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Mass Spectrometry Imaging for Nephrotoxicity Evaluation of Different Doxorubicin Formulations

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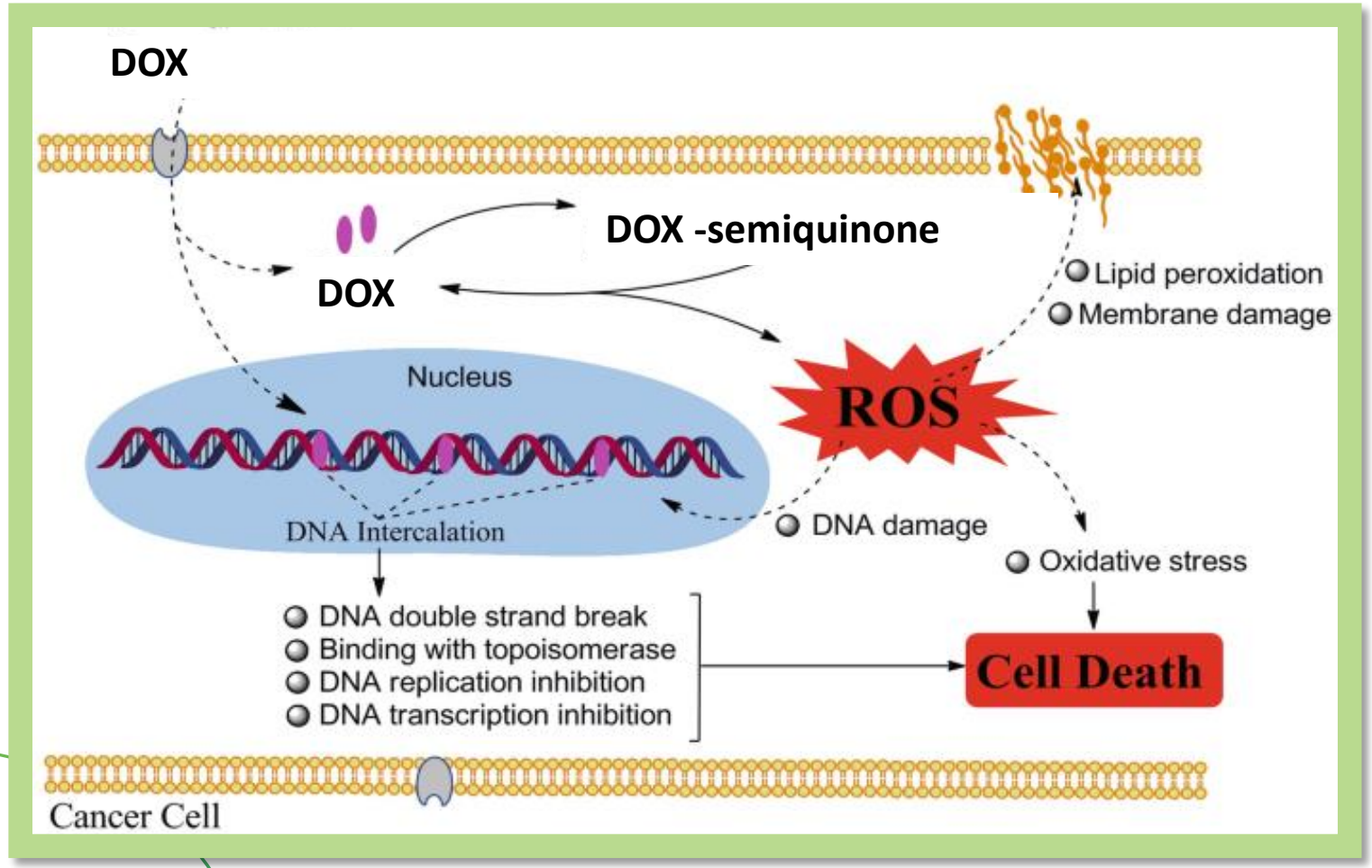
DOXORUBICIN (DOX)

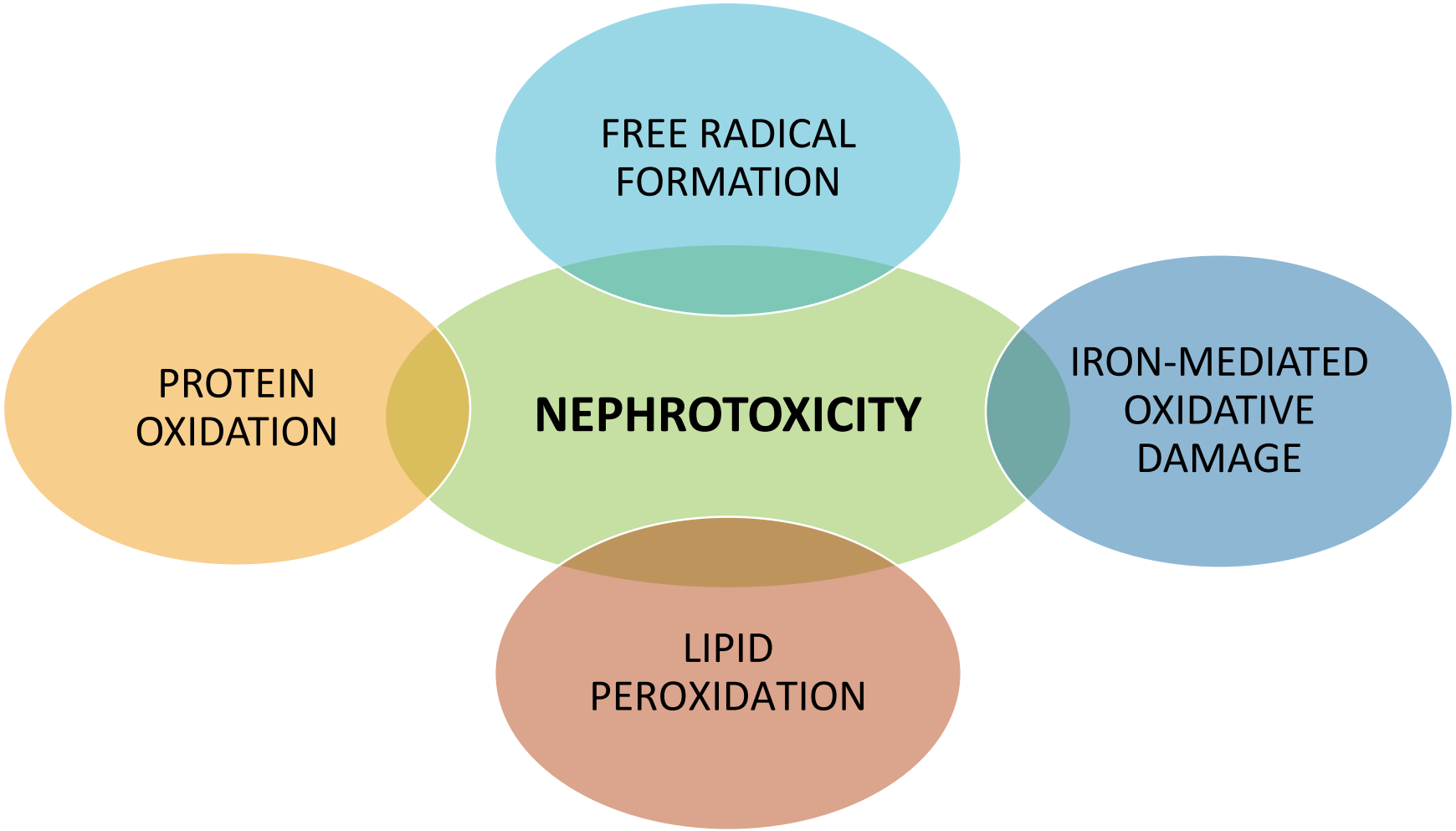
- effective antineoplastic agents for the treatment of a variety of adult cancers

MECHANISM OF ACTION:



DOX LIMITATIONS

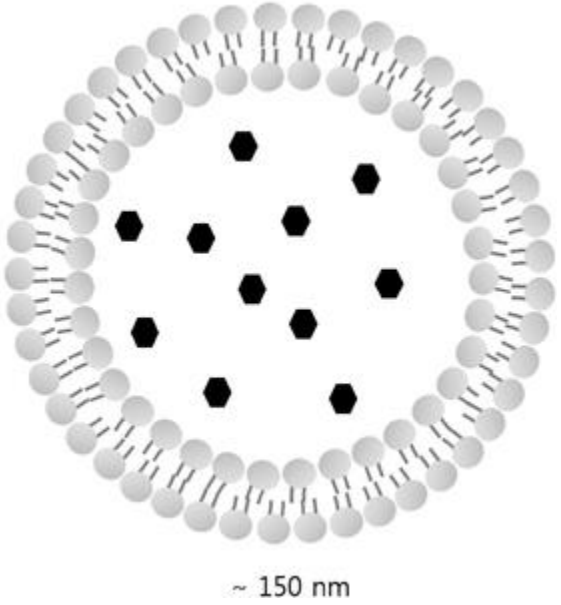
- lack of selectivity for target, short biological half-life, development of DOX resistance, and significant adverse effects including cardiotoxicity and **nephrotoxicity**



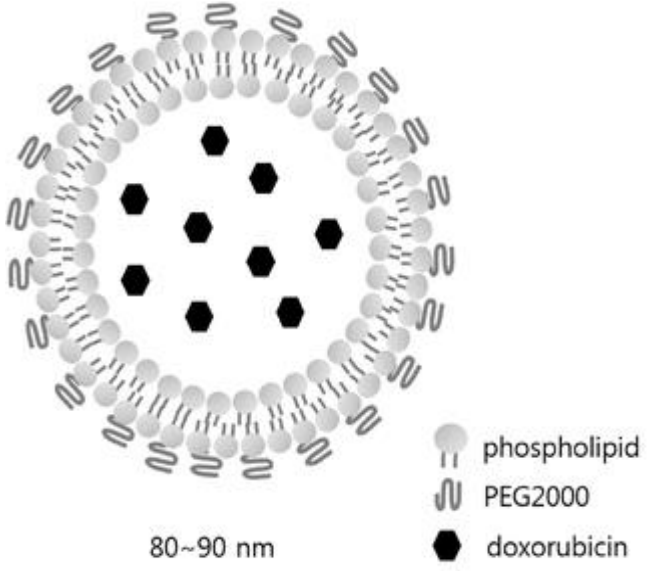
NEW APPROACHES

- encapsulation of DOX into liposomal nanoparticles (liposomes)

NON-PEGYLATED LIPOSOMAL DOX (Myocet™)



PEGYLATED LIPOSOMAL DOX (Doxil®)



NEW FORMULATION

DOX loaded in PLGA nanoparticles (nanoDOX)

MyBiotech

PLGA - poly(lactic-co-glycolic acid)

NON-PEGYLATED LIPOSOMAL DOX, Myocet™ (lipoDOX)

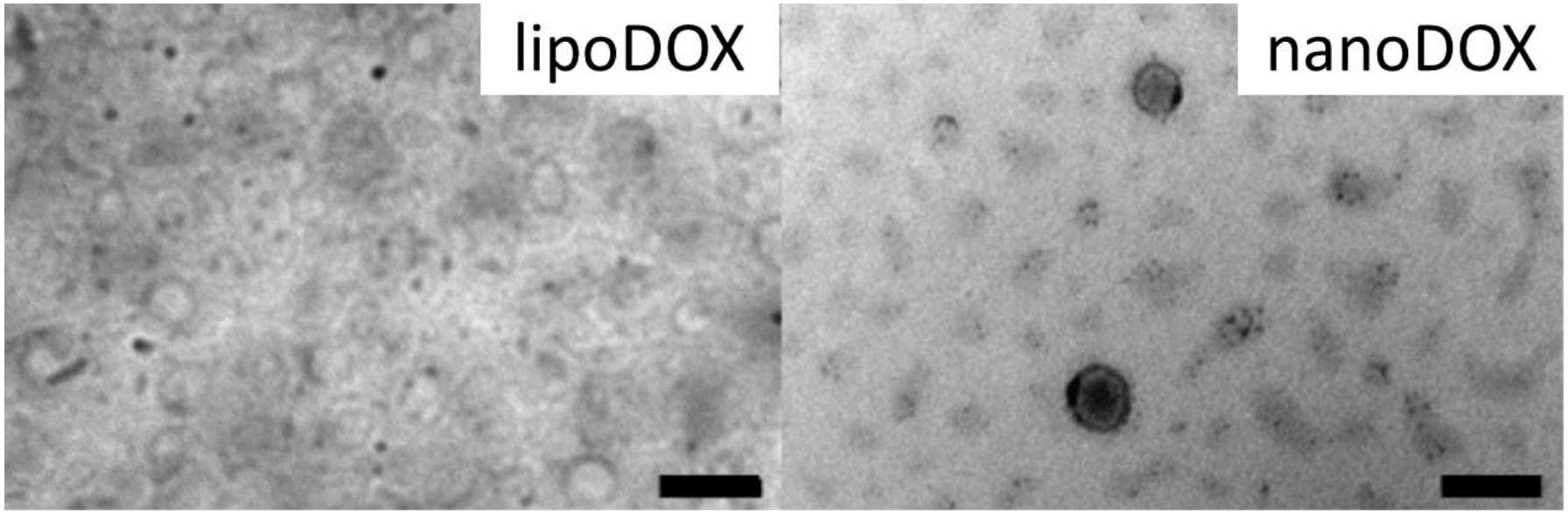
PLIVA teva

CONVENTIONAL DOX (convDOX)

PLIVA teva

CHARACTERIZATION

Transmission electron micrographs:

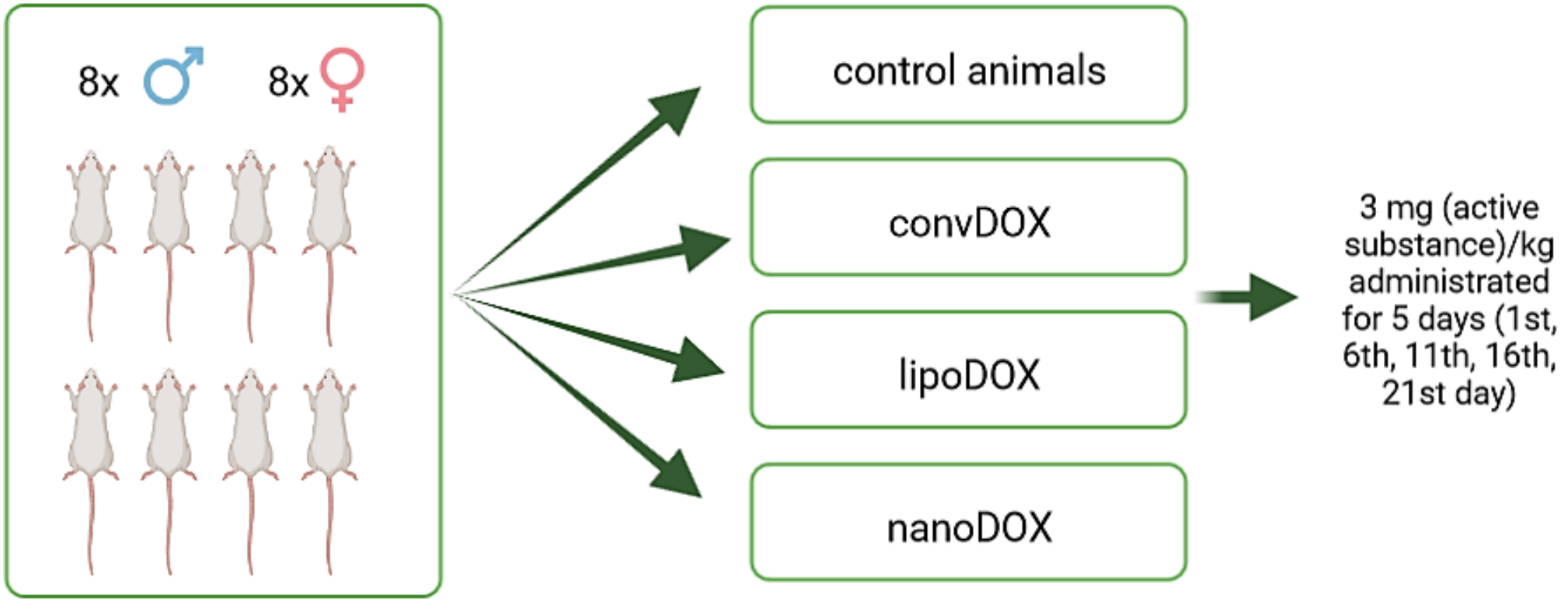


Hydrodynamic diameter (d_H) obtained by DLS and ζ potential obtained by ELS method:

Formulation	d_H / nm (% intensity)	ζ potential /mV
lipoDOX	126.2 ± 1.4 (100 %)	-48.3 ± 0.8
nanoDOX	252.4 ± 12.7 (100 %)	-12.9 ± 0.4

IN VIVO TESTING

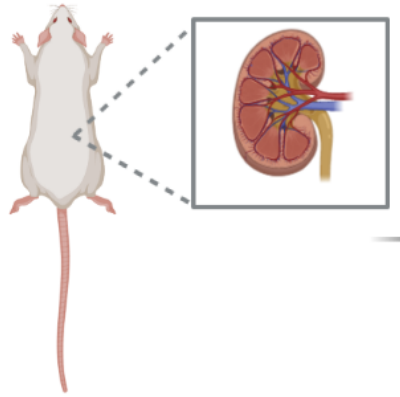
DESIGN OF ANIMAL EXPERIMENTS:



MALDI-TOF MASS SPECTROMETRY IMAGING (MSI)

- In MALDI-TOF mass spectrometry, the ion source is matrix-assisted laser desorption/ionization (MALDI), and the mass analyzer is time-of-flight (TOF) analyzer
- MALDI is appropriate to analyze biomolecules like peptides, lipids, saccharides, or other organic macromolecules
- provide a wide variety of information for the safety profiling of drugs

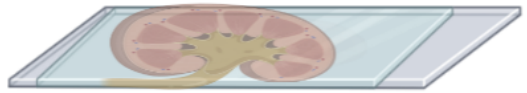
The principle of MALDI- TOF MSI:



1. Tissue harvesting

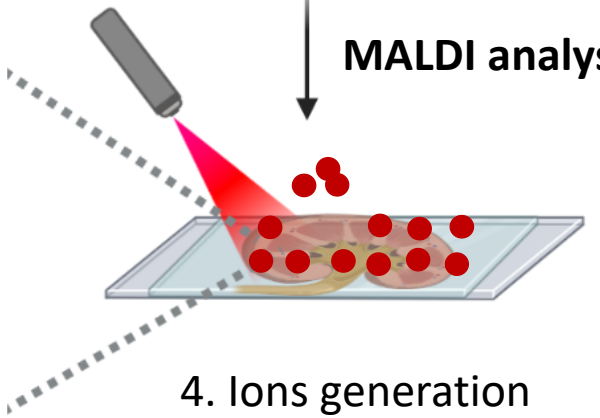
2. Cryosectioning

Transfer on ITO slides

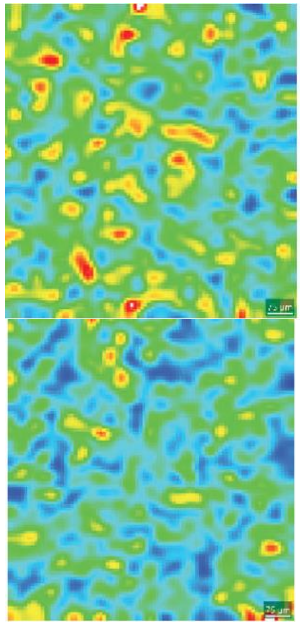


3. Matrix application

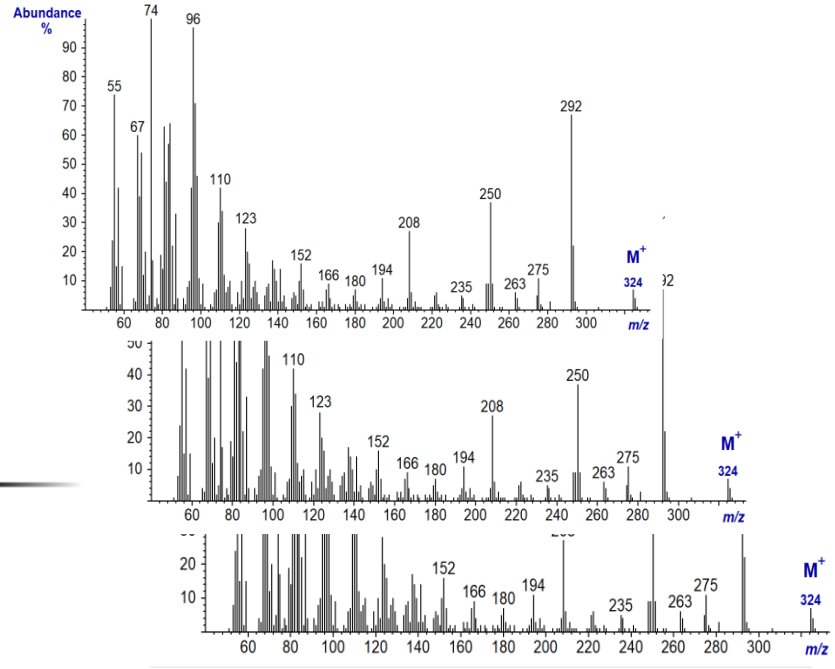
MALDI analysis



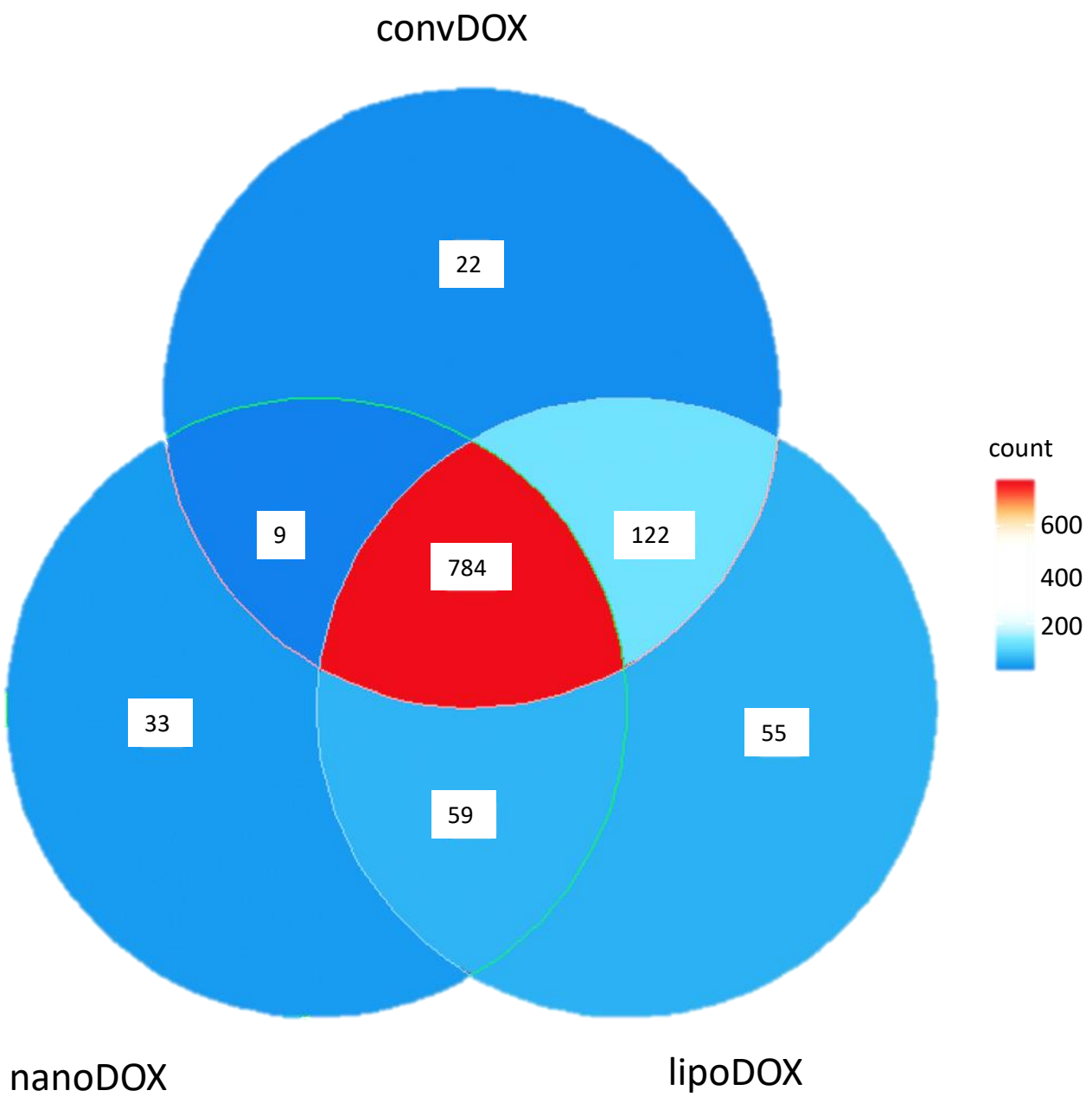
4. Ions generation



MSI images at selected m/z



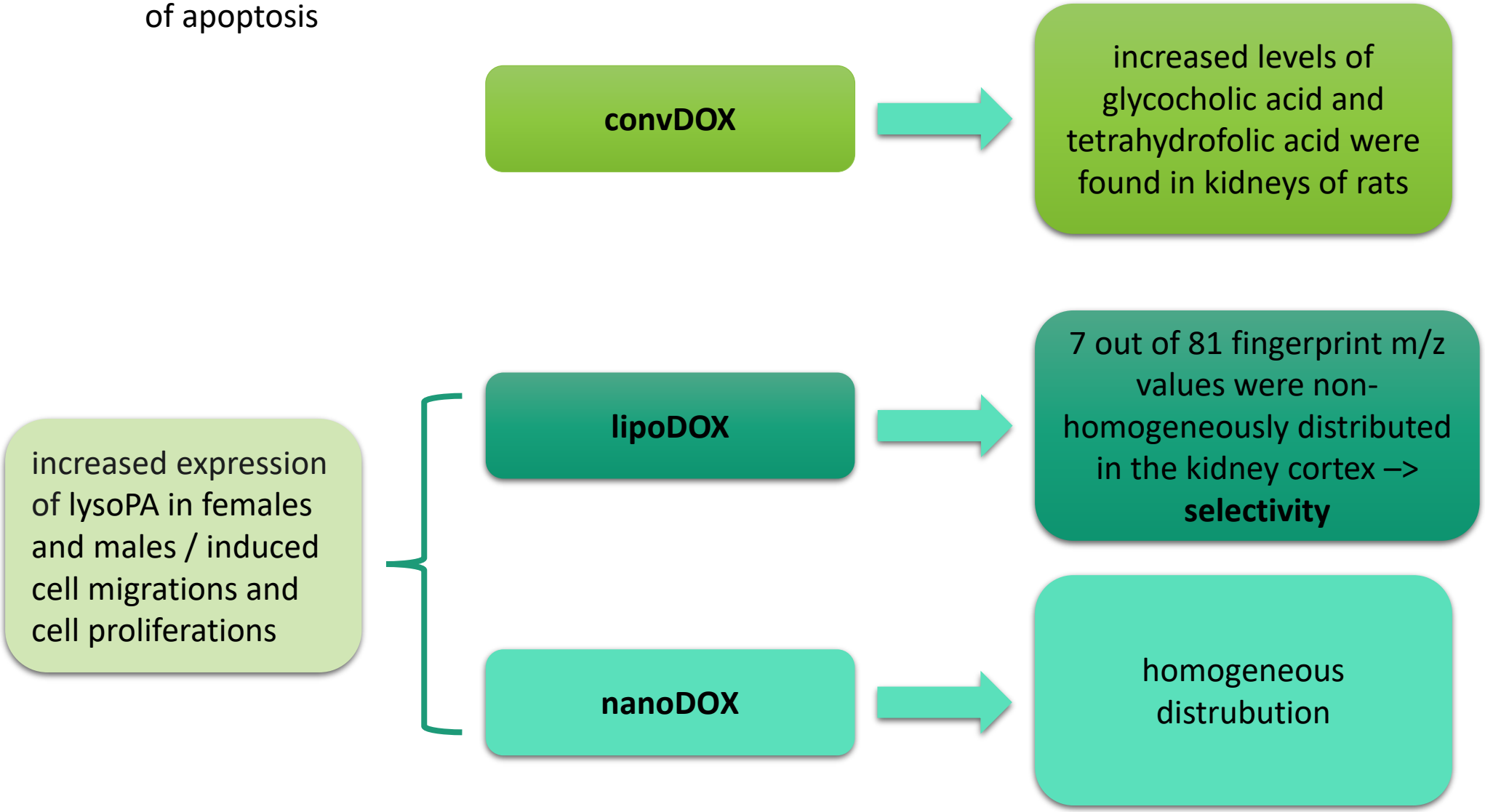
MS spectrum



- 1084 m/z values were significantly changed → the most significantly changed m/z values were shared by all DOX formulations
- 22 significantly changed m/z values formed **convDOX-specific histochemical fingerprints**
- 59 m/z values formed the **nanoformulations-specific histochemical fingerprints**
- nanoDOX and convDOX treatments shared 122 m/z values

HUMAN METABOLOME DATABASE (HMDB)

- 11 unique endogenous metabolites were found → the majority were associated primarily with different mechanisms of apoptosis



CONCLUSIONS

- observed chemical changes in nontargeted tissue were divided into convDOX-specific and nanoformulation-specific fingerprints.
- analysis yielded 22 significant m/z values forming convDOX-specific histochemical fingerprint and 59 significant m/z values that form the nanoformulation-specific fingerprint.
- some of these values were associated with apoptosis in the kidney cortex, an effect shared by all DOX formulations.
- cell migrations and cell proliferations were nanoformulation-specific.
- nanoDOX effects were more similar to the effects of convDOX and were characterized by greater histochemical alterations.
- in contrast to convDOX and nanoDOX, lipoDOX formulation showed some level of selectivity for kidney cortex substructures and it induced the least number of histochemical changes.

FUTURE GOAL: to overcome the lack of selectivity targeting agents, such as aptamers, peptides and monoclonal antibodies should be applied for the delivery of DOX as this approach was found to be successful in improvement of therapeutic index of DOX.

ACKNOWLEDGEMENTS

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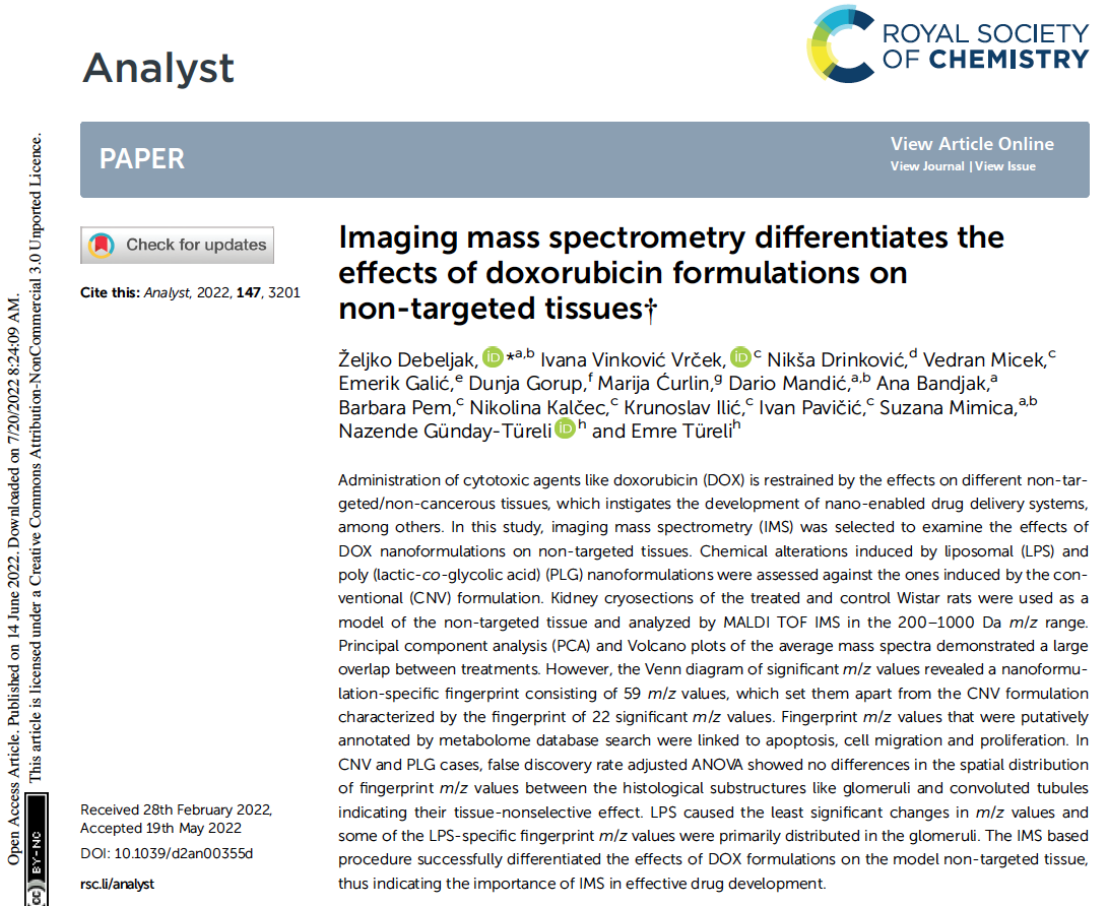

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Imaging mass spectrometry differentiates the effects of doxorubicin formulations on non-targeted tissues†

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Administration of cytotoxic agents like doxorubicin (DOX) is restrained by the effects on different non-targeted/non-cancerous tissues, which instigates the development of nano-enabled drug delivery systems, among others. In this study, imaging mass spectrometry (IMS) was selected to examine the effects of DOX nanoformulations on non-targeted tissues. Chemical alterations induced by liposomal (LPS) and poly (lactic-*co*-glycolic acid) (PLG) nanoformulations were assessed against the ones induced by the conventional (CNV) formulation. Kidney cryosections of the treated and control Wistar rats were used as a model of the non-targeted tissue and analyzed by MALDI TOF IMS in the 200–1000 Da *m/z* range. Principal component analysis (PCA) and Volcano plots of the average mass spectra demonstrated a large overlap between treatments. However, the Venn diagram of significant *m/z* values revealed a nanoformulation-specific fingerprint consisting of 59 *m/z* values, which set them apart from the CNV formulation characterized by the fingerprint of 22 significant *m/z* values. Fingerprint *m/z* values that were putatively annotated by metabolome database search were linked to apoptosis, cell migration and proliferation. In CNV and PLG cases, false discovery rate adjusted ANOVA showed no differences in the spatial distribution of fingerprint *m/z* values between the histological substructures like glomeruli and convoluted tubules indicating their tissue-nonselective effect. LPS caused the least significant changes in *m/z* values and some of the LPS-specific fingerprint *m/z* values were primarily distributed in the glomeruli. The IMS based procedure successfully differentiated the effects of DOX formulations on the model non-targeted tissue, thus indicating the importance of IMS in effective drug development.

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