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

Caitlin M. Tressler1, Hong Liang2, James R. Eshleman2,3, Katherine A Stumpo2, and Kristine Glunde1,3

1Rush-H. Morgan Department of Radiology and Radiologic Science, Division of Cancer Imaging Research, 2Department of Pathology, 3Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Department of Chemistry, University of Scranton, Scranton, PA 18510

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 intratumoral phosphorunlabeled 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.

Results: Quantitation with 1,5-DAN

Results: Tumor Imaging

The authors would like to acknowledge the Johns Hopkins Applied Imaging Mass Spectrometry (AIMS) Core facility at the Johns Hopkins Department of Radiology and Radiologic Science. We would like to thank the National Institutes of Health (NIH R01 CA239352) as well as the Sol Goldman Cancer Research Center Pilot Funding for support.

Acknowledgements

References


Jansen
drugs
two
at
a
tissue
metabolites
in
between
observed
enters
diameter,
mice
renal
would
homogenate
M
13
was
layer
(top)
and
Average
a
Beijnen
while
xenograft
in
at
200
quantitate
various
tumor
Unsupervised
of
1000
we
were
are
in
observed
citrate
2
observed
associated
its
and
mice
shows
50
[gemcitabine
1
gemcitabine
2]
[cm
a
polymerase,
triphosphate
model
mostly
mimetic
(PDAC)
to
which
linear
analysis
size
[264
of
tissue
dFdCMP
to
with
tumor,
model
in
observed
using
MALDI mass spectrometry imaging.

Introduction

Gemcitabine (dFdC) is a slowly utilized chemotherapeutic agent that has been studied by mass spectrometry (1-3) and mass spectrometry imaging (MSI) (4). Gemcitabine triphosphate is the most active form. Gemcitabine monophosphate (dFdMP) and gemcitabine diphosphate (dFdDP) are also metabolites of gemcitabine treatment. Previous work identified that gemcitabine (1.5 DAN) uptake

Methods

Tissue Mimetic Model Preparation

Tissue mimetic model was prepared from mouse liver tissue. Tissue was inserted, freeze-dried, and pulverized on liquid nitrogen. Tissue for the flat homogenate were filled with 205 mg of tissue and homogenated. After initial homogenization, 10 µL of gemcitabine monostearate solution from a 0.1M stock solution in water was added and the tubes were homogenized for a second time. A 100 µL of transferred 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 thawed-mounted on 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/g gemcitabine phosphate buffered saline was administered by tail vein injection. After 0.5, 1, 2, or 24 hours, mice were sacrificed and the following tissues were harvested: Panc-1 tumor, pancreas, kidney, spleen, and liver. Corresponding control tissues were harvested from untreated mice as well. Tissues were frozen in cryo in liquid nitrogen and cryo-sectioned at a tissue thickness of 70 µm slides. Slides were sprayed with 15% -dithiotrethiol (DTT) matrix in 50% acetonitrile and 2-0m cryo-cooled glass transporters (AuNP) in 50% methanol using an HTS MS sprayer prior to imaging. Tissues were imaged in both reflection positive and reflection negative modes with 200 microns raster size and 50 micron laser spot size, scanning a range from m/z 200 to 3000 in a Bruker Autoject-MALDI-TOF/TOF instrument.

Results: Quantitation with AuNP

Results: Tumor Imaging

Figure 10: Average spectra of xenograft pancreatic tumor tissue. m/z 264 at m/z 286 overlaid with a matrix peak, while [dFdCMP]+ 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]+ is well resolved (mass filter shown in orange), while the ionic form overlaid with a matrix peak.

Figure 13: Increasing amounts of AuNP (blue) 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]+, tumor concentration, with a hot spot in the tumor center.

Table 3: Unsupervised S254 segmentation analysis shows gemcitabine metabolite and lipid regions (m/z 230 to 500) in control 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 shown).

We have developed a spraying method on the HTS MS sprayer that maximizes the detection of gemcitabine and its metabolites from various tissues of gemcitabine-treated pancreatic tumors 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 quantify gemcitabine to a lower concentration than is possible with 1,5-DAN.

Our MALDI imaging study shows that gemcitabine and gemcitabine triphosphate, the relevant metabolites for DNA damage resulting in anti-tumor activity, are present in the tumors of Panc-1 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 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 images show substantial changes in both the tumor and pancreas, but not in the kidneys 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 be able 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 identification 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 hematolunin (blue) and thionin 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 quantification of purified gemcitabine metabolites, including gemcitabine triphosphate using similar approaches as described for gemcitabine.