THE ANTIMICROBIAL PEPTIDE SET-M33: *IN VIVO* EFFICACY AND TOXICITY IN DIFFERENT ANIMAL SPECIES AND ENCAPSULATION IN INHALABLE POLYMERIC NANOPARTICLES FOR PULMONARY DELIVERY

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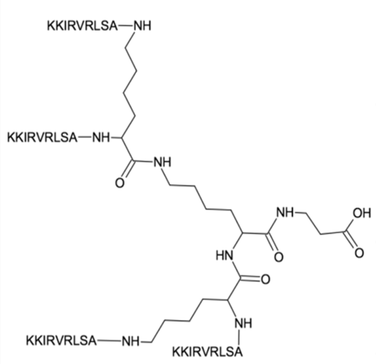
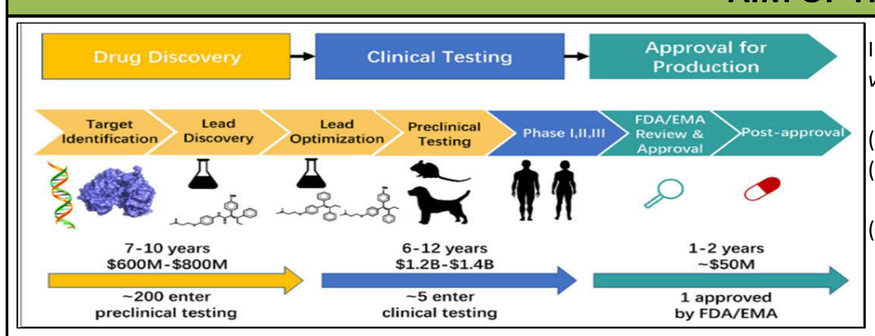
BACKGROUND



- Non-natural 9-aa cationic peptide synthetized in a tetrabranched form with four sequences linked by a lysine core
- Multiple Antigen Peptides (MAPs) acquire high resistance to protease and peptidase activity making these molecules good candidates for *in vivo* use.
- SET-M33 showed strong resistance to proteolytic degradation when synthetized in a tetrabranched form
- Strong antimicrobial activity against Gram-negative bacteria and MDR bacteria

AIM OF THE STUDIES



In recent years we focused on the pre-clinical development of the peptide in terms of *in vivo* efficacy and toxicity. In particular, we analysed:

(1) The local and systemic toxicity of SET-M33 in an inhalation study in mice;
(2) The efficacy of aerosolized SET-M33 in a mice model of endotoxin (LPS)-induced pulmonary inflammation;
(3) The toxicity study of SET-M33 by intravenous administration in rats and dogs, two animal species recommended as rodent and non-rodent test systems, respectively, by international guidelines.

Considering the certain degree of toxicity emerged by these studies, we concentrated our research on strategies to reduce the toxic effects at the local level, while maintaining its efficacy. With this purpose, the idea of peptide encapsulation in biocompatible nanoparticles (NPs) came up. A promising formulation approach is the encapsulation in a poly(lactide-co-glycolide) nanoparticles that mask the charges of SET-M33, preserve its bioactive structure, reduce side effects, facilitate transport to bacteria and achieve a prolonged therapeutic effect.

EFFICACY AND TOXICITY IN A MURINE MODEL OF PULMONARY INFLAMMATION

International Journal of *Molecular Sciences*

MDPI

Article

In Vivo Efficacy and Toxicity of an Antimicrobial Peptide in a Model of Endotoxin-Induced Pulmonary Inflammation

Laura Cresti [1,2], Giovanni Cappello [2,3], Silvia Vailati [4], Elsa Melloni [4], Jlenia Brunetti [2], Chiara Falciani [2], Luisa Bracci [1,2] and Alessandro Pini [1,2,*]

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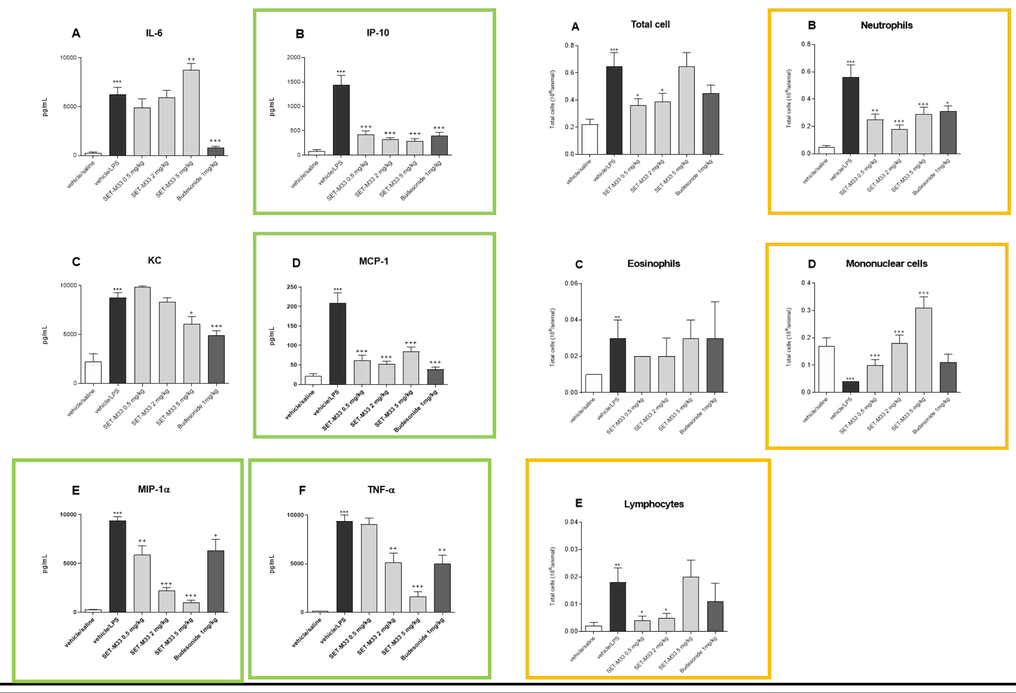
Crl:CD-1(ICR) mice (for toxicity study)
Balb/c mice (for efficacy study)

1) Toxicity study of SET-M33 administered by snout inhalation exposure for 1 h/day for 7 days at doses of 5 and 20 mg/kg/day

2) Efficacy study of the peptide in an endotoxin (LPS)-induced pulmonary inflammation model. Intratracheal administration of SET-M33 at 0.5, 2 and 5 mg/kg



The no observable adverse effect level (**NOAEL**) for aerosol administration was considered to be **5 mg/kg/day in murine model**

The outcome of the *in vivo* toxicity and efficacy studies showed that a value ≤ NOAEL dose is compatible with anti-inflammatory efficacy

SAFETY EVALUATIONS IN RATS AND DOGS OF SET-M33 ADMINISTERED INTRAVENOUSLY

Sprague Dawley rats

Naïve beagle dogs

scientific reports

OPEN **Safety evaluations of a synthetic antimicrobial peptide administered intravenously in rats and dogs**

Laura Cresti[1,3], Chiara Falciani[2], Giovanni Cappello[2,3], Jlenia Brunetti[2], Silvia Vailati[4], Elsa Melloni[4], Luisa Bracci[1,2] & Alessandro Pini[1,2]

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4-week daily administration by infusion for the evaluation of macroscopic and clinical signs of toxicity with a recovery period of 2 weeks for rats and 4 weeks for dogs.

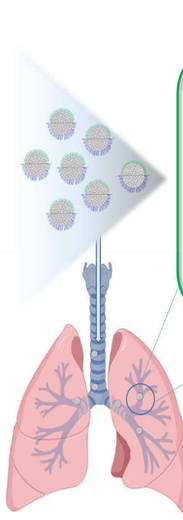
Parameters evaluated: dose ranging finding (DRF), neurological toxicity, respiratory function, cardiac parameters, clinical laboratory investigations, bio-analytics and toxicokinetic, histopathology.

1) Daily administration of SET-M33 at the highest doses (15 mg/kg/day and 4mg/kg/day for rats and dogs, respectively) caused renal effects, such as tubule degeneration/regeneration, elevated blood concentrations of urea and creatinine, high glucose (only in rats), all of these identifying the **kidneys** as a target for toxic effects, thus suggesting a functional deficit. This confirms the bio-distribution and excretion data obtained previously with radio-iodinate SET-M33, which showed an evident uptake of the peptide by kidneys and bladder after intravenous administration of the peptide to mice (Brunetti J. *et al*, Sci Rep. 2016;6:26077. doi: 10.1038/srep26077).

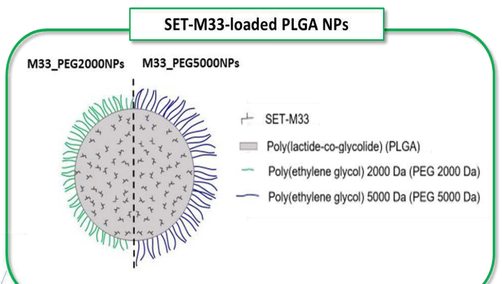
2) The bioanalytical evaluation of blood parameters and the histological analysis did not suggest any possible toxic effects on other organs in rats and dogs. The lower doses did not provoke any detectable side effect for all organs evaluated.

3) The results obtained with SET-M33 administered intravenously indicate that the **NOAEL** resulted <1 mg/Kg/day.

INHALABLE POLYMERIC NANOPARTICLES FOR PULMONARY DELIVERY OF SET-M33: ANTIBACTERIAL ACTIVITY AND TOXICITY *IN VITRO* AND *IN VIVO*

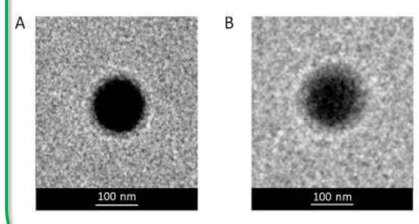


SET-M33-loaded PLGA NPs



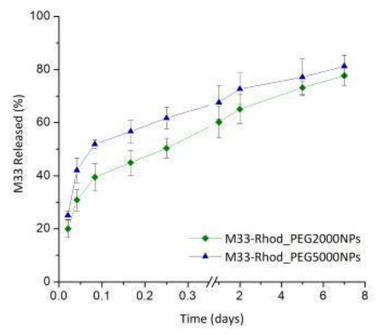
Production of nanoparticles

PLGA-based nanoparticles (NPs) containing SET-M33 peptide is prepared by a modified emulsion/solvent diffusion technique at 4% theoretical loading (4mg of M33 *per* 100 mg of NP). Formulation was prepared adding poly(lactic-co-glycolic acid) (PLGA) conjugated with polyethylene glycol (PEG) at two different molecular weight (2000 Da and 5000 Da) to the organic phase containing PLGA



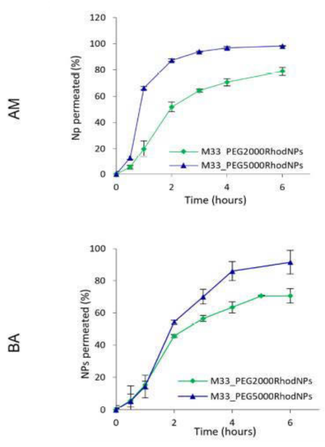
TEM images of M33-loaded NPs: M33_PEG2000 NPs (A) and M33_PEG5000 NPs (B).

In vitro release of SET-M33 peptide from nanoparticles

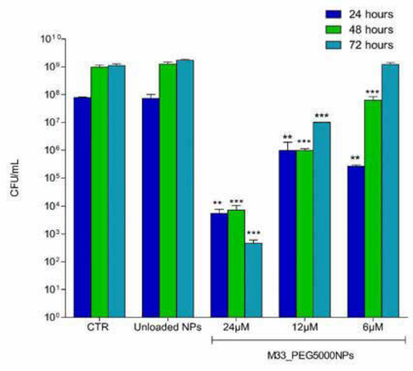


The two NPs formulations showed typical controlled release of the peptide lasting about 7 days.
The mechanism of release can be related to the diffusion process

In vitro diffusion studies across simulated lung barriers

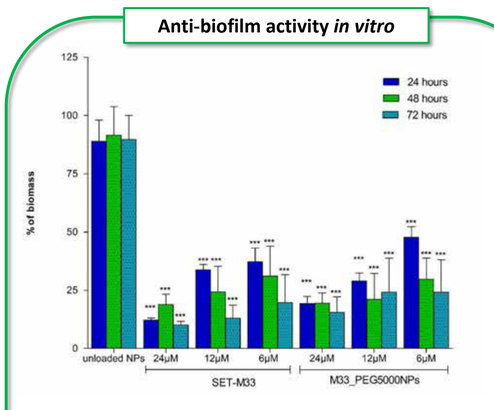


Anti-bacterial activity *in vitro*



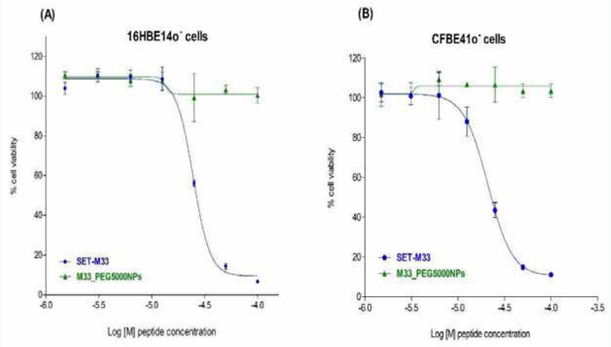
Antibacterial activity persisted up to 72 hours when M33_PEG5000 NPs were loaded at the higher concentrations and up to 48 hours with M33_PEG5000 NPs loaded with 6 μM SET-M33. Evident dose-dependent manner.

Anti-biofilm activity *in vitro*



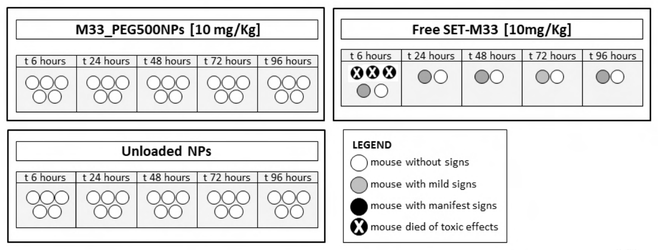
The profile of anti-biofilm activity of M33_PEG5000 NPs was very similar to that of the free peptide; it had a dose-dependent trend and the greatest reduction in biomass was at 24 μM. Like the free form of the peptide, M33_PEG5000 NP activity was strongest after 72 hours.

Cytotoxicity

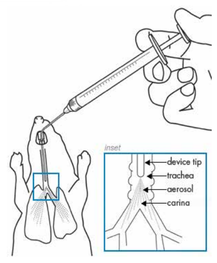


No toxicity was detected for M33_PEG5000 NPs, whereas the free peptide at the same concentrations as in the NPs showed an EC_{50} of ≈22μM on both cell lineage.

In vivo Acute Toxicity



Mice were treated via i.t. administration of free SET-M33 and M33_PEG5000 NPs at the same peptide concentration (10 mg/Kg). Mice were checked for signs of toxicity for up to 96 h. No signs of toxicity were observed in mice treated via i.t. administration of M33_PEG5000 NPs and unloaded nanoparticles, confirming the absence of toxicity already seen in cytotoxicity tests, and the high biocompatibility of the material used.
i.t. administration of free SET-M33 caused three deaths within 6 h, mild signs in one mouse and no signs in another mouse.



pharmaceutics

MDPI

Article

Inhalable Polymeric Nanoparticles for Pulmonary Delivery of Antimicrobial Peptide SET-M33: Antibacterial Activity and Toxicity In Vitro and In Vivo

Laura Cresti [1,2,3], Gemma Conte [4], Giovanni Cappello [2,3], Jlenia Brunetti [3], Chiara Falciani [3], Luisa Bracci [1,3], Fabiana Quaglia [5], Francesca Ungaro [5], Ivana d'Angelo [4,*] and Alessandro Pini [1,3,*]

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