Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.178*

Unravelling the Role of Membrane Active Peptide CorTS 1

Aditi Arora[1], Sujithra Shankar[1], Sushmita G Shah[2], and Archana Chugh[1]*
[1]Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi, 110016 India;
[2]Senior Consultant Ophthalmologist, Eye Life Hospital, Mumbai, 400052 India

Introduction

Quality of human life is significantly affected by impaired vision. According to a recent WHO estimate, at least 2.2 billion people around the world suffer from vision impairment or blindness, out of which in almost 1.2 billion people the occurrence of the condition could have been prevented or treated [1]. Human eye is a unique organ with its intricate anatomical structure involving complex arrangement of tissue layers. Several physiological and anatomical barriers such as ocular static and dynamic barriers, nasolachryimal drainage, tear turnover, reflex blinking prevent permeation of any exogenous material into ocular tissues. Consequently, it becomes difficult to achieve desirable therapeutic drug concentration in different eye segments [2]. Hence, various strategies are being developed for efficient drug delivery to deeper ocular tissues.

Cell penetrating peptides (CPPs)-based therapeutics is one such promising approach that employs bioactive peptides with cell permeating potential and the capacity to enhance the curative effects of drugs. Cell penetrating peptides have been mainly employed for intracellular delivery of cargoes such as nanosized nucleic acids, small molecule drugs and drug carrying nanoparticles. Recently studies have demonstrated successful application of CPPs for efficient drug delivery to ocular tissues as well [3-6]. Investigations into the immunomodulatory effects of CPPs in ocular diseases have also been reported [7-10]. Hence, further research in different aspects of CPP functions can facilitate the development of optimized therapy against various infectious and non-infectious ocular diseases.

Our group has developed a novel corneal targeting CPP- CorTS 1 (Corneal Targeting Sequence 1) for efficient drug delivery to anterior eye segment [11]. CorTS 1 has been designed by substituting Arginine residue in lieu of X in conserved LRR motifs- LXXLXLXXNXL. Based on the sequence composition it was hypothesized that the arginine residues will confer the peptide with cell permeating potential and presence of leucine residues will aid interaction of peptide with collagen rich extracellular matrix of stromal layer of cornea. Thus, aiding in development of a corneal targeting cell penetrating peptide. The peptide shows a dose dependent uptake via both energy dependent and energy independent mechanism in Human Corneal Epithelial cell line (HCE) without any effect on cell viability. Interestingly, potent antimicrobial action against MRSA and *Fusarium dimerum* has also been shown by the peptide [11]. Encouraged by this finding we are further investigating the role of CorTS 1 in immune-modulation by evaluating its effect on proinflammatory cytokines. Moreover, interaction of peptide with plasma membrane of HCE cells has been also assessed using scanning electron microscopy (SEM) to gain better understanding of internalization pathway.

Results and Discussion

In our previous study we have reported that for its internalization in HCE cells, CorTS 1 employs both ATP dependent (such as via endocytosis) and independent pathways. Herein, to gain further insight into the mechanism of uptake we have performed co-localization study with lysotracker DND-99 dye and FITC labelled CorTS 1. As shown in Figure 1, both yellow (from superimposition of green labelled peptide and red lysotracker stain) and green colored vesicular structures are observed in CorTS 1 treated cells suggesting that the peptide is utilizing both endosome dependent and independent pathways for its translocation in HCE cells. Thus, corroborating the results obtained in our prior study.

Furthermore, we have investigated the nature of interaction between the peptide and cell membrane of HCE cells using SEM. Interestingly, no significant changes in the topology of cell membrane were observed on treatment with CorTS 1 (Figure 2). Therefore, further experiments are required for better understanding of nature of direct penetration pathways that CorTS 1 utilises for its entry in HCE cells.

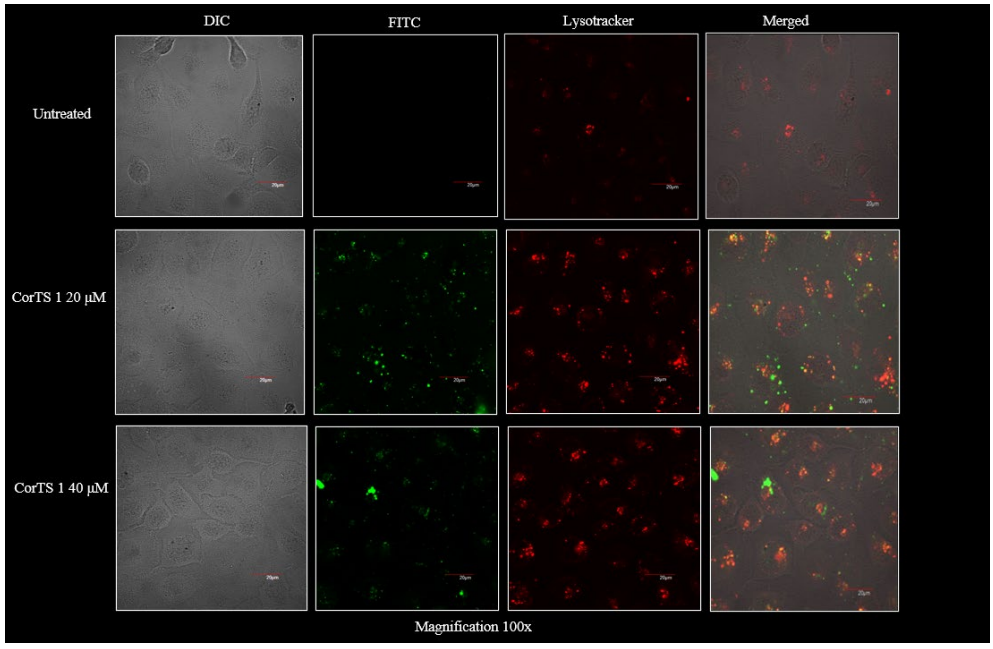

Fig. 1. Subcellular localization of CorTS 1 peptide in HCE cells as examined by staining the cells with lysotracker red DND-99.

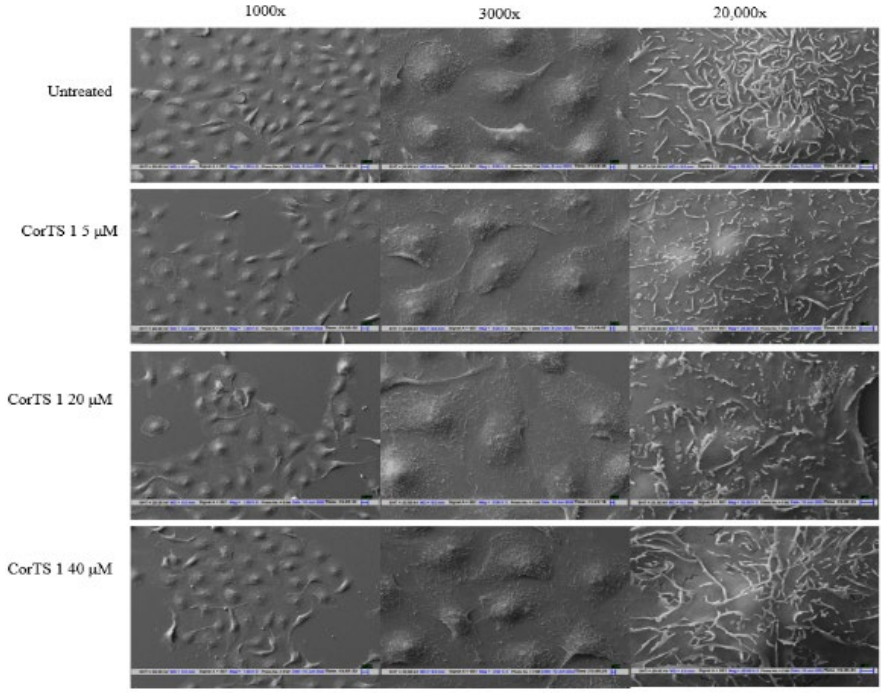

Fig. 2. SEM analysis of HCE cells after CorTS 1 treatment for 15 minutes at 37℃ and 5% CO_2.

CorTS 1 has also demonstrated strong antimicrobial action against deadly microbes including MRSA and *Fusarium dimerum* with MIC values of 10.53µM and 21.05 µM, respectively [11]. Based on these findings we were encouraged to investigate if the peptide also possesses immunomodulation potential. For this purpose, the effect of peptide treatment on the release of TNF-α by LPS stimulated THP1 cells was evaluated using ELISA. Preliminary studies have shown an approximately 2.3 times reduction in the TNF-α levels on application of CorTS 1 at a concentration of 20 µM.

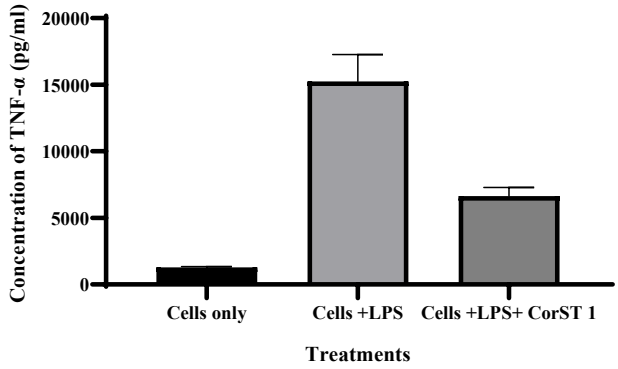

Fig. 3. Evaluation of TNF-α levels in LPS stimulated THP1 cells on CorTS 1 treatment.

Additionally, further investigation into the role of CorTS 1 in immunoregulation and management of microbial keratitis are currently underway.

Acknowledgements

Authors are thankful to the Central Research Facility of IIT Delhi for providing facilities of Scanning electron microscopy and Confocal microscopy. The authors are also thankful to Dr Vivek Singh, LV Prasad Eye Institute, Hyderabad for providing HCE cell line for the study. AA is thankful to Indian Institute of Technology, Delhi; Department of Biotechnology- Conference, Travel, Exhibition and Popular Lectures (CTEP) programme and European Peptide Society for providing financial support and GATE fellowship; travel award and registration grant respectively to attend this symposium.

References

1. "Blindness and vision impairment," 2021, [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment
2. Gaudana, R., Ananthula, H.K., Parenky, A., and Mitra, A.K. *AAPS J.* **12**(3), 48-360 (2010), https://doi.org/10.1208/s12248-010-9183-3
3. Johnson, L.N., Cashman, S.M., and Kumar-Singh, R. *Mol. Ther.* **16**(1), 107-114 (2008), https://doi.org/10.1038/sj.mt.6300324
4. Jain, A., Shah, S.G., and Chugh, A. *Pharm. Res*. **32**(6), 1920-1930 (2015), https://doi.org/10.1007/s11095-014-1586-x
5. Chu, Y., et al. *Int. J. Nanomedicine* **12**, 353 (2017), https://doi.org/10.2147/IJN.S126865
6. Wang, Y., et al. *Nat. Commun*. **10**(1), 804 (2019), https://doi.org/10.1038/s41467-019-08690-4
7. Ildefonso, C.J., et al. *Invest. Ophthalmol.* Vis. Sci. **57**(2), 372-386 (2016), https://doi.org/10.1167/iovs.15-17703
8. Ahmed, C.M., Massengill, M.T., Brown, E.E., Ildefonso, C.J., Johnson, H.M., and Lewin, A.S. *Exp. Eye Res.***177**, 12-22 (2018), https://doi.org/10.1016/j.exer.2018.07.020
9. Marques, R.E., et al. *J. Neuroinflammation* **14**(1), 1-14 (2017), http://dx.doi.org/10.1186/s12974-019-1686-y
10. Ahmed, C.M., Ildefonso, C.J., Johnson, H.M., and Lewin, A.S. *PLoS One* **15**(2), e0227524 (2020), https://doi.org/10.1371/journal.pone.0227524
11. Shankar, S., Shah, S.G., Yadav, S., and Chugh, A. *Eur. J. Pharm. Biopharm*. **166**, 216-226 (2021), https://doi.org/10.1016/j.ejpb.2021.06.014