Understanding the mechanism of action of the lanthipeptide NAI-112, an antinociceptive agent

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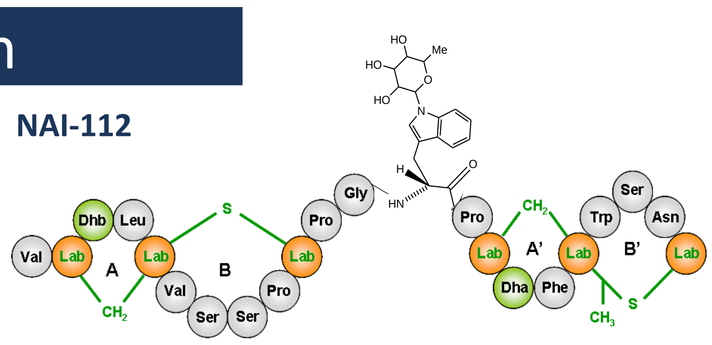
Introduction

***Actinoplanes* sp. DSM24059**

NAI-112



NAI-112 is a ribosomally synthesized, post-translationally modified peptide produced by the actinomycete *Actinoplanes* sp. DSM24059. NAI-112 consists of 22 amino acids and contains an N-terminal labionin and a C-terminal methyl-labionin. Unique among lanthipeptides, it carries a 6-deoxyhexose moiety[1].

NAI-112 *in vivo* antibacterial activity

- Modest activity on *Staphylococcus aureus* (growth inhibition at 16 μg/mL)

NAI-112 *in vivo* antinociceptive activity in mouse model

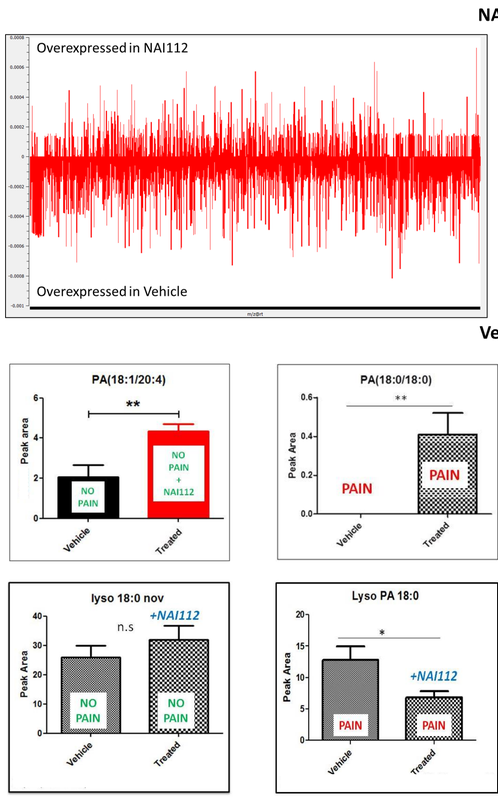
- Dose-dependent reduction of formalin-induced pain behavior in early and late phase
- Dose-dependent reduction of hyperalgesia and allodynia in chronic constriction injury of the sciatic nerve model
- Sensitivity to TRPV1 antagonist AMG9810: hypothesis of NAI-112 engagement in vanilloid-sensitive pathway

Aim of the work is to understand the mode of action of the lanthipeptide NAI-112 by identifying the molecular targets in two *in vivo* systems sensitive to NAI-112: the neuropathic pain model in mouse **(1)** and the bacterial cell model **(2)**

Results: neuropathic pain model in mouse (1)

1.1. Lipidome analysis in spinal cord

- Mice were treated with 30 mg/kg IP NAI-112
- Spinal cord samples were collected after 2 hours from injury
- Lipidome composition was analyzed in HR-LCMS



Different lipid profiles with or without NAI-112 after pain stimulus

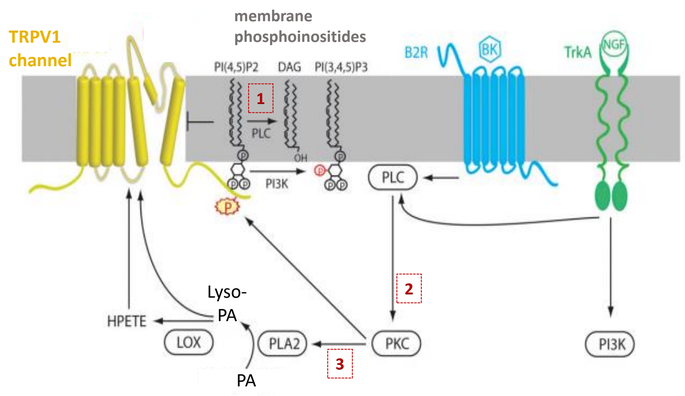
Increase of Phosphatidic Acids (PA) in the presence of NAI-112 in both no pain and pain model

Decrease of Lyso-Phosphatidic Acids (Lyso-PA) in pain model

1.2 Effects of NAI-112 on TRPV1-activating enzymes

Since no *in vitro* antagonistic activity of NAI-112 on TRPV1 channel was observed, we then analyzed the effects of NAI-112 on key enzymes for TRPV1 activation

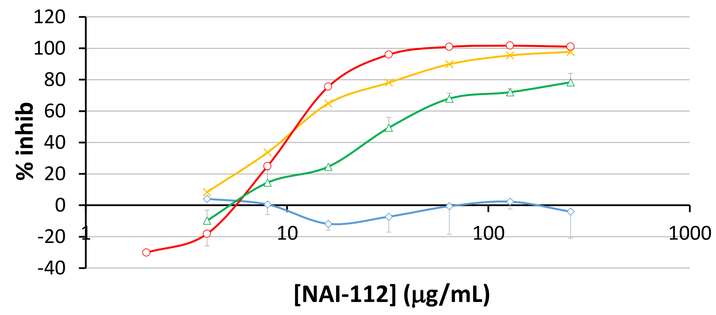
- Model depicting sensitization of TRPV1 by lipid metabolism[3]



Activation of B2R and TrkA receptors by Bradikinin (BK) and NGF after pain stimulus leads to the activation of Phospholipase C (PLC) that acts through three different mechanisms to activate the TRPV1 channel :

1. depletion of membrane phosphoinositides
2. activation of Protein Kinase C (PKC) which phosphorylates and activates TRPV1
3. PKC-mediated activation of Phospholipase A_2 (PLA_2) which generates lyso-PA

- Enzyme activity assays in presence of serial dilution of NAI-112



	PLA_1	PLC	PKC
IC_{50} (μM)	5.37	5.26	16.35

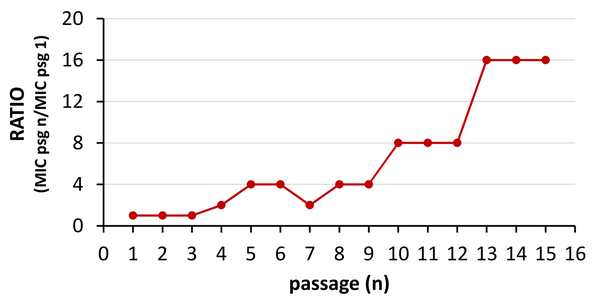
The presence of NAI-112 affects lipid metabolism with a major effect on PA and Lyso-PA levels and, interestingly, Lyso-PA have been defined as the chemical signature of neuropathic pain[2] since they activate the TRPV1 receptor, triggering pain sensation.

NAI-112 has an inhibitory effect on Phospholipase A_1, Phospholipase C and Protein Kinase C. No inhibition was observed on Phospholipase A_2. PKC inhibition was confirmed in other systems, even if at modest levels (27%). NAI-112 effects on phospholipases will be further analyzed.

Results: bacterial cell model (2)

2.1 Isolation of NAI-112 resistant *S. aureus* ATCC6538P

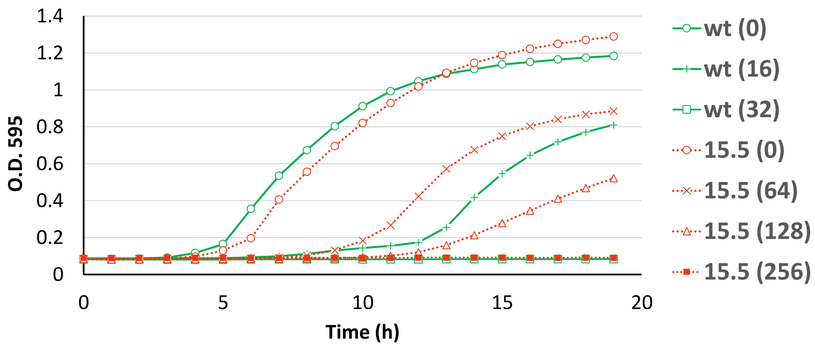
- No spontaneously resistant mutants were obtained (<10^9 *S. aureus*)
- Serial passages in the presence of increasing NAI-112 concentration:



after 15 passages *S. aureus* cultures growing in the presence of 128-256 μg/ml of NAI-112 were selected

2.2 Characterization of NAI-112 resistant mutants

- 5 colonies from resistant cultures at passage 15 (15.1 – 15.5) were isolated and analyzed in inhibition curves at different NAI-112 concentrations (μg/ml)



Growth inhibition:
at 16 μg/ml for wt
at 64-128 μg/ml for 15.5 (and 15.1-15.4 mutants)

NAI-112 resistant *S. aureus* cultures were isolated, even if with relatively modest MIC increase (8 to 16-fold), and colonies from the same culture showed a similar susceptibility to NAI-112. Cross-resistance to other antibiotics and genome sequencing with SNPs analysis are on-going.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720776.

Conclusions

Alteration of PA and Lyso-PA levels in neuropathic pain mouse model and *in vitro* inhibition of PKC, PLA_1 and PLC were observed in the presence of NAI-112. Stable NAI-112 resistant *S.aureus* mutants were isolated and will be analyzed to identify potential molecular targets.

References

1. Iorio *et al*. (2014) *ACS Chemical Biology* 9, 398-404;
2. Ueda *et al*. (2013) *Biochimica et Biophysica Acta* 1831, 61-73;
3. Cao *et al*. (2013) *Neuron* 77, 667-679.