

## Tigecycline attenuates polymorphonuclear leukocyte (PMN) receptors but not functions

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Tigecycline achieves high intracellular concentrations in polymorphonuclear leukocytes (PMNs). To evaluate the effects of tigecycline on human PMNs, PMNs were incubated with tigecycline dilutions (0.1 to 100 mg L<sup>-1</sup>). Phagocytosis-associated PMN Fcγ- and complement receptors as well as phagocytosis and oxidative burst induced by *Staphylococcus aureus* were measured by flow cytometry. Incubation with tigecycline caused small but significant decreases in the density of complement receptors CD11b and CD35 (all concentrations) and Fcγ receptors CD16 and CD32 (high concentrations), but not in the percentages of receptor-bearing cells, except for small reductions in the proportions of CD16 positive cells at high concentrations. Tigecycline had no effect on phagocytosis or oxidative burst induced by *S. aureus*. Tigecycline was thus associated with decreased density of PMN complement and (at high concentrations) Fcγ receptors. Although statistically significant, the differences were small and did not influence the PMN function as measured by phagocytosis and oxidative burst.

**Keywords:** tigecycline, leukocyte, phagocytosis, oxidative burst

Accepted June 16, 2011

Tigecycline, a new glycylicycline antibiotic, is the 9-*t*-butyl-glycylamido semisynthetic derivative of tetracycline minocycline (1). Tigecycline is moderately bound to human plasma proteins, penetrates highly into inflammatory fluid (2) and exhibits high activity against a broad array of bacteria (3). High intracellular concentrations within polymorphonuclear leukocytes (PMNs) were demonstrated after 1 h of incubation (4). Different antibiotics may have divergent effects on cells of the immune system (5). We had previously found some, but not all, tetracyclines to influence human PMN receptors and functions (6–8). We now studied normal PMNs after incubation with tigecycline to see if the high intracellular concentrations of this tetracycline derived antibiotic interfered with phagocytosis-associated surface receptors on PMNs and with the PMN function as measured by phagocytosis and oxidative burst after incubation with *Staphylococcus aureus*.

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## EXPERIMENTAL

### *Tigecycline*

Tigecycline (Wyeth Pharmaceuticals, USA) was dissolved in Dulbecco's phosphate-buffered saline supplemented with glucose and bovine serum albumin (DPBS-GA) and used the same day.

### *Cells and incubation*

PMNs were obtained from heparinized venous blood of healthy donors. Erythrocytes were lysed and the leukocyte fraction was washed twice, resuspended in DPBS-GA and adjusted to a concentration of  $1.25 \times 10^7$  nonlymphocytes (PMNs and monocytes) per mL (9). Viability was > 98 % as measured by trypan blue exclusion.

Leukocytes were preincubated for 30 min at 37 °C in different concentrations of tigecycline in DPBS-GA in sterile and nonpyrogenic microtiter plates (Costar® 3790 96 Well Cell Culture Cluster, Corning, USA) in a temperature-controlled shaker. Control cells were incubated in DPBS-GA without tigecycline.

### *Receptor studies*

PMNs were marked with the following mouse anti-human monoclonal antibodies according to the instructions of the manufacturers: CD11b-PE (BD Biosciences, USA), CD16-PC5, CD32-PE, CD35-FITC, CD64-FITC and isotype controls IgG1-FITC, IgG1-PC5 and IgG2a-PE (Beckman Coulter, UK). Results are presented as mean fluorescence intensity (MFI) in arbitrary units.

### *Phagocytosis and oxidative burst*

*S. aureus* bacteria (Cowan III, NCTC 8532, National Collection of Type Cultures, UK) opsonized with pooled human serum were used as targets for phagocytosis and stimuli for oxidative burst. For phagocytosis, bacteria were labelled with rhodamine green X, and oxidative burst substrate was dihydrorhodamine 123.

For phagocytosis, leukocytes were preincubated as described. Preopsonized rhodamine green X-labelled *S. aureus* (20 per nonlymphocyte) were added to the leukocyte wells for 7.5 min. The reaction was stopped by adding 0.2 mL of cold DPBS with 0.02 % EDTA to each well. The plates were then put on ice for 30 min, diluted 1:5 in DPBS-EDTA and phagocytosing and non-phagocytosing cells were counted by flow cytometry. The results are presented as phagocytic index, defined as the percentage of phagocytosing nonlymphocytes multiplied by the mean number of bacteria per phagocytosing cell.

For oxidative burst quantitation, preopsonized, unlabelled *S. aureus* (20 per nonlymphocyte) and  $10 \mu\text{g mL}^{-1}$  of dihydrorhodamine 123 were added to each leukocyte well, the final concentration of dihydrorhodamine being  $2 \mu\text{g mL}^{-1}$ . The resulting conversion to fluorescent rhodamine was measured by flow cytometry (10). Results are presented in arbitrary units.

Flow cytometry was performed on a Coulter Epics XL-MCL flow cytometer (Beckman Coulter) (10).

### Statistical analysis

Data are presented as mean  $\pm$  SD. Student's paired sample *t*-test (SPSS) was used to determine the significance of differences.

### Ethics

The study was approved by the Regional Committee for Medical Research Ethics, Western Norway.

## RESULTS AND DISCUSSION

CD11b and CD35 density on the cells was reduced with all tigecycline concentrations (Fig. 1). CD16 density was reduced with 100 mg L<sup>-1</sup> of tigecycline only (Fig. 2), and CD32 with 10 and 100 mg L<sup>-1</sup> (Fig. 3).

The proportions of PMNs bearing complement (CD11b, CD35) or Fc $\gamma$ II (CD32) receptors were not influenced by incubation with tigecycline and were between 98.9 % and 100.0 % (mean values) for all tigecycline concentrations as well as for the controls. The proportion of CD16 positive cells was slightly lower (mean 94.4 % without tigecycline), and decreased to a mean value of 86.8 % with 100 mg L<sup>-1</sup> of tigecycline ( $p < 0.05$ , data not shown). No significant differences were found in either of these cases.

Fig. 1. Density of complement receptors CD11b (squares) and CD35 (triangles) after incubation in tigecycline. Bars represent standard deviations of the means of 12 samples. Incubation in tigecycline (all concentrations) was associated with a significant (\*:  $p < 0.05$ ) reduction of receptor density. MFI – mean fluorescence intensity.

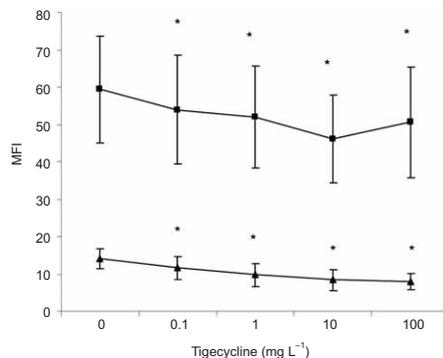
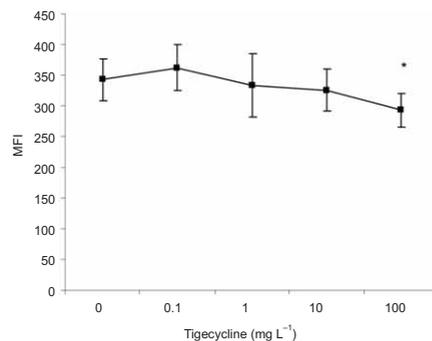


Fig. 2. Density of Fc $\gamma$  receptor III (CD16) after incubation in tigecycline. Bars represent standard deviations of the means of 12 samples. Cells incubated in 100 mg L<sup>-1</sup> of tigecycline showed a significantly (\*:  $p < 0.05$ ) decreased receptor expression. MFI – mean fluorescence intensity.



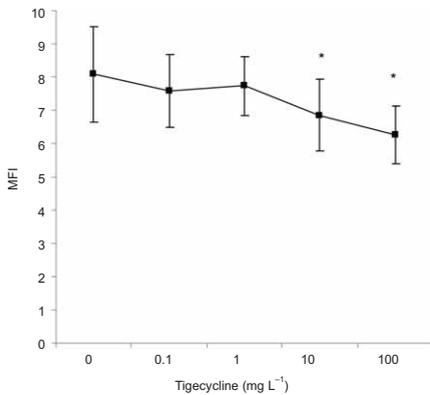


Fig. 3. Density of Fc $\gamma$  receptor II (CD32) after incubation in tigecycline. Bars represent standard deviations of the means of 12 samples. Cells incubated in 10 and 100 mg L<sup>-1</sup> of tigecycline showed a significantly (\*;  $p < 0.05$ ) decreased receptor expression. MFI – mean fluorescence intensity.

Phagocytosis of *S. aureus* was not influenced by incubation in 0.1, 1, 10, or 100 mg L<sup>-1</sup> of tigecycline (Fig. 4). Oxidative burst was not affected neither (Fig. 5).

We found incubation of PMNs with tigecycline to be associated with small but statistically significant decreases in the density of complement and, at high concentrations, of Fc $\gamma$  receptors on the cells. However, with the exception of a small decrease in the percentages of cells bearing the CD16 receptor after incubation in high concentrations of tigecycline (10 and 100 mg L<sup>-1</sup>), the proportions of cells bearing these receptors were not affected, and neither were phagocytosis and oxidative burst. This suggests that tigecycline, at this wide range of concentrations, does not affect human PMN function as measured by these parameters.

*S. aureus* are normally phagocytosed *via* complement receptors. The fact that significant decreases in CD11b density on the phagocytes were not associated with attenuated phagocytosis of *S. aureus* suggests an inherent over-capacity of this part of the immune system.

To our knowledge, the effects of tigecycline on PMN function have not been previously studied. However, tigecycline is a derivative of tetracycline, minocycline, and deleterious effects of tetracyclines on PMN phagocytosis have been known for more than three decades (11). We have previously found diverging effects of different tetracyclines on PMN functions; while high concentrations of doxycycline and minocycline were

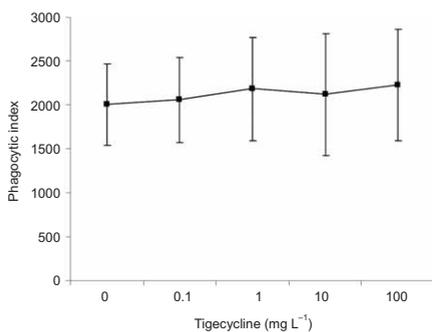


Fig. 4. Phagocytosis of *S. aureus* without and with 0.1, 1, 10 and 100 mg L<sup>-1</sup> of tigecycline. The results are presented as phagocytic index, defined as the percentage of phagocytosing nonlymphocytes multiplied by the mean number of bacteria per phagocytosing cell. Bars represent standard deviations of the means of 12 samples. No significant differences were observed.

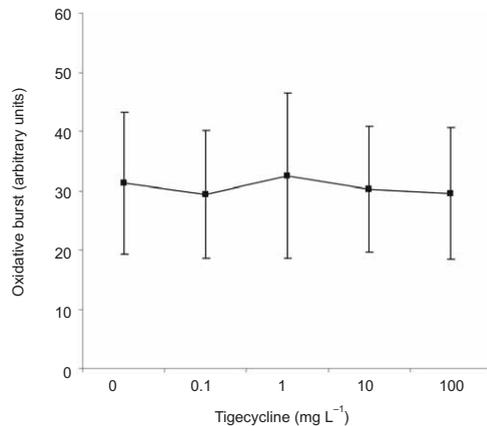


Fig. 5. Oxidative burst induced by *S. aureus* without and with 0.1, 1, 10 and 100 mg L<sup>-1</sup> of tigecycline. Bars represent standard deviations of the means of 12 samples.

found to impair PMN chemiluminescence and glucose oxidation, this was not the case with oxytetracycline (6). Incubation in doxycycline was also associated with a decreased percentage of Fc $\gamma$  receptor bearing PMNs and lymphocytes (7), and patients with acute myocardial infarction receiving doxycycline were found to have reduced PMN activity (12). However, a decrease in PMN chemiluminescence with increasing concentrations of doxycycline could not be reproduced using a commercial doxycycline preparation, suggesting that additives may neutralize this effect in some *i.v.* preparations (8).

## CONCLUSIONS

High intracellular concentrations of tigecycline in PMNs (4) may be a therapeutic advantage (1). While the transport of tigecycline into the cells may be associated with changes in the density of surface receptors, as suggested by the results of the present study, with small but significant decreases in the density of complement and Fc $\gamma$  receptors, we found no indication of interference with the antimicrobial function of the cells, not even at supratherapeutic concentrations of the drug.

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## S A Ž E T A K

### Tigeciklin slabi receptore za polimorfonuklearne leukocite (PMN) ali ne i njihovu funkciju

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Tigeciklin postiže visoku intracelularnu koncentraciju u polimorfonuklearnim leukocitima (PMN). Da se ispita učinak tigeciklina na humane PMN, PMN su inkubirani s različitim koncentracijama tigeciklina (0,1 do 100 mg L<sup>-1</sup>). Protočnom citometrijom praćeni su s fagocitozom povezani PMN Fc $\gamma$ - i komplementarni receptori, fagocitoza i oksidativno prsnuće inducirano bakterijom *Staphylococcus aureus*. Inkubacija s tigeciklinom utjecala je na malo, ali značajno smanjenje gustoće komplementarnih receptora CD11b i CD35 (sve koncentracije) i Fc $\gamma$  receptora CD16 i CD32 (visoke koncentracije), ali ne i na postotak stanica s receptorom, osim malog smanjenja udjela CD16 pozitivnih stanica pri visokim koncentracijama. Tigeciklin nije imao učinak na fagocitozu ni na oksidativno prsnuće inducirano bakterijom *S. aureus*. Zbog toga je tigeciklin povezan samo sa smanjenjem gustoće PMN komplementa i u visokim koncentracijama Fc $\gamma$  receptora. Iako je razlika statistički značajna, bila je mala i nije utjecala na funkciju PMN u pogledu fagocitoze i oksidativnog prsnuća.

*Ključne riječi:* tigeciklin, leukocit, fagocitiza, oksidativno prsnuće

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