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

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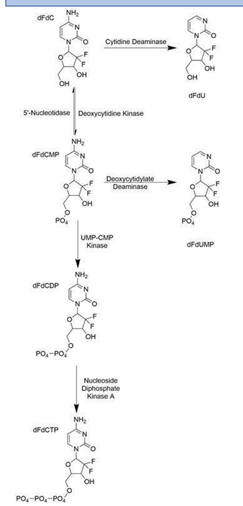
Abstract

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 penetrating the tumor. Previous work showed that intracellular phosphorylation of gemcitabine to its di- and tri-phosphates 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



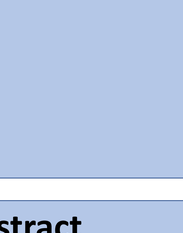
Gemcitabine (dFdC) is a clinically utilized chemotherapeutic agent that has been studied by mass spectrometry [1-3] and mass spectrometry imaging (MSI) [4]. Gemcitabine enters the cell and can be deaminated into 2',2'-difluorodeoxyuridine (dFdU), which is inactive. Alternatively, it can be phosphorylated by deoxycytidine kinase to gemcitabine monophosphate (dFdCMP). dFdCMP can also be deaminated into 2',2'-difluorodeoxyuridine monophosphate (dFdUMP), which is also inactive. Alternatively, dFdCMP can be phosphorylated twice more in a step-wise fashion to generate the two active metabolites gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP). dFdCTP is known to bind to DNA polymerase, shutting down DNA transcription in cancer cells [3]. 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.

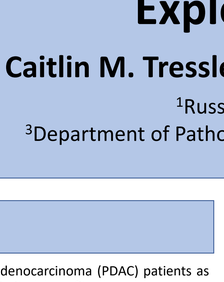
Compound	Abbreviation	m/z [M+H$^+$]	m/z [M+Na$^+$]	Polarity
Gemcitabine	dFdC	264	286	Positive
Gemcitabine Monophosphate	dFdCMP	344	366	Positive
Gemcitabine Diphosphate	dFdCDP	424	446	Positive
Gemcitabine Triphosphate	dFdCTP	504	526	Positive
2',2'-Difluorodeoxyuridine	dFdU	263	N/A	Negative
2',2'-Difluorodeoxyuridine Monophosphate	dFdUMP	345	367	Positive

Methods

Tissue Mimetic Model Preparation

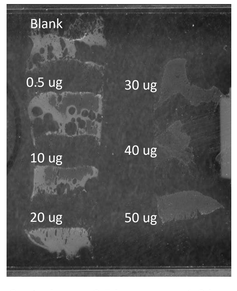
Tissue mimetic model was prepared from mouse liver tissue. Tissue was harvested, freeze-clamped, and pulverized on liquid nitrogen. Tubes for the Precellys Bead Homogenizer were filled with 300 mg of tissue and homogenized. After initial homogenization, 10 µL of gemcitabine stock solutions diluted from a 10 mM stock solution in water was added and the tubes were homogenized for a second time. A positive displacement pipette was used to transfer homogenate to a cryotube and each layer was frozen at -80 ˚C. The model was then cryo-sectioned at 10 microns and thaw-mounted onto cleaned indium tin oxide (ITO) slides.





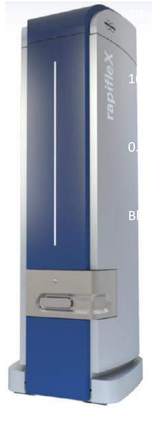
Animal Experiments and MALDI Imaging

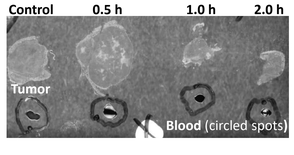
Athymic nude mice were inoculated with Panc1 PDAC cells in the left flank. Once tumors had grown to about 0.5 cm in diameter, 200 mg/kg gemcitabine in phosphate buffered saline was administered by tail vein injection. After 0.5, 1.0, or 2.0 hours, mice were sacrificed and the following tissues were harvested: Panc1 flank tumor, pancreas, blood, liver, and kidney. Corresponding control tissues were harvested from untreated mice as well. Tissues were frozen in floats on liquid nitrogen and cryo-sectioned at 10-micron thickness ITO slides. Slides were sprayed with 1,5-diaminonaphtalene (DAN) matrix in 50% acetonitrile or 2-nm citrate-capped gold nanoparticles (AuNP) in 50% methanol using an HTX M5 sprayer prior to imaging. Tissues were imaged in both reflectron positive and reflection negative mode with 100-micron raster size and 50-micron laser spot size, scanning a range from m/z 200 to 1000 on a Bruker Rapiflex MALDI TOF/TOF instrument.



Matrix Application

Spraying Methods:
10 mg/ml 1,5-DAN
50% ACN with 0.1% TFA
8 passes
Or:
Citrate-capped 2-nm AuNP
50% MeOH/50% Water
1 pass





Results: Quantitation with 1,5-DAN

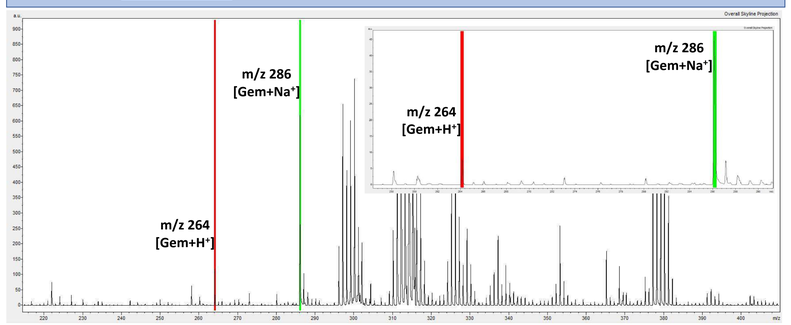


Figure 1: Average spectra of tissue mimetic model imaged with 1,5-DAN. Inset show mass filters of m/z 264 [Gem+H$^+$] (red) and m/z 286 [Gem+Na$^+$] (green). Low relative intensity of Gemcitabine peaks is observed due to high signal intensity from 1,5-DAN matrix.

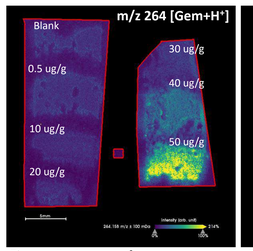


Figure 2: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 264 [Gem+H$^+$].

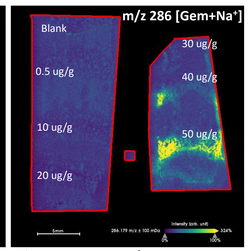


Figure 3: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 286 [Gem+Na$^+$].

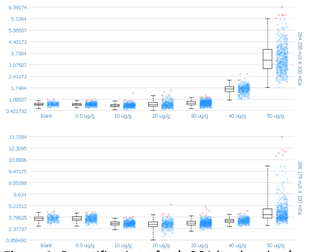


Figure 4: Quantification of m/z 264 (top) and m/z 286 (bottom) in SCiLS Lab, demonstrating linear correlation between signal intensity *versus* concentration at higher concentrations.

Results: Quantitation with AuNP

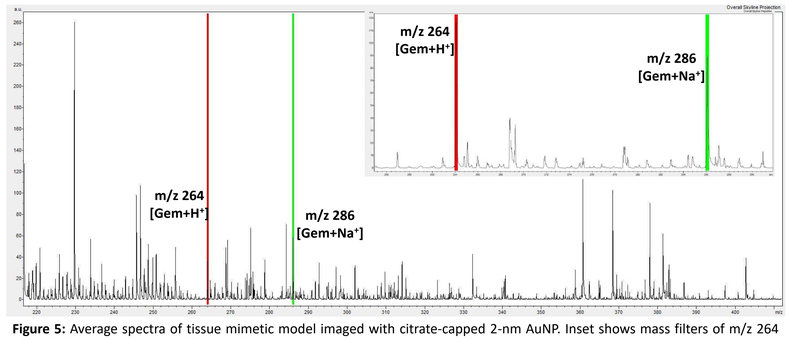


Figure 5: Average spectra of tissue mimetic model imaged with citrate-capped 2-nm AuNP. Inset shows mass filters of m/z 264 [Gem+H$^+$] (red) and m/z 286 [Gem+Na$^+$] (green).

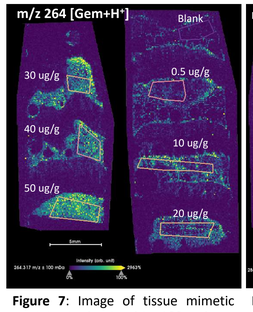


Figure 7: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 264 [Gem+H$^+$].

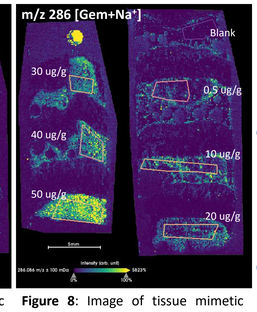


Figure 8: Image of tissue mimetic model obtained with 100 micron pixel size at m/z 286 [Gem+Na$^+$].

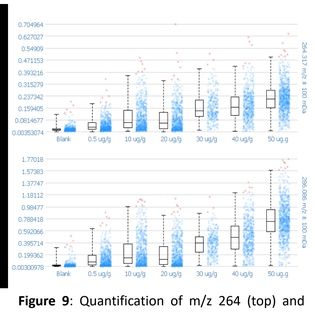


Figure 9: Quantification of m/z 264 (top) and m/z 286 (bottom) in SCiLS Lab, demonstrating linear correlation between signal intensity *versus* Gemcitabine concentration.

Acknowledgements

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Results: Tumor Imaging

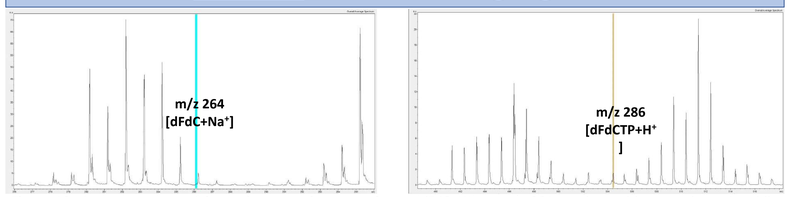

Figure 10: Average spectra of imaged pancreatic tumor tissue. [dFdC+H$^+$] at m/z 264 overlaps with a matrix peak, while [dFdC+Na$^+$] at m/z 286 is well resolved. The mass filter used for image display and quantification is shown in blue. In the case of the active drug metabolite gemcitabine triphosphate, [dFdCTP+H$^+$] is well resolved (mass filter shown in orange), while the sodiated form overlaps with a matrix peak.

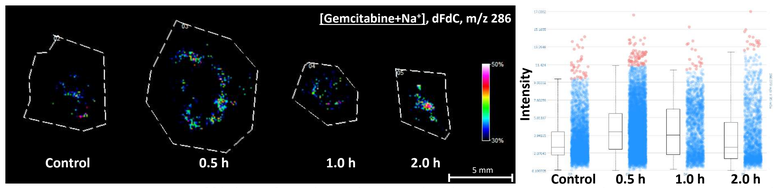

Figure 11: Increasing amounts of [dFdC+Na$^+$] are observed at 0.5 and 1.0 h post-treatment. By 2.0 h post-treatment, near basal-level of signal is observed for overall [dFdC+Na$^+$] tumor concentration, with a hot-spot in the tumor center.

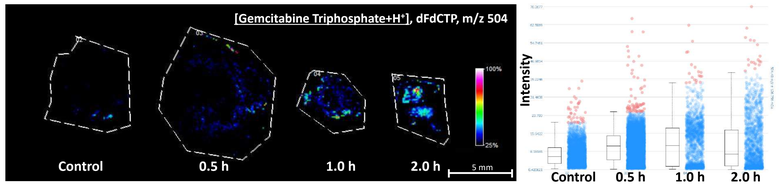

Figure 12: Increasing amounts of [dFdCTP+H$^+$] are observed at 0.5 and 1.0 h post-treatment. By 2.0 h post-treatment, near basal-level of signal is observed, for overall [dFdCTP+H$^+$] concentration, with hot-spot regions observed in central tumor regions.

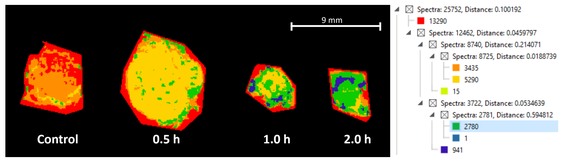

Figure 13: Unsupervised SCiLS segmentation analysis shows substantial changes in the metabolite and lipid regions (m/z 200 to 1000) in the tumor tissue starting at 0.5 h post-treatment, and continuing to be more pronounced at 1.0 h and 2.0 h post-treatment.

Conclusions and Future Directions

- We show that both 1,5-DAN and 2-nm citrate-capped AuNPs are appropriate matrices for MALDI imaging of gemcitabine and its metabolites in positive and negative ion mode.
- 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 method on the HTX M5 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 gemcitabine imaging using tissue mimetic models. Our data show that 2-nm citrate-capped AuNPs allow us to quantitate gemcitabine at 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 Panc1 pancreatic 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-based mass spectrometry studies [1-3]. Gemcitabine is known to be excreted through the renal system.
- Unsupervised analysis of all tissues shows substantial changes in both the tumor and pancreas, but not 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 biomolecules 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 generate quantitative maps of gemcitabine and its metabolites with 2-nm 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 MS/MS imaging of all tissues to validate our results.
- We will also hematoxylin-and-eosin (H&E) and trichrome 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 sodiate all ions to improve our quantification.
- We are also pursuing quantitation of purified gemcitabine metabolites, including gemcitabine triphosphate using similar approaches as described for gemcitabine.

References

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