Pancreas tumor model in rabbit imaged by perfusion CT scans

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ABSTRACT

The goal of this work was to develop and validate a pancreas tumor animal model to investigate the relationship between photodynamic therapy (PDT) effectiveness and photosensitizer drug delivery. More specifically, this work lays the foundation for investigating the utility of dynamic contrast enhanced blood perfusion imaging to be used to inform subsequent PDT. A VX2 carcinoma rabbit cell line was grown in the tail of the pancreas of three New Zealand White rabbits and approximately 3-4 weeks after implantation the rabbits were imaged on a CT scanner using a contrast enhanced perfusion protocol, providing parametric maps of blood flow, blood volume, mean transit time, and vascular permeability surface area product.

Keywords: Photodynamic therapy, dosimetry, cancer, tumor model

1. INTRODUCTION

Photodynamic Therapy (PDT) is being investigated for treatment of pancreatic cancer in an ongoing clinical trial using verteporfin and light treatment. Pancreatic cancer PDT involves injection and uptake of the photosensitizer (here verteporfin) and 690 nm light to active it at approximately 20 J/cm² delivered through a fiber. However, measurements of drug uptake in the tumor and pancreas are challenging and in vivo light dosimetry is perhaps even more prohibitive in the human trial due to the complexity and location of the disease. Thus there is considerable value in the study of drug delivery and light delivery in a large animal model if it could mimic the architecture and vascular-stromal-epithelial structure of human pancreatic adenocarcinoma. In particular, in this study, one major hypothesis being studied is if the perfusion CT scans of a tumor can indicate the efficiency of photosensitizer delivery during treatment. If true, the CT scan could provide a surrogate measurement for clinical treatment planning in future work.

In the current study, the goal was to design a model to study uptake of verteporfin with an in vivo-ex vivo approach that could be effectively compared to in vivo perfusion CT scans. The perfusion acquisition sequence provides parametric images of blood flow, blood volume, mean transit time, and vascular permeability, which have stronger logistical potential to correlate with delivery of the photosensitizer. Preliminary studies in murine models showed that this was possible using contrast MRI, but murine models are not well suited for whole body CT scan studies, and so the results do not translate well into clinical studies.

The goal here was to complete the study with a whole body multi-slice CT scanner designed for clinical use. There are physical limitations on the long scan time required with small animals that makes it impossible to get useful data with human iodinated contrast agents. Thus a larger animal model was needed that would allow use of a standard human contrast agent and whole body scanner. Rabbits are a routinely used option for use with the well published model of VX2 carcinoma. Here VX2 was implanted into the pancreas as a section of tumor, and a whole body CT scanner was then employed to detect contrast agent uptake in the tumor. The results have potential to directly translate into clinical use in our ongoing collaborator study.
2. MATERIALS AND METHODS

Pre-operative care: The rabbits were injected with Ketaporfen or Buprenorphine 1 h prior to surgery. They were then anesthetized with a Ketamine/Xylazine cocktail. Once anesthetized, the animals were intubated and isoflurane was administered to maintain anesthesia. The abdomen of the animals was then shaved and prepped with chlorhexiderm before any incision were made. All surgical procedures were carried out under sterile conditions. The animals were maintained in a light to moderate plane of anesthesia to reduce movement and stress on the animal for the duration of the study via isoflurane.

SC Tumor Implantation: The right flank of Rabbits will be prepared by sterilizing with chlorhexiderm after prior shaving. Bupivacaine was injected around the site of the incision, and a small incision (1 cm) was made with sterile scissors in the flank of the rabbit, and small pieces of solid VX2 tumor were placed into a pocket formed just under the skin. The skin was then closed with a continuous buried suture using 3-0 nylon dexon absorbable suture.

From the time the incision was made until it was closed with the suture, the surgery lasted approximately 10 minutes. Surgery was performed on a heating pad to prevent hypothermia. One rabbit was needed to initially grow the SC tumor, and was used for imaging here. The rabbits were survived for 3-4 weeks, and were monitored daily for size of tumor as well as for any effects caused by surgery or of tumor growth as described.

Pancreatic tumor Implantation: A surgical incision was made with a sterile scalpel through the upper median line of the abdomen. Finding the pancreas just below and attached to the spleen, a small stainless steel suture (28 gauge) is placed in the head of the pancreas 0.5-1 cm from the suture. 2 small pieces of VX2 tumor were implanted per site, with 2 sites per animal into the pancreas. Briefly a small hole is made in the pancreas and a “pocket” made to make space for the chunks. After chunks are placed in the pancreas and Surgicel absorbable hemostat is put over the incision site. The abdominal wall was closed with a 6-0 Dexon nylon suture and skin was closed with a different 4-0 Dexon nylon suture.

From the time the abdomen is open until the time the abdomen wall is closed with the suture, the surgery lasted approximately 5-10 minutes, and rabbits were maintained in a sterile environment on a heat pad.

Animal Preparation for CT Scanning: Animals were anesthetized with Ketamine/Xylazine (35/5 mg/kg). Once sedated the animals were intubated and maintained in a light to moderate plane of anesthesia to reduce movement and stress on the animal for the duration of the imaging. Animals were scanned using a CT scanner for up to 60 minutes. Animals were under direct view at all times, and were monitored for respiration, body temperature, SPO2 levels, and heart rate using EKG throughout the procedure. Body temperature was maintained by placing the animal in a blanketed environment. Once CT scanning was complete, animals were injected iv with verteporfin. IV injections were done in a peripheral vein (Ear) using a 22 gauge IV catheter.

The scan was designed to achieve normal capnia with a breathing cycle of 20 breaths per min. Then when anesthesia was confirmed, a dose of 0.07 ml of vecuronium bromide (2 mg/ml) was given to provide a high level of muscle relaxant, avoiding the rabbit from moving involuntarily during the breath hold phase. This dose was intended to last between 15 - 30 mins making the animal completely reliant on ventilated breathing, which would be sufficient for the full CT scan sequence.

For the scan, a two phase protocol was used including 1) a breath-hold phase for 30 seconds, followed by 2) a breathing phase imaging for 2 minutes. Prior to the injection of contrast, the ventilator was turned off to initiate the breath hold. The 30 sec CINE scan was completed coincidentally with the injection of the contrast. A 20 sec prep delay between the 1st and 2nd phase protocol was included, giving sufficient time to turn the ventilator back on to restore breathing. The second phase of the scan protocol followed this, using a cycle of 20 breaths per min, and a 4.0 sec burst of CINE scan performed every 10 sec for a period of 2 min total. The images were then reconstructed at 0.2 sec intervals, resulting in 19 images per slice location for each cine scan.
Figure 1. Surgical images are shown of the resection of the pancreas (a), the stainless steel suture and opening of the pancreas for tumor implantation (b) and implanted tumor in the pancreas (c). In (d) the rabbit is shown intubated and within the CT scanner for imaging.

**CT Perfusion Scan Analysis:** The first step of in creating parametric maps was to remove images from the second phase of CT imaging that were not in the same breathing phase and the breath-hold images collected in the first phase. This was done manually by visual comparison of anatomical landmarks (1). The remaining time-stamped images were imported into the parametric mapping software package, CT Perfusion 4 (GE Healthcare). While running the software, an arterial input function was selected by drawing a region of interest over the descending aorta and used as an input to so-called adiabatic approximation to the tissue-homogeneity model (2). Essentially, using the arterial input function a *a priori* information, this model converts the uptake curve of CT contrast measured in every 2-by-2 pixel region of the field of view to a vector of four hemodynamic parameters: blood flow, blood volume, mean transit time, and permeability surface area product.

3. RESULTS

Figure 2 presents a time series of CT images from the abdomen of the rabbit as the CT contrast agent perfused into the tissue. While it is possible to recognize major blood vessels and a kidney better after contrast injection it is not obvious from an observational standpoint what the hemodynamic infrastructure of the tumor is, which happened to cover a large area of the top right side of the abdomen. However, by analyzing these data with perfusion software to create maps of vascular permeability, blood volume, and blood flow (Fig. 3 b-d, respectively), it was much easier to make initial conclusions about the vascularity and leakage properties of the VX2 carcinoma when implanted in the rabbit pancreas. The first obvious observation from Fig 3d is that the blood flow in the tumor is considerably lower than the surrounding healthy tissue, with only a few small regions of moderate flow. This result suggests that the tumor in this rabbit has large areas of necrosis or avascular stroma. This is promising since most human pancreatic cancers are characterized by their low vascularity and large necrotic regions.

While blood flow and blood volume certainly play a significant role in understanding the delivery of a photosensitizer during PDT, they do not provide the whole picture since uptake will also be regulated by the permeability or leakiness of the tumor's blood vessels. The permeability surface area product map in Fig 3b tells an interesting story in this regard. While both blood flow and blood volume were low throughout the tumor, there were large sections of the tumor that appeared to particularly leaky, which could enhance drug delivery. It will be very interesting going forward to investigate the relative affects both PS and blood flow/volume have on photosensitizer uptake. It may be that one parameter will be more informative than the others or a combination of these parameters may best inform uptake. In the future we plan to explore tracer kinetic modeling to better understand the relationship between CT Perfusion parameters and drug delivery.

Though some interesting hypotheses could be made from the pancreatic cancer rabbit, the tumor in this rabbit grew to an extent that would not be common clinically (typically cancers would be diagnosed before they got to this stage). To better visualize the hemodynamic characteristic of the VX2 tumor when at a more manageable size, Fig. 4 presents CT perfusion parametric maps collected from the rabbit that was used to grow the tumor in the thigh. These
results demonstrate the hyperemic rim (rim of high blood flow) and hypoxic core (low flow, low volume interior) most often associated with the VX2 carcinoma (3). The PS product in this tumor appeared to be similar to the surrounding healthy tissue and relatively homogeneous over the volume of the tumor.

**Figure 2.** The temporal sequence of a single slice from one rabbit is shown with the contrast injection occurring between images c and d. The contrast uptake in the kidney (left) and the tumor mass (right) can be seen.

The contrast CT imaging studies were processed for perfusion recovered parameters of 1) permeability*surface area product, 2) blood volume (BV), 3) blood flow (BF). An example scan is shown in figure below.

**Figure 3.** The temporal sequence of a single vessel is shown (a) with the contrast injection occurring several seconds into the scan. The increase and decrease of the bolus is obvious. The images were used to calculate parametric maps of (b) Permeability* surface area, (c) blood volume, (d) blood flow are show for the tumor shown in the previous figure.
Figure 4. Parametric maps of (a) Permeability* surface area, (b) blood volume, (c) blood flow are shown for the initial subcutaneous tumor with close up views showing the tumor heterogeneity with high flow and permeability in the periphery of the tumor and likely low flow and permeability in the center.

4. DISCUSSION

The implantation and propagation of the tumor line was considered quite successful in this pilot phase of the work. The tumor grew aggressively and within 3 weeks was too large to be useful in the peritoneal cavity. The heterogeneity of the tumor was very high, with large areas of necrosis and fluid pooling, large areas of vascularity and stroma. From this standpoint it was considered a potentially successful emulation of a human pancreatic disease, although admittedly not fully characterized yet.

The perfusion scans worked well, following the protocol developed by Stewart et al (1). The image shown in Figure 4 is typical with a permeability which is relatively homogeneous throughout the tissue, but blood volume and flow regions being maximal nearest the growing periphery of the tumor. Work is ongoing to analyze these tumors in a systematic manner and compare these images to pathology verification.

5. ACKNOWLEDGMENTS

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6. REFERENCES

