Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Therapeutic Efficacy of Linezolid and Rifampicin against Experimentally Induced MRSA Mediastinitis in Rabbits

Muhammad Nadeem Asi*, Ghulam Muhammad, Farah Deeba and Faqir Muhammad[1]

Department of Clinical Medicine and Surgery; [1]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad-38040, Pakistan
*Corresponding author: mnadeemasi@gmail.com

ARTICLE HISTORY (14-170)

Received: April 09, 2014
Revised: August 19, 2014
Accepted: December 23, 2014

Key words:
Induced mediastinitis
Linezolid
Methicillin-resistant
MRSA
Rabbit
Rifampicin
Staphylococcus aureus

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant challenge for human and veterinary health professions especially in the developing nations. Resistance to wide array of antibiotics is a major hurdle in treating MRSA infections including mediastinitis. In present study, therapeutic efficacy of linezolid alone and in combination with rifampicin was evaluated in experimentally induced MRSA mediastinitis in rabbits. Experimental upper median sternotomy was performed on 56 rabbits divided into seven groups. Out of these seven groups, one was taken as uncontaminated control group; one as untreated contaminated group; two groups were given different doses of linezolid, one group was treated with rifampicin alone whereas remaining two groups were given combinational therapy of linezolid and rifampicin. Both 50 mg/kg and 100 mg/kg dose rates of linezolid were found to be effective whereas no added benefit was observed in rabbits given a combination of rifampicin and linezolid. Linezolid can be used in serious MRSA infections especially in those patients not responding to conventional antimicrobial therapy but the selection of correct dose of linezolid is important in each patient.

To Cite This Article: Asi MN, G Muhammad, F Deeba and F Muhammad, 2015. Therapeutic efficacy of linezolid and rifampicin against experimentally induced MRSA mediastinitis in rabbits. Pak Vet J, 35(2): 159-162.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) infections remain to be a significant challenge for human healthcare profession (Dulon *et al.,* 2011; David *et al.,* 2012; Khan *et al.*, 2013). During the past two decades, pockets of methicillin resistant *Staphylococcus aureus* (MRSA) infections have been recognized in both developed (Brugnaro *et al*., 2009, Jarvis *et al*., 2012; Aidah *et al*., 2014) and developing countries including Pakistan (Assma *et al*., 2009). MRSA infections have been associated with high mortality, and morbidity with high therapeutic costs (Cosgrove *et al*., 2003; Engemann *et al*., 2003). To date, scanty data are available regarding prevalence of MRSA infections in Pakistan (Hakim *et al*., 2007). Antibiotics continue to play an iconic role in the prevention and cure of staphylococcal infections but resistance to conventional antibiotics can lead to treatment failure as may be seen in patients with MRSA infections. Patients undergoing cardiothoracic surgery are at higher risk of developing postoperative mediastinitis and MRSA mediastinitis is not uncommon among these patients. Higher mortality rates have been reported due to MRSA mediastinitis after open-heart surgery procedures (Braxton *et al*., 2000; Miralem *et al.,* 2004; Memon *et al*., 2013).

Vancomycin (a glycopeptides antibiotic) has been the first line therapy for the treatment of MRSA infections. However, increase in minimum inhibitory concentration (MIC) of vancomycin against MRSA isolates and notable association between these isolates with higher rates of treatment failure and mortality has prompted investigators to research for other treatment options. Linezolid offers the promise of an ideal antibiotic for the treatment of MRSA infections (Watkins *et al*., 2012). It is a synthetic oxazolidinone antibiotic endowed with an excellent antimicrobial activity against *Staphylococcus aureus* resistant to methicillin and vancomycin (Rybak *et al*., 2000, Jinjian *et al*., 2013), while rifampicin has also been used in combinational therapy against MRSA (Forrest and Tamura, 2010). The present study was planned to evaluate the efficacy of two frontline antibiotics viz. linezolid and rifampicin against MRSA infection in a rabbit model.

MATERIALS AND METHODS

Two frontline drugs viz., linezolid and rifampicin were evaluated for their therapeutic efficacy against experimentally induced MRSA mediastinitis in rabbits.

Confirmation of MRSA isolate and susceptibility testing: The MRSA isolate used in the present was recovered from the human patient with surgical site infection and was confirmed by the composite of Gram staining, catalase reaction (using freshly prepared 3% hydrogen peroxide solution), β hemolysis on blood agar, growth on CHROMagar MRSA (CHROMagar Microbiology, Paris, France), tube coagulase test (using lypholyzed rabbit plasma), clumping factor (bound coagulase), protein A (Staphytect Plus, Oxoid Ltd, Basingstoke, UK) and analytical profile index (api® Staph, bioMériux Sa, France). Further confirmation was based on latex agglutination test (PBP2' test kit; Oxoid Ltd, Basingstoke, UK), resistance to oxacillin on Mueller-Hinton agar plates (by disc diffusion method) and molecular detection of *mecA* gene as per Brown *et al.* (2005). MIC of the strain was determined by E-test (AB Biodisk, Solna, Sweden) in accordance to Anonymous (2006).

Experimental rabbits and grouping: A total of 56 adult male rabbits weighing between 1.5-2 kg were randomly divided into 7 groups viz., A, B, C, D, E, F and G, having 8 rabbits each. The rabbits of each group were housed in separate cages and were provided with water and feed *ad libitum*. Group A and B were regarded as uncontaminated and contaminated untreated controls respectively. The subsequent groups (C, D, E, F and G) were subjected to MRSA contamination in order to induce mediastinitis. Rabbits in group C and D were treated with linezolid @ 50 and 100 mg/kg, IV, b.i.d respectively whereas rabbits in group E received rifampicin @ 40 mg/kg orally b.i.d. Group F and G received a combination therapy (linezolid 50 mg/kg + rifampicin 40 mg/kg) and (linezolid 100 mg/kg + rifampicin 40 mg/kg) twice a day, respectively.

Experimental contamination of mediastinum with MRSA in rabbits: Each rabbit was anesthetized with combination of medetomidine (@ 0.15-0.25 mg/kg) and ketamine (@ 10-15 mg/kg) intramuscularly as per Meredith and Flecknell (2006). After surgical preparation of cranial thorax of each rabbit, the incision was made over cranio-dorsal area of sternum. Both skin and pre-sternal layers were incised and partial upper median sternotomy was performed without opening the pleural space (Sacar *et al*, 2008). All rabbits (n=48; group B thru G) received 0.5 ml of 10^8 colony forming units (CFU)/ml of MRSA directly in to the wound (mediastinal and sternal layers). Following inoculation, the skin layers were immediately closed with surgical silk (2-0). All subjects (n=56) were examined twice daily for physical parameters (temperature, pulse rate, respiration rate).

Evaluation of the infection: Twelve hour post-therapy, all rabbits (n=48) of groups B-G were euthanized by injecting 120 mg of pentobarbital as per guidelines of Ethics Committee, University of Agriculture, Faisalabad Pakistan. Sternotomy was performed on each rabbit and swabs were obtained from mediastinum. Swabs samples were placed in tubes containing 1 ml of phosphate buffered saline and vortexed for 3 minutes. In order to quantitate MRSA isolates, serial 10-fold dilutions (0.1 ml) of bacterial suspensions were streaked on sheep blood agar plate, incubated for 37°C for 48 hours. All plates were evaluated for presence of MRSA growth and number of CFUs per plate was counted.

Statistical analysis: Data were analyzed with SPSS for windows 17.0. Arithmetic mean±SD of CFU was calculated for quantitative culture results. One way analysis of variance (ANOVA) was used to calculate the difference among the groups. Difference were recorded significant if $P<0.05$.

RESULTS

The clinical isolate used in the present study was found to be susceptible to linezolid and rifampicin using the microdilution broth method. The minimal inhibitory concentrations (MICs) for both rifampicin and linezolid were found to be 0.01 µg/ml and 2 µg/ml, respectively. All (n=8) rabbits in group B (contaminated, untreated) showed evidence of mediastinitis. All rabbits (n=40) in groups receiving antibiotic therapy, except 4 (group C), 6 (group D), 1 (group E), 5 (group F) and 6 (group G) rabbits showed evidence of mediastinitis. Culture negative rates and mean bacterial counts are shown in Table. The 50 mg/kg dose of linezolid alone or in combination with rifampicin was found effective in comparison with the contaminated untreated group ($P>0.05$). Quantitative mean bacterial counts were found significantly lower in comparison with contaminated untreated group receiving 50 mg/kg linezolid alone or in combination with rifampicin (group C and F). Gross difference in overall mean bacterial count could be observed in groups receiving 100 mg/kg linezolid alone or in combination with rifampicin (group D and G) in comparison with untreated contaminated group ($P<0.05$). However, in group E where 40 mg/kg rifampicin was used, the results were not as promising as in groups receiving linezolid alone or in combination with rifampicin. On the other hand, in terms of mean bacterial count, no statistically significant difference was found between groups receiving linezolid alone or in combination with rifampicin.

DISCUSSION

The aim of the present study was to evaluate the efficacy of linezolid alone or in combination with rifampicin in the treatment of post-sternotomy mediastinitis in experimentally induced infections of MRSA in rabbit model. Moreover, efficacy of rifampicin along with linezolid was also evaluated accordingly. Mediastinitis model were selected in rabbits with experimental induction of MRSA since analogous infections in human are not uncommon and generally need in time aggressive treatment and long-term antimicrobial therapy. In present study authors used both 50 mg/kg and 100 mg/kg doses of linezolid as 25 mg/kg dose has been reported to be ineffective in such infections (Sacar *et al*., 2008). Similarly one report (Oramas-Shirey *et al*., 2001) documented that 25 mg/kg IV dose of linezolid given thrice a day was found to be ineffective in treating *S. aureus* infections. Post-cardiovascular surgery infections are life threatening and add up to post-surgical complications leading to increased rates of morbidity and

Table: 1: Outcome of 7-day treatment with linezolid and rifampicin in experimentally induced mediastinitis (MRSA) in rabbits

Groups	Drug and dose	No. culture negative/Total	Mean bacterial count[1]
Group A (Uncontaminated Control)	No treatment	8/8	0.0
Group B (Contaminated Control)	No treatment	0/8	7.78±0.18
Group C (linezolid @ 50 mg/kg)	Linezolid (50)	4/8	3.14±0.11
Group D (linezolid (@100 mg/kg)	Linezolid (100)	6/8	1.22±0.16
Group E (rifampicin (@ 40 mg/Kg)	Rifampicin (40)	1/8	7.14±0.22
Group F (linezolid @ 50 mg/Kg + rifampicin @ 40 mg/Kg)	Linezolid (50) + Rifampicin (40)	5/8	3.01±0.17
Group G (linezolid @ 100 mg/Kg + rifampicin @ 40 mg/Kg)	Linezolid (100) + Rifampicin (40)	6/8	1.16±1.18

[1]Log 10 CFU/ml±SD; Values in column are significantly different, F=331.584, df=6, P<0.001.

mortality. It has been reported that MRSA infections are often related with worse clinical outcome (Mekontso-Dessap *et al*., 2001). Vancomycin has commonly been used in patients with serious post-cardiovascular surgery MRSA infections. However, since there are number of reports concerning vancomycin resistant MRSA isolates (Sakoulas *et al*., 2007; Van Hal and Fowler, 2013) vancomycin is not widely being used in treating MRSA infections.

In the present study, linezolid both at dose rate of 50 mg/kg and 100 mg/kg was found to be effective as significant (P<0.05) reduction was observed in overall bacterial count in mediastinum. Moreover, combinational therapy using linezolid and rifampicin was found to carry no advantage over use of linezolid alone. Our results are in line with those described by Sacar *et al*. (2008) where they reported that linezolid alone or in combination with rifampicin was effective against MRSA strain without any added benefit of using rifampicin. This study has shown indifference regarding combinational therapy using linezolid and rifampicin versus linezolid alone. This finding in our results is in line with that described by Dailey *et al*. (2003). Linezolid both at 50 mg/kg and 100 mg/kg found to be effective alone and in combination with rifampicin. Nevertheless, there are numerous reports (Diekema and Jones, 2001; Meka *et al*., 2004; Gu *et al*., 2013) of development of resistance against this agent and therefore its use should be restricted only for patients with serious life-threatening infections. Linezolid alone or in combination with rifampicin was found to be effective in treating the experimentally induced MRSA mediastinitis in experimental rabbits. However, adding the rifampicin with linezolid did not show any beneficial response in treating experimentally induced MRSA mediastinitis in experimental rabbit infection model.

Conclusion: Linezolid both at 50 and 100 mg/kg dose was found effective in reducing the overall bacterial count in MRSA mediastinitis in experimental rabbits. However, addition of rifampicin did not show any additional benefits thus use of linezolid alone in serious MRSA infections might be more judicious to avoid unnecessary use of rifampicin and also to prevent the development of rapid resistance against rifampicin.

REFERENCES

Aidah N, N Abdullah, E Oskoueian, CC Sieo and WZ Saad, 2014. Membrane-active antibacterial compounds in methanolic extracts of jatropha curcas and their mode of action against *Staphylococcus aureus* S1434 and *Escherichia coli* E216. Int J Agric Biol, 16: 723-730.

Anonymous, 2006. 2006. Performance Standards for Antimicrobial Susceptibility Testing; 16th informational supplement M100-S 16 Clinical and Laboratory Standards Institute, (CLSI), Wayne, PA, USA.

Assma N, Ajmal, M Farzana and A Maleeha, 2009. Nosocomial methicillin-resistant *Staphylococcus aureus* frequency in a tertiary care hospital, Lahore, Pakistan. Biomedica, 25: 97-100.

Gu B, T Kelesidis, S Tsiodras, J Hindler and RM Humphries, 2012. The emerging problem of linezolid-resistant staphylococcus. J Antimicrob Chemother, 68: 4-11.

Braxton JH, CA Marrin, PD McGrath, CS Ross, JR Morton, M Norotsky, DC Charlesworth, SJ Lahey, RA Clough and GT O'Connor, 2000. Mediastinitis and long-term survival after coronary artery bypass grafting surgery. Ann Thorac Surg, 70: 2004-2007.

Brown DF, DI Edwards, PM Hawkey, D Morrison, GL Ridgway and KJ Towner, 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemothr, 56: 1000-1018.

Brugnaro P, U Fedeli, G Pellizzer, D Buonfrate, M Rassu, C Boldrin, SG Parisi, A Grossato, G Palu and P Spolaroe, 2009. Clustering and risk factors of methicillin-resistant *Staphylococcus aureus* carriage in two Italian long-term care facilities. Infection, 37: 216-221.

Cosgrove SE, G Sakoulas, EN Perencevich, MJ Schwaber, AW Karchmer and Y Carmeli, 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis, 36: 53-59.

Dailey CF, PJ Pagano, LV Buchanan, JA Paquette, JV Haas and JK Gibson, 2003. Efficacy of linezolid plus rifampicin in an experimental model of methicillin-susceptible *Staphylococcus aureus* endocarditis. Antimicrob Agents Chemother, 47: 2655-2658.

David MZ, S Medvedev, SF Hohmann, B Ewigman and RS Daum, 2012. Increasing burden of methicillin-resistant *Staphylococcus aureus* hospitalizations at US academic medical centers, 2003-2008. Infect Control Hosp Epidemiol, 33: 782-789.

Diekema DJ, MA Pfaller, FJ Schmitz, J Smayevsky, J Bell, RN Jones and M Beach, 2001. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis, 32: 114-132.

Dulon M, F Haamann, C Peters, A Schablon and A Nienhaus, 2011. MRSA prevalence in European healthcare settings: a review. BMC Infect Dis, 11: 138.

Engemann JJ, Y Carmeli, SE Cosgrove, VG Fowler, MZ Bronstein, SL Trivette, JP Briggs, DJ Sexton and KS Kaye, 2003. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin Infect Dis, 36: 592-598.

Forrest GN and K Tamura, 2010. Rifampicin combination therapy for nonmycobacterial infections. Clin Micro Rev, 10: 14-34.

Jinjian FU, YE Xiaohua, CHA Chen and CHASidong, 2013. The efficacy and safety of linezolid and Glycopeptides in the treatment of *Staphylococcus aureus* infections. PLos ONE, 8:1

Hakim ST, S Arshed, M Iqbal and SG Javaid, 2007. Vancomycin sensitivity of *Staphylococcus aureus* isolates from hospital patients in Karachi, Pakistan. Libyan J Med, 2: 176-179.

Jarvis WR, AA Jarvis and RY Chinn, 2012. National prevalence of methicillin-resistant *Staphylococcus aureus* in patients at United States health care facilities. Am J Infect Control, 40: 194-200.

Khan A, R Hussain, MT Javed and F Mahmood, 2013. Molecular analysis of virulent genes (*coa* and *spa*) of *Staphylococcus aureus* involved in natural cases of bovine mastitis. Pak J Agric Sci, 50: 739-743.

Meka VG, Sk Pillai, G Sakoulas, C Wennersten, L Venkataraman, PC DeGirolami, GM Eliopoulos, RC Moellering and HS Gold, 2004. Linezolid resistance in sequential *Staphylococcus aureus* isolates

associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. J Infect Dis, 190: 311-317.

Mekontso-Dessap A, M Kirsch, C Brun-Buisson and D Loisance, 2001. Poststernotomy mediastinitis due to *Staphylococcus aureus*: comparison of methicillin-resistant and methicillin-susceptible cases. Clin Infect Dis, 32: 877-883.

Memon J, Y Yang, J Kashif, M Yaqoob, R Buriro, J Soomro, W Liping and F Hongjie, 2013. Genotypes, virulence factors and antimicrobial resistance genes of *Staphylococcus aureus* isolated in bovine subclinical mastitis from Eastern China. Pak Vet J, 33: 486-491.

Meredith A and P Flecknell, 2006. Manual of Rabbit Medicine and Surgery. BSAVA, Telford Way, Quedgeley, Gloucester, UK.

Miralem P, S Raymond and H Roland, 2004. Successful treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) mediastinitis in a heart transplant recipient. Eur J Cardiothorac Surg, 25: 1127-1128.

Oramas-Shirey MP, LV Buchanan, CL Dileto-Fang, CF Dailey, CW Ford, DH Batts and JK Gibson, 2001. Efficacy of linezolid in a staphylococcal endocarditis rabbit model. J Antimicrob Chemother, 47: 349-352.

Rybak MJ, E Hershberger, T Moldovan and RG Grucz, 2000. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and resistant strains. Antimicrob Agents Chemother, 44: 1062-1066.

Sacar M, S Suzan, K Illknur, C Nural, Z Cevahir, S Teke, K Toprak, A Ali, O Faruk, A Aytekin, T Huseyin, 2008. Efficacy of linezolid in the treatment of mediastinitis due to methicillin-resistant *Staphylococcus aureus*: an experimental study. Int J Inf Dis, 12: 396-401.

Sakoulas G, C Robert and Jr Moellering, 2008. Increasing antibiotic resistance among methicillin-resistant *Staphylococcus aureus* strains. Clin Infect Dis, 46: 360-367.

Van Hal SJ and VG JR Fowler, 2013. Is it time to replace vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* infections? Clin Infect Dis, 12: 1779-1788.

Watkins RR, TL Lemonovich and TM File, 2012. An evidence-based review of linezolid for the treatment of methicillin-resistant Staphylococcus aureus (MRSA): place in therapy. Core Evid, 7: 131-43.