ROVANCE – Resistance Overcoming Antibiotics New Chemical Entities: A Platform Technology to Overcome Bacterial Resistance

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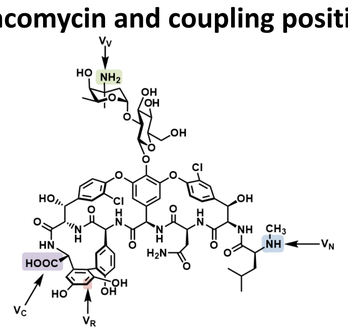
Introduction

Vancomycin still represents the gold standard antibiotic for treatment of multi-drug resistant infections caused by Gram-positive bacteria [1]. Due to the high prevalence of infections caused by MRSA, the application of vancomycin dramatically increased [2]. This is followed by a tremendously increase of resistant pathogens which is one of the most important problems of modern medicine. The WHO classified vancomycin-resistant *Enterococci* as "high priority pathogens".

One strategy to circumvent bacterial resistance is the modification of established antibiotics. We developed novel vancomycin derivatives that overcome vancomycin-resistance in the main types (*vanA*, *vanB*, *vanC*) [3-5]. In this project, we conjugated polycationic peptides to four different sites of vancomycin (called V_N, V_V, V_C, V_R). All modification sites were able to improve the antimicrobial activity. The V_N-, V_V-, V_C-position were even able to break vancomycin resistance. For further tests the lead candidate, FU002, a polyarginine peptide coupled to the V_N-position, was used, due to its easier synthesis and the promising antimicrobial activity values. In consequence to this new modification, improved pharmacokinetics in comparison to vancomycin were obtained.

Besides vancomycin, the strategy could be transferred to other glycopeptide and cell wall targeting antibiotics indicating its potential applicability as platform technology (referred to as ROVANCE technology).

Vancomycin and coupling position



Coupling position and MIC

Ethylenediamine linking (+/-)	Peptide	MIC [mol/ml]
+	R6	8.1×10^{-9}

Ethylenediamine linking (+/-)	Peptide	MIC [mol/ml]
-	R6	5.1×10^{-10}
-	R9	5.3×10^{-10}
+	R6	1.9×10^{-9}
+	R9	1.1×10^{-9}
+	R3Myr	2.0×10^{-9}

Ethylenediamine linking (+/-)	Peptide	MIC [mol/ml]
+	R6	5.1×10^{-10}
+	R9	2.7×10^{-10}
+	R3Myr	2.5×10^{-10}

Ethylenediamine linking (+/-)	Peptide	MIC [mol/ml]
+	R6	1.0×10^{-9}
+	R9	5.3×10^{-10}
+	R3Myr	1.5×10^{-9}

Methods

Synthesis

For the synthesis of the conjugates, vancomycin was coupled with a linker (Sulfo-SMCC). In parallel, the peptide was synthesized by standard solid phase peptide synthesis. In a next step the purified vancomycin-linker-conjugate was coupled to the peptide. Afterwards, purification was performed by preparative HPLC.

SPECT

For radiolabeling, a tyrosine-modified derivative was used and labeled with radioactive iodine-125 (^{125}I) using the chloramine T method according to Crim et al. [6].

Systemic mouse infection model

For the *in vivo* efficacy assessment, an infection model with the MRSA strain *S. aureus USA300 lac* was performed over 5 days. Therefore, the infected mice were treated intravenous with either 0.9% NaCl as negative control, vancomycin as positive control or FU002 twice daily with a dose corresponding to 30 mg/kg/d vancomycin.

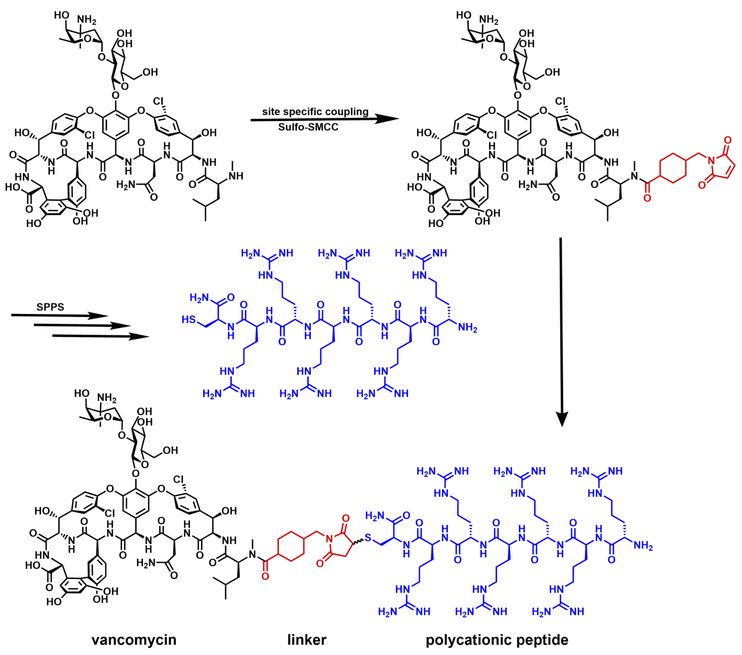


Figure 2. Synthesis of the peptide conjugates, representatively shown for the lead candidate FU002.

Conclusion

In conclusion, the conjugation of polycationic peptides was proven to represent a viable strategy for the reactivation of vancomycin and daptomycin. The lead candidate FU002 (Arginine-modified vancomycin) was proven to be effective *in vitro* and *in vivo*.

With respect to daptomycin, most importantly, the lead candidate DAP-R6 was able to overcome the daptomycin resistance in the well-characterized, daptomycin-resistant *S. aureus* strain HG001-DRSA. The possibility to also modulate the biodistribution profile allows for an application-based design of novel conjugates. This raises the hope for novel options to combat multidrug-resistant bacteria.

Figure 7. The pool of potential antibiotics and the influence of resistance development on its fate. Potential antibiotics primarily comprise natural products and synthetically generated compounds. Our peptide modification strategy enables to generate highly active compounds with drug-like properties.

References

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Results

Antimicrobial activity *in vitro*

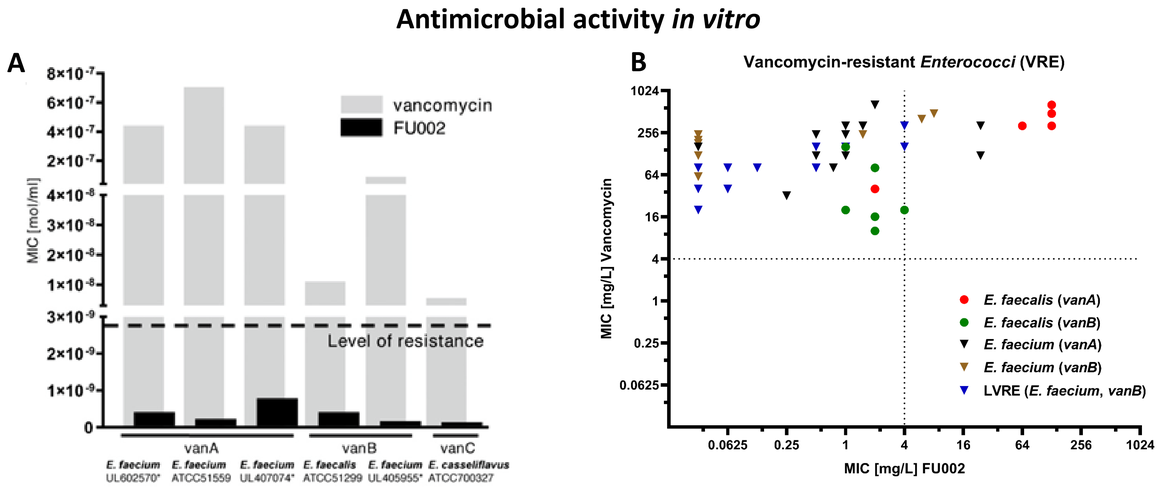

Figure 3. Antimicrobial activity of vancomycin and its derivative FU002. The determination of the minimum inhibitory concentrations (MIC) shows that FU002 breaks all levels of vancomycin resistance [3]. **A)** FU002 in comparison to vancomycin on selected vancomycin-resistant *Enterococci*, B) MIC of vancomycin and FU002 on over 50 vancomycin-resistant *Enterococci* (VRE), including vanA and vanB-type resistant *E. faecalis* and *E. faecium*. The dotted lines indicate the concentration (4 mg/L) at which bacterial strains are resistant towards vancomycin.

Biodistribution studies in Wistar rats

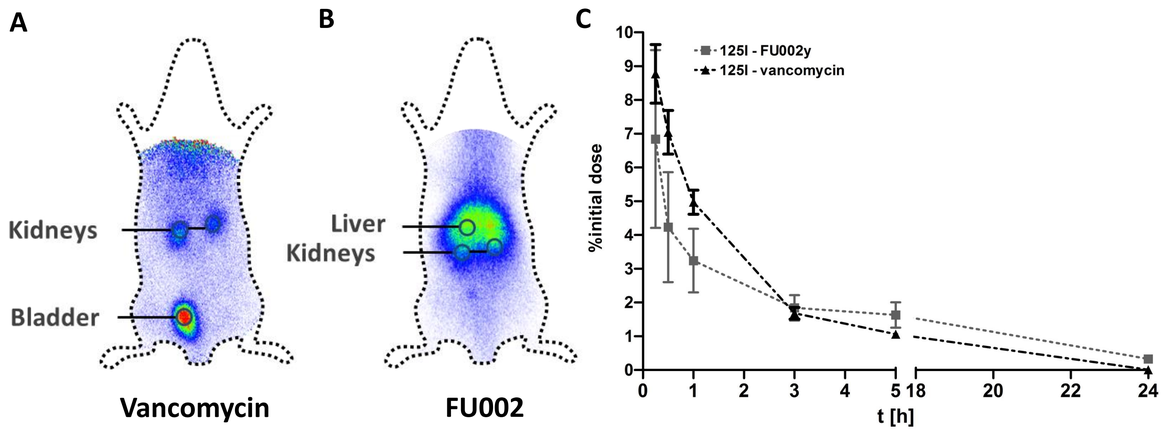

Figure 4. *In vivo* comparison of vancomycin with FU002. A) Planar scintigraphy 10 min p.i. reveals that the rapid excretion and kidney accumulation of ^{125}I-labeled vancomycin can be overcome by **B)** modulation of the pharmacokinetics to achieve liver specificity in the case of FU002 [3]. **C)** Pharmacokinetics of FU002 show a rapid blood elimination of the drug after i.v. injection.

Systemic mouse infection model

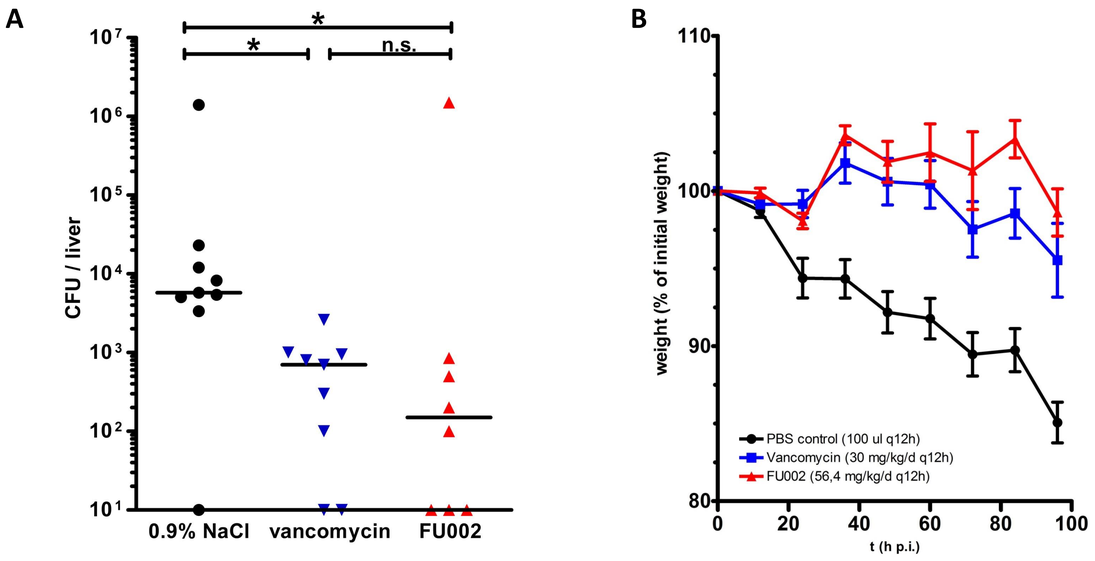

Figure 5. *In vivo* sepsis model in mice. Comparison of vancomycin and FU002. A) FU002 (56.4 mg/kg/d) leads to significant reduction of colony forming units of vancomycin-sensitive *Staphylococcus aureus* USA300 lac in liver in comparison to vancomycin (30 mg/kg/d) [3]. **B)** The body weight of infected mice over treatment stayed constant [3].

ROVANCE as platform technology

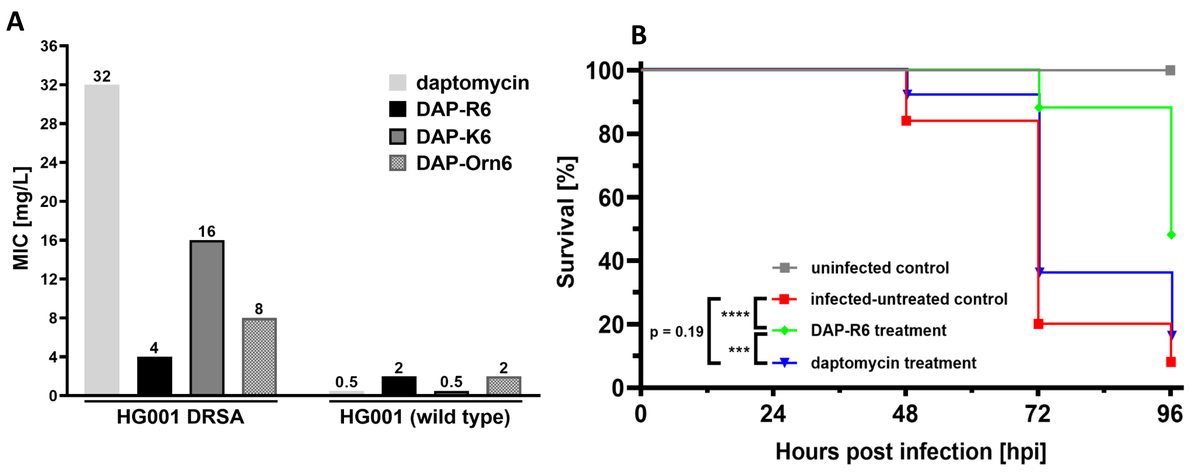

Figure 6. Transferability of the modification strategy to daptomycin. A) Comparison of MIC values of daptomycin conjugates bearing a net charge of +6. For HG001-DRSA, hexa-arginine (DAP-R6) outperforms hexa-lysine (DAP-K6) and hexa-ornithine (DAP-Orn6) conjugates. For wild type HG001, the antimicrobial potentials of the conjugates match daptomycin. **B)** Kaplan Meier plot of DRSA-infected zebrafish embryos after treatment with daptomycin and DAP-R6. Treatment with DAP-R6 led to a prolonged survival of DRSA infected larvae compared to treatment with daptomycin. Significance calculation using the log-rank (Mantel-Cox) test: *** = 0.0007; **** = <0.0001.