MALDI Mass Spectrometry Imaging of Gemcitabine Treatment in Pancreatic Cancer: Exploring Multiple Matrices to See the Whole Picture

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Abstract
Gemcitabine is one of the first-line chemotherapeutic drugs for treating pancreatic ductal adenocarcinoma (PDAC) patients as adjuvant chemotherapy or those with metastatic disease. However, this treatment has limited benefits due to poor drug delivery to PDAC, which may be a result of dense desmoplastic tumor stroma and low levels of tumor vascularization, preventing chemotherapeutic drugs from permeating the tumor. Previous work showed that intracellular phosphorilation of gemcitabine to its deaminated and triphosphates inhibits DNA synthesis, leading to cancer cell death. To date, gemcitabine delivery and metabolism have been studied in tissue homogenates, which limits the assessment of tissue distribution of gemcitabine and its metabolites (1–3). Here we have used matrix-assisted laser desorption/ionization (MALDI) imaging to quantitatively evaluate the tissue distribution of gemcitabine in mouse models of pancreatic cancer.

Introduction
Gemcitabine (dFdC) is a clinically utilized chemotherapeutic agent that has been studied by mass spectrometry (1-3) and mass spectrometry imaging (MSI) (4). Gemcitabine is effective in vivo and can be deaminated into 2’,2’-diaminonaphtalene monophosphate (dA5NMP), which is inactive. Alternatively, dFEMP can be phosphorylated to form a triphosphate triester via the adenosine triphosphate (ATP) pathway to form the triphosphate (dFdCTP), which is known to bind to DNA polymerase, shutting down DNA transcription in cancer cells (5). In this study, we sought to determine the spatial distribution of gemcitabine, as well as its metabolites, in pancreatic tumors and various other tissues from a mouse model of pancreatic cancer using MALDI mass spectrometry imaging.

Methods
Tissue Mimetic Model Preparation
Tissue mimetic models were prepared from mouse liver tissue. Tissue was homogenized, frozen, clamped, and pulsed on liquid nitrogen. Tubes for the freeze-dried homogenate were filled with 100 ml of tissue and homogenized. After initial homogenization, 10 ml of gemcitabine monophosphate solution shared from a 30 ml stock solution in water was added and the tubes were homogenized for a second time. A polysiloxane displacement pipet was used to transfer homogenate to a cryotube and each layer was frozen at -40°C. The model was then cryo-sectioned at 10 microns and thaw-mounted onto cleaned indium oxide (ITO) slides.

Animal Experiments and MALDI Imaging
Athymic nude mice were inoculated with Panc-1 PDAC cells in the left flank. Once tumors had grown to about 0.5 cm in diameter, 200 mg/kg gemcitabine in phosphate buffered saline was administered by tail vein injection. After 0.5, 1.0, or 2.0 hours, mice were narcotized and the following tissues were harvested: Panc-1 Rant tumor, pancreas, kidney, heart, and liver. Corresponding control tissues were harvested from unirradiated mice as well. Tissue were frozen in liquid nitrogen and cryo-sectioned at 10 micron thickness (ITO slides). Slides were sprayed with 1:1 phosphorilated (dA5NMP) matrix in 0.5% acetic acid 1:200 nylon-capped glass transparencies (AuNPs) in 50% methanol using an HTS MS sprayer prior to imaging. Tissues were imaged in both reflection positive and reflection negative modes with 20X, 50X, and 100X microscope objectives and 50 mm laser spot size, scanning a range from m/z 200 to 2000 in a Bruker Autoflex-Nash MALDI TOF/TOF environment.

Results: Quantitation with 1,5-DAN

Figure 2: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 264 [Gem+Na]+. Figure 3: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 286 [Gem+Na]+.

Results: Quantitation with AuNP

Figure 4: Quantification of m/z 264 (top) and m/z 286 (bottom) in SQUID Lab, demonstrating linear correlation between signal intensity versus gemcitabine concentration at higher concentrations.

Figure 5: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 264 [Gem+Na]+. Figure 6: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 286 [Gem+Na]+.

Results: Tumor Imaging

Figure 7: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 264 [Gem+Na]+. Figure 8: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 286 [Gem+Na]+.

Conclusions and Future Directions
We show that both 1,5-DAN and 2-mm citrate-capped AuNPs are appropriate matrices for MALDI imaging of gemcitabine and its metabolites in positive and negative ion modes. We have observed an improved linear correlation between gemcitabine concentration and relative intensity using a tissue mimetic model as compared to tissue spotting (data not show).

We have developed a spraying protocol on the HTS MS sprayer that maximizes the detection of gemcitabine and its metabolites from various tissues of gemcitabine-treated pancreatic tumor xenograft-bearing mice.

We have begun to explore quantitative imaging using tissue mimetic models. Our data show that 2-mm citrate-capped AuNPs allow us to quantitatively gemcitabine in a lower concentration than is possible with 1.5-DAN.

Our MALDI imaging study shows that gemcitabine and gemcitabine triphosphate, the relevant metabolite for DNA damage resulting in anti-tumor activity, are present in the tumors of Panc-1 tumor xenograft models at 0.5, 1.0, and 2.0 h following intravenous injection of 200 mg/kg gemcitabine.

Gemcitabine and gemcitabine triphosphate are observed in low concentrations in both the kidney and the liver (data not shown), as expected from previous homogenate mass spectrometry studies (5–7). Gemcitabine is known to be excreted through the renal system.

Unique spatial analysis of tissue sections shows substantial changes in both the tumor and pancreas, but not in the kidney in the mass range between m/z 200-1000, which typically contains mostly metabolites and lipids. Further analysis will be performed to characterize changes in biomarkers in the tumors and organs, which are associated with gemcitabine treatment.

In our next experiments, we will image both the tissue mimetic model and tumor tissue on the same slide to be able to generate quantitative maps of gemcitabine and its metabolites with 2-mm citrate-capped AuNPs.

Gemcitabine and its metabolites have been well documented using mass spectrometry from tissue homogenates (1–3). To confirm the identifications of gemcitabine and its metabolites from the tissues in our study, we plan to complete MALDI imaging of all tissues to validate our results.

We will also harvest and lyophilize (SMD) and Triton X-100 stain all tissues following mass spectrometry imaging to determine if distributions of gemcitabine or its metabolites correlate with vasculature, extracellular matrix or other features within the tumor microenvironment.

We are also exploring protocols to solubilize all ions to improve our quantification.

We are also pursuing quantization of purified gemcitabine metabolites, including gemcitabine triphosphate using similar approaches as described for gemcitabine.

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