Tikrit Journal of Pharmaceutical Sciences 2009 5(2):203-207

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

Waseem Ali Hasan
Department of Pharmacology and Toxicology, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq
Received 15 / 6 / 2009 accepted 3/8 /2009

Abstract
The glycopeptide antibiotics vancomycin and teicoplanin (targocid) are widely used in the treatment of infections caused by gram positive bacteria. Vancomyc 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 MBC for the same strains were 8-32 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.

دراسه تفريقية لفعاليه المضادات الحيويه للفانكومايسين والتاركوسيد ضد سلالات من المكورات الذهبيه العنقوديه والمكورات السبحيه

وسيم علي حسن

المستخلص
ان المضادات الحيويه والتي تشمل الفانكومايسين والتيكوبلانين (التاركوسيد) تستخدم على نطاق واسع في علاج الاصابات الناتجه عن البكتريا الموجبه كرام. ان الفانكومايسين يثبط تفاعلات ارتباط المجاميع السكريه بسلاسل الكلايكان والارتباط المستعرض لسلاسل الببتيدات خلال عملية تجميع الببتيدوكلايكين، التاركوسيد يثبط بلمرة الببتيدوكلايكين والذي ينتج عنه تثبيط تكوين جدار البكتريا وموت الخليه. في هذه الدراسه عشرون سلاله من بكتريا المكورات السبحيه القيحيه والكورات السبحيه الرئويه وخمسه وعشرون سلاله من المكورات الذهبيه العنقوديه تم جمعها من اصابات مختلفه. وتم تحديد التركيز الادنى المثبط و التركيز الادنى القاتل للفانكومايسين و التاركوسيد ضد السلالات البكتيريه، وقد وجد ان التركيز الادنى المثبط للفانكومايسين ضد المكورات السبحيه كان ٤-٨ مايكروغرام لكل مل وبالمقارنه ٨- ١٦ مايكروغرام لكل مل للتاركوسيد و التركيز الادنى المثبط للتاركوسيد ضد المكورات العنقوديه كان ٤- ١٦ مايكروغرام لكل مل بينما التركيز الادنى القاتل لبعض السلالات كان ٨- ٣٢ مايكروغرام لكل مل. ان التاركوسيد هو اقل فعاليه من الفانكومايسين ضد المكورات العنقوديه ولكن مساوي او اكثر فعاليه ضد المكورات السبحيه وخصوصا عندما اظهر فعاليه ممتازه ضد عتر المكورات السبحيه الرئويه.

Introduction

Sever 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 aglycopeptide antibiotic, obtained in 1978 by fermentation from *actinoplanes teichomceticus*, 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 *streprococcus pyogens* and *streptococcus pneumonia*e 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, Mannitol salt agar and coagulase test [8].

Antimicrobial activity of vancomycin and teichoplanin (targocid):

Vancomycin and teichoplanin 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% Co_2 [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 suceptebility (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 [20] 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 Novellos study [21].

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

M.o.	Antibiotic	Number of strains have MIC (µg/ml)equal to														
		≥ 64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.15	0.007	≤0.003
S. pyogenes N= 13	Vancomycin				11	2										
	Targocid			9	4											
S. pneumonia N= 7	Vancomycin				5	1	1									
	Targocid				6	1										
Staph. aureus N= 20	Vancomycin				13	2	2	1	2							
	Targocid			16	2	2										

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 Noviellos 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

M.o.	Antibiotic	Number of strains have MBC (µg/ml)equal to:													
		≥ 64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.15	0.007	≤0.003
S. pyogenes N= 13	Vancomycin			1	10	2										
	Targocid		1	9	3											
S. pneumonia N= 7	Vancomycin			1	5	1										
	Targocid				6	1										
Staph. aureus N= 20	Vancomycin			2	10	4	4									
	Targocid		3	15	2											

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 Maritza [15] results (MBCs of Vancomycin = 16 µg/ml and 8 µg/ml in Jennifer study [16]. Table 2 showed that the vancomycin and Teichplanin 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.

References

1- Chang Fy, peacocl JE, Musher DM, et al staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study, medicine 2003, 82: 333- 339.

2- Shurland S, Zhan M, BradhamDD. Comparison of mortality risk associated with bacteremia due to methicillin-susceptible staphylococcus aureus. Infect control hosp Epidemiol 2007, 28: 273- 279.

3- Thalia I M, Deborah L M, Jan. EF, David Ap, et al. Semi synthetic glycopeptide antibiotic deprival from Ly264824 active against vancomycin resistant enterococci. American society of microbiology, 1996; 40 (9): 2194- 2199.

4- Meylan PR, Franciolip and Glauser MP. Discrepancies between MBC and Actual killing of viridaus Group streptococci by cell- wall- active antibiotics Antimicrobial Agents and chemotherapy Mar. 1986, 29 (3) = 418- 423.

5- Knudesen. J. D, Funrsted. K., E spersen, F. et al, activities of vancomycin and teicoplanin agnist penicillin- resistant pneumonia invitro and invivo and correlation to pharmokinetic parameters in the mouse peritonitis model. Anti microbial agents and chemotherapy 1997, 41, 1910-5.

6- Anezka , teicoplanin (targocid). Forum , mrsaresources. Com 2006 Ju 131.

7- Gerald JC, Barrie PM, Andrew GF, and Anthony Simmons. Practical medical microbiology. Longman Singapore publishing 1996.

8- Ellen JB, Lanco RP, Sydney MF. Diagnostic microbiology. 9th ed St. Lowis: Mosby, 1994: 321- 360.

9- Jenny DR, Kurt f, susan R et al. pharmacodynamics of Glycopeptides pneumonia or staphylococcus aureus infection. Antimicrobial agents and chemotherapy 2000, 44(5): 1247- 1254,.

10- Hawkery PM, Lewis DA Medical bacteriology a practical approach. New York: IRL press, 1989: 167- 193.

11- Kulgman, KP. Activity of vancomycin and teicoplanin against penicillin resistant pneumococci, Europe J. clinical microbial infection, 1994 dis. 13: 1-2

12- Williams , A. H., and Gruneberg R N. Teicoplanin J. antimicrob. Chemotherapy. 1984; 14: 441- 445.

13- Lister PD. Multiply- resistant pneumococcus: therapeutic problems in the management of serious infection. Euro. J. clin. Microbial Infection Dis.: 1995 dis. 14 (1): 18- 25.

14- Michel J. Rybak- steven, leonard L, Kerri L et. al, characterization of vancomycin- hetero resistant staphylococcus aureus from the metropolitan area of Detroit, michigun, over a 22 years period (1986 to 2007), J. clin/ microbial 2008 sep. 46 (9); 2950-2954.

15- Martiza BM, Cristius Paul M.T and Francoise VB. Pharmacodynamic evaluation after intracellular activities of antibiotics against staphylococcus aureus in a model of THP- 1 Macrophages. J. antimicrobial agents chemotherapy 2006 march, 50 (3); 841-851.

16- Jennifer and Larisac. Dalbavancin (Zeven), a noval glycopeptides for resistant gram- positive organisms. Drug forecast. 2008 Jan, 33(1): 42- 45.

17- Clinical and Laboratory standard institute 200. Performance standards for antimicrobial susceptibility testing 15th informational supplements, approved standard, CLSI document M100- S15. Clinical and laboratory standards Institute, wayne, PA

18- Pierre V, Patrice F, Brigitte B and Daniel P. In vivo emergence of subpopulations expressing teicoplanin or vancomycin resistance phenotype in aglycopeptide susceptible, methicillin-resistant strain of staphylococcus aureus. J. antimicro. Chemother. 2001, 47; 163-170.

19- Hirmatsu K. Vancomycin resistance in staphylococcus. Drug resistance. 1985,1; 135- 50.

20- Lopez FF, Martinez FG, Perez JS. Comparative study of treatment with penicillin, cefotrixon, trovafloxacin, quinupristin- dalfopristin and vancomycin in experimental endocarditis due to penicillin- and ceftriaxone- resistant streptococcus pneumonia. J. Antimicro. Chemother. 2001, 47 (S); 623- 629.

21- Noviellos, Ianniello F and Espositos. Invitro activity of Ly333328 (oritavancin) against gram- positive aerobic cocci and synergy with ciprofloxacin against enterococci. J. antimicrob. Chemother. 2001 48 (2), 283-286.

22- Perry J D, Jones Al, G Frances K. Glycopeptide tolerance in bacteria causing endocarditis. J. Antimicrob. Chemotherapy 1999, 44 (1); 121- 124.