FIN/DEB, described previously, using an ABI377 sequencer (Applied Biosystems, Foster City, CA, USA). This allowed detection of mutations at nucleotide position 913, and also confirmed PCR-RFLP results.

PCR-RFLP analysis discriminated the blaTEM genes into three restriction linkage groups (according to polymorphic sequence variations) (Table 1): the 'TEM-1A type' (n = 1), the 'TEM-1B type' (n = 14) and the 'TEM-1C type' (n = 2). Nucleotide sequencing of blaTEM genes representative of each group confirmed this classification. The linkage groups of these blaTEM genes from animal sources were identical to those of the corresponding parental blaTEM genes from human specimens. All linkage groups had C32 in the promoter region, indicating a weak promoter. However, some of the strains presented resistance phenotypes (with MICs of amoxicillin 1024 → 2048 mg/L, cefalothin 16 → 32 mg/L and mecillinam 0.5 → 2.56 mg/L, data not shown). This may be due to enzyme hyperproduction as a result of the gene being on multicopy plasmids, tandem-genes in a single plasmid and/or reduced permeability of outer cell membrane to antibiotics.

Analysis of the molecular diversity of blaTEM genes encoding β-lactamases contributes to our understanding of this mechanism of resistance in Enterobacteriaceae isolated from humans, or animals (this study). We demonstrated the genetic relatedness between blaTEM genes in pathogenic E. coli strains isolated from humans, and animal species (this study). This genetic proximity is in agreement with the known zoonotic potential of uropathogenic E. coli strains: these strains display PapA subunit diversity and share the same papG alleles coding for the Pap fimbriae adhesin, both of which are virulence factors.

Acknowledgements

This work was supported by POCTI/CVT/36253/99 (FEDER) grant from Fundação para a Ciência e a Tecnologia, Lisbon, Portugal.

References


Subinhibitory concentrations of florfenicol enhance the adherence of florfenicol-susceptible and florfenicol-resistant Staphylococcus aureus

Maren Blickwede1, Peter Valentin-Weigand2 and Stefan Schwarz*1

1Institut für Tierzucht, Bundesforschungsanstalt für Landwirtschaft (FAL), Höltystrasse 10, 31535 Neustadt-Mariensee; 2Institut für Mikrobiologie, Zentrum für Infektionsmedizin, Tierärztliche Hochschule Hannover, Bischhofsholer Damm 15, 30173 Hannover, Germany

Keywords: invasion, fibronectin, surface hydrophobicity

*Corresponding author. Tel: +49-5034-871-241; Fax: +49-5034-871-246;
E-mail: stefan.schwarz@fal.de

Sir,

The ability of Staphylococcus aureus to adhere to surfaces of host tissues is thought to be essential for colonization and establishment of infections. There is increasing evidence that subinhibitory concentrations of antibiotics interfere with microbial adherence to host cells. In this study, the effect of subinhibitory concentrations of the fluorinated chloramphenicol derivative florfenicol on adherence properties of florfenicol-susceptible and florfenicol-resistant S. aureus was investigated.

The florfenicol-susceptible strain S. aureus Newman (ATCC 25904) and its florfenicol-resistant derivative carrying the cfr-erm(33)-sre multiresistance plasmid pSCFS1 from Staphylococcus sciuri were cultivated for 20 h at 37°C in brain heart infusion broth (BHI, Oxoid, Wesel, Germany) and in BHI supplemented with either 0.2 mg/L florfenicol (Essex Tierarznei, Munich, Germany) or 2 mg/L florfenicol (= 1/20 and 1/2 MIC for S. aureus Newman, respectively). In addition, S. aureus Newman:pSCFS1 was cultivated in BHI supplemented with 64 mg/L florfenicol (= 1/2 MIC for S. aureus Newman:pSCFS1). MICs of florfenicol were determined by broth microdilution according to the NCCLS guideline M31-A2. Adherence and invasion assays using 5 × 10^6 bacteria per experiment and confluent monolayers of HEP-2 cells (~3 × 10^5 cells per well) were carried out as described by Dziewanowska et al. The results were recorded as percentage of cfu of adherent or intracellular bacteria. For microscopic confirmation, adherence assays were carried out on culture slides containing ~2 × 10^5 HEP-2 cells. Washed monolayers with adherent bacteria were fixed with 0.37% formaldehyde (Sigma, Taufkirchen, Germany) at 4°C, washed with PBS and covered with blocking buffer [10% fetal calf serum (Sigma) in PBS] for 1 h. Adherent staphylococci were detected by use of a rabbit-anti-staphylococcal antibody, an FITC-conjugated goat-anti-rabbit IgG antibody (Dianova, Hamburg, Germany) and subsequent immunofluorescence microscopy. Bacteria-epithelial cell ratios were determined by counting manually the number of bacteria adherent to
Correspondence

Table 1. Effect of subinhibitory concentrations of florfenicol on adherence and invasion properties of florfenicol-susceptible *S. aureus* strain Newman and florfenicol-resistant *S. aureus* strain Newman:pSCFS1

<table>
<thead>
<tr>
<th></th>
<th><em>S. aureus</em> Newman</th>
<th></th>
<th><em>S. aureus</em> Newman:pSCFS1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>0.2 mg/L Ff</td>
<td>2 mg/L Ff</td>
</tr>
<tr>
<td>Adherence to HEp-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cells (%)</td>
<td>4.15(±1.24)</td>
<td>5.06(±0.92)</td>
<td>15.11(±1.15)</td>
</tr>
<tr>
<td>Adherence to HEp-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cells (bacteria/cell)</td>
<td>1.3(±0.2)</td>
<td>1.3(±0.1)</td>
<td>5.6(±1.1)</td>
</tr>
<tr>
<td>Invasion (%)</td>
<td>0.04(±0.04)</td>
<td>0.07(±0.09)</td>
<td>0.1(±0.1)</td>
</tr>
<tr>
<td>Adherence to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heptadecane (%)</td>
<td>38.1(±10.5)</td>
<td>31.2(±4.1)</td>
<td>29.2(±20.5)</td>
</tr>
<tr>
<td>Adherence to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibronectin (%)</td>
<td>3.15(±2.14)</td>
<td>3.71(±2.57)</td>
<td>16.71(±9.55)</td>
</tr>
</tbody>
</table>

The results are given as mean values of three independent tests, processed in duplicate (± standard deviations). Ff, florfenicol.

*Values differ significantly (*P* ≤ 0.05, unpaired two-sided Student’s *t*-test) from the untreated controls.

epithelial cells in three microscopic fields with ~ 50 epithelial cells per field.

Growth in the presence of low subinhibitory concentrations of florfenicol, such as 0.2 mg/L for *S. aureus* Newman as well as 0.2 and 2 mg/L for *S. aureus* Newman:pSCFS1, did not change significantly the adherence patterns of both strains compared with the untreated controls. However, when grown in the presence of the strain-specific 1/2 MIC, both strains adhered significantly better (*P* ≤ 0.05) to HEp-2 cells (Table 1). Although invasion also increased significantly when the bacteria were treated with the strain-specific 1/2 MIC, the percentages of invading bacteria ranged between 0.1% and 0.28% (Table 1) and thus were negligible in view of the percentages of adherent bacteria. This observation was in good accordance with that of Dzwiekanowska et al., who showed that *S. aureus* strain Newman is less invasive than other *S. aureus* strains which exhibit comparable adherence to epithelial cells. Thus, virtually all bacteria detected after release from the epithelial cells could be considered as truly adherent bacteria. Quantification of the adherent bacteria per epithelial cell by immunochemical microscopic evaluation yielded results that correlated well with those obtained by the plating technique. A four-fold increase in the number of adherent bacteria per epithelial cell was detected in case of the florfenicol-resistant *S. aureus* Newman whereas an eight-fold increase was seen in the case of the florfenicol-susceptible *S. aureus* Newman:pSCFS1 (Table 1).

Bacterial adhesion can be the result of either hydrophobic interactions between the bacteria and the host cells, binding of bacteria to specific ligands, e.g. fibronectin, or a combination of both. Therefore, florfenicol-treated staphylococci and untreated controls were investigated for their surface hydrophobicity by a hydrocarbon adherence assay and for their ability to bind to fibronectin-coated micotitre plates (1 µg of fibronectin per well). Independently of the florfenicol concentration used, both strains showed a non-significant decrease in their surface hydrophobicity (Table 1). In contrast, the fibronectin-binding profiles of both strains grown in the presence of the strain-specific 1/2 MIC of florfenicol exhibited significant four- to five-fold increases (Table 1). Based on this finding, we assume that binding to fibronectin plays a relevant role in the increased adherence of the staphylococci to HEp-2 cells, rather than non-specific hydrophobic interactions.

The observations made in this study differ from those of a previous study on tetracycline-treated *S. aureus* in which increased adherence was directly correlated with significantly increased hydrophobicity. However, the results of our study also showed parallels to a study in which pretreatment of *S. aureus* with sub-MIC levels of ciprofloxacin gave an induced expression of fibronectin binding proteins and also resulted in increased adhesion of the staphylococci. Although further work is needed to elucidate the mechanisms leading to the observed changes in the adherence patterns, our data demonstrate that florfenicol concentrations in the range of strain-specific 1/2 MIC enhance the adherence of florfenicol-susceptible and also florfenicol-resistant *S. aureus* to epithelial cells, and thus may alter virulence properties of this pathogen.

Acknowledgements

We wish to thank Laurentiu Benga for his expert introduction into the adherence experiments and Gunter Amtsberg for the staphylococcal antibody. This work was supported by the Deutsche Forschungsgemeinschaft (GK 745).

References


Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh288
Advance Access publication 9 June 2004

Inhibition of TNF-α production in THP-1 macrophages by glatiramer acetate does not alter their susceptibility to infection by Listeria monocytogenes and does not impair the efficacy of ampicillin or moxifloxacin against intracellular bacteria

Halim Ben Abdelaziz, Sandrine Lemaire, Stéphane Carryn†, Françoise Van Bambeke, Marie-Paule Mingeot-Leclercq and Paul M. Tulkens*

Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, 73.70 avenue E. Mounier 73, B-1200 Brussels, Belgium

Keywords: L. monocytogenes, listeriosis

*Corresponding author. Tel: +32-2-762-21-36; Fax: +32-2-764-73-73; E-mail: tulkens@facm.ucl.ac.be

Sir,

Listeriosis is one of the potential adverse effects of TNF-α-neutralizing treatments.1 Glatiramer (copolymer 1; COPAXONE), a mixed, random polymer of Ala, Glu, Lys and Tyr used in the treatment of relapsing–remitting multiple sclerosis2 blocks the secretion of TNF-α from IFN-γ- and endotoxin-stimulated THP-1 macrophages.3 We have, therefore, examined the influence of glatiramer on the ability of IFN-γ to contain Listeria infection and on the activity of ampicillin and moxifloxacin to kill intraphagocytic bacteria in THP-1 macrophages. Glatiramer [CAS Registry no. 147 245-92-9; batch no. 242908102: average molecular weight, 7500Da (limits, 4200–16 350); amino acid content (molecular fraction) L-Glu, 0.139; L-Ala, 0.432; L-Tyr, 0.091; L-Lys, 0.338; total amino acid residue content, 87.9%; bacterial endotoxin content, <0.25 endotoxin units/mg] was kindly received from Teva Pharmaceuticals Industries (Petah Tiqua, Israel). All experimental procedures and assay methods have been described in our previous publications.4,5 We used a concentration of glatiramer of 20 mg/L, which was both non-toxic (based on lactate dehydrogenase release) and effective in blocking the production of TNF-α in THP-1 cells.6

Glatiramer (20 mg/L) did not influence the intrinsic antimicrobial activity of ampicillin or moxifloxacin towards L. monocytogenes, based on MIC determinations in broth [0.3 ± 0.1 and 0.5 ± 0.1 mg/L (arithmetic dilutions) for ampicillin and moxifloxacin, respectively]. Unstimulated cells produced only negligible amounts of TNF-α and glatiramer did not alter this behaviour. In contrast, the medium of cells exposed to IFN-γ (100 units/mL; 24 h) contained 38.3 ± 0.6 ng/L of TNF-α, and this concentration was decreased by ~2/3 in the presence of glatiramer. With infected cells, TNF-α production remained low in unstimulated cells and unaffected by the presence of glatiramer, whereas it amounted to 31.6 ± 1.2 ng/L (5 h post-phagocytosis) in IFN-γ-stimulated cells (24 h prior to infection). This production was again decreased by 2/3 if glatiramer was present. Glatiramer did not significantly modify the capacity of THP-1 macrophages to phagocyte L. monocytogenes. Figure 1 shows that glatiramer did not modify the growth of intracellular L. monocytogenes compared with untreated cells in the 24 h model (this model uses gentamicin at a concentration of 2 × its MIC to prevent the extracellular growth of L. monocytogenes). No change was seen either in the 5 h model (data not shown). As previously described,6 IFN-γ impaired the intracellular growth of

![Figure 1. Influence of glatiramer on the course of L. monocytogenes intracellular infection in J774 macrophages. Cells were exposed to L. monocytogenes for 1 h (phagocytosis) and then returned to fresh medium for 24 h before cell collection and enumeration of cell-associated bacteria. The growth of extracellular bacteria, which would otherwise occur after 5–6 h through the release of dying cells, was prevented by addition of gentamicin [2 mg/L (2 × MIC); this concentration does not prevent the intracellular growth of L. monocytogenes, data not shown]. The four left blocks refer to cells unexposed to ampicillin and treated as follows: ctrl (control), no treatment; gtr (glatiramer), cells incubated with glatiramer (20 mg/L) for 24 h, and then maintained in the presence of the same concentration of glatiramer during the phagocytosis of L. monocytogenes as well as during the post-phagocytosis period (fresh solutions of glatiramer were used each time); IFN (IFN-γ), cells exposed to IFN-γ (100 units/mL) for 24 h before phagocytosis (IFN-γ was not present during phagocytosis or during the post-phagocytosis period); gtr + IFN, cells treated as for the IFN group but with glatiramer present throughout the experiment as in the gtr group. The four right blocks refer to experiments with the same design but for which ampicillin (50 mg/L) was added during the 24 h post-phagocytosis period. The ordinate shows the change in bacterial counts from original inoculum (post-phagocytosis). All data points are the mean of three determinations ± S.D. Blocks with the same letters denote groups that are not significantly different from one another by one-way ANOVA (P>0.05). This experiment was performed twice with essentially similar results.

288