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Commentary: "Evaluation of Small Molecule Drug Uptake in Patient-Derived Prostate Cancer Explants by Mass Spectrometry"

Ryan Nguyen[1], Cody J. Peer[1], William D. Figg[1]*

1Clinical Pharmacology Program, Office of the Clinical Director, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

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***Correspondence:**
Dr. William D. Figg, 10 Center Drive, Building 10/Room 5A03, Bethesda, Maryland, 20892, USA; Telephone No: +1-240-760-6179; Fax No: +1-240-858-3020; Email: figgw@mail.nih.gov.

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ABSTRACT

Herein, the strengths and weaknesses of a study previously published in Scientific Reports will be discussed. The major aim of this study was to investigate the spatial uptake of enzalutamide by intact prostate tissue using matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) and histological staining. Additionally, quantitative analysis of *in situ* tissue enzalutamide concentration was achieved by conventional liquid chromatography-tandem mass spectrometry (LC-MS/MS). The authors demonstrated that enzalutamide rapidly permeates prostate tissue and accumulates in regions containing high epithelial cell counts. While these findings have potential to inform future pharmacodynamic experimental designs and endpoints, this study is limited in its scope by the current state of MALDI-MSI technology, which is not yet sensitive enough to investigate clinically relevant *in situ* enzalutamide concentrations.

Introduction

In a conventional pharmacokinetic assay, drug-containing tissue samples are completely homogenized prior to any drug extraction and sample clean-up steps. Subsequent drug measurements via liquid chromatography-tandem mass spectrometry (LC-MS/MS) are therefore representative of the whole sample, and no conclusions can be drawn as to where the compound of interest has distributed within the intact tissue. Indeed, drug distribution is often highly variable and may not be easily predicted from plasma concentrations or dose[1,2]. An understanding of drug uptake by tissue, which could aid in the elucidation of drug mechanism and resistance, has recently been supplemented by matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI). During a typical MALDI-MSI experimental setup, thin sections of snap-frozen tissue are uniformly coated with a predetermined MALDI matrix and then irradiated with a laser to liberate atoms and molecules. Ideally, the matrix efficiently absorbs the incident laser energy via vibrational (infrared) or electronic (ultraviolet) excitation and will also contain polar functional groups to enhance solubility in aqueous solutions[3]. In addition, the matrix can provide a proton source for the analyte(s) it comes into contact with, thus promoting formation of the positive ions that are mass analyzed. Single mass spectra are generated as the laser moves across the tissue sample and irradiates the matrix, and the resulting scans can then be analyzed by selecting for the desired mass-to-charge (m/z) ratio in each pixel. This process, for which several types of open-source imaging software are currently available[4], including MATLAB

and ImageJ, yields an intensity map showing the relative abundance of a selected ion across the sample. Additionally, nearly all software available for MSI can perform standard preprocessing steps such as normalization and baseline correction[4]. These tools facilitate subsequent data analysis and minimize experimental variance.

In this paper[5], the authors implemented MALDI-MSI to investigate the spatial uptake of enzalutamide, an androgen receptor (AR) antagonist, in prostate tissue. Enzalutamide is currently indicated for the first-line treatment of metastatic castration resistant prostate cancer and was recently approved for high-risk localized disease. It is believed to block the binding of androgens to the AR via competitive inhibition, thus disrupting a key process in the progression of prostate cancer. In addition, enzalutamide has been shown to prevent AR nuclear translocation and binding of the androgen-AR complex to DNA[6]. As enzalutamide engages directly with its AR target, the extent to which it can penetrate tumor tissue, as well as how it distributes once inside the tissue, could inform the development of clinically-relevant pharmacodynamic assays[5].

MALDI-MSI Reveals Enzalutamide Accumulates in Epithelium-Rich Regions of Prostate Tissue

Mutuku et al first obtained intact human prostate tissue samples, both benign and malignant, from enzalutamide-naïve patients (n−8) and cultured them over a 24 hour period on a gelatin sponge containing 50 μM (approximately 25 μg/mL) enzalutamide. One half of each sample was removed at the time of harvest and used for LC-MS/MS, which afforded the absolute quantitation of enzalutamide content within the homogenized tissue. The authors reported a time-dependent increase in drug uptake, with the most distribution evident by 6–24 hours after exposure to enzalutamide. MALDI-MSI was performed on the remaining half of each sample, all of which underwent haematoxylin and eosin (H&E) staining prior to imaging. Comparison of the resulting MALDI-MSI intensity maps to the H&E staining showed a distinct accumulation of enzalutamide signal in tissue regions with elevated levels of both malignant and benign epithelial cells, which are known to highly express the AR. As such, the authors proposed that *in situ* enzalutamide concentration is likely linked to a patient's AR expression. While a reasonable conclusion given the data, it is essential to also consider other factors that affect drug penetration, such as the tumor microenvironment. Disordered vascular architecture, aberrant blood flow, and accumulation of stroma proteins in the extracellular matrix are common characteristics that can obstruct the passage of molecules into the tumor[2]. The physicochemical properties of the drug in question also determine tumor tissue accessibility, as small, hydrophilic compounds are more readily dissolved in the extracellular matrix and therefore likely to distribute in the junctions between cells[2]. Highly lipophilic compounds may penetrate the lipid membrane with ease but can also exhibit a high degree of protein binding, thus preventing their rapid delivery to an intracellular target[1]. A more accurate assessment of the MALDI-MSI enzalutamide findings would therefore append AR expression to the aforementioned parameters.

The authors concluded that drug penetration and distribution within tissue are crucial parameters in the development of pharmacodynamic experiments involving solid tumors. As this paper reports, the ability of enzalutamide to rapidly permeate cultured prostate tissue could inform both the design and interpretation of a pharmacodynamic assay investigating enzalutamide activity and/or resistance.

MALDI-MSI Lacks the Sensitivity Required for Clinically Relevant Pharmacokinetic Investigations

While Mutuku et al successfully demonstrated the heterogeneous nature of enzalutamide uptake by prostate tumors, their study also reveals several limitations of MALDI-MSI. For example, prior to selecting a 50 μM enzalutamide treatment, the authors first chose to investigate a 10 μM dose (4.6 μg/mL) as this value is consistent with reported plasma concentrations found in patients receiving the recommended clinical dose (160 mg orally once daily) [7]. Subsequent MALDI-MSI studies of the 10 μM samples, however, showed insufficient enzalutamide signal intensity for accurate quantitation. A higher dose of 50 μM was consequently selected for tissue treatment, and as such the reported MALDI-MSI data corresponds to higher doses (360 mg/day) rather than the FDA recommended 160 mg/day dose for all indications. It is also crucial to consider that enzalutamide is 97% plasma protein bound, and a clinically-relevant diagnostic tool must therefore be sensitive enough to account for the significantly lower fraction of unbound, active drug that is free to distribute into tissues such as tumor[7]. Based on steady-state pharmacokinetics of 160 mg qd, the mean peak plasma concentration (C_{max}) and mean trough (pre-dose) plasma concentration (C_{min}) of enzalutamide are 16.6 μg/mL and 12.0 μg/mL, respectively[8]. Unbound enzalutamide plasma concentrations can therefore be expected to fall roughly within the range of 300 to 500 ng/mL, which is significantly lower than both the 10 μM (4.6 μg/mL) and 50 μM (approximately 25 μg/mL) enzalutamide treatments investigated.

In addition to this lack of sensitivity, only relative quantitation was possible from the MALDI-MSI analysis, and as such Mutuku et al incorporated an LC-MS/MS assay to perform absolute quantitation of the enzalutamide signals. Unlike the MALDI-MSI studies, this LC-MS/MS assay was sensitive enough to capture clinically relevant

in situ enzalutamide concentrations and was capable of quantitating values as low as 3 ng/mL. While efforts to increase the sensitivity of MALDI-MSI have been reported using alternative ionization enhancement techniques, the steps involved are often time-consuming[9,10]. For example, chemical derivatization of a target analyte is commonly employed to boost ionization efficiency but can take several hours to complete. Other studies have attempted to avoid this issue with post-ionization strategies[9,11], but these methods often require expensive secondary laser sources. Finally, the implementation of quantitative MALDI-MSI has also recently been reported[12-14], but this technique still lacks the sensitivity of conventional LC-MS/MS assays and suffers from experimental variance. Thus, further studies are warranted to increase the sensitivity and reliability of MALDI-MSI using cheaper and more efficient techniques.

Conclusion

This paper investigated the uptake of enzalutamide in prostate tissue via conventional LC-MS/MS and MALDI-MSI. The authors reported that enzalutamide within conditioned medium rapidly suffuses prostate tissue, providing a viable experimental design for future pharmacodynamic studies. Additionally, the implementation of MALDI-MSI and histological staining revealed that enzalutamide accumulates in tissue regions containing high epithelial cell counts. The authors reasonably posited that because epithelial cells are known to highly express the AR, it is likely that an individual's *in situ* enzalutamide content is linked to AR expression. What is neglected from this argument, however, is that a multitude of other factors such as tumor microenvironment and drug physiochemistry also influence drug uptake. The MALDI-MSI implemented in this study was not sensitive enough to even approach clinically relevant enzalutamide concentrations in tissue, and conventional LC-MS/MS was also required for absolute quantitation. More research is warranted to improve the sensitivity and quantitative power of existing MALDI-MSI technology, and going forward, the development of new technology will be needed to reduce both experimental costs and time.

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