Improving on-tissue detection of doxorubicin by using Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging

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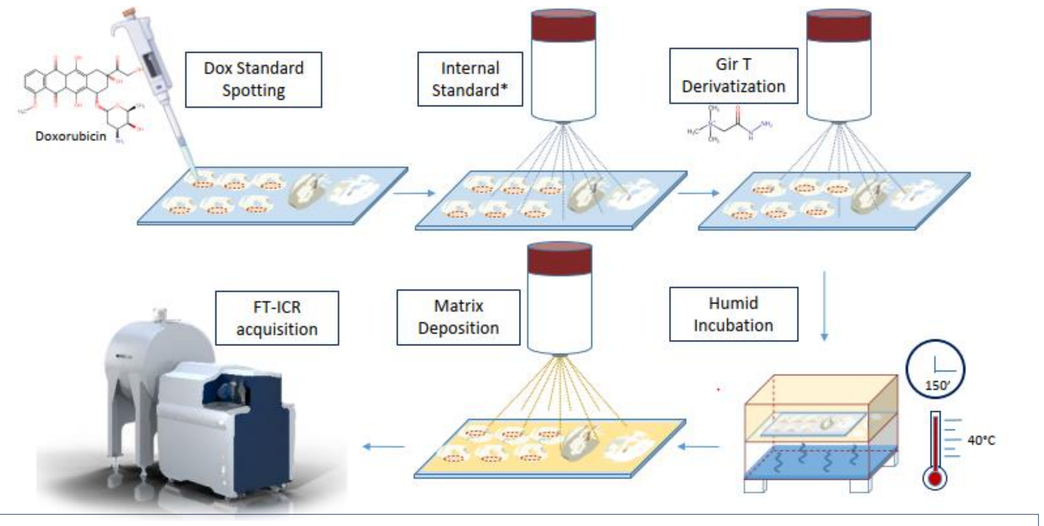
Introduction

Doxorubicin (Dox) is one of the most prescribed anti-tumor drugs for several types of cancer including breast, lung, and hematologic malignancies[1]. However, Dox is toxic to the heart and can cause asymptomatic left ventricular dysfunction or even heart failure[2]. The molecular mechanism of doxorubicin has been previously described as a DNA intercalant that leads to the formation of free radicals causing direct DNA damage, calcium homeostasis dysregulation, but also structural DNA damage via inhibition of Topoisomerase[3]. Despite the fact that it has been widely studied, the organ-specific distribution of Dox is not fully understood yet. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) offers the possibility to evaluate the abundance and the spatial distribution of biomolecules in tissues. MALDI-MSI provides the advantages of being non-destructive for the sample, relatively fast, and does not require any labeling, unlike other techniques such as autoradiography[4]. To the best of our knowledge, the distribution of doxorubicin in treated animals has never been studied. In this work, therefore, we investigated the organ-specific distribution of doxorubicin in heart and kidney in a dosed animal. We developed an approach that allowed us to increase the limit of detection while keeping the tissue integrity for in-situ MALDI-Imaging of Dox by using on-tissue chemical derivatization (OTCD). After protocol optimization, the limit of detection was improved 100-fold using the OTCD strategy, which permitted relative quantification of the drug. Later, an additional step using an internal standard was added to the workflow to perform absolute quantification at clinically relevant concentrations. The new approach will allow a better understanding of organ-specific toxicity induced by this chemotherapeutic drug.

Method

Doxorubicin-treated organs (heart and kidney) were taken from mice that have been injected with 4 mg/kg concentration of Dox. Tissue sections from two timepoints (3h post- and 24h post-injection of Doxorubicin) have been mounted onto ITO slides. A 8 points Dox standard curve was manually spotted onto control tissues to perform quantification. 7 layers of Girard reagent T (Gir T in MeOH 0.2% TFA) have been applied using a M3+ sprayer (HTX Technologies). After 150 min incubation time at 40 degrees, 10 layers of CHCA matrix were applied using a TM sprayer. MALDI-MSI images have been acquired with a FT-ICR instrument (Solarix, Bruker), using CASI mode at 50 µm spatial resolution. Quantification has been performed using LipostarMSI software Molecular Horizon). These experiments have been done in triplicate.



Results

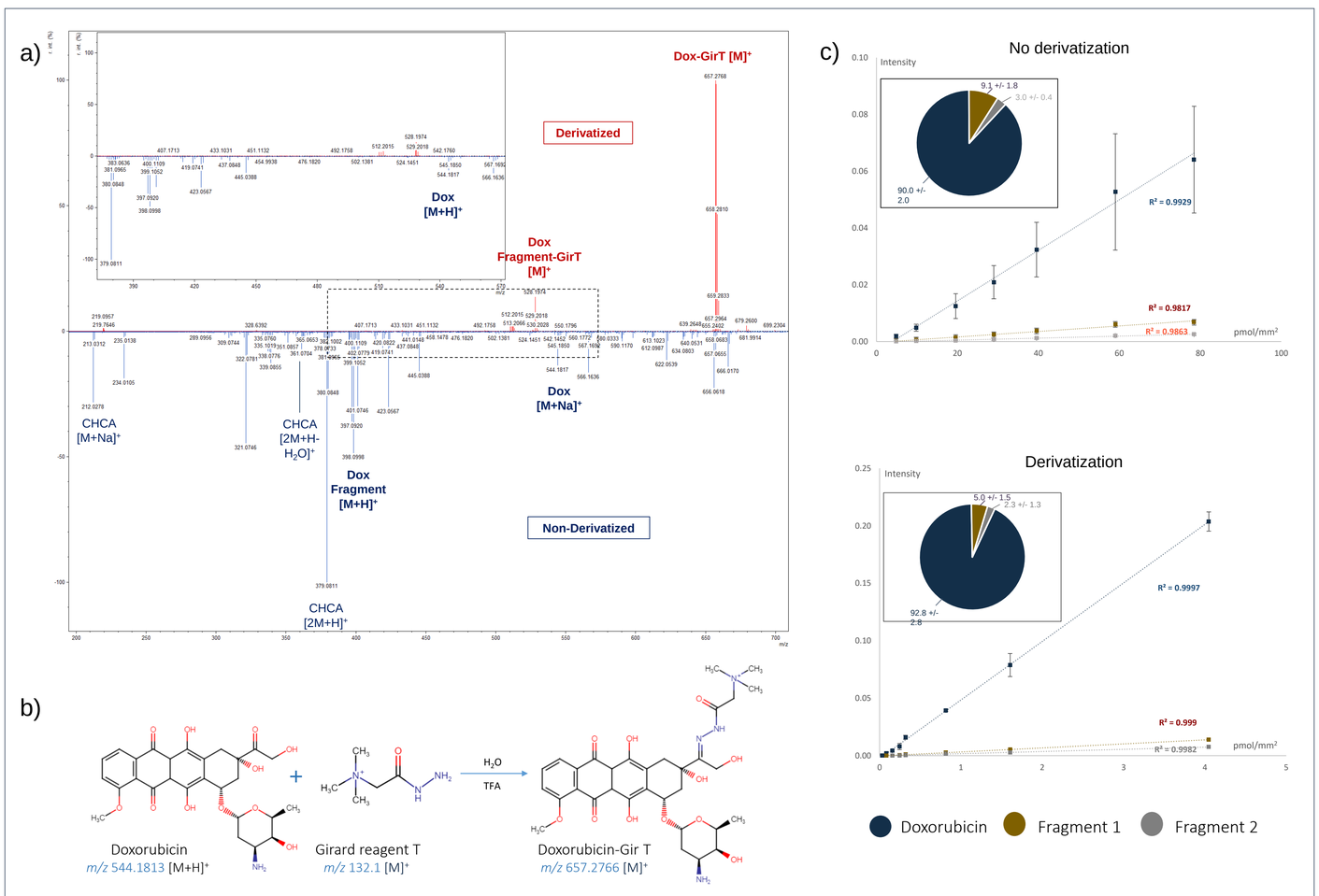


Figure 1. Derivatization of Doxorubicin (Dox) with Girard's reagent T (GirT) on ITO slide. Dox derivatization versus non derivatization was tested on ITO slide by spotting 0.5 µl of 50 µM Dox solution (mean spectrum of spot). Non-derivatized Dox was detectable at m/z 544.1802 $[M+H]^+$, as well as m/z 566.1436 $[M+Na]^+$. The most intense signal (100 % relative intensity base peak) of the spectrum was for the Doxorubicin-GirT at m/z 657.2750 $[M]^+$ (a). Chemical reaction between the Doxorubicin and the Girard's reagent T in humid and acid environment (b). Effect of the Dox derivatization by GirT on the Limit of Detection (LOD). The potassiated adduct m/z 582.1372 $[M+K]^+$ and Dox-fragments were plotted to evaluate the LOD (c). Using GirT derivatization, $[M]^+$ radical form of Doxorubicin and GirT-fragments were plotted to create the calibration curve. Dox fragmentation was observed and averaged for the 8 dilution points and averaged in the pie chart. Each experiment was performed in triplicate.

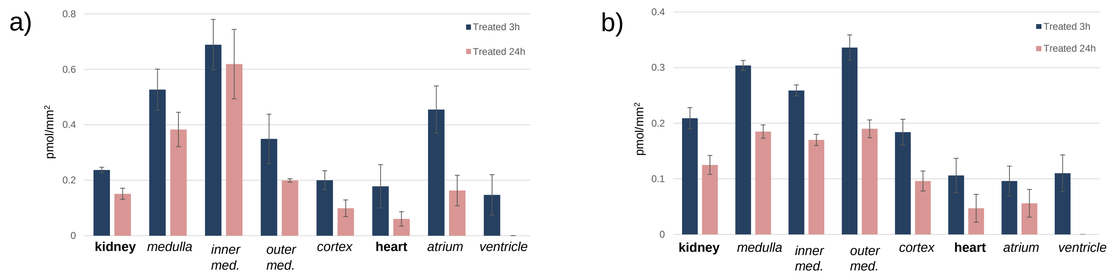


Figure 3. Dox-GirT quantification in 3h/24h treated organs. Dox concentrations calculated using the standard curves created previously. Relative quantification was performed using RMS normalized data (a), while absolute quantification was using IS normalized data (b). Experiments were performed in triplicate.

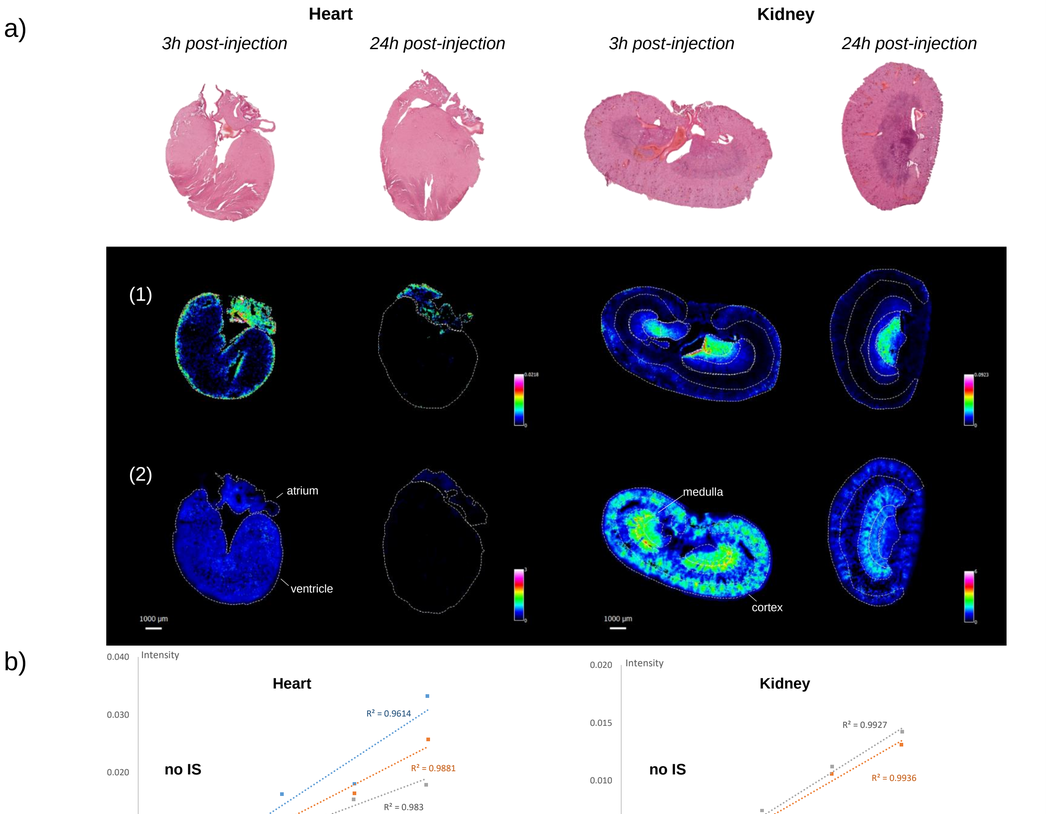


Figure 2. MALDI-MSI distribution of Dox-GirT (m/z 657.2766 [M]+). The image depicts the Dox-GirT spatial distribution in 3h-treated and 24h-treated heart/kidney sections (a). RMS normalization was used for relative quantification (1), and internal standard (13C, D3-Dox) normalization for absolute quantification (2). Calibration curves were created by spotting seven concentrations of Dox on control tissues and used for further quantification (b). Experiments were performed in triplicate. Calibration curve 1 is represented in blue, calibration curve 2 is represented in orange, and calibration curve 3 is represented in grey.

Conclusions

- Quantification results showed significant differences in Dox concentrations according to the normalization method. While RMS normalization showed inter-variability between organ subregions (medulla vs cortex for instance), the IS normalization corrected the concentration differences. As expected, lower concentration was found in the 24h post-injection organs.
- Dox maximum concentration was found in the kidney (*e.g.* outer medulla with 0.3 pmol/mm² IS normalized) at 3h post-injection while Dox minimum concentration was found in the heart (lower than 0.1 pmol/mm² at 24h post-injection IS normalized). The optimized OTCD and subsequent quantification create a strong tool to study longer treatment time points that would provide additional information about doxorubicin distribution and the associated effects at clinically relevant concentrations.

Acknowledgements

This research has been made possible with the support of the Dutch Province of Limburg.
This research has been made possible with the support of the European INSPIRE project (agreement No GA 858070)

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