS), plasma volume fraction (vp), and extra-cellular extravascular (interstitial) volume fraction (ve). Pearson correlation was used to investigate the relationship between central IFP (as identified by CT imaging), tumor perfusion (F, PS, vp) and the total CT-Liposome tumor concentration divided by the concentration in blood (tumor to blood ratio; TBR).

RESULTS AND DISCUSSION
A strong and close to statistically significant negative correlation (r=-0.80, p=0.05) between the central tumor IFP and the TBR measured at 48hrs (Figure 1). This suggests that elevated central IFP results in decreased liposome accumulation, which is consistent
with theoretical predictions (9). There were no strong correlations between $F$ or $PS$ and TBR. However, a positive but not statistically significant correlation ($r=0.61$, $p=0.20$) was observed between $v_p$ and the TBR. These observations suggest that a greater contribution from IFP compared to tumor perfusion in mediating the total tumor accumulation of CT-liposomes in the MDA-MD-231 orthotopic xenograft model.

IFP did not correlate with $v_p$, or $PS$. However, a strong but not statistically significant positive correlation ($r=0.73$, $p=0.10$) was found between IFP and $F$. This result raises the question as to why a relationship between $F$ and TBR was not observed. The small sample size used in this study may have been an issue. However, it may also be that elevated IFP results in elevated and spatially heterogeneous tumor blood flow (i.e., the ‘steal’ phenomena). Indeed it was observed that tumor perfusion was heterogeneous across the tumor volume and significantly higher perfusion was observed at the periphery of all tumors. Therefore, the whole tumor average analysis method presented here may not be sensitive enough to relate IFP, tumor blood flow, and liposome accumulation. We have previously demonstrated a strong correlation between intra-tumoral perfusion and CT-liposome accumulation in an orthotopic cervix xenograft tumor model (10). Therefore, future work will be to investigate the intra-tumoral relationship between IFP, perfusion, and liposome accumulation.

**CONCLUSION**

The results of the study suggest that IFP plays a more important role compared to tumor perfusion in the total tumor accumulation of CT-liposomes in the MDA-MD-231 orthotopic xenograft model. Future work will be to investigate the intra-tumoral relationship between IFP, tumor blood flow, and liposome accumulation to further elucidate the relative importance of IFP and tumor perfusion in mediating liposome accumulation.

**REFERENCES**


![Figure 1. Central tumor IFP as a function of the TBR demonstrating a strong negative correlation between the two parameters.](image-url)