IOCN 2025 Conference
The 5th International Online Conference on Nanomaterials
22–24 September 2025 | Online
MDPI

Microfluidic production of amiodarone loaded nanoparticles and application in drug repositioning in ovarian cancer

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INTRODUCTION & AIM

Amiodarone (currently used as an antiarrhythmic agent) repositioning in cancer treatment is promising, however toxicity limits seem to arise[1], constraining its exploitability. Notably, amiodarone has been investigated for the treatment of ovarian cancer, a tumor known for metastasizing within the peritoneal cavity. This is associated with an increase of fatty acid oxidation, which strongly depends on CPT1A, a transport protein which has been found overexpressed in ovarian cancer[2,3]. Amiodarone is an inhibitor of CPT1A but its role still has to be explored. The aims of this study is to confirm amiodarone activity over ovarian cancer cell lines, focusing on consequently lipids alteration and the development of drug delivery systems through microfluidics to overcome amiodarone toxicity.

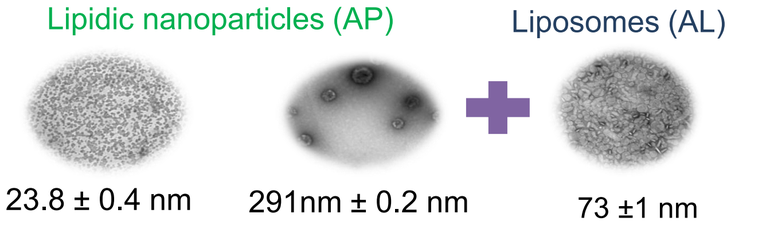
METHOD

Doxil® formulation (HEPC: CHO: DSPE-PEG molar ratio 55:40:5) was used to produce amiodarone loaded liposomes. The lipid mixture was dissolved in ethanol, together with amiodarone, and mixed in the herringbone micromixer glass chip with DPBS. Samples collected from microfluidics appeared as milky solutions, after dialysis and centrifugation it was possible to obtain clear solution of amiodarone liposomes (AL) and milky solution of amiodarone particles (AP). Amiodarone efficacy was assessed in ovarian cancer cells (A2780, Kuramochi and OVCAR-5).It was also tested in anokis resistant cells, i.e. cells forced to grown in suspension. Indeed, anoikis resistant cells are considered to overexpress CPT1A and increase lipid metabolism[3]. Effects of amiodarone on the lipid metabolism of epithelial ovarian cancer cells were evaluated to better explore its potential as an inhibitor of CPT1A, confirming its activity.

RESULTS & DISCUSSION

Amiodarone delivery systems

Lipidic nanoparticles (AP) — Liposomes (AL)

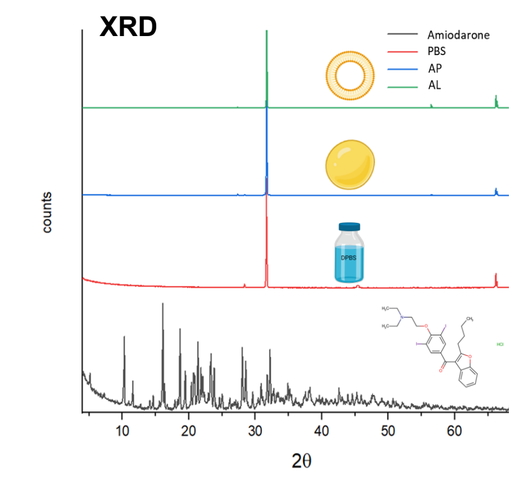


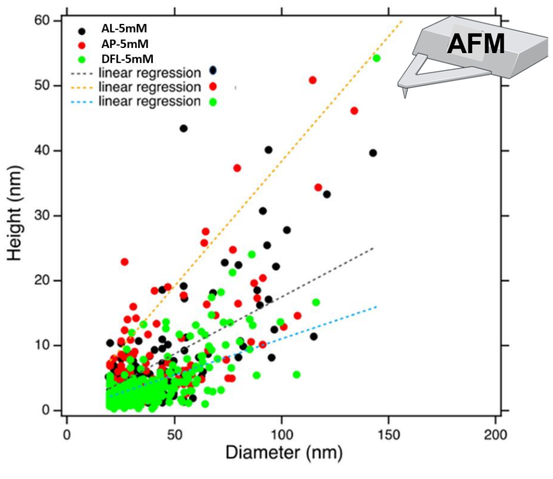
23.8 ± 0.4 nm — 291nm ± 0.2 nm — 73 ±1 nm

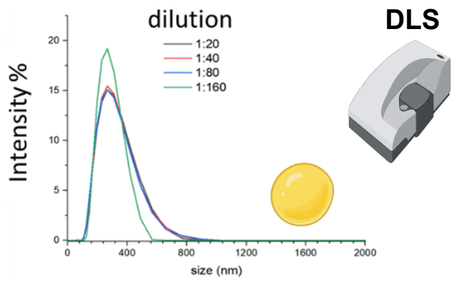
PdI 0.22 ± 0.07 nm — PdI 0.18 ± 0.02 nm

Drug loading > 100%

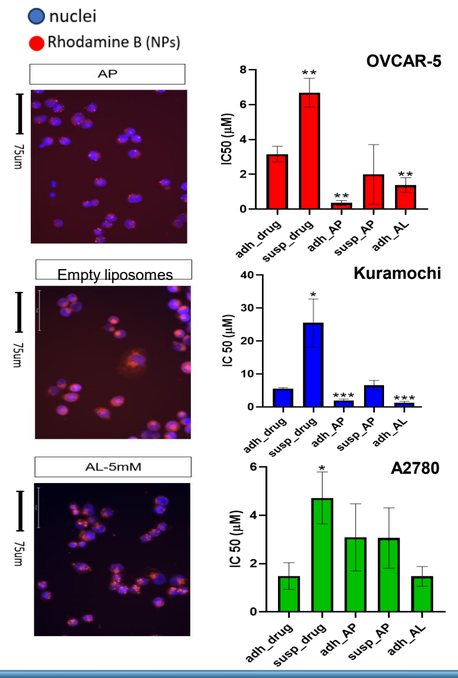
The Amiodarone (AM) encapsulation into AL reached drug to lipid ratio value about 15% w/w (16 mol%) which is analogues to doxorubicin amount in Doxil® formulation (12% w/w), moreover Doxil® lipid composition was maintained.







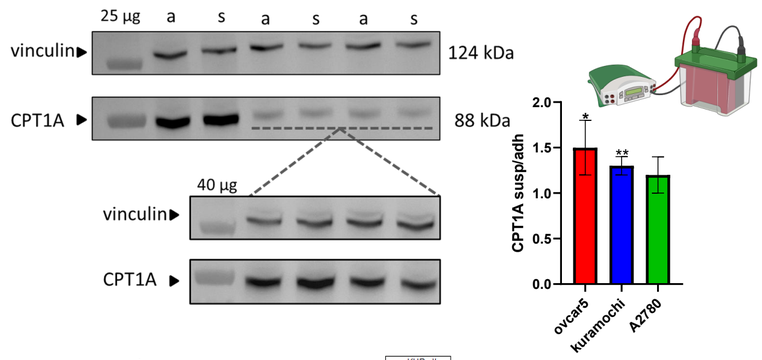
The loading of the drug change elasticity of liposome membranes suggesting that AM is encapsulated into the bilayer. The formation of NPs with strong tendency toward aggregation was proved, with DLS and AFM. The nano-components of the aggregates in AP samples showed stability over dilution. The amorphous state of the drug was proved in both nanoparticles formulation by X ray diffraction analysis.

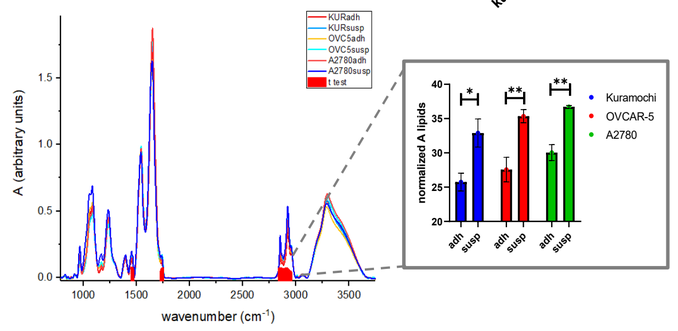


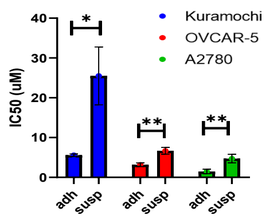
AP in suspension leads to IC50 values comparable to ones obtained of free drug in adhesion cultures. AL were active in adhesion culture, where both NPs formulations decreased the IC50 values for Kuramochi and OVCAR-5, while IC50s were comparable to the one of the free drug for A2780. SKOV3 are known to form spheroids when forced to grow in suspension, hence it can represent a good model to evaluate the permeability of AP. Therefore, AP were tested on SKOV3 spheroids obtaining an IC50 value equal to 2±1μM comparable with the one obtained for the other cell lines, while the free drug seems to be ineffective.

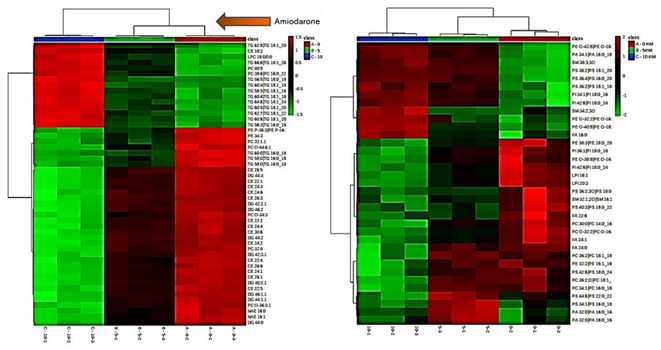
Internalization of lipidic particles was evaluated by incubation of A2780 treated by fluorescently labelled particles. All the tested particles have been proved to be internalized given the red fluorescence coming from the cytosolic compartment of cells.

CPT1A and Amiodarone









To evaluate if the CPT1A was overexpressed in suspension cultures, western blot analysis was performed. Western blot results revealed that OVCAR-5 has higher level of CPT1A compared to A2780 and Kuramochi. Comparing the two different cultures, a modest increase of expression of CPT1A for suspension culture was determined for OVCAR-5 and Kuramochi. Fourier transformed infrared spectroscopy (FTIR) analysis revealed significantly different profile for adhesion and suspension cultures, with differences in spectral areas that can all be related to lipids.

Amiodarone cytotoxicity was evaluated both in adhesion and suspension culture obtaining IC50 values in line with a previous study and analogue to the values obtained for cisplatin[4]. To evaluate if amiodarone acts as an inhibitor of CPT1A and therefore influence lipids, lipidomics analysis was performed on A2780 cultured in adhesion treated for 96 hours with the drug at concentration equal to 5 and 10 μM. AM determined a significant alteration of the lipidome of cells.

CONCLUSION

Amiodarone was encapsulated into lipidic delivery systems trough microfluidics obtaining simultaneously liposomes with high drug content and nanoparticles mainly composed by the drug. The role of CPT1A in the amiodarone efficacy over epithelial ovarian cancer models was investigated. CPT1A was proven by western blot to be overexpressed in suspension cultures of A2780, Kuramochi, OVCAR-5 even if the increment is only determinable after densitometry analysis since the fold increase is modest. This result is also confirmed by FT-IR, which revealed a significative different profile for adhesion and suspension culture with lipids peaks being the main responsible of the difference. The amiodarone resulted efficient in reducing cell viability, however its effect is lower in suspension condition. Lipidomics profile of treated and not treated cells revealed a significant decrease of lipids content in treated cells which is in line with the inhibition of CPT1A. However, in order to better evaluate amiodarone CPT1A inhibition features the evaluation of fatty acid oxidation rate, such as by SeaHorse Flux Analyzer, would be useful. The reduced ability of amiodarone in suspension cultures could be determined by cell aggregate resistance to drug diffusion. Nanoparticles has been proven to be particularly useful to restore amiodarone effect in suspension cultures and hence overcome diffusion limit, while liposomes were effective only in adhesion culture[5]. However, these two formulations could be suitable for different administration routes. In vivo studies would be useful to fully understand the potential clinical used of these amiodarone delivery systems.

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