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Cytotoxicity and phototoxicity effects of modified temporin analogs with unnatural aminoacids

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Introduction

Antimicrobial peptides (AMPs) are promising candidates for a new generation of anti-infective agents. Temporins are known to be particularly active against Gram-positive bacteria and Gram-negative bacteria. In addition to antimicrobial activity, some kinds of AMPs exert rapid and selective cytotoxicity against malignant cells but show relatively lower cytotoxicity against untransformed proliferating cells. In this study the cytotoxicity and phototoxicity effects of novel Temporin A analogs with modification of Arg residue in position 7 with unnatural Cit (Phe-Leu-Pro-Leu-Ile-Gly-Cit-Val-Leu-Ser-Gly-Ile-Leu-NH2; DTCit) and Orn (Phe-Leu-Pro-Leu-Ile-Gly-Orn-Val-Leu-Ser-Gly-Ile-Leu-NH2; DTOrn) on BALB/3T3 cell lines were investigated.

Materials & Methods

The cell lines BALB 3T3 (mouse embryonic fibroblasts). Cell lines were obtained from the American Type Cultures Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Sigma-Aldrich, Schnelldorf, Germany) in an incubator at 37 ◦C, 5% CO_2 and 95% humidity. Plastic flasks 75 cm2 (Biologix, Lenexa, KS, USA) were used to grow the cells.
The neutral red uptake (NRU) assay is a cell viability assay that allows in vitro quantification of modified peptide analogs-induced cytotoxicity. The assay relies on the ability of living cells to incorporate and bind neutral red, a weak cationic dye, in lysosomes. Cells were plated at a density of $1*10^4$ cells in a 100 µL culture medium in each well of 96-well microplates and allowed to adhere for 24 h. Peptides were dissolved in DMSO and diluted in culture medium to a concentration range 10 to 1000 µL were then added and the cell cultures were incubated for an additional 24 h. After treatment with Neutral Red medium, the absorption was measured on a microplate reader at wavelength 540 nm. The phototoxicity test was performed in parallel on the second 96-well plate, which was irradiated with a solar simulator (LED lamp) for 10 minutes.

Results

The cytotoxicity expressed in % relative to the negative control was determined. Dose–response dependence was observed for all peptide analogs (Fig. 1). At a concentration of 60 µg/mL, no cytotoxic effect was observed on the test peptides. CC50 values (50% cytotoxic concentration) were calculated through nonlinear regression analysis (Table 1). The peptide analogs tested had significantly lower toxicity ($p < 0.001$) than the natural peptide Temporin A. The exception was DTF, where increased toxicity (CC50 = 92.40 ± 2.82 µM) was observed. To evaluate the phototoxic potential of the investigated peptide analogs, we used the photo irritation factor (PIF), which was calculated using the following formula:

PIF = CC50 − Irr/CC50 + Irr.

Table 1 Cytotoxicity/phototoxicity in BALB 3T3, CC50 values and PIF factor

Compounds	Structure	Mean CC_{50} ± SD (µM)		PIF*
		- Irr	+ Irr	
DTA	Phe-Leu-Pro-Leu-Ile-Gly-**Arg**-Val-Leu-Ser-Gly-Ile-Leu-NH_2	106.32 ± 4.18	105.40 ± 4.88	1.01
DTF	Phe-Leu-Pro-Leu-Ile-Gly-**Lys**-Val-Leu-Ser-Gly-Ile-Leu-NH_2	92.40 ± 2.82	88.35 ± 1.48	1.05
DTCit	Phe-Leu-Pro-Leu-Ile-Gly-**Cit**-Val-Leu-Ser-Gly-Ile-Leu-NH_2	356.09 ± 7.64	331.78 ± 12.30	1.07
DTOrn	Phe-Leu-Pro-Leu-Ile-Gly-**Orn**-Val-Leu-Ser-Gly-Ile-Leu-NH_2	337.27 ± 7.09	345.1 ± 6.41	0.98

*Photo Irritation Factor: PIF < 2 not phototoxic, 2 < PIF < 5 probable phototoxicity, PIF > 5 phototoxic

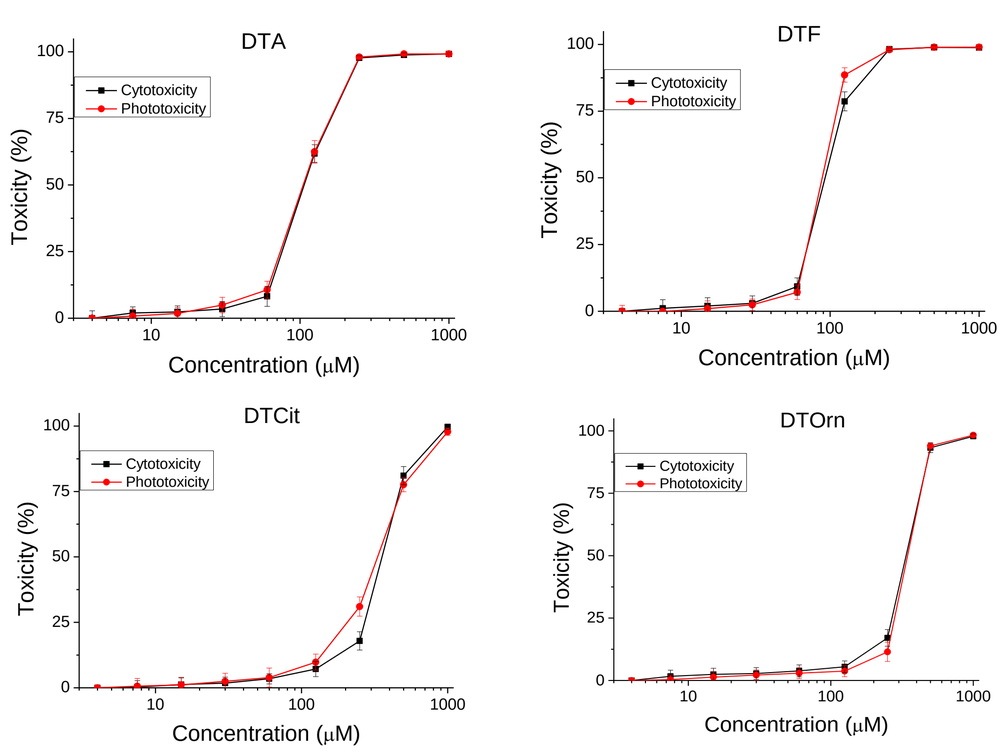

Fig. 1 Parent-peptide DTA and synthesized analogs DTF, DTDab and DTDap: Cytotoxicity/phototoxicity of peptide analogues determined in the cell line BALB/c 3T3 clone A31, n = 6

Conclusions

All modified temporin analogs do not show phototoxicity - no statistically significant difference in the CC_{50} values was observed for the irradiated peptides compared to the non-irradiated ones. The cytotoxic test shows that DTCit is the less toxic agent in comparison to Temporin A. materials proved efficient for use in both biosorption and tissue engineering applications.

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