Differential Study of Antimicrobial Activity of Vancomycin and Teicoplanin (Targocid) against Strains of Staphylococcus aureus and Streptococci sp.

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Abstract
The glycopeptide antibiotics vancomycin and teicoplanin (targocid) are widely used in the treatment of infections caused by gram positive bacteria. Vancomycin inhibits both transglycosylation and transpeptidation reaction during peptidoglycan assembly, targocid inhibits peptidoglycan polymerization, resulting in inhibition of bacterial cell wall synthesis and cell death. In this study twenty strains of streptococcus pyogenes and streptococcus pneumoniae and twenty five strains of staphylococcus aureus were collected from different infections. MICs and MBCs of vancomycin and targocid were determined against the bacterial strains, the MICs of vancomycin for the streptococcus strains were 4-8 µg/ml compared with 8-16 µg/ml for targocid and MICs of targocid for staphylococcus aureus were 4-16 µg/ml. the conclusion of this study was that targocid was less active than vancomycin against staphylococci but equal or more active against streptococci specifically it presents excellent in vitro activity against streptococcus pneumonia strains.

دراسة تفريقيّة لفعالية المضادات الحيوية للفاعكومايسين والتاركوكسيد ضد سلالات من المكورات الذهبيه العنقودية والمكورات السببية

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المستخلص

إن المضادات الحيوية والتي تشمل الفانكومايسين والتيموكاليتين (التاركوكسيد) تستخدم على نطاق واسع في علاج الإصابات الناتجة عن البكتيريا الموجه كرم. أن الفانكومايسين يرتبط فيفاعً لموضوع المجاميع السكرية سلالات клакاجان والأرباط المتعرض لسلوك الـ ببتيدات خلال عملية تجميع الببتيدوكلاكين، التاركوكسيد يرتبط بثمرة الببتيدوكلاكين والذي ينتج عنه تثبيط تكوين جدار البكتيريا وموت الخلايا. في هذه الدراسة عاشرون سلالة من بكتيريا المكورات السببية الفيروسية والـ الكورات السببية النورية وخمسة عشرون سلالة من المكورات الذهبيه العنقودية تم جمعها من إصابات مختلفة. وقد وجد إن التركيز الإدائي المثبط للفاعكومايسين ضد المكورات السببية كان 10-8 ميكروغرام لكل مل ونسبة مكالمات 8-16 ميكروغرام لكل مل للتاركوكسيد وتركيز الإدائي المثبط للتاركوكسيد ضد المكورات السببية كان 4-16 ميكروغرام لكل مل بينما التركيز الإدائي القاتل لمبهم السلالات كان 32 ميكروغرام لكل مل. أن التاركوكسيد هو أقل فعالية من الفانكومايسين ضد المكورات العنقودية ولكن مساوي أو أكثر فعالية ضد المكورات السببية وخصوصا عندما أظهر فعالية ممتازة ضد المكورات السببية النورية.
Introduction
Severe infection associated with gram-positive organism such as staphylococcus aureus and streptococcus sp. contribute to significant morbidity and mortality examples include bacteremia, skin and soft tissue infections, hospital- acquired pneumonia, endocarditis, meningitis, bone and joint infections and catheter- related blood stream infections (1,2). The glycopeptide antibiotics vancomycin and teicoplanin are widely used in the treatment of infections caused by gram-positive bacteria. Vancomycin is the drug that is consistently active against methicillin-resistant staphylococcus aureus and is the preferred treatment for methicillin- resistant Staphylococci, while resistance to vancomycin did not emerge until almost 30 years after its introduction (3). Vancomycin inhibits both transglycosylation and transpeptidation reaction during peptidoglycan assembly. It makes 5 hydrogen bonds to the D-Ala-D-Ala dipeptide terminus of each uncross linked peptidoglycan side chain, inhibiting both the transglycosilase and transpeptidase activity of PBPs (4).
Teicoplanin is a glycopeptide antibiotic, obtained in 1978 by fermentation from actinoplanes teichomyceticus, bacteria have an external cell wall that is reinforced by molecules called peptidoglycans, the cell wall is vital for protection against the normal environment of the body in which the bacteria live (5). Teicoplanin works by blocking the fermentation of these peptidoglycans. By doing this walls of the bacteria become weak and this results in the death of the bacteria. Teicoplanin is used to treat serious infections of the heart and blood, it is not absorbed from gut and therefore only given by injection or infusion (6).

Materials and Methods
Bacterial Strains:
Twenty strains of streproccus pyogens and streptococcus pneumoniae were collected from patients with different infection in Tikrit teaching hospital and tested for gram stain morphology, colony morphology on blood agar, hemolysis on sheep blood agar, optochin susceptibility test, bacitracin susceptibility test, susceptibility in deoxycholate (bile), salt carbohydrate utilization, miniaturization manual systems such as the APi 20 strep system. There were 13 streptococcus pyogenes strains, seven streptococcus pneumonia strains. Strains were stored in human blood and subcultured on 5% human blood agar plates (7). Also twenty five strains of staphylococcus aureus were collected from different patients with different infections and tested for gram stain morphology, colony morphology on nutrient and blood agar, Mannitl salt agar and coagulase test (8).

Antimicrobial activity of vancomycin and teicoplanin (targocid):
Vancomycin and teicoplanin were dissolved in sterile water at a concentration 125 μg/ml. determination of MIC was carried out using the standard methods of serial two fold dilution of vancomycin and targocid in Muller- hinton broth (Difco) (9). Antimicrobial concentrations ranged from 0.013 to 64 μg/ml. By using an automatic pipette, 0.1 ml of 10^-2 cfu/ml bacterial suspension was added to 0.1 ml of each antibiotic dilution in 0.8 ml of Muller- Hinton broth. Gentle mixing was carried out by repeated aspirations and flushing in order to avoid splashing the bacteria against the tube walls, which in turn allow them to escape exposure the antibiotic (9). The colony count of the final inoculums was determined by plating an appropriate dilution of the control tube containing no antibiotic. The tubes inoculated with bacteria were incubated for 18-24 hr. at 37°C and the MIC was determined as the lowest antibiotic concentration preventing visible growth (10). For MBC, the exact number of surviving organisms at 24h. in each dilution tube was determine by sampling 10 μl from all tubes without visible growth, and from the first tube with growth, as well as from the control tube.
containing no antibiotic. Each 10 μl sample was spread with a glass rod over the entire surface of a blood agar plate. Colony counts on blood agar plates were read after 24 hr of incubation at 37°C in 5% CO₂ (4).

**Results and Discussion**

The glycopeptides has been used in the treatment of infections in human since the introduction of vancomycin is 1956. beside vancomycin, other glycopeptides have been tested. Only teichoplanin introduced in 1984, has been widely used until now for the treatment of human in most parts of world (11,12). The glycopeptides have been reserved as second line antibiotics for infections caused by β-Lactam-resistant gram positive bacteria (13). As shown in table 1, the vancomycin MICs ranges for 20 isolates of *staphylococcus aureus* tested were 0.5-8 μg/ml in another studies the MICs values were ranged from 0.5-4 μg/ml in thalia and Michael (3,14), 1 μg/ml in jenny (9), Maritza (15) and 8 μg/ml in Jennifer studies (16). Teichplanin MICs for 20 isolates of *staphylococcus aureus* were ranged from 4-16 μg/ml and according to comitedel antibiogramme de la societe Francaise De microbiologie (17), this result represented as two isolates were susceptible to teichoplanin (MICs ≤ 4 μg/ml ), two isolates showed intermediate susceptibility (MICs= 8 μg/ml) and 16 isolates were resistant (MICs= 16 μg/ml). Also table 1 shows the vancomycin MICs ranges for seven isolates of *streptococcus pneumonia*, which were 2-8 μg/ml, In another studies the MICs values were ranged from 0.12 μg/ml (5), 0.5 μg/ml (25) and 0.03-0.25 μg/ml (21). While the teichoplanin MICs for the same isolates of *streptococcus pneumoniae* tested were ranged from 4-8 μg/ml compared with 0.062 μg/ml in Jenny study (9) and 0.03 μg/ml for both streptococcus *pneumoniae* penicillin susceptible and streptococcus *pneumoniae* penicillin resistant in Noviello's study (21).

**Table (1):- MICs of Bacterial strains to Vancomycin and Targocid**

<table>
<thead>
<tr>
<th>M.o.</th>
<th>Antibiotic</th>
<th>Number of strains have MIC (μg/ml) equal to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥ 64</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>Vancomycin</td>
<td>11</td>
</tr>
<tr>
<td>N=13</td>
<td>Targocid</td>
<td>9</td>
</tr>
<tr>
<td><em>S. pneumonia</em></td>
<td>Vancomycin</td>
<td>5</td>
</tr>
<tr>
<td>N=7</td>
<td>Targocid</td>
<td>6</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>Vancomycin</td>
<td>13</td>
</tr>
<tr>
<td>N=20</td>
<td>Targocid</td>
<td>16</td>
</tr>
</tbody>
</table>

Also the table showed that the vancomycin MICs for thirteen *streptococcus pyogenes* isolates were ranged from 4-8 μg/ml while the teichoplanin MICs were 8-16 μg/ml, in previous study Perry et al (22) found that vancomycin MICs for *streptococcus pyogenes* isolates were 5 mg/l and teichoplanin MICs for the same isolates were 0.125 μg/ml while Jennifer (16) found the vancomycin MICs for *streptococcus pyogenes* (erythromycin- susceptible) were 0.5 μg/ml and teicoplanin MICs 0.06 μg/ml for *streptococcus pyogenes* (erythromycin-resistant) the MICs were 0.5 μg/ml, while the teicoplanin 0.03 μg/ml in Noviello's study (21). The vancomycin MICs for *streptococcus pyogenes* were 0.25 μg/ml and for teicoplanin ranged between 0.03-0.06 μg/ml.
Table (2): MBCs different isolates of bacterial strains to Vancomycin and Targocid

<table>
<thead>
<tr>
<th>M.o.</th>
<th>Antibiotic</th>
<th>Number of strains have MBC (μg/ml)=equal to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥64</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>Vancomycin</td>
<td>1</td>
</tr>
<tr>
<td>N=13</td>
<td>Targocid</td>
<td>1</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>Vancomycin</td>
<td>1</td>
</tr>
<tr>
<td>N=7</td>
<td>Targocid</td>
<td>6</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Vancomycin</td>
<td>2</td>
</tr>
<tr>
<td>N=20</td>
<td>Targocid</td>
<td>3</td>
</tr>
</tbody>
</table>

MBC is the lower concentration of the drug necessary for elimination of 99.9% of the M.o. tested, according to this definition, the vancomycin and teichoplanin MBCs for 20 isolates of staphylococcus tested were 2-16 μg/ml, 8-32 μg/ml respectively. In comparison with Marita (15) results (MBCs of Vancomycin = 16 μg/ml and 8 μg/ml in Jennifer study (16)). Table 2 showed that the vancomycin and Teichoplanin MBCs for seven streptococcus pneumonia isolates and the MBCs of vancomycin ranges were 4-16 μg/ml and these higher values may be due to involvement of impairment autolysin regulations or modification in the cell wall composition (5). In table 2 the study found that the values of vancomycin MBCs for the same isolates of streptococcus pyogenes were ranged from 4-16 μg/ml and for the teicoplanin MBCs were ranged from 8-32 μg/ml. Novillos (21) found the values of vancomycin, teicoplanin MBCs for streptococcus pyogenes isolates were 0.5 μg/ml, 0.06-0.25 μg/ml respectively, while in Perry study (22) the values of vancomycin, teicoplanin were > 64 μg/ml for both of them and in Jennifer study the vancomycin MBCs for streptococcus pyogenes (erythromycin susceptible) were 0.5-2 μg/ml and for teicoplanin were 0.06 -0.5 μg/ml and the vancomycin MBCs for streptococcus pyogenes (erythromycin-resistant) were 0.5 μg/ml and for teicoplanin were 0.03 μg/ml. The tube dilution method suffers several technical problem, such as carry-over of antibiotics, bacterial splashed on the wall of the test tubes, unbuffered medium or bacterial growth phase, all of which have been shown to affect the values of MICs and MBCs.

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