Melittin: a new aim in cancer therapy

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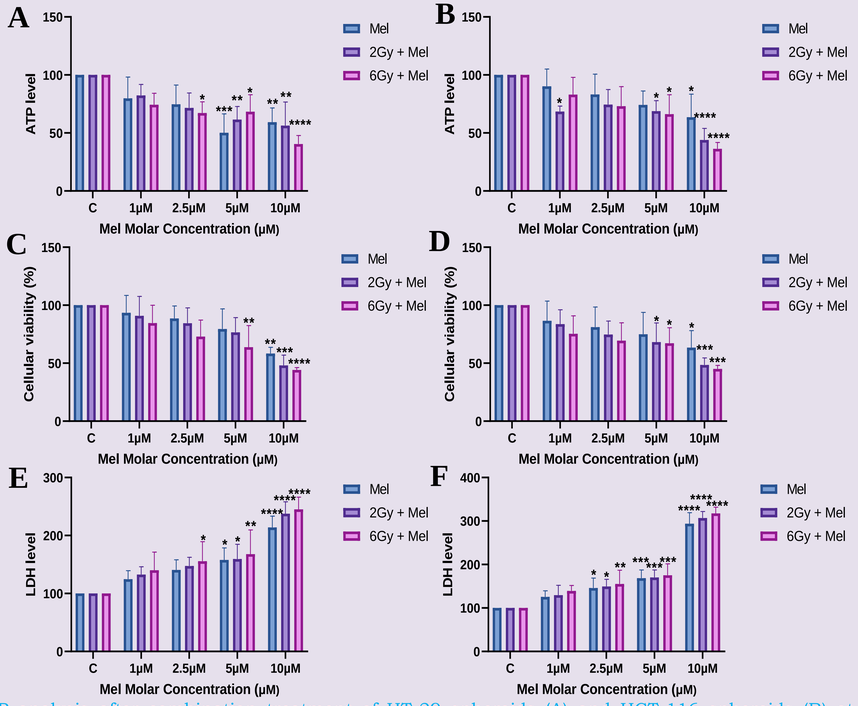
Introduction

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]. MEL (Mellitin) is the main component of BV and the principal toxin which constitutes approximately 50% of its dry matter. MEL is a small linear basic peptide with the chemical formula $C_{13}1H_{228}N_{38}O_{32}$ consisting of the known 26 amino acid sequence, weighing 2847.5 Da, which has a powerful haemolytic activity. Moon et al. (2008) showed that MEL induced apoptosis in leukemic cells through the up-regulation of Bax and caspase3 activation and down-regulation of Bcl-2 and the inhibitor of apoptosis protein (IAP) family members [2]. In vivo MEL resulted in a significant inhibition of the tumour cell growth in Balb/c nude mice. Using hepatocellular carcinoma cell lines (metastatic HCC cell) and an animal model (nude mice), it was found that MEL inhibited tumour cell metastasis by reducing cell motility and migration via the suppression of Rac1-dependent pathway. These findings suggested that MEL could also be used as a chemotherapeutic agent against tumours in vivo [3]. 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) ATP analysis after combination treatment of HT-29 spheroids (A) and HCT-116 spheroids (B) at 48h), with Melittin (Mel) (1, 2.5, 5 and 10 μM) and proton irradiation (2 and 6 Gy). (C)(D) MTT measurement after combination of treatment (proton radiation and Mel) of HT-29 spheroids (C) and HCT-116 spheroids (D) after 48h . The spheroids were irradiated with 2 and 6Gy and then treated with Mel (1, 2.5, 5 and 10 μM). (E)(G) LDH analysis after combination treatment of HT-29 spheroids (E) and HCT-116 spheroids (F) at 48h, with Melittin (Mel) (1, 2.5, 5 and 10 μM) and proton irradiation (2 and 6 Gy). Data are expressed as mean ±SD (n = 3).

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

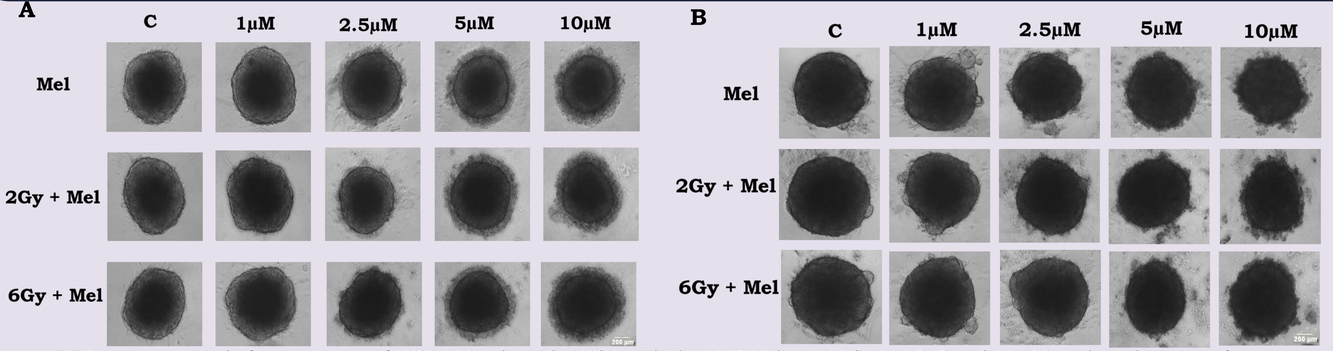
Cell line: HCT116 is a human colon cancer cell line used in therapeutic research and drug screenings. HCT116 cells are used in a variety of biomedical studies involving colon cancer proliferation and corresponding inhibitors. The cell line has been used in tumorigenicity studies. Cells were grown in 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.

LDH Assay: In the LDH assay protocol, LDH reduces NAD to NADH, which then interacts with a specific probe to produce a color (OD max = 450 nm). The kit can detect 1 - 100 mU/mL of LDH directly in samples. The assay is quick, convenient, and sensitive.



(A) Images acquired after treatment of HCT-116 spheroids (48h), with the AMP Melittin (Mel) (1, 2.5, 5 and 10 μM) and combination of treatment (proton radiation and Mel). The irradiated spheroids at 2Gy show the presence of a cloud of detached cells and at 6Gy massive necrotized areas. (B) Images acquired after treatment of HT-29 spheroids (48h), with the AMP Melittin (Mel) (1, 2.5, 5 and 10 μM) and combination (proton radiation and Mel). The spheroids that were subjected to proton radiation and Mel show a massive level of deterioration of the outer layer which confirms 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 Melittin 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

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Acknowledgments

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