3D tumour spheroids for the prediction of the effects of radiation and treatment with antimicrobial peptides

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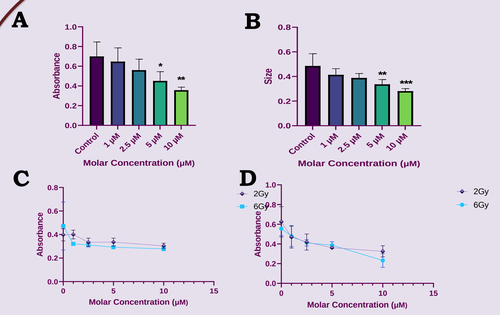
Introduction

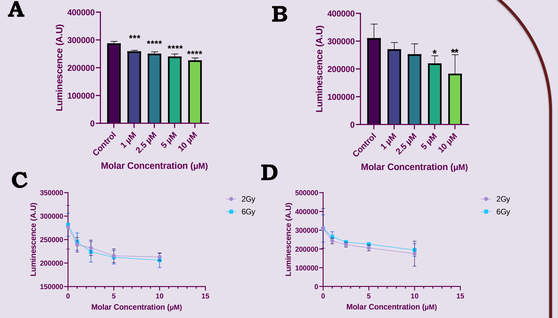
In the last years, the indiscriminate use of conventional antibiotics has generated a worrisome increase of resistant pathogens. Antimicrobial peptides (AMPs) are considered a plausible alternative therapy against pathogens due to their structural and functional characteristics, as well as their low toxicity against eukaryotic cells and their broad spectrum of action against different pathogens, including Gram-negative and Gram-positive bacteria, fungi, parasite and virus[1]. Interestingly, AMPs also have the capability to recognize certain types of plasma membranes, and this selectivity allows differential recognition of normal cells, non-malignant tumor cells and malignant tumor cells; thereby the use of these AMPs could be a viable alternative for cancer treatment. These peptides can be isolated from different organisms, such as microorganisms, plants and animals. Such peptides are amphipathic and cationic molecules of low molecular weight and they have a low probability to generate resistance. Therefore these natural peptides have been utilized as the base for synthetizing new analog peptides with chemical or structural modifications for improving their antimicrobial stability and efficiency [2].

In our study, we focused on a Cecropin A-Melittin hybrid and proton irradiation treatment combination to observe its possible use as a cancer treatment.

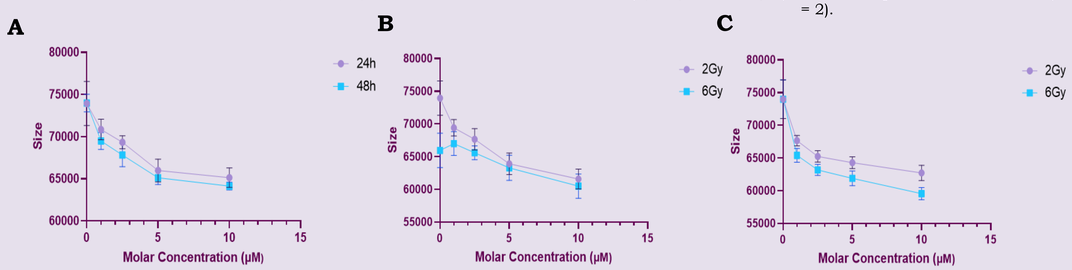
Viability of spheroids after treatment



(A)(B) MTT analysis after treatment of HCT-116 spheroids (24 and 48h), with the hybrid Cecropin A – Melittin (CA-Mel) (1, 2.5, 5 and 10 μM).(C)(D) MTT measued after combination of treatment (proton radiation and CA-Mel) after 24h (C) and 48h (D). The spheroids were irradiated with 2 and 6Gy and then treated with CA-Mel (1, 2.5, 5 and 10 μM).



(A)(B) ATP analysis after treatment of HCT-116 spheroids (24 and 48h), with the hybrid Cecropin A – Melittin (CA-Mel) (1, 2.5, 5 and 10 μM).(C)(D) ATP measured after combination of treatment (proton radiation and CA-Mel) after 24h (C) and 48h (D). The spheroids were irradiated with 2 and 6Gy and then treated with CA-Mel (1, 2.5, 5 and 10 μM). Data are expressed as mean ±SD (n = 2).



(A) Size measure after treatment of HCT-116 spheroids (day 5 of their evolution), with the hybrid Cecropin A – Melittin (CA-Mel) (1, 2.5, 5 and 10 μM).(B) Combination of treatment (proton radiation and CA-Mel) after 24h. The spheroids were irradiated with 2 and 6Gy and then treated with CA-Mel (1, 2.5, 5 and 10 μM).(C) And the same combination treatment after 48h.

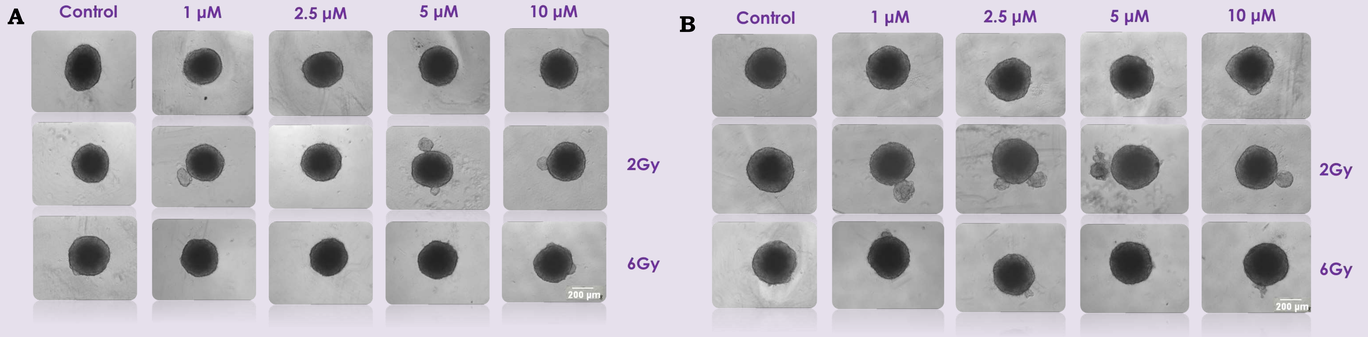
Cytotoxic effects of CA-Mel and proton radiation on HCT-116 tumoral spheroids

Cell line: HCT116 is a human colon cancer cell line used in therapeutic research and drug screenings. HCT116 cells are used in a variety of biomedical studies involving colon cancer proliferation and corresponding inhibitors. The cell line has been used in tumorigenicity studies. Cells were grown in DMEM (Dulbecco's Minimum Essential Medium) supplemented with 10 % fetal calf serum (FCS), 100 units/mL of penicillin and 100 μg/mL of streptomycin at 37 °C in a humidified incubator under an atmosphere containing 5 % CO_2.

MTT analysis: The cell viability was assessed using a MTT assay. First, the BJ cells were plated into 24 well plates. After 24h of growing the cells in the presence of the nanoplatforms, the medium and the discs were removed from the wells, which were then incubated with a final concentration of 1 mg/mL of MTT. After 4 h, the medium was removed and DMSO was added to dissolve the formed crystals. The optical absorbance was recorded at 570 nm using the plate reader Mithras LB 940 (Berthold, Germany) and the absorbance values of blank wells (only DMSO) were extracted in order to calculate the cell viability.

Spheroid Formation and Analysis : A concentration of 5000 cells/well of HCT-116 cells was seeded. A final volume of 200 μL of cell suspension was placed in each well of a clear, round bottom, ultra-low attachment 96-well microplate (Corning, NY, USA). After this, the plate was centrifuged for 2 min and then incubated at 37 ◦C for up to 5 days. Spheroid formation was confirmed by observing the plate under a light microscope (Olympus CX23 Binocular Microscope, Düsseldorf, Germany). Spheroids were monitored daily and the incubation medium was replaced every 3 days.

ATP Assay: ATP levels in the treated spheroids were assessed, as will be described below. Here, 100 μL of medium was removed from each well, then the remaining 100 μL with the spheroid was transferred into an opaque 96-well plate. After this, 100 μL of CellTiter-Glo® reagent (Promega, Madison, WI, USA) was added onto the spheroids, which were incubated at room temperature for 10–15 min under thorough shaking to make sure that the spheroids were broken. Finally, the luminescence of the cells was measured using the plate reader.



(A) Images acquired after treatment of HCT-116 spheroids (24h), with the hybrid Cecropin A – Melittin (CA-Mel) (1, 2.5, 5 and 10 μM) and combination of treatment (proton radiation and CA-Mel). The irradiated spheroids at 2Gy show the presence of vesicles and at 6Gy massive necrotized areas. (B) Images acquired after treatment of HCT-116 spheroids (48h), with the hybrid Cecropin A – Melittin (CA-Mel) (1, 2.5, 5 and 10 μM) and combination (proton radiation and CA-Mel). The spheroids that were subjected to proton radiation and CA-Mel show multiple vesicles and slight deterioration of the outer layer which confirm the possible destructive effects of the sinergy between our two types of treatment.

Conclusions

- The formed spheroids of tumoral nature were affected significantly by our scheme of treatment showing the benefits that occur with the use of combination treatments which can reduce toxicity and possible appearance of internal lesions.
- The antimicrobial activity of the hybrid CA-Mel peptide applied on the spheroids was proved to be significantly enhanced by the presence of proton irradiation. Moreover, the sinergy between the two types of treatment should be studied more in case other combinations could prove even more appropriate.

References

[1] Zasloff, M. (2002) Nature 415, 389–395.
[2] Baindara, P., Korpole, S., and Grover, V. (2018), Appl. Microbiol. Biotechnol. 102, 10393–10408.

Acknowledgments

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