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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A2.6

Impact of erythrocytes and thrombocytes on bacterial growth and antimicrobial activity of selected antibiotics

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Background: The efficacy of antibiotics is often predicted by *in vitro* pharmacodynamic (PD) models. Host factors such as protein binding, temperature and pH have already shown that they influence the reliability of these existing models. However, the impact of corpuscular blood compounds is not well understood and therefore often neglected.

Methods: We set out to investigate if addition of human erythrocytes or thrombocytes to standard growth media (Mueller-Hinton Broth, MHB) has an influence on bacterial growth behavior and on the efficacy of antibiotics by using bacterial growth assays and time–kill curves of selected strains; *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. Experiments were performed in triplicates over 24 hours. The final concentration of the corpuscular blood components in our experiments was set to physiological concentrations in blood of healthy human, *i.e.* an erythrocyte concentration of 3×10^6 cells/µl in cation-adjusted MHB and 2.5×10^5 thrombocytes/µl in MHB; 20 mM Ca^{2+} were used. At an infection site, thrombocytes are found to be activated. Calcium is capable to activate thrombocytes; therefore, an adjusted calcium level was set in the MHB to resemble accurate conditions. Meropenem, ciprofloxacin and tigecycline were tested as representative of broad-spectrum antibiotics with very different chemical and pharmacokinetic characteristics. Concentrations several-fold above and below the minimal inhibitory concentration (MIC) were simulated.

Results: We could confirm that erythrocytes slightly promoted bacterial growth (between 0.32 and 0.80 $\log_{10}$) and decreased antibiotic efficacy in dependence on bacterial species and the type of antibiotic in most assays. Addition of erythrocytes to MHB decreased bacterial killing after 24 hours (ratio of 24 h *vs.* baseline) for most bacterial species and tested antibiotics (delta 0.46 to 4.76 $\log_{10}$). *P. aeruginosa* tested with meropenem is an exception and showed the opposite effect with a delta of −1.27 $\log_{10}$. Tests with thrombocytes revealed no difference in bacterial growth assays. Likewise, addition of 20 mM Ca^{2+} to MHB did not influence bacterial growth. Addition of thrombocytes led to a reduction of antimicrobial activity of meropenem and ciprofloxacin for all bacterial strains. Addition of thrombocytes to MHB decreased bacterial killing after 24 hours in all bacterial species tested with meropenem and Ciprofloxacin (delta 1.30 to 2.37 $\log_{10}$). Experiments with tigecycline showed delta values around zero. Tests with *P. aeruginosa* followed the trend of a decrease in bacterial killing with a delta of 0.42 $\log_{10}$. A slightly positive effect on bacterial killing was seen with *E. coli* (delta −0.2 $\log_{10}$) and *S. aureus* (delta −0.94 $\log_{10}$).

Discussion: It can be hypothesized that decreased antimicrobial activity of antibiotics in the presence of erythrocytes may be caused by binding or intracellular accumulation in erythrocytes. The reduced activity caused by thrombocytes might also be due to binding and thereby reduced free levels of antibiotics. Whether activation and the consecutive change in confirmation or agglutination enhances this impact remains to be investigated. In conclusion, we have demonstrated that corpuscular blood components influence antimicrobial activity, which might have an impact with regard to extrapolation of *in vitro* activity testing to *in vivo* efficacy in patients.

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