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NANYANG TECHNOLOGICAL UNIVERSITY

URECA
Undergraduate Research Experience on CAMpus

Category: Biomedical and Medicine **Student: Koh Phay Chung** **Project ID: LKCM15014**
Lee Kong Chian School of Medicine

Preclinical safety and immunogenicity evaluation of bioactive antimicrobial peptides

Background

Bioactive peptides with antimicrobial properties have many potential applications, ranging from food preservation to the treatment of drug-resistant infections. However, some of these peptides may induce unwanted toxicity or immunogenicity. Regulatory agencies, therefore, require rigours safety assessments of all the therapeutic peptides using appropriate biological model systems.

Our collaborating group has developed a novel series of AMPs with broad spectrum antimicrobial activities.

Aim

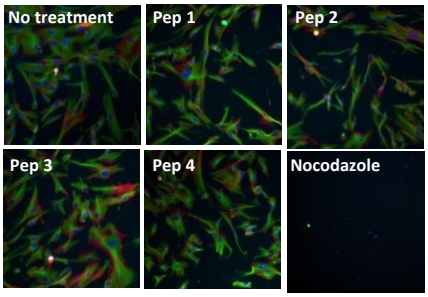
To examine possible cytotoxicity and immunogenicity (if any) of the four AMPs (Pep 1, Pep 2, Pep 3, & Pep 4) using cell culture model systems .

Methodology

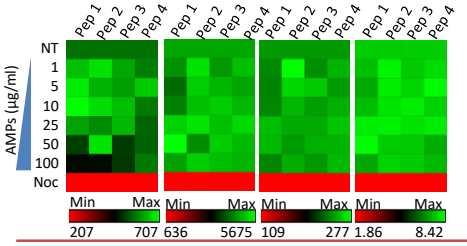
1. Human primary T-cells, dermal fibroblasts, HEK293 kidney cells and A549 lung epithelial cells.
2. Multi-parametric High Content Analysis using INCell Analyzer 2200 automated microscope.
3. MTT-based cell viability assay.
4. Impedance-based real-time cell proliferation monitoring.
5. Enzyme-Linked Immuno-Sorbent Assay (ELISA) to quantify secreted levels of cytokines (IL-2, IFNγ, and IL-4).

Results

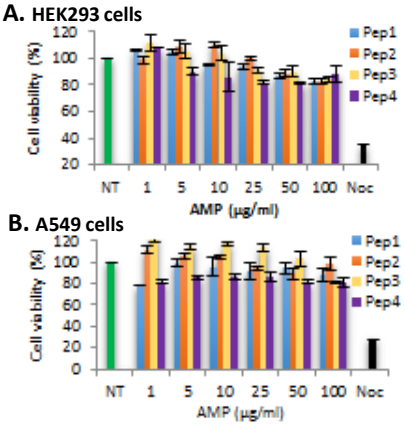
1. The selected four AMPs did not interfere with the morphology or cytoskeletal systems in human dermal fibroblasts. Cells were treated with Pep 1, Pep 2, Pep 3 or Pep 4 up to 100 µg/ml for 24h, fluorescently stained for α-Tubulin (green), Actin (red) & Nucleus (blue), and imaged using an automated microscope.



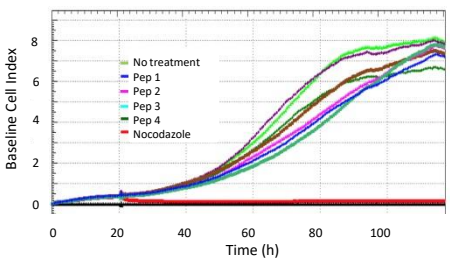
2. High Content Analysis showed no detectable alteration in cellular phenotypes in dermal fibroblasts due to AMPs. Cells were treated with increasing concentrations of AMPs for 24h and cell phenotypic parameters were quantified. *NT*, untreated cells; *Noc*, nocodazole (control).



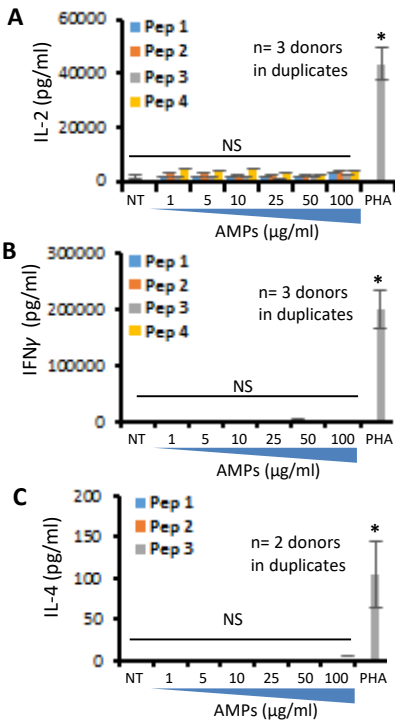
3. All the four AMPs were non-cytotoxic. HEK293 (**A**) and A549 (**B**) cells were treated with AMPs (1 to 100 µg/ml, 24h) and cell viability was analysed by MTT assay.



4. AMPs did not interfere with cell adhesion or proliferation. Live cell proliferation monitoring of A549 cells treated with 100 µg/ml AMPs for up to 96h using impedance-based real-time measurements.



5. AMPs did not induce the secretion of pro-inflammatory cytokines in primary human T-cells. Cells were treated with AMPs (1 to 100 µg/ml) and supernatant media were collected after 24h. Secreted levels of IL-2 (**A**), IFNγ (**B**) and IL-4 (**C**) were determined by ELISA. *NT*, no treatment; *PHA* 1.5% used as controls. *NS*, not significant; *, $p > 0.01$



Conclusion

- All the tested AMPs are non-cytotoxic and non-immunogenic.
- The above AMPs are safe for potential *in vivo* applications.

On-going and Future Work

- Comprehensive assessment using multiple model systems is under progress. Possibility of anti-drug antibody response needs to be tested *in vivo*.

Project Title: Preclinical safety and immunogenicity evaluation of bioactive antimicrobial peptides.

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