RESEARCH ARTICLE

In-vitro and *In-vivo* Therapeutic Effects of Vancomycin on Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

The present study aimed to evaluate the therapeutic influence of vancomycin on methicillin-resistant *Staphylococcus aureus* (MRSA) i*n-vitro* and *in-vivo*. Thirty rabbits were divided equally into three groups; group A served as a negative control, group B serve as positive control given MRSA at 2.7 x 10^6 CFU/mL, group C infected with 2.7 × 10^6 CFU/mL MRSA isolates that treated by Vancomycin (40) mg/kg-S/C daily after two days of infection for five days. The results showed that different vancomycin concentrations different degrees of inhibition against areas MRSA were applied on media well diffusion. The inhibition zone scale was varied according to antibacterial agent concentration and, when the agent concentration increased, the inhibition zone increased proportionally. The gross examination was performed on all groups for five days post-infection. Subcutaneous injection with MRSA resulted in pus-filled lesions after 24 hours of infection with a mean diameter (5.7 cm^3) in all infected groups following the lesions reaching maximum diameter (8.3 cm^3) after10 days post-infection in the infected non-treated group. The treated group with vancomycin showed decreased lesion volume but not a significant difference compared with the untreated group after 48 hours of treatment. Gross inspection of sacrificed animals of infected untreated classes, five days after infection, showed shin area with MRSA with severe ulcerative pyogenic lesions and a significant increase in lesion volume.

In conclusion, Vancomycin showed good activity against MRSA *in-vivo* and *in-vitro*.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), Rabbit, Vancomycin.

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INTRODUCTION

The mainstay of anti-MRSA therapy has long been Vancomycin, a glycopeptide discovered in 1956. Intravenous access is needed for administration because Vancomycin has low oral bioavailability.[1] Glycopeptides, by a mechanism independent of PBP, inhibit cell wall synthesis and are thus active against MRSA.[1,2] Although glycopeptide resistance is not unusual in vancomycin resistance enterococcus (VRE), multidrug-resistant MRSA has remained susceptible.[1] The first Staph is resistant to Vancomycin. In 2002 was launched. vancomycin-resistant *Staphylococcus aureus* (VRSA) collected from at least eight more cases in the U.S.A. (Michigan) were recognized.[3,4]

VanA was the first VRSA positive and is believed to have gained vanA via the colonization of VRE; VanA mutual move from VRE to S. Aureus was achieved *in vitro* to support this assumption.[5] Increased resistance to other medicinal products, increased rates of post-surgical infection, longer time and treatment failure have also been linked to higher vancomycin isolate levels than VRSA.[6]

Vancomycin is heterogeneous in many cases, showing good response in some cases and while suffering prolonged bacteraemia in some others although using fit doses. Many reports are showed that MRSA is thought which causes response failure. The most common refractory bacteremia cases are susceptibility by classical technics of

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Table 1: Diameter of inhibition zone vancomycin *in-vitro* antibacterial activity at various levels (µg /mL) with MRSA (mm)

Conc. µg/mL Zone of Inhibition (mm)	*2 µg/mL*	*4 µg/mL*	*6 µg/mL*	*8 µg/mL*	*10 µg/mL*
Vancomycin	0.00 ± 0.00	10.18 ± 0.4	14.9 ± 0.25	16.3 ± 0.1	18.1 ± 0.8
	Ae	Ad	Ac	Ab	Aa
D.W	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba	0.00 ± 0.00 Ba

testing with reduced vancomycin susceptibility remains small.[7]

By passing many *S. aureus* in broth media (that composed of Vancomycin), our results showed that difficulty microbial growth in media composed of vancomycin>10 mg/L.[8]

The Aim of the study was to investigate the therapeutic influence of Vancomycin against MRSA *in-vivo* and *in-vitro.*

MATERIALS AND METHODS

McFarland at (0.5) was used to calculate the average viable MRSA cells per mL of the stock suspension. In taking 1 mL of Staphylococcus aureus hose from overnight cultivation (Brain heart infusion broth), wash with Peptone water 9 mL, take 1 mL and dilute serial 10-fold.

Vancomycin (powder) was prepared by dissolving 1 gm of each one in 10 mL of distilled water as a stock solution. Then make series of dilutions in many levels (2,4,6,8,10) µg/mL. Using these concentrations to determine the methicillin resistance staphylococcus aureus sensitivity to these antibiotics,[9] the agar well diffusion was done.

Bacterial inoculums used to induce infection (skin infection) was (2.7 x 10^6 CFU/mL) of (MRSA); preparations for the inoculums standardize using serial Dix-fold dilutions according to a feasible method-pour counting technique. The 0.2 mL was given to every rabbit's left flank subcutaneously, and the symptoms of inflammation were watched. The dilution in the rabbits that displayed signs of infectiveness the rabbit during the entire infection was used.[10]

Thirty rabbits were divided equally into three groups:

- *Group (A):* not infected group which given normal saline S/C for 7 days (negative control).
- *Group (B):* infected with MRSA (positive control).
- *Group (C):* infected with MRSA then treated with Vancomycin (40) mg/kg. B.W S/C daily after two days of infection for five days.

RESULTS AND DISCUSSION

Different vancomycin concentrations were used in the agar well dissemination test, causing varying degrees of the inhibition areas against (MRSA). Depending on the concentration of antibacterial agents, the inhibition region was of a different size, increasing agents proportionally greater Table 1.

Vancomycin has been the critical treatment of transpeptidation. Still, side effects, a required IV access, and increasing resistance restrict its use by attaching the D alanyl D-alanine residue of the bacterial wall.[11] Distilled water (D.W) was used as control, no region of inhibition was found, D.W was used in in-vitro and in-vivo pedic studies as a solvent for Vancomycin. Concentration based on all the antibacterial activities is found, which is not similar to the results of Tomar *et al.*[12]

Vancomycin is shown to be (2.0 µg/mL) in the M.I.C. results calculated by tube dilution process. These findings come similar to Entenza *et al*.,[13] wherever found vancomycin M.I.C.s of MRSA was 2 µg/mL. The volume of 0.2 mL of bacterial suspension was given S/C in the flank region after the adjustment time for all animal groups after preparing a bacterial suspension of MRSA. In contrast, animals were observed for 24 hours. Haut lesions occurred 24 to 48 hours later.

The gross examination was performed on all groups for five days post-infection. Subcutaneous injection with MRSA resulted in pus-filled lesions after 24 hours of infection with the mean diameter (5.7 cm^3) in all infected groups following the lesions reaching maximum diameter (8.3 cm^3) after ten days post-infection in the infected non-treated group.

The treated group with Vancomycin showed decreased lesion volume but not a significant difference compared with the untreated group after 48 hours of treatment. The gross examination after five days post-infection performed to the sacrificed animals of infected non-treated group showed shin area with MRSA with severe ulcerative pyogenic lesions and a significant increase in lesion volume. These findings were in agreement with Fernandez *et al.*[14] that demonstrated that using Vancomycin produced in dose-proportional reduce the lesion in all doses (31–68)% as compared with the control group. According to Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), Vancomycin can be used to treat methicillin-resistant Staphylococcus aureus infections in adults and children.[15]

Due to the extensive use of Vancomycin, the Methyl Isocyanate (M.I.C.) of Vancomycin against MRSA is increasing every year. The 2011 Guideline for Clinical Application of Antibiotics in China states that Vancomycin is an antibiotic meant for the particular use. Vancomycin is a drug and must be considered the last line of defence against MRSA.[16]

CONCLUSION

Vancomycin gives an excellent therapeutic effect against MRSA both *in vivo* and *in vitro.*

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