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RESEARCH PAPER

Pharmacodynamic and response surface analysis of linezolid or vancomycin combined with meropenem against Staphylococcus aureus

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ABSTRACT

Purpose To systematically assess the impact of pharmacodynamic interactions when adding either linezolid or vancomycin to meropenem on the antibacterial activity against methicillin-susceptible *Staphylococcus aureus* (MSSA). These regimens are frequently used in empiric therapy when risk factors for MRSA are present, but MSSA will often turn out as pathogen.

Methods Checkerboard and time-kill curve studies were performed against three strains of MSSA covering clinically relevant concentrations of all antibiotics. We newly elaborated a response surface analysis (RSA) to quantify the extent of the pharmacodynamic interactions.

Results The most prominent result was that linezolid fully antagonised the rapid (4–6 h) bactericidal effect of meropenem against MSSA to bacteriostasis at clinically relevant concentrations of both drugs. This interaction was invisible in the conventional checkerboard analysis (insensitive turbidity threshold). RSA quantified a 1.5–3.2 log_{10}-fold higher bacterial load compared to expected additivity for linezolid and meropenem. Vancomycin and meropenem interacted partly synergistic (subinhibitory) or additive (inhibitory combinations) being bactericidal after 24 h.

Conclusions Standard doses of linezolid and meropenem will provide inhibitory concentrations and thus pharmacodynamic antagonism throughout the whole dosing interval for MSSA. Further data is required to assess the clinical significance of this interaction.

KEY WORDS antibiotic combination therapy · checkerboard · pharmacodynamic drug-drug interactions · response surface analysis · time-kill curve studies

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ABBREVIATIONS

ATCC	American type culture collection
BI	Bliss independence
C	Drug concentration
CaMHB	Cation-adjusted Mueller Hinton broth
CFU	Colony forming unit
E	Antibacterial effect
EC_{50}	Drug concentration at which the half-maximum effect is observed
Emax	Maximum drug effect
GC	Growth control
H	Hill factor expressing the steepness of the concentration-effect relationship
IE	Intensity of the antibacterial effect
LZD	Linezolid
MER	Meropenem
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
PD	Pharmacodynamic
PK	Pharmacokinetic
RSA	Response surface analysis
$T_{>MIC}$	Time period that drug concentrations exceed the minimal inhibitory concentration
TKC	Time-kill curve
VAN	Vancomycin

INTRODUCTION

Combinatory regimens of meropenem (MER) with either linezolid (LZD) or vancomycin (VAN) are frequently employed for initial treatment of severe nosocomial infections (e.g. pneumonia), because they provide considerably large and partly complementary antibacterial spectra including multi-drug

resistant gram-positive (e.g. MRSA) and gram-negative pathogens (e.g. *P. aeruginosa*) (1). The combination of LZD and carbapenems was studied extensively with MRSA and found synergistic *in vitro* and in animal models (2,3). Targeted therapy of ventilator-associated pneumonia caused by MRSA with linezolid in combination with a carbapenem is under clinical evaluation (clinicaltrials.gov; NCT01356472).

To our knowledge, the combination of LZD with a carbapenem against MSSA has not been evaluated systematically, although LZD combined with MER vs. MSSA is a commonly utilised treatment option in patients with risk factors until MRSA is ruled out (1). An *in vitro* interaction study between the alternative antibiotic VAN and MER indicated no unfavourable interaction when subinhibitory concentrations of VAN were added to MER (4), but evidence about potential drug-drug interactions at higher concentrations of VAN is lacking. *In vitro* settings such as checkerboard (5) and particularly time-kill curve (TKC) studies (6) are powerful tools at an early stage to systematically investigate such drug effects alone and in combination and eventually generate hypotheses.

Hence, the objective of the present study was to assess the impact of adding either LZD or VAN to MER at clinically relevant concentrations of all antibiotics in TKC studies. Further, the results and conclusions of the less laborious conventional checkerboard analysis (evaluated by turbidity) were compared with those of the 'dynamic' checkerboard (3) (with quantification of bacteria in each cavity of the well-plate) and the TKC studies. Modelling and simulation techniques were utilised to provide quantitative measures for the drug (inter)action studies: To capture the individual effects, we performed pharmacodynamic (PD) concentration-effect modelling to the checkerboard and TKC data. Possible PD interactions between the investigated antibiotics were assessed by a newly elaborated response surface analysis (RSA).

MATERIALS AND METHODS

Bacterial Strains

A reference strain (ATCC 29213, American Type Culture Collection, Manassas, VA/USA), and two clinical isolates of MSSA originating from tracheal secretion (MV 13391) and sputum (MV 13488) (Institute of Microbiology and Hygiene, Charité University Hospital, Berlin/Germany) were evaluated.

Antimicrobials and Susceptibility Testing

Linezolid (Pfizer, LOT: PF-00184033) and meropenem (Astra-Zeneca, LOT: 111202) were kindly provided by the respective manufacturers. Vancomycin was USP grade (Sigma-Aldrich, Steinheim/Germany, LOT: SLBB4575V). Preliminary experiments indicated that the drug solutions at their respective final concentrations in broth did not change the pH value of the broth (pH 7.4). The minimal inhibitory concentration (MIC) was determined in triplicate for each antibiotic according to the CLSI guideline (7).

Conventional and Dynamic Checkerboard

The conventional checkerboard experiment was performed in a 48-well plate in triplicate. 100 µL of appropriately diluted drug solutions of LZD and/or MER were added to 890 µL (single drug) or 790 µL (combinations) of cation-adjusted Mueller-Hinton broth (CaMHB, Sigma-Aldrich, Steinheim/Germany) along the horizontal and vertical path of the well plate, respectively, in order to yield final drug concentrations covering both MICs and clinically relevant concentrations of both drugs (8,9). 10 µL of a suspension of ATCC 29213 from an overnight subculture were added to yield a final inoculum size of approx. 10^6 CFU/mL. After 20 h of incubation at 37°C, the well plate was evaluated visually for turbidity. In addition, for the 'dynamic checkerboard', bacteria were quantified in each well with the developed quantification assay (s. 'Quantification of S. aureus' below).

Time-kill Curve Experiments

TKC experiments were performed in 50 mL cell culture flaks with vented caps (BD, Le Pont de Claix/France) in $n \geq 2$ replicates. 1.0 mL of appropriately diluted drug solutions was added to 8.9 mL (single drug) or as double combinations of LZD or VAN and MER to 7.9 mL (combinations) of CaMHB (Oxoid, Basingstoke/UK), respectively. Final drug concentrations covered the clinically relevant ranges from 0.015 to 8 mg/L for MER (9), 0.5–32 mg/L for LZD (8) and 0.06–16 mg/L for VAN (10), alone and in selected combinations. An inoculum size of approximately 10^6 CFU/mL was utilised. The inoculated flasks were incubated for 24 h at 37°C while shaking at 1 Hz at ambient air.

Quantification of S. aureus

The 100 µL sample drawn from the culture flask or cavity of the well plate was subjected to serial dilutions in phosphate-buffered saline with peptone (8.5 g NaCl, 0.3 g KH_2PO_4, 0.6 g Na_2HPO_4 and 1.0 g peptone (from meat, tryptic, Sigma Aldrich, Steinheim/Germany) per litre of Milli-Q™ water, pH = 7.0). Antibiotic carryover was avoided by either direct dilution to subinhibitory concentrations, or by a centrifugation/washing method (up to 3 cycles at 610 g for 10 min). Preliminary experiments indicated equivalence of both processing methods. Columbia agar plates (Carl Roth, Karlsruhe/Germany) were spot-inoculated with the prepared

dilutions (5× or 10×10 µL) and subsequently incubated for 18–24 h at 37°C. Agar plates were read visually and CFU/mL were calculated. A ≥3 $\log_{10}$-fold reduction of bacteria was defined as 'bactericidal', whilst smaller reductions were referred to as 'bacteriostatic' (11).

Pharmacodynamic and Response Surface Analysis

The PD of the antibiotics against MSSA was first assessed individually using a sigmoidal maximum effect model (Eq. 1):

$$E(C) = \frac{Emax \times C^H}{EC_{50}{}^H + C^H} \quad (1)$$

For the dynamic checkerboard, the bacterial concentration in mean of $\log_{10}$ CFU/mL at the end of $n=3$ experiments was utilised as effect measure. The antibiotic was assumed to reduce the bacterial concentration at maximum by *Emax*. The drug concentration (C) at which the half-maximum effect (EC_{50}) was observed and the Hill factor (H) expressing the steepness of the concentration-effect relationship characterised the shape of the concentration-effect curves.

For the TKC studies, the area between bacterial killing curve and growth control (GC) curve (intensity of the effect, *IE*), as described by Firsov and colleagues (12), was calculated by the trapezoidal rule as PD effect. Drug effects were also evaluated by Eq. 1 and were assumed to augment the *IE* in a concentration-dependent manner.

PD modelling and simulations were performed in 'R' (RStudio with 'R' version 3.0.2, RCore Team, Vienna/Austria). Parameter estimation was performed using ordinary least squares regression; standard errors were calculated from the variance-covariance matrix.

For quantification of the extent of the drug interactions between either LZD or VAN with MER, we adapted a RSA described by Prichard and colleagues (13) using Bliss independence (BI). The expected additive effect $E_{comb,BI}$ was calculated based upon the two individual drug effects E_A and E_B using the final parameter estimates of the PD analyses of each drug (sigmoidal Emax models): $E_{comb,BI}=E_A+E_B-E_A\times E_B$. For antibiotics with a different maximum effect, we elaborated the conventional BI equation as follows: As BI was originally derived from probability theory, the maximum effect to be evaluated by BI is limited to 1.0. Hence, the effect of the more effective drug (A), i.e. E_A was normalised to 1.0, whilst the effect of the individually less effective drug (B), i.e. E_B was set to the fraction of 1.0, i.e. $Emax_B/Emax_A$ (term in large brackets in Eq. 2). The modified BI term was then scaled to the maximum effect $Emax_A$ of the more effective drug (A) to apply the elaborated equation to the experimental data:

$$E_{comb,BI}(C_A, C_B) = Emax_A \times \left(E_A(C_A) + \frac{Emax_B}{Emax_A} \times E_B(C_B) - E_A(C_A) \times \frac{Emax_B}{Emax_A} \times E_B(C_B) \right) \quad (2)$$

To quantify an interaction, we compared the measured, i.e. observed, combined effect $E_{comb,obs}$ to the predicted additive effect $E_{comb,BI}$ of Eq. 2. $E_{comb,obs} > E_{comb,BI}$ indicated synergy and vice versa antagonism. Deviations from additivity were reported either in $\log_{10}$ CFU/mL (checkerboard) or in changes in *IE* (TKC studies). For the checkerboard, Bliss antagonism or Bliss synergy were tested for significant deviation (t-test, Bonferroni-corrected, alpha = 0.05) from the expected additivity surface accounting for variability of both $E_{comb,obs}$ (variance of experimental data) and $E_{comb,BI}$ (variance of additivity response surface computed by the delta-method (14)). As the calculation of the variance for the *IE* is cumbersome, we chose to report the 'range' as a conservative measure of dispersion, which should not overlap with the 95% confidence interval of the residual error of the additivity surface for a 'significant' deviation from additivity. Moreover, a sensitivity analysis was performed to identify potentially highly influential experimental scenarios.

RESULTS

Susceptibility Testing

For MER, the MICs were 0.13 mg/L for ATCC 29213 and MV 13391 and 0.06 mg/L for MV 13488. The MICs were 2 mg/L for LZD and 1 mg/L for VAN for all studied organisms.

Conventional and Dynamic Checkerboard

Evaluation of the checkerboard by turbidity revealed no interaction between LZD and MER (Fig. 1a), particularly at inhibitory concentrations of both agents. Notably, the MIC (indicated by $^+$ in Fig. 1) for MER was 0.06 mg/L in the utilised CaMHB (Sigma-Aldrich) in comparison to the CaMHB (Oxoid) used for susceptibility testing and performance of the TKC studies in which the MIC was 0.13 mg/L. Figure 1c illustrates the result of the 'dynamic' checkerboard study when bacteria were additionally quantified. MER alone (≥0.25 mg/L) reduced the bacteria by >3 $\log_{10}$ CFU/mL. LZD alone reduced the bacteria at maximum by

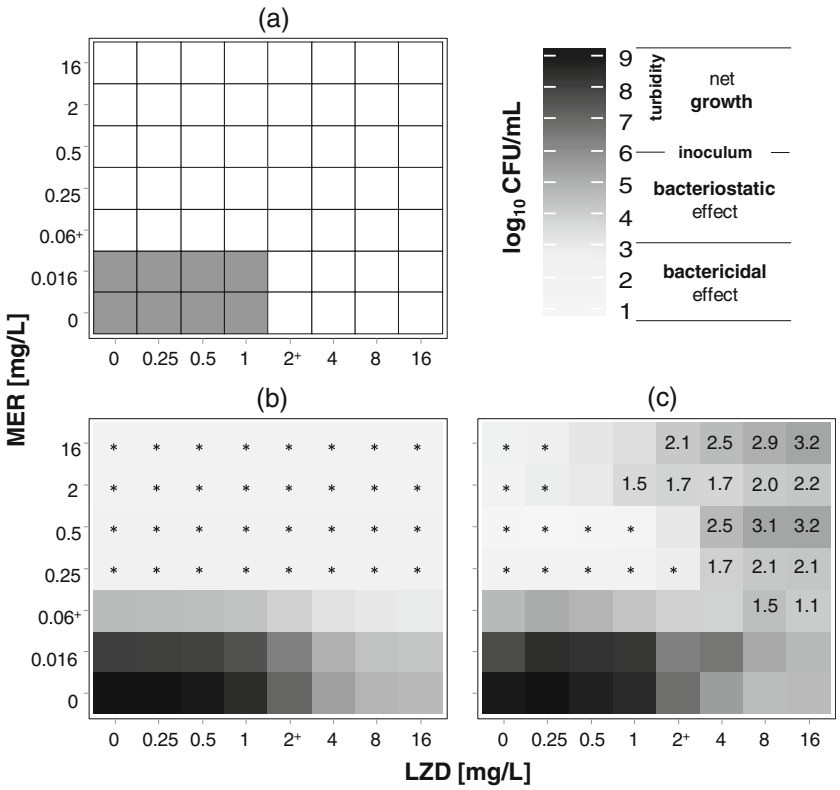

Fig. 1 Conventional checkerboard (**a**) with turbid (*grey*) and clear (*white*) cavities compared to heat map of expected, calculated additive response surface based on Bliss independence (**b**) and the observed, experimental data of the dynamic checkerboard (**c**) for each investigated combination of LZD and MER: Gradient represents bacterial counts after 18 h of incubation (mean of $\log_{10}$ CFU/mL, $n = 3$); (*) indicates bactericidal effect, ($^{+}$) indicates the MIC. Net growth (to turbidity), bacteriostatic and bactericidal effects are visualised along with the respective $\log_{10}$ CFU/mL. Significant Bliss antagonism is reported as Δ $\log_{10}$ CFU/mL directly in the heat map; no Bliss synergy was observed.

ca. 1.5 $\log_{10}$ CFU/mL. In combination, if drug concentrations exceeded the MIC, the bactericidal effect of MER was antagonised and the combinatory effect corresponded to the bacteriostatic effect of LZD alone.

Time-kill Curve Experiments

For ATCC 29213, MER alone was rapidly bactericidal after 4–6 h at concentrations up to 0.5 mg/L. Compared to MER at 0.25–0.5 mg/L, higher concentrations (2–8 mg/L) revealed a lower initial effect of MER (paradoxical effect of beta-lactams; Eagle effect (15)), but resulted in similar bacterial concentrations at 24 h (Fig. 2a). LZD was bacteriostatic and reduced the bacterial concentration at maximum by ca. 1 $\log_{10}$ CFU/mL (Fig. 2b). VAN was bactericidal after 24 h at concentrations ≥2 mg/L (Fig. 2c). Regrowth was observed up to 0.13 mg/L (MER) or 1 mg/L (VAN), respectively.

In combination, LZD at 4–32 mg/L antagonised the bactericidal effect of MER at 0.5–8 mg/L (MER) with the combinatory effect being decreased to the effect of LZD alone (Fig. 2d). The combination of VAN and MER was bactericidal after 24 h if the concentrations exceeded 0.5 mg/L for VAN and 0.13 mg/L for MER (Fig. 2e). Combinations of MER at higher concentrations (2–8 mg/L) and VAN (2–16 mg/L) resulted in the same killing profile as observed with the individual antibiotics alone (Fig. 2f).

TKC studies with the clinical isolates (MV 13488, MV 13391) confirmed the antagonism between LZD and MER (Fig. 3a, 3b) and the indifferent interaction between VAN and MER (Fig. 3c, 3d).

Pharmacodynamic and Response Surface Analysis

The PD parameter estimates of the sigmoidal Emax models of the analysis of the individual effects are presented in Table I, showing substantial differences in maximum effects *Emax* values and shape parameters EC_{50} and *H*. All PD parameters were estimated with good precision and were used to describe the concentration-effect relationship of the dynamic checkerboard (Fig. 4a) and TKC study (Fig. 4b).

For the dynamic checkerboard, the expected additivity response surface based on BI is illustrated in Fig. 1b. RSA revealed that subinhibitory LZD and MER interacted predominantly additively. Bliss antagonism was observed for combinations with inhibitory concentrations of LZD and MER (Fig. 1c).

For the TKC study, the RSA detected Bliss antagonism for all studied inhibitory concentrations of LZD with MER (Fig. 5). LZD and subinhibitory MER were Bliss additive. For VAN with MER, a trend towards Bliss synergy was observed at subinhibitory concentrations; inhibitory VAN and MER were Bliss additive (Fig. 5).

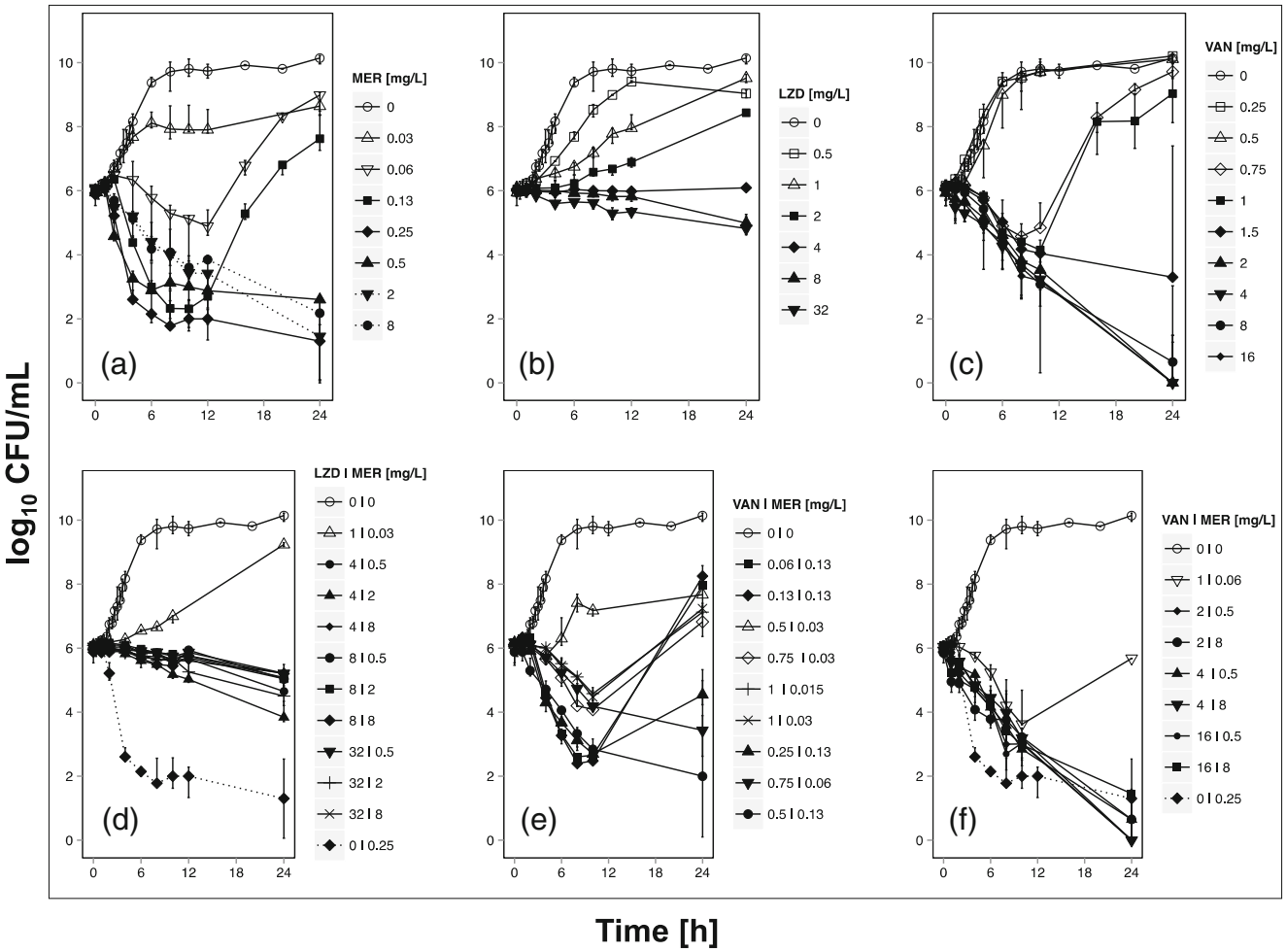

Fig. 2 Time-kill curve studies with MER (**a**), LZD (**b**) and VAN (**c**) against MSSA ATCC 29213; *open symbols* indicate subinhibitory and filled symbols inhibitory drug concentrations; *dashed lines* in (**a**) indicate TKCs in which paradoxical effects of MER were present. Time-kill curves with combinations of LZD (**d**) or VAN (**e**, **f**) with MER against MSSA ATCC 29213 (*straight lines*); *dashed lines* in (**d**) and (**f**) indicate the maximum individual effect of MER from (**a**) for better comparison. (median and range of 4–6 determinations from at least 2 separate experiments).

The sensitivity analysis revealed that the estimate of *Emax* of MER was influenced by the scenarios with high MER concentrations that displayed the paradoxical 'Eagle'-effect (i.e. 2 and 8 mg/L). In total, however, neither exclusion of the scenarios with high concentrations (i.e. 2 and 8 mg/L) nor with the maximally effective concentrations of MER (i.e. 0.25 and 0.5 mg/L) influenced the conclusions on the antagonism between LZD and MER and the additive interaction between VAN and MER.

DISCUSSION

In the present study, we systematically investigated the *in vitro* activity of combinations for PD drug-drug interactions of MER and either LZD or VAN against MSSA. The most prominent result in our experiments was that the rapid bactericidal effect of MER against MSSA was fully antagonised by LZD utilising concentrations in the clinically relevant range for both drugs (8,9) particularly matching concentrations observed at the target site, e.g. in the lung: Steady state LZD concentrations in epithelial lining fluid ranged from 2.6±1.7 to 14.4±5.6 mg/L after 600 mg intravenous LZD infusion twice daily (16). Steady state MER concentrations up to 11.4±10.9 mg/L were measured in lung tissue applying microdialysis after 1 g MER every 8 h administered via a short-term infusion (17). MER, as a cell-wall antibiotic, exerts its effect against actively replicating bacteria. As a protein-synthesis inhibitor, LZD might growth-arrest the bacteria and thus could preclude the effect of MER. To our knowledge, this is the first time that antagonism between LZD and a beta-lactam against MSSA is reported. Moreover, MSSA does not at all exhibit the same interaction pattern as MRSA, for which synergy between carbapenem and linezolid has been described (2,3).

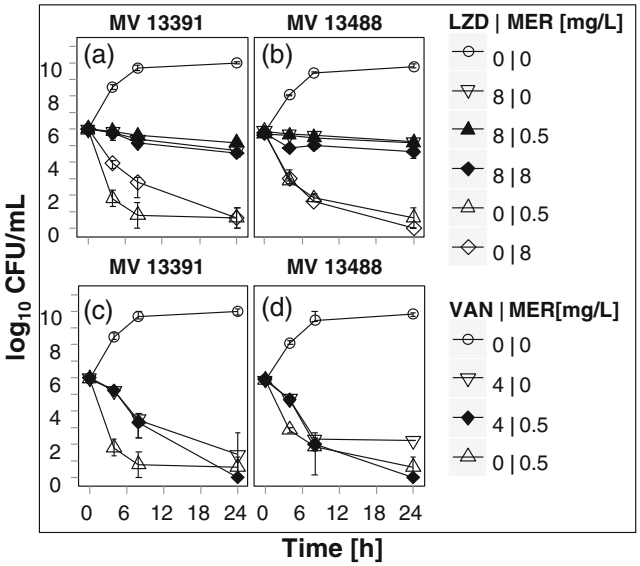

Fig. 3 Time-kill curve studies of LZD (**a**, **b**) or VAN (**c**, **d**) and MER against clinical isolates MV 13391 (**a**, **c**) and MV 13488 (**b**, **d**) of MSSA. *Open symbols* indicate single drug effects or growth control, *filled symbols* represent drug combinations. (median and range of 4–6 determinations from at least 2 separate experiments).

Combinations of VAN and MER resulted in an indifferent interaction at inhibitory concentrations of both drugs (9,10), which adds knowledge to previous studies that only investigated subinhibitory concentrations (4). VAN with MER was bactericidal when drug concentrations exceeded 0.13 mg/L (MER) and 0.5 mg/L (VAN). A trend towards synergy was confirmed when subinhibitory VAN and MER were combined. Also for VAN, the investigated drug concentrations matched the clinically observed ones at the target site: VAN concentrations in epithelial lining fluid of the lung ranged from 0.4 to 8.1 mg/L after trough concentration-adjusted (15–20 mg/L) multiple dosing (18). VAN with MER was bactericidal when drug concentrations exceeded 0.13 mg/L (MER) and 0.5 mg/L (VAN).

Table I PD Parameter Estimates (Relative Standard Error, %) From the PD Analysis of the Individual Drug Antibacterial Effects. (n.d. = not determined)

	MER	LZD	VAN
Checkerboard study			
Emax [$\log_{10}$ CFU/mL]	6.9 (5.2)	4.42 (2.6)	n.d.
EC50 [mg/L]	0.043 (22.7)	2.19 (5.1)	n.d.
H	1.77 (29.7)	2.45 (9.8)	n.d.
Time-kill curve study			
Emax [($\log_{10}$ CFU/mL) · h]	148 (4.6)	96 (1.9)	154 (2.8)
EC50 [mg/L]	0.067 (13.4)	1.55 (4.8)	1.02 (4.6)
H	2.21 (21.9)	1.33 (5.7)	3.17 (11.5)

Checkerboard experiments have been criticised (19), e.g. regarding their reproducibility (20) or interpretation (5). Our experiments underline the limitations of the 'conventional' checkerboard method when only turbidity is used as evaluation criterion. The turbidity threshold ($>10^7$ CFU/mL) was insensitive to detect the interaction between LZD and MER, possibly a reason why the interaction had not been detected in a large study applying this technique (21). However, we advocate the 'dynamic' checkerboard with quantification of bacteria in combination with modelling and simulation techniques, as applied in our study, being powerful tools for screening and hypotheses generation.

For the interaction analysis, we derived the BI RSA (13,22) to investigate drug combinations with different individual maximum effects, but a mutual maximum possible effect. The properties of the modified BI equation can be illustrated if the combined effect is evaluated at the EC_{50} values of both drugs. For the conventional BI with the same *Emax* value (i.e. 1.0) for both drugs the combined effect would result in 75% of *Emax* ($0.5+0.5-(0.5\times0.5)$). If the maximum effect of drug B was 50% of that of drug A, according to the modified BI equation the combined effect would result in 62.5% of $Emax_A$ due to the minor contribution of drug B to the combined effect [$1.0\times(0.5+0.25-(0.5\times0.25))$]. At concentrations of *Emax* of both drugs, the effect will eventually reach $Emax_A$, the maximum effect of the more effective drug [$1.0\times(1.0+0.5-(1.0\times0.5))$]. Moreover, we also considered the uncertainty of the expected additivity response surface for decision-making, which is frequently neglected (13,22–24). The presented approach allows for an individual quantitative analysis of every investigated scenario and does not assume a constant interaction type over the whole drug concentration range as other approaches do (23,25). Further, it enabled us to provide measures for the scenarios in which the interaction was not obvious (i.e. to discriminate between synergy and additivity from unprocessed TKC data). However, we have to acknowledge some limitations of our analysis originating from the utilised definition of additivity: There is some controversy which of the additivity criteria is reflecting the situation best, and appropriate definition of additivity still remains a controversial issue (26). We had to choose BI against Loewe additivity, since the latter equation is not defined for drug effects between the maximum effects if the equation is modified for drugs exhibiting different maximum effects (27). Moreover, BI, but also Loewe additivity are 'black-box' approaches that do not take into account the mechanism of (inter)action of the investigated antibiotics. Further research is necessary to provide PK/PD models to assess drug combinations in a more mechanistic way.

Whereas PK drug-drug interaction studies typically investigate the clinically relevant drug concentration range, this has rarely been exploited for PD drug-drug interactions but is crucial for assessing the interaction potential of the frequently

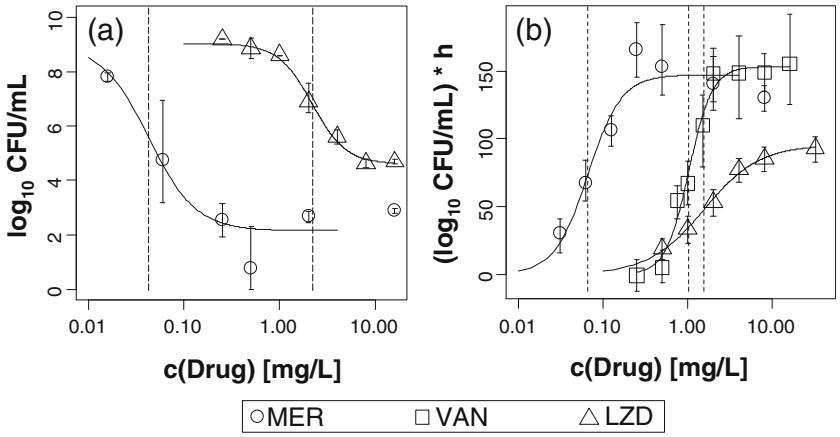

Fig. 4 Individual drug concentration-effect relationships of MER, LZD and VAN based on sigmoidal Emax models for the dynamic checkerboard (**a**) and TKC studies (**b**): symbols represent the experimental data (*error bars*: range), *curves* the predicted relationship based on the PD parameter estimates of the model; *vertical dashed lines* represent EC_{50} values of the respective antibiotics.

used antibiotic combination therapies: Exposure of bacteria to subinhibitory concentrations—as very often only used in interaction experiments—generated an additive interaction between LZD and MER, which is obviously converse at inhibitory concentrations. For both drugs, $T_{>MIC}$ is the relevant PK/PD index, i.e. sustained plasma concentrations above the MIC are generally desirable. For the comparatively low MICs of MSSA, standard doses of both LZD and MER will provide inhibitory concentrations and thus pharmacodynamic antagonism throughout the whole dosing interval.

Timely killing of bacteria is clearly the purpose of antibacterial therapy. Highly standardised TKC studies are powerful to compare differences in killing kinetics and generated the hypothesis that in MSSA VAN seems superior to LZD when combined with MER. Further research is required to clinically assess the different interactions between MER combined with LZD or VAN, whether kinetics of bacterial killing *in vivo* are similar to *in vitro* or whether additional factors play a role, e.g. the manifold actions of the immune system. Furthermore, in addition to the reduction of viable bacteria other factors may contribute to the therapeutic success, e.g. the inhibition of toxin synthesis. Yet, patients with nosocomial infections receiving LZD or VAN with MER are critically ill and likely to be immunocompromised: A suboptimal (bacteriostatic) treatment might not be sufficient for these patients and bactericidal action is considered beneficial (11). Relevant effects observed in further preclinical experiments (particularly animal studies) and ultimately clinical studies could influence the controversy on whether to use LZD or VAN for empiric combination therapy with MER (or other broad spectrum beta-lactams), since efficacy against MRSA would need to be balanced against potential differences in efficacy against MSSA and other pathogens.

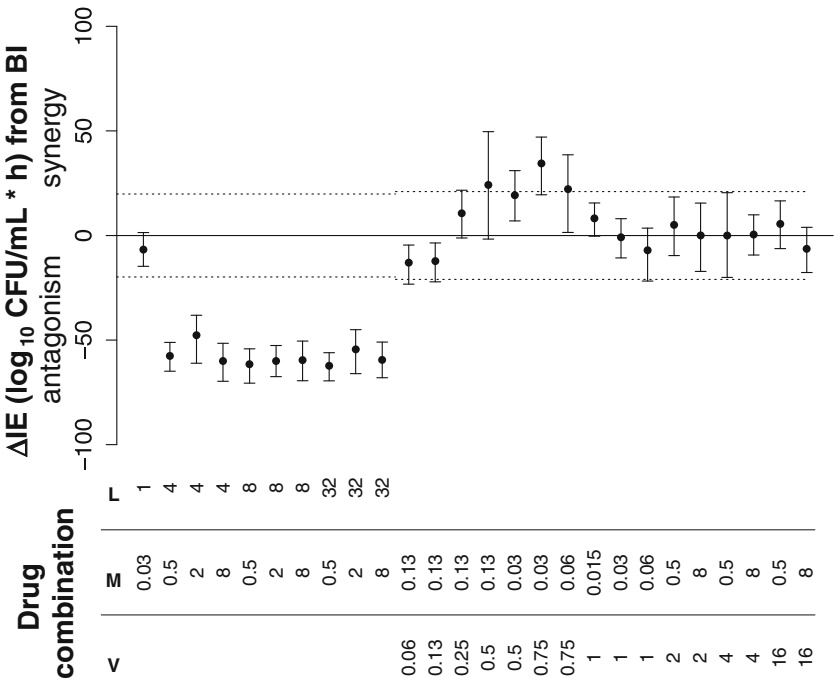

Fig. 5 Deviation of intensity of effect (*IE*) from expected Bliss independence (BI) ± range for the scenarios studied in the TKC studies (L / M / V = concentration of linezolid, meropenem and vancomycin in mg/L, respectively). 'Significant' negative or positive deviation from Bliss independence (non-overlap of range with 95% confidence interval of residual error of expected additivity response surfaces represented by *dashed lines*) indicated Bliss antagonism or synergy, respectively.

CONCLUSION

In summary, we detected antagonism between LZD and MER against MSSA, limiting the combined effect to bacteriostasis, whereas MER alone and the combination of VAN and MER were bactericidal. Moreover, our study illustrates the usefulness of combining quantitative pharmacodynamic data with modelling and simulation techniques to gain much more knowledge and insight from microbiological experiments than provided by 'traditional' (e.g. turbidity-based) methods. Further preclinical and ultimately clinical studies are warranted to further elucidate the clinical significance of our results.

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