Antimicrobial peptides: New therapy protocol that enhances the effects of radiotherapy

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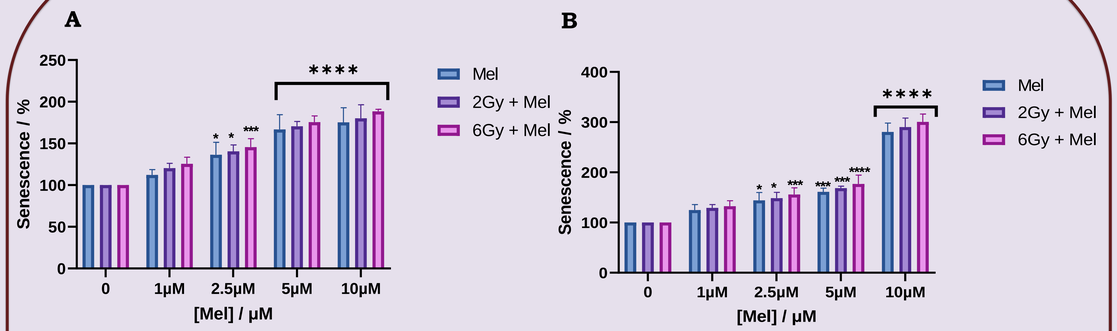
Introduction

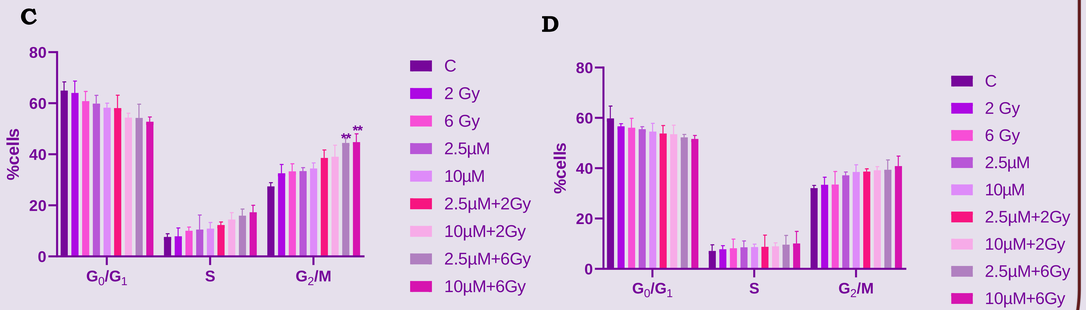
In the last years, the indiscriminate use of conventional antibiotics has generated a worrisome increase of resistant pathogens. Antimicrobial peptides (AMPs) are considered a plausible alternative therapy against pathogens due to their structural and functional characteristics, as well as their low 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 Melittin and proton irradiation treatment combination to observe its possible use as a cancer treatment.

Viability of spheroids after treatment



(A)(B) Senescence analysis after treatment of HCT-116 spheroids, (A)-24 and (B)- 48h, with Melittin (Mel) and also with proton irradiation. The spheroids were irradiated with 2 and 6Gy and then treated with Mel (1, 2.5, 5 and 10 µM).



(A)(B) Cell cycle analysis after treatment of HCT-116 spheroids, (A)-24 and (B)- 48h, with Melittin (Mel) and also with proton irradiation. The spheroids were irradiated with 2 and 6Gy and then treated with Mel (1, 2.5, 5 and 10 µM).

Cytotoxic effects of 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.

Spheroid Formation and Analysis: A cell density of 5000 cells/well of HCT-116 cells was seeded. A final volume of 200 µL of cell suspension was placed in the wells 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 4 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.

Irradiation setup: Proton beams were delivered by a TR-19 cyclotron, built by Advanced Cyclotron Systems Inc., (Vancouver, Canada). It is a versatile, fully automated device that vertically accelerates negative ions (H-) from an external source to an energy of 14-19 MeV. TR-19 is equipped with two proton extraction ports by stripping hydrogen ions using pyrolytic carbon sheets, which can operate simultaneously. The proton beam is previously spread on metal foils and a small number of protons are selected to obtain dose rates of the order of 1Gy / min. The doses which proved most effective were 2 and 6 Gy.

Senescence measurements: After the treatment (24 and 48 h) the spheroids were washed with PBS, fixated with a 2% paraformaldehyde solution for 10 minutes. Washed the spheroids in a 10% BSA solution in order to remove the fixation solution and then proceeded to stain the spheroids with the CellEvent™ Senescence Green Probe provided by the CellEvent™ Senescence Green Detection Kit (ThermoFisher Scientific, Waltham, MA USA) and incubated for 2 and half hours at 37 °C without CO2 . After incubation wash the spheroids with PBS and image using an Alexa Fluor™ 488/FITC filter set using a plate reader (Mithras LB 940).

Cell cycle analysis: After 24 and 48 h of proton irradiation, the spheroids were harvested, trypsinized to detach the cells from the spheroidal shape and fixed with pre-chilled 70% ethanol at −20°C. Following, the cells were incubated with 0.2 mg/ml RNase and 20 µg/ml propidium iodide (PI) in a 0.1% Triton TX-100 solution in the dark for 30 min at 37 °C. Cell cycle distribution was analysed by flow cytometry using a Beckman Coulter Cell Lab Quanta SC Flow Cytometer, 771917 Laser, Arc, MPL flow cytometer and data analysed using the Quanta Analysis software.

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 applied melittin peptide on 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

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[2] Baindara, P., Korpole, S., and Grover, V. (2018), Appl. Microbiol. Biotechnol. 102, 10393–10408.

Acknowledgments

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