Releasing antimicrobial peptides through liposomal nanocarriers to fight multidrug-resistant bacteria

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Multidrug-resistant bacterial infections are expected to **surpass the lethality of cancer** by 2050 [1]

No new class of antibiotic has been discovered for many years – arguably not since the 70's. [2]

Drugs used for last resort therapies are rapidly becoming **today's last line of defense**. [1]

Background

As a result of the rise of antibiotic resistance, increasingly exotic and abandoned treatments are being seriously considered to fight multidrug-resistant (MDR) bacterial infections. One of the revived candidates – colistin (see fig. 1), an antimicrobial peptide – shows great promise towards treating gram-negative infections. While formerly used mostly as a last-resort salvage therapy, it is now proving increasingly attractive for the treatment of gram-negative infections, despite the nephrotoxic side-effects it induces in humans. One way to negate this toxicity is by encapsulating colistin in liposomal nanoparticles, encouraging a slow release rather than a high-dose burst. [3-6].

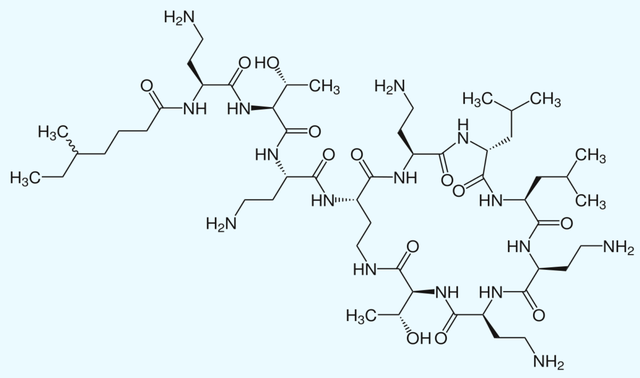

fig. 1. Structure of polymyxin E1, showcasing the heptacyclic ring, exocyclic tripeptide, and the attached fatty acid (6-methyloctanoic acid)

Colistin readily interacts with the outer membranes of these bacteria by displacing cations, binding to the lipopolysaccharide layers, destabilizing the membrane, increasing its permeability and causing cell death (See fig. 2).

Aim of study

A liquid-chromatography tandem mass spectrometry (LC-MS/MS) method was to be established for the measurement of colistin using an internal standard, polymyxin B1, to assess the release kinetics between colistin from liposomal nanoparticles.

Results

An LC-ESI-MS/MS method was successfully developed for the separation of colistin and polymyxin B1 (See fig. 2). The MS/MS fragmentation spectrum obtained by direct injection can be seen in fig. 4. Mobile phase A consisted of H_2O + 0.1 % formic acid, whereas mobile phase B consisted of acetonitrile 0 1 % formic acid. Separation was performed on an ACE C18 column from VWR (100 mm x 2.1 mm inner diameter, 3 µm particle size). Isocratic elution was conducted with 17 % B for three minutes, followed by a 90 % B washing step for 0.5 minutes, prior to a 4-minute reconditioning of the column.

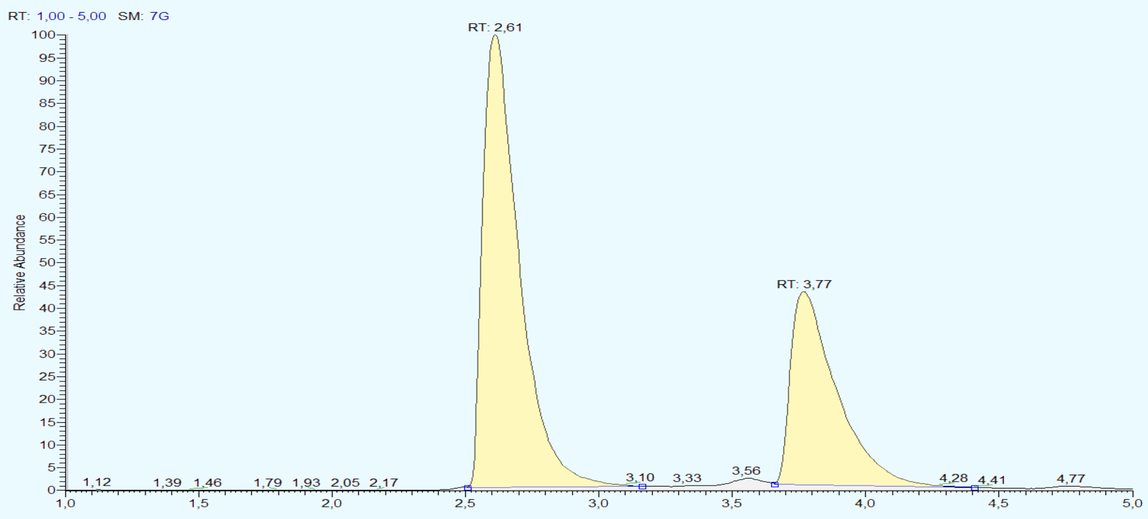

fig. 2. Representative chromatogram for the LC method used in this work, obtained from a sample containing both colistin (first peak) and polymyxin B1 (second peak).

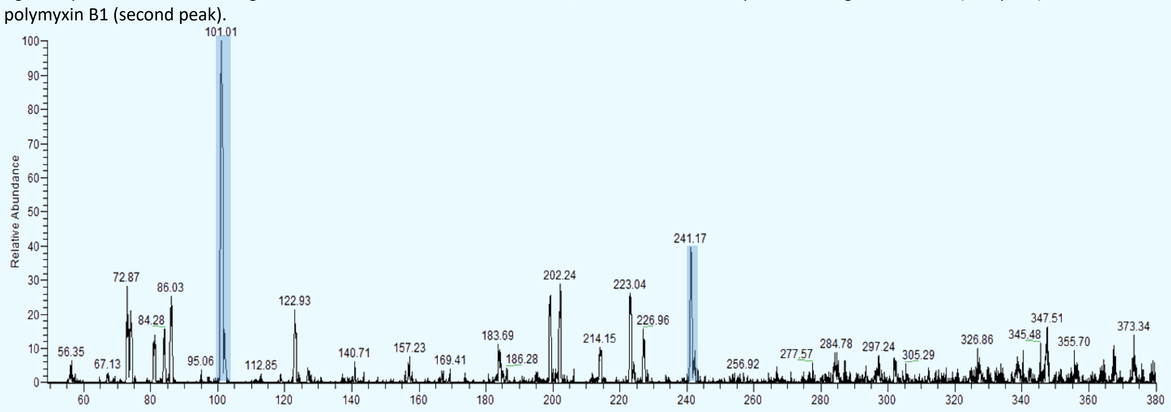

fig. 2. MS/MS spectrum of polymyxin E1 $[M+3H]^{3+}$, obtained through direct injection onto the MS. *m/z* values used with MRM - marked in blue - were used for quantification *(m/z 101)* and qualification *(m/z 241)*

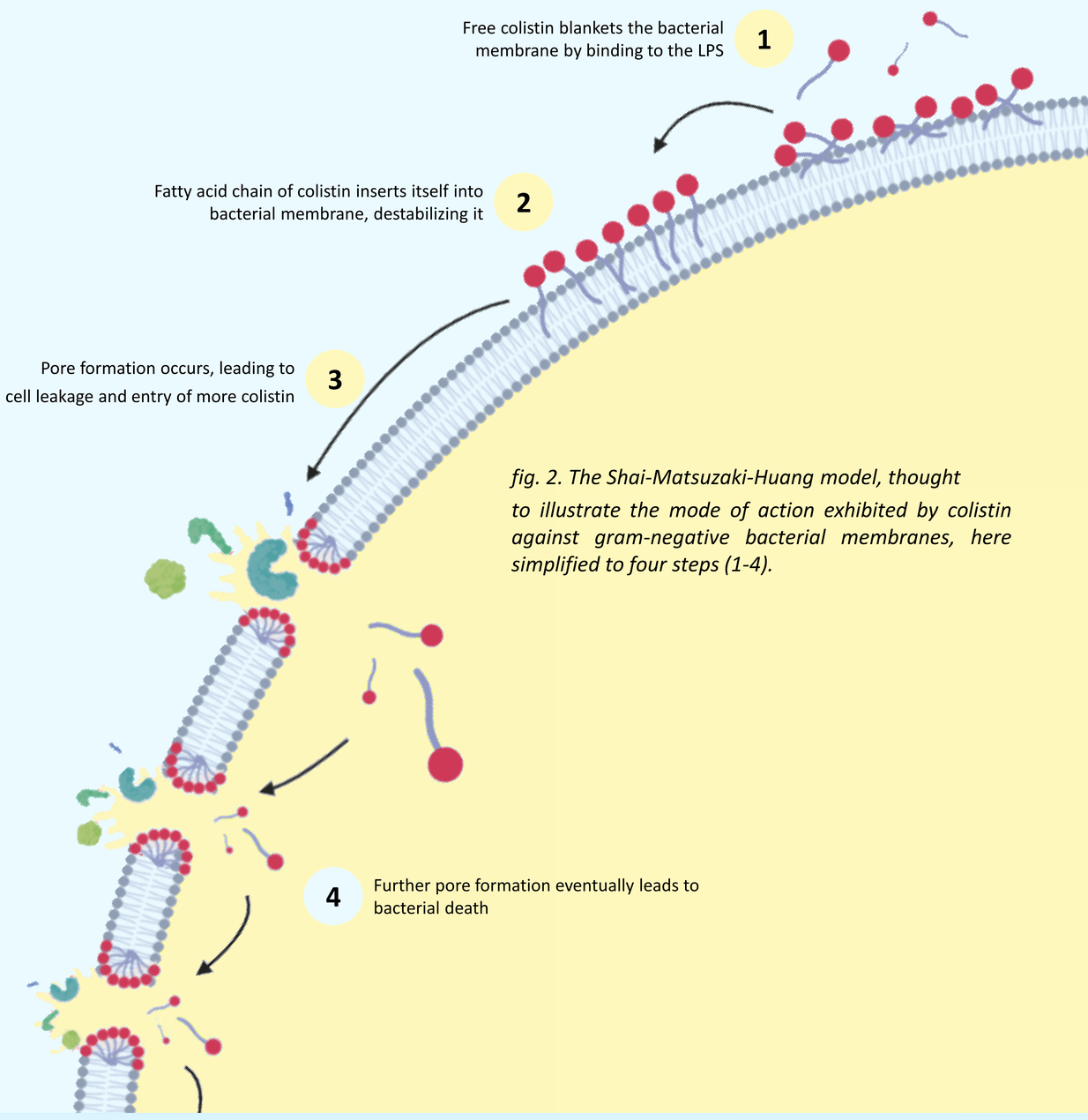

fig. 2. The Shai-Matsuzaki-Huang model, thought to illustrate the mode of action exhibited by colistin against gram-negative bacterial membranes, here simplified to four steps (1-4).

Applied method

A time-release study was conducted to evaluate the rate at which colistin was released into free solution from loaded liposomes. The liposomes were loaded with a concentration of 1.8 mg/ml, and data points were sampled via. dialysis at 1.5, 4, 24 and 96 hours post-liposome preparation (See fig. 5). The results showed the release curve with the expected release of colistin from the liposomes.

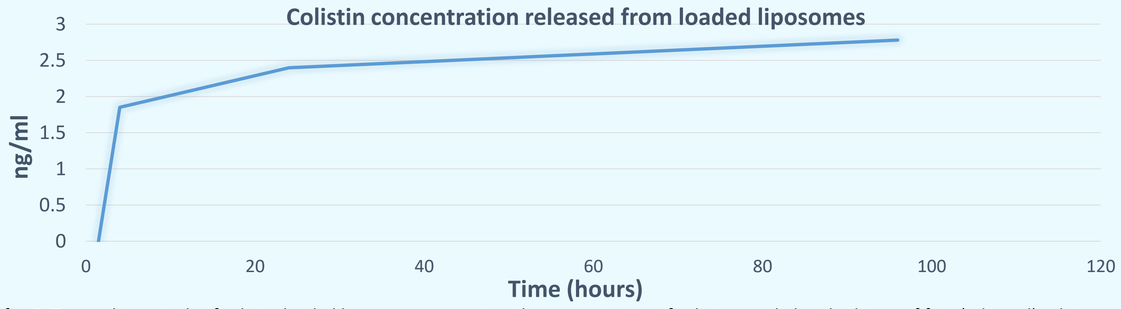

fig. 5. Time-release study of colistin-loaded liposomes, measuring the concentration of colistin in a dialyzed solution of free (released) colistin and loaded liposomes.

Conclusions and further work

Following the successful method development and preliminary release study, a more elaborate release time study is desired in order to obtain a more detailed information on the release. Furthermore, loaded liposomal leakage will be evaluated by size exclusion chromatography (SEC) followed by fractionation instead of dialysis, to separate loaded, intact liposomes from free, released colistin. Finally, organoids – miniature biological representations of live organs – will be used to compare the toxicity of liposomally encapsulated colistin, versus free colistin, by tracking levels of biomarkers known to correspond with colistin-induced renal damage.

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