

Distribution of Irinotecan in Liver and Model Human Tumor by Tissue Imaging Mass Spectrometry

Maria C. Prieto Conaway¹, Shousong Cao², Farukh Durrani², Youcef Rustum², Ping Wang³, Khin Marlar³, Latif Kazim³

¹Thermo Fisher Scientific, San Jose, CA, USA; Roswell Park Cancer Institute, Dept of Cancer Biology, Buffalo, NY, USA; Roswell Park Cancer Institute, Dept. of Cell Stress Biology, Buffalo, NY, USA Buffalo, NY.

Overview

Purpose: To determine irinotecan metabolites and their distribution in human tumors directly from tissue by MALDI Imaging MS. To establish MALDI ion trap technology as ideally suited for direct tissue analysis of drugs in tissue.

Methods: Nude mice with implanted human head and neck tumors were treated with either irinotecan, methylselenocysteine (MSC), both drugs combination, or non-treated (control). Frozen tumors and livers were sliced to ~12 µm thick and thaw-mounted on to non-conductive glass slides. Irinotecan metabolites were investigated by Single Reaction Monitoring or CRM with an LTQ XL™ coupled to a MALDI source.

Results: Metabolites previously identified in human urine samples were detected directly off tissue in all irinotecan treated tissues and those with both irinotecan plus MSC. SN-38-G, a known irinotecan metabolite, seems more uniformly distributed in the tumor treated with irinotecan plus MSC.

Introduction

Irinotecan, isolated from the Chinese tree *Camptotheca acuminata*, has shown strong antitumor activity against a wide variety of human solid tumor xenografts in mice. Irinotecan is a DNA topoisomerase I inhibitor that binds to and prevents dissociation of the DNA-topoisomerase I complex (involved in DNA replication), thereby inhibiting enzyme activity.

Lack of microvessels has been shown to offer resistance to irinotecan therapy in human head and squamous carcinomas¹. Two types of tumor xenografts, FaDu and A253, with high vascular and avascular regions, respectively, were used in the above referenced study. The purpose of our study is: 1) to demonstrate that MALDI ion trap is ideally suited for direct tissue analysis of drugs in tissue; and 2) to utilize imaging capabilities for looking at regions that are accessible/inaccessible to the drug in the tumor in order to establish MALDI MS-based imaging as a viable technique in cancer therapy research.

Irinotecan and its active metabolites have been previously identified (Fig. 1) by HPLC separation coupled to electrospray mass spectrometry detection from human urine samples².

Methods Steps for the study of drug distribution in dosed animals:

1) **MALDI mass spectrometric studies of the drug in solution:** The parent drug, irinotecan, was purchased from Pfizer Inc. (NY, NY, USA) and studied in positive ion mode. MALDI matrix was 2,5-DHB (Laser Biolabs, France). MALDI fragmentation patterns were compared to LC-MS results¹ and direct infusion electrospray ionization (ESI, spectra provided by L. Kazim, RPCI).

2) **Dosing:** Nude mice were transplanted with the human head and neck tumor FaDu. The mice underwent four different treatments: untreated control, MSC alone, irinotecan alone, and irinotecan + MSC. Mice were treated with MSC orally, 0.2 mg/mouse/day daily for 7 days week, then irinotecan (100 mg/kg) was given by i.v. injection, either alone or added to MSC-treated mice.

3) **Sample Preparation:** The liver and tumor were excised, flash-frozen and kept at -80 °C until sliced to ~12-15 micron thick sections in a cryotome (Thermo Fisher Scientific, MA, USA) and thaw-mounted on to glass plus slides (Fisher Scientific, USA). Glass slides with tissue samples were rinsed in ethanol for 30 sec, air dried by shaking, and placed under vacuum for ~30 min. This seemed to take care of excess lipids and salts that interfered and suppressed MS signals. 0.1 µL of 6-aza-2-thiothymine (ATT, SIGMA™, St. Louis, MO, USA) matrix were spotted on top of the tissue or sprayed with a commercial airbrush. Matrix solvent was either 50/50 (v/v) acetonitrile/0.1% TFA or 70/30 (v/v) methanol/0.1% TFA.

4) **Instrumentation:** An LTQ XL (Thermo Fisher Scientific) coupled to a MALDI source was used for imaging mass spectrometry, with custom data acquisition software to raster the tissue in the X and Y directions. The data was analyzed with in-house software (ImageQuest 1.0) to visualize the distribution of the drugs within the tumors. A 60 Hz nitrogen laser (LTB, Germany) with beam diameter of ~100 µm impinges directly on to the MALDI plate (at a 30° angle). Both ion activation techniques, collision-induced-dissociation (CID) and pulsed Q dissociation (PQD), were employed. PQD effectively lowers the low mass range in ion traps to m/z 50.

FIGURE 1. Metabolism of irinotecan extracted from human

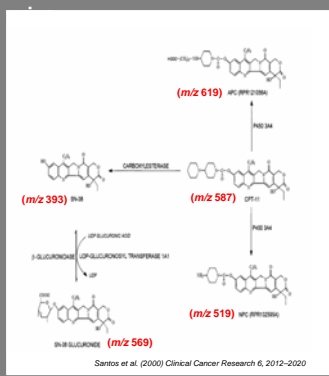
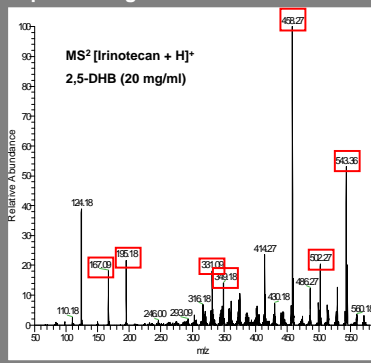


FIGURE 2. Fragment ion spectrum of solution irinotecan by PQD of m/z 587. Expected fragment ions in red.



Results

Solution studies: The MALDI fragmentation spectrum (Fig. 2) shows all major irinotecan ion fragments (marked in red) as found by electrospray ionization (data not shown) and LC-MS published work².

Metabolites found in treated tissue (ATT spotted tissue): Unwashed tissue did not produce any significant results as the MS² and MS³ fragmentation spectra of known metabolites were weak. Rinsing with ethanol for 30 sec. prior to matrix application decreased the amount of phospholipids and salts that can interfere or suppress analytes of interest³.

We were able to detect the following metabolites (see Fig. 1) in FaDu tumors treated with irinotecan and irinotecan plus MSC and not in the controls (not treated or MSC-treated only): SN-38 (m/z 393), SN-38-G (m/z 569), isobaric M1 and/or M2 (m/z 603), and the parent drug irinotecan (m/z 587). Metabolites M1 and M2 are the oxidation of the CPT moiety and of the terminal piperidine, respectively². The MS/MS of m/z 603 contains weak fragment ions at 518 (-85, loss of piperidine) and m/z 502 (-101, loss of oxidized piperidine), which would indicate that both M1 and M2 metabolites are present in tissue, respectively. However, the MS³ of m/z 518, which would confirm the presence of M1 is weak and not conclusive. The peak at m/z 603 appears to be mostly M2, due to the prominent m/z 559 peak (-CO₂), with MS³ data to confirm this (data not shown).

We were not able to detect the NPC metabolite (m/z 519) directly, an indication of *in vivo* metabolized loss of a terminal piperidine, although loss of piperidine was evident through collision induced dissociation in the ion trap. Figure 3 shows the need for MS³ to truly isolate a specific precursor when working with complex tissue samples.

Results (continued)

ATT matrix sprayed on tissue: Unmetabolized irinotecan was detected in the liver and FaDu tumors (data not shown) by imaging mass spectrometry. Parent drug and metabolites SN-38-G and M2 were convincingly identified from tissue by MS/MS and MS³ tandem mass spectrometry in positive ion mode. Figure 4 shows the distribution of two metabolites (SN-38 and its glucuronide form) in FaDu tumors treated with irinotecan alone and irinotecan plus MSC. MSC has been shown to increase irinotecan efficacy against tumors and found to be more effective in highly vascularized tumors such as FaDu than in avascular tumors (A253)⁴. Results from Figure 4 show m/z 349 more uniformly distributed in FaDu tumors treated with both MSC and irinotecan than in those treated with irinotecan alone, where the analyte appears clumped in regions. These results were consistent in 3 different tissue samples: two acquired at 100 µm resolution (MS² and MS²/MS³ data), one at 50 µm resolution (MS² only), possibly indicating that coating variability during matrix application was not to blame. Results from the MS³ image are more specific and truly indicative of the metabolite, as controls showed some signal for MS² of m/z 393 (data not shown).

FIGURE 3. Ability of MS³ for effectively isolating intended precursor: MS² includes undesired phospholipid (m/z 184) while MS³ shows the right metabolite fragment (m/z 349) in irinotecan treated tumor

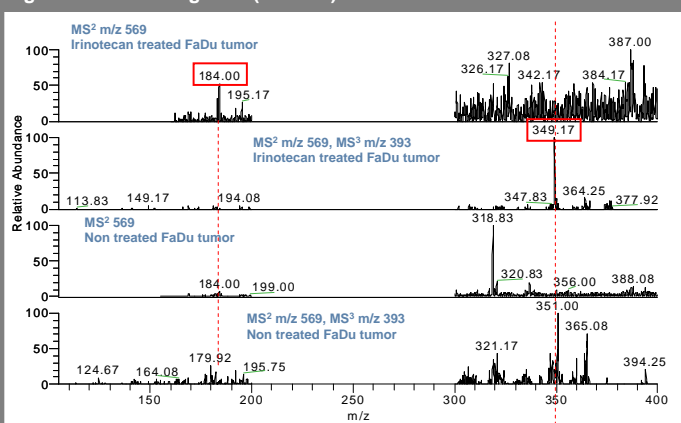
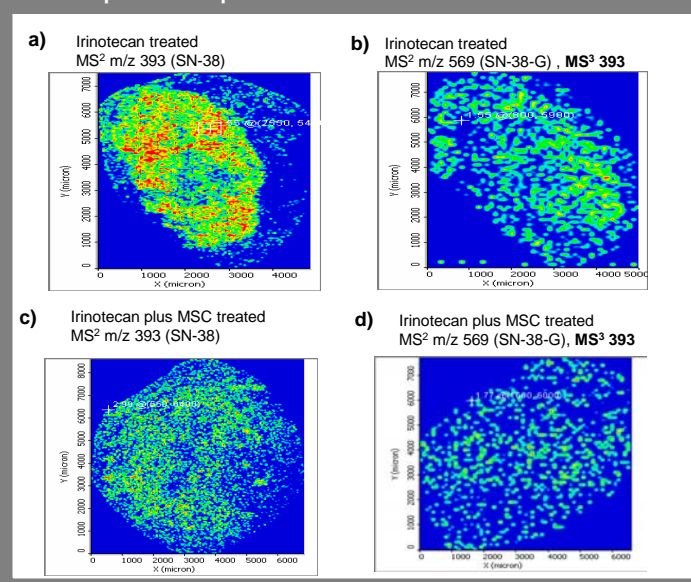


FIGURE 4. Distribution of m/z 349 fragment ion from fragmentation of SN-38 and SN-38-G metabolites in two FaDu tumor samples treated with either irinotecan (a, b) or irinotecan plus MSC (c, d). Figures a and c acquired at 50 µm resolution



Conclusions

- Most known irinotecan metabolites were identified by MS/MS and confirmed by MS³ using the LTQ XL with MALDI source directly from drug-treated tumor samples.
- The irinotecan metabolite SN-38-G seemed more uniformly distributed in FaDu tumors treated with both MSC and irinotecan than in those treated with irinotecan alone, where the analyte appears clumped in regions (MS² and MS³ data).
- Rastering a commercial laser with beam of ~100 µm at a spacing of 50 µm does improve the resolution of the 2-dimensional image⁵ (Fig. 4 a, c).
- Given the complexity of tissue samples, MS³ is truly needed in MALDI tissue imaging to filter interfering and isobaric species that are part of the baseline chemical noise and isolated during MS².
- Future work using MALDI tissue imaging will compare the results presented here in highly vascularized FaDu tumors with avascular tumors such as A253.

References

- 1 A. Bhattacharya, K. et al. (2004). Lack of Microvessels in Well-Differentiated Regions of Human Head and Neck Squamous Cell Carcinoma A253 Associated with Functional Magnetic Resonance Imaging Detectable Hypoxia, Limited Drug Delivery, and Resistance to Irinotecan Therapy, *Clinical Cancer Research* 10, 8005-8017.
- 2 A. Santos, S. Zanetta, T. Cresteil, A. Deroussent, F. Pein, E. Raymond, L. Vernillet, M-L. Risse, V. Boige, A. Gouyette, and G. Vassal (2000). Metabolism of Irinotecan by CYP3A4 and CYP3A5 in Humans, *Clinical Cancer Research* 6, 2012-2020.
- 3 BJ Xu, RM Caprioli, ME Sanders, RA Jensen (2002). MALDI MS Analysis of Laser Microdissected Cells, *J Am Soc Mass Spectrom*, 13, 1292-1297.
- 4 Cao (2004). Selective modulation of the therapeutic efficacy of anticancer drugs by selenium containing compounds against human tumor xenografts. *Clinical Cancer Research* 10, 2561-2569.
- 5 J. C. Jurchen, S. S. Rubakhin, J. V. Sweedler, J. Am. Soc. Mass Spectrom. 16 (2005) 1654-1659.

© 2007 Thermo Fisher Scientific Inc. All rights reserved. SIGMA is a trademark of Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.