Mitigation of the Cathelicidin Peptide LL-37 Cytotoxicity Induced by Interaction with the Polysulfonated Drug Suramin

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INTRODUCTION

Human host defense peptides (HDPs), includes:

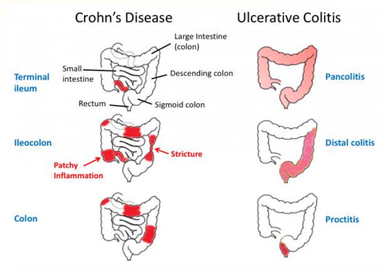
- Histatins
- Defensins
- Cathelicidins: In humans are only represented by the peptide **LL-37.** These are located at the surface of epithelial tissues for impeding the invasion of pathogenic microorganisms.

LL-37 has been detected in a variety of tissues and body fluids, including:
◈ Airway surfaces ◈ Skin ◈ Gastrointestinal/urogenital track ◈ Bone marrow

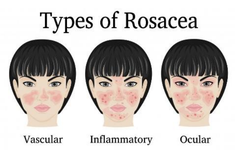
Although LL-37 preferentially **perturbs bacterial membranes**, it interacts also with eukaryotic cell membranes and **high peptide concentrations can cause cytotoxicity** and its overexpression has been associated with harmful inflammatory responses and apoptosis.

Overexpression of the peptide LL-37 leads to activation of inflammatory pathways.

Higher levels of LL-37 have been detected in the intestinal mucosa of patients affected by:



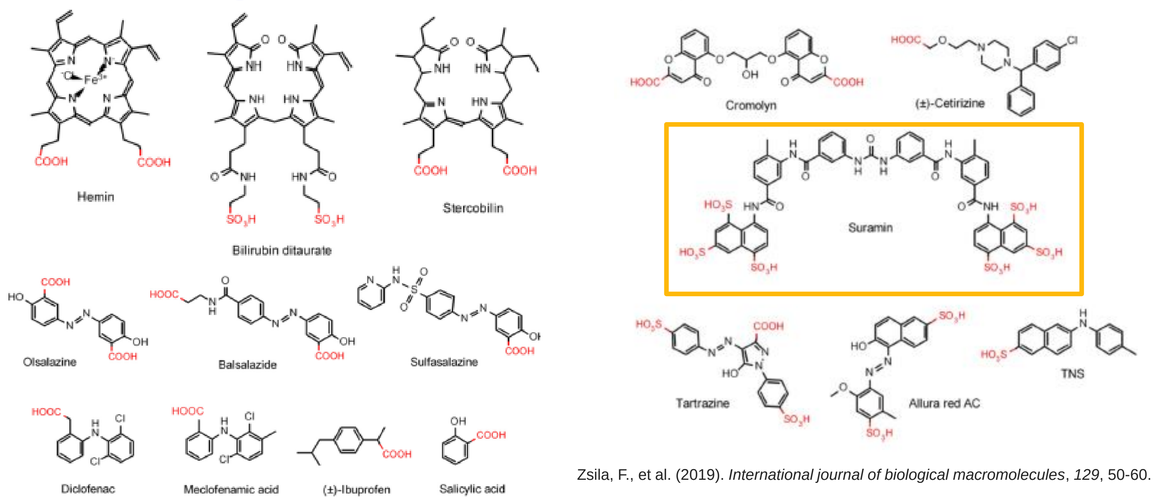
Dysfunctional overexpression of LL-37 can amplify local inflammatory response in common skin diseases:



https://tmedweb.tulane.edu/pharmwiki/doku.php/inflammatory_bowel_disease_ibd

The development of strategies aimed to reduce the harmful effect of LL-37 dysregulated expression is highly needed.

Previous studies in our research group demonstrated that the **interaction with several small molecules**, including anti-inflammatory drugs, porphyrin pigments, bile salts and food dyes may perturb LL-37 mediated pathways, especially in the **gastrointestinal tract** with currently unknown outcomes.



Zsila, F., et al. (2019). *International journal of biological macromolecules*, *129*, 50-60.

MAIN GOAL

Investigate the potential of **suramin** in **lowering LL-37 cytotoxicity**, which would be beneficial in pathological conditions characterized by **upregulated peptide expression.**

METHODS

Immunoregulator peptides used:

Peptide	Sequence	Length	Net charge
LL-37	LLGDFFRKSK EKIGKE**FKRI VQRIKDFLRN LV**PRTES	37	+ 6
FK-16	FKRIVQRIKD FLRNLV	16	+ 5

Folding inducer used:

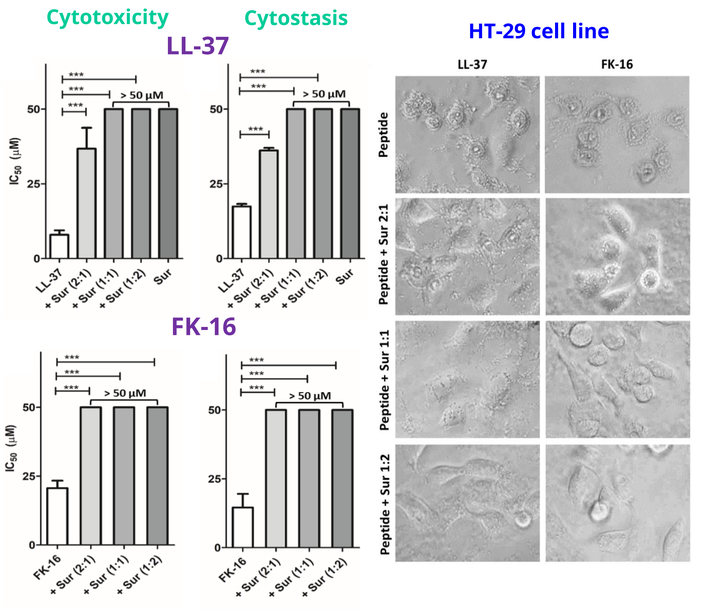
Experimental Techniques used:

Biophysical methods:
- ✓Circular dichroism (**CD**) spectroscopy
- ✓Dynamic Light Scattering (**DLS**)
- ✓Fourier-transform infrared spectroscopy (**FTIR**)

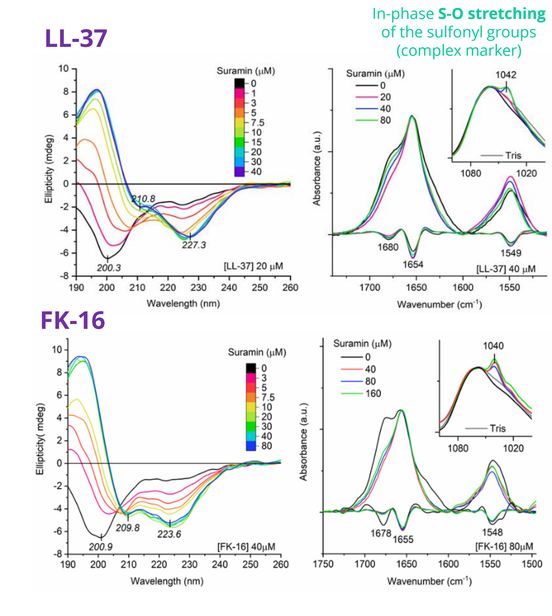
Functional *in vitro* assays:
- ✓Cytotoxicity/Cytostasis effect: **MonoMac6,** and **HT29.**
- ✓Cellular uptake evaluation by flow cytometry on HT29 cells.
- ✓Confocal microscopy on H29 cells.

RESULTS

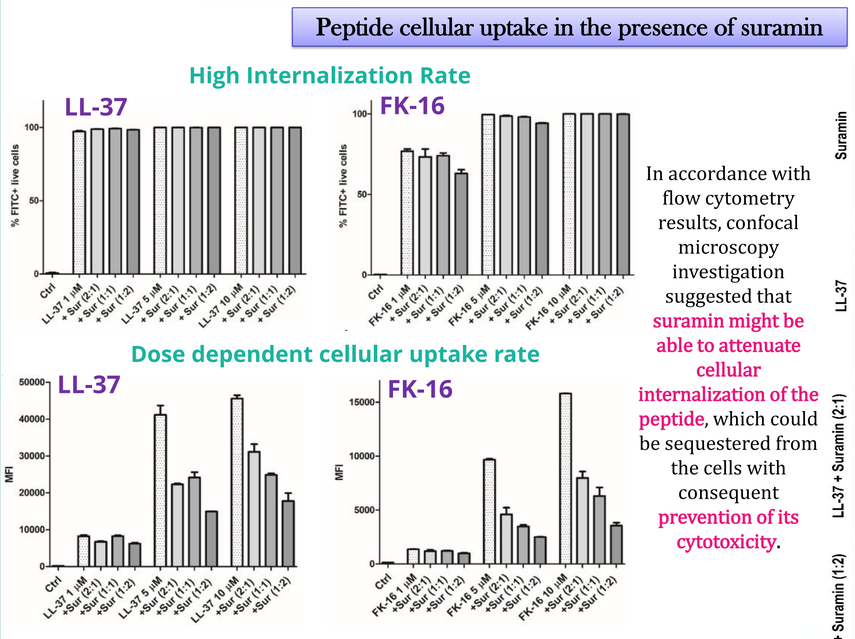
Effect of suramin on LL-37 and its fragment cytotoxicity



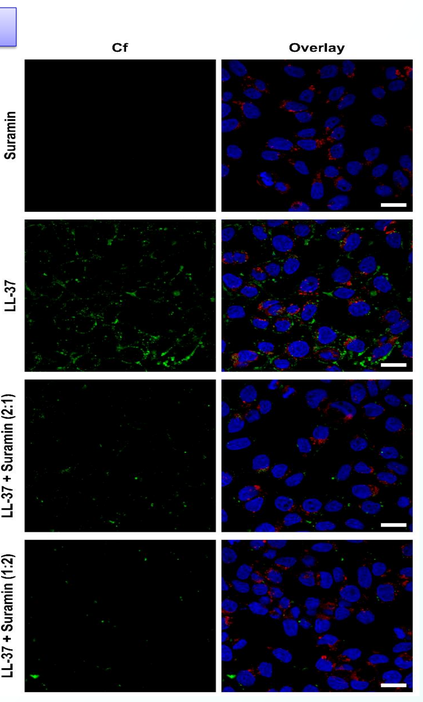
Impact of suramin on the peptide secondary structure



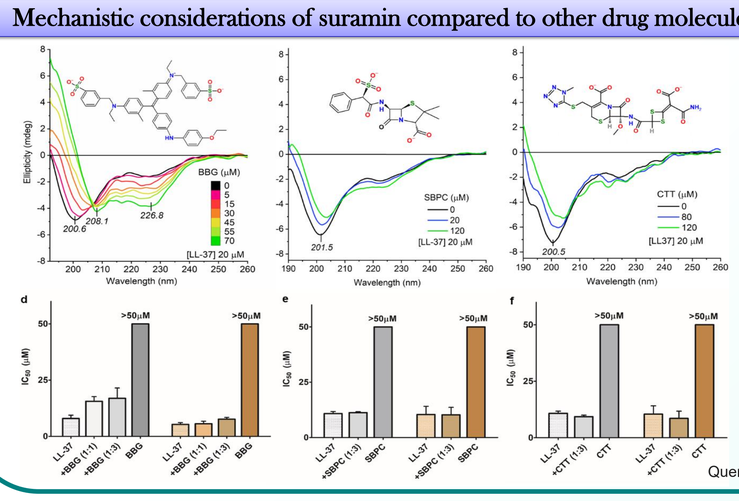
Peptide cellular uptake in the presence of suramin



In accordance with flow cytometry results, confocal microscopy investigation suggested that **suramin might be able to attenuate cellular internalization of the peptide**, which could be sequestered from the cells with consequent **prevention of its cytotoxicity.**



Mechanistic considerations of suramin compared to other drug molecules



The investigation of peptide interaction with other **negatively charged small molecules** with peculiar structural elements, suggested that the **unique action exerted by suramin** might be related not only to its polyanionic nature but also to the presence of a hydrophobic central part and rotatable bonds.

Quemé-Peña, M., et al. (2020). ACS Pharmacology & Translational Science.

CONCLUSIONS

1. In this study, we have shown that the multi-purpose drug **suramin was able to reduce cellular internalization** and consequent toxic effect of the cathelicidin peptide LL-37 on the viability and growth of colon and monocytic cell lines.
2. Molecular insights of this action were obtained from the combination of different spectroscopic methods, which indicated folding of the peptide secondary structure upon interaction with suramin and formation of drug-peptide complexes.
3. Based on analogous measurements with FK-16, it is strongly suggested that the corresponding sequential region of LL-37 most likely binds suramin with high affinity.
4. This new action of suramin on LL-37 cytotoxicity **could potentially be exploited for novel repurposing strategies** aimed to prevent inflammatory responses occurring in several disorders as a consequence of the cathelicidin dysregulated overexpression.

REFERENCES

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2. Zsila, F., et al. (2019). "Disorder-to-helix conformational conversion of the human immunomodulatory peptide LL-37 induced by antiinflammatory drugs, food dyes and some metabolites." International journal of biological macromolecules 129 (2019): 50-60.
3. Zsila, Ferenc, and Tamás Beke-Somfai. "Human host-defense peptide LL-37 targets stealth siderophores." Biochemical and biophysical research communications 526.3 (2020): 780-785.

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