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Experimental Lysostaphin therapy in methicillin resistant *Staphylococcus aureus* induced keratitis in Rabbits: A comparative evaluation

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ABSTRACT

Objective: A comparative evaluation of topical lysostaphin therapy with vancomycin and azithromycin in methicillin-resistant Staphylococcus aureus (MRSA) keratitis in a rabbit model. **Methods**: This study was conducted on 18 eyes of 18 rabbits. All rabbits were divided into 3 groups with 6 rabbits each. Right eyes were experimental eyes. Keratitis was induced by aseptically injecting the corneal stroma with 100 colony forming unit (CFU) of MRSA strain (MU-50). Each group was treated 5 hours post injection with one drop of 5% vancomycin, 0.28% lysostaphin or 1% azythromycin, every half an hour for 5 hours. Seven ocular parameters (conjunctival injection, chemosis, corneal infiltrate, corneal edema, flare in the anterior chamber, hypopyon formation, and iritis) were graded on a scale of 0 to 4 on slit lamp examination of rabbit eyes. Rabbits were sacrificed and corneas were excised and cultured to determine the number of CFU per cornea. **Results**: Lysostaphin significantly lowered the total slit lamp examination scoring of the experimental eye as compared to vancomycin and azithromycin (p-value 0.014) group. None of the eyes treated with vancomycin and azithromycin were sterile whereas four out of six eyes became sterile in lysostaphin treated group. There was significant lowering of mean log CFU/cornea in lysostaphin group as compared to vancomycin and azithromycin (p-value 0.008). Also, there was no statistically significant difference in slit lamp examination scoring between vancomycin and azithromycin groups. **Conclusion**: Lysostaphin has a greater potency to treat methicillin-resistant Staphylococcus aureus induced keratitis as compared to azithromycin and vancomycin.

Keywords: Azithromycin, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus keratitis*, vancomycin

Introduction

Bacterial keratitis is one of the leading causes of monocular disability in the developing world. Amongst different bacterial species, *Staphylococcus aureus*is a prevalent cause of bacterial keratitis, especiallyamong individuals with a previously compromised cornea[1-4].Topical cefazolin often used in combination with an aminoglycoside or a fluoroquinoloneare the antibiotics most often prescribed for treating *Staphylococcus* keratitis[5-8]. At present the antibioticof choice for treatment of Methicillin-resistant *Staphylococcus aureus*(MRSA) keratitis is vancomycin,but accumulating mutations in *Staphylococcusaureus* have led to resistance to vancomycin[9-12]. The continuing emergence of antibiotic-resistant *Staphylococcus aureus* infections has spurred the need for new antimicrobial agents to treat these infections. Lysostaphin is a potent anti-staphylococcal agent and has potential as a therapeutic agent against *Staphylococcus aureus* infections[13, 14]. Lysostaphin has not yet been approved as a therapy for staphylococcus keratitis.The present study is aimed at investigating topical lysostaphin therapy with recently introduced antibacterial antibiotics viz. vancomycin and azithromycin in MRSA induced keratitis in rabbits.

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Material and method

The present study was conducted on 18 eyes of 18 rabbits at the Institute of Ophthalmology, JNMC, AMU, Aligarh, after obtaining the ethical clearance from the animal house of JNMC. No author has financial interest in this study or the product. Equipment used: (1) tissue Homogeniser- employed for homogenising the cornea during its processing. (2)

Laminar flow bench- based on work principle involving double filtration of air. (3) Spectrophotometer. (4) Slit Lamp Biomicroscope

Lysostaphin (Sigma Aldrich, Germany) is a protein complex with highly specific lytic activity against staphylococcus species and it is produced by recombinant technology by expressing it in E coli. It is supplied in the form of lyophilized powder with activity of >3000 unit/mg of solid in 5mg vial, shipped in dry ice & stored at a temperature of 20°C.

Culture used: nutrient agar, nutrient broth, human blood agar, saline solution 0.85%.

Drug used: vancomycin hydrochloride, lysostaphin, azithromycin,proparacaine hydrochloride, ketamine hydrochloride injection**.**

MRSA strains Mu 50 employed in the present study were kindly provided by Prof. Keiichi Hiramatsu and his team of Prof. Teruyo Ito, Juntendo University, Tokyo, Japan.

Albino rabbits, *Oryctolaguscuniculus*, of both sexes were used. Rabbits were divided into 3 groups (Group A, B and C). These groups comprised of 6 rabbits in each. Right eyes (RE) were used as experimental eyes. They were sedated by i.m injection of ketamine (22.5 mg/kg).Keratitis was induced by aseptically injecting the corneal stroma with 100 colony forming unit (CFU) of given MRSA strain (per 10µl) with 1ml syringe having 30 gauge hypodermic needle**[6]**(figure 1). Five hours post injection, all rabbits developed keratitis. Group A, B and C were treated by with one drop (45µl) of topical vancomycin(5.0%), lysostaphin(0.28%) and azythromycin(1%) dropsrespectively, every half an hour for 5 hours.The results were compared 10 hours post injection in terms ofslit lamp examination scoring of rabbit's eyes(Table 1,figure 2) and CFU of *Staphylococcus aureus*per cornea. This methodology was adapted from the work of Dajcs*et al*, (2000) with minor modifications[15].

Table 1: Slit Lamp Biomicroscopy Scoring of Rabbit's Eyes

S.no	Parameters	Grades Grade 0 →no change.
1)	CONGESTION OF CONJUNCTIVA	1-Flushed reddish, slight perilimbal injection in1quadrant. 2-Bright red, 2 quadrants. 3-Dark beefy red, 3 quadrants. 4-Diffuse congestion all around.
2)	CHEMOSIS OF CONJUNCTIVA	1-Swelling above normal, no lid eversion. 2-Swelling with misalignment of lids upper>lower. 3-Swelling with partial eversion, upper=lower. 4-Marked eversion, Upper>lower.
3)	CORNEAL INFILTRATES	1-Only upto epithelial surface. 2-May be dense but superficial and limited to ulcer base. 3-Dense infiltrates extending to mid stroma. 4-Dense extending deeper than mid stroma / upto sclera.
4)	CORNEAL EDEMA	1-Striped change of cornea. 2-Limited corneal edema. 3-Obvious corneal edema. 4-Diffuse corneal edema.
5)	FLARE	1-Faint barely detectable. 2-Moderate, iris and lens details clear. 3-Marked, iris and lens details hazy. 4-Intense flare, fibrinousexudate.
6)	HYPOPYON	1-Upto 1 mm. 2-More than 1 - 2 mm. 3-More than 2 – 3 mm. 4-More than 3 mm.
7)	IRITIS	1-Slight congestion of iris vessels, only part of iris. 2-Mild congestion, all vessels of iris involved. 3-Moderate congestion, all vessels of iris involved. 4-Marked congestion, all vessels, little normal tissue.

Each of seven ocular parameters (conjunctival injection, chemosis, corneal infiltrate, corneal edema, flare in the anterior chamber, hypopyon formation and iritis) were graded on a scale of 0 to 4. The parameter grades were added to produce a single total slit lamp examination score ranging from 0 (normal eye) to 28 (maximally inflamed eye)[6].

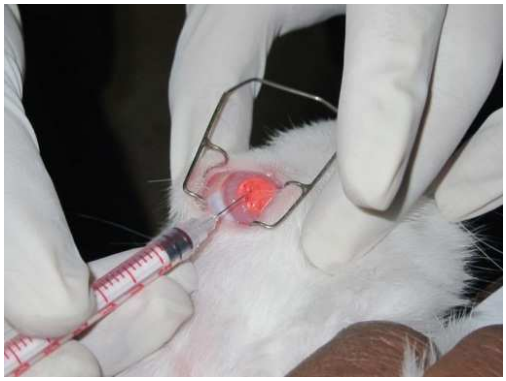

Figure 1: Intrastromal injection of MRSA strain in rabbit cornea to induce keratitis

Figure 2: Slit lamp examination of rabbit eye

The data was analysed by paired t-test, p-values of ≤ 0.05 were considered significant.

Results and observations

Slit lamp examination scoring

During the course of study, topical use of lysostaphin treated group showed significantly lower total slit lamp examination scoring as compared tovancomycin group (p-value 0.015) and azithromycin group (p-value 0.014) (Table 2 and Figure3). Lysostaphin significantly lowered slit lamp examination scoring in three out of the seven parameters studied in the present study viz. corneal infiltrate, corneal edema and flare. (Figure 4-6)

Table 2 &Figure 3: Total score - Slit Lamp Examination scoring after 10 hours post injection with 100 colony forming units of bacterial test strain Methicillin-Resistant *Staphylococcus aureus* Mu-50.

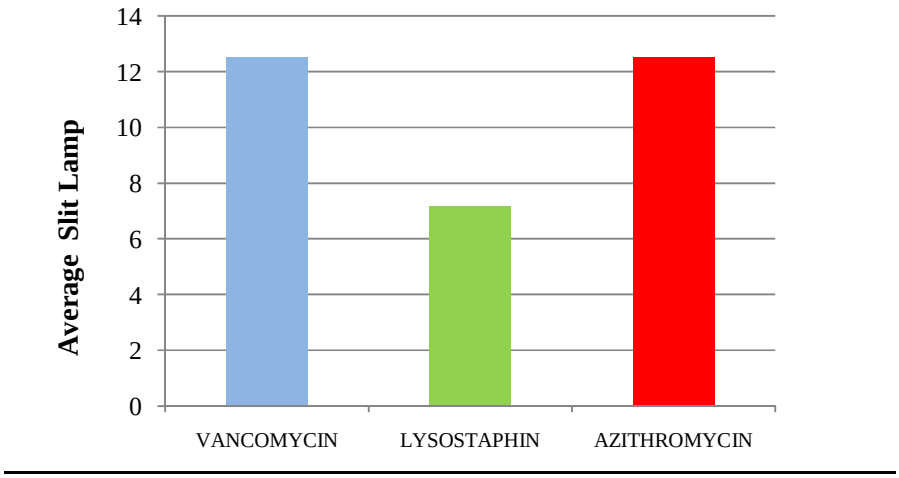

Figure 3:Total slit lamp examination scoring

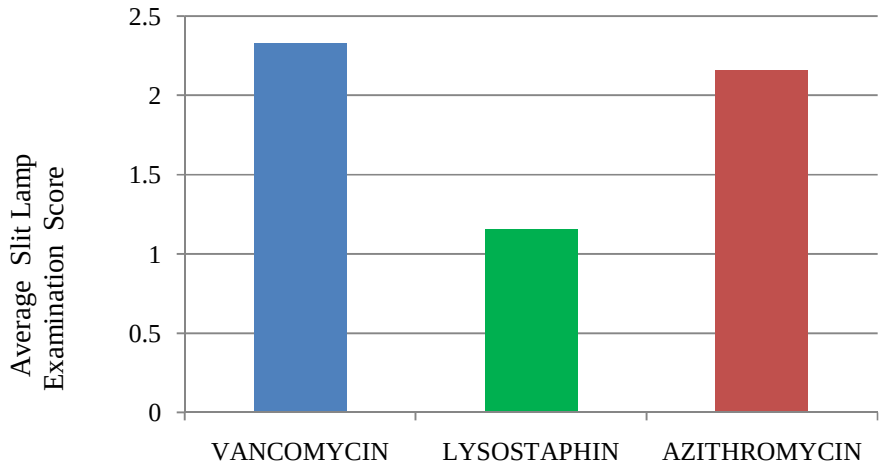

Figure 4: Corneal Infiltrates- Slit Lamp Examination Scoring after 10 hours post injection with 100 colony forming units of bacterial test strain Methicillin-Resistant *Staphylococcusaureus*Mu-50.

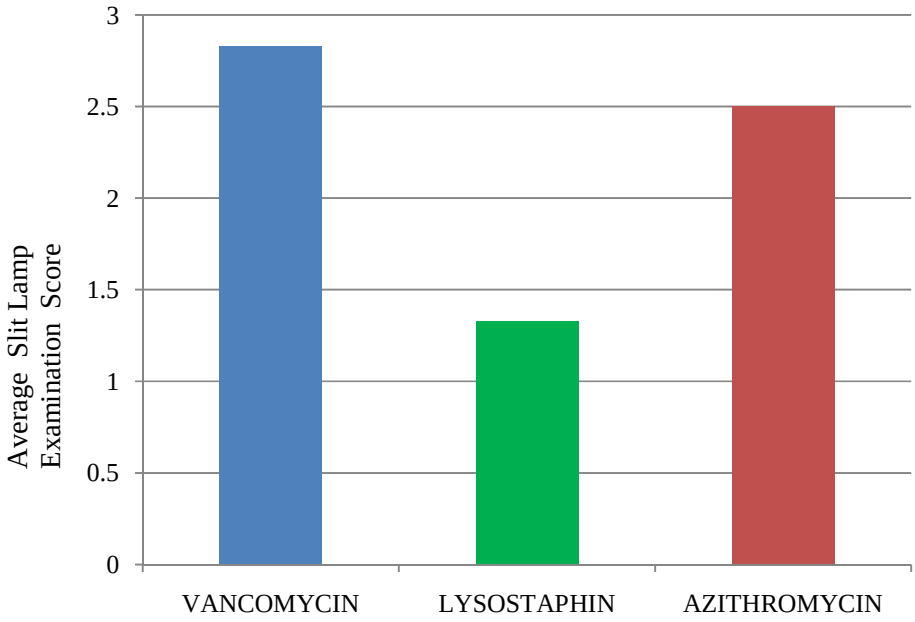

Figure 5:Corneal Edema- Slit Lamp Examination Scoring after 10 hours post injection with 100 colony forming units of bacterial test strain Methicillin-Resistant *Staphylococcus aureus* Mu-50.

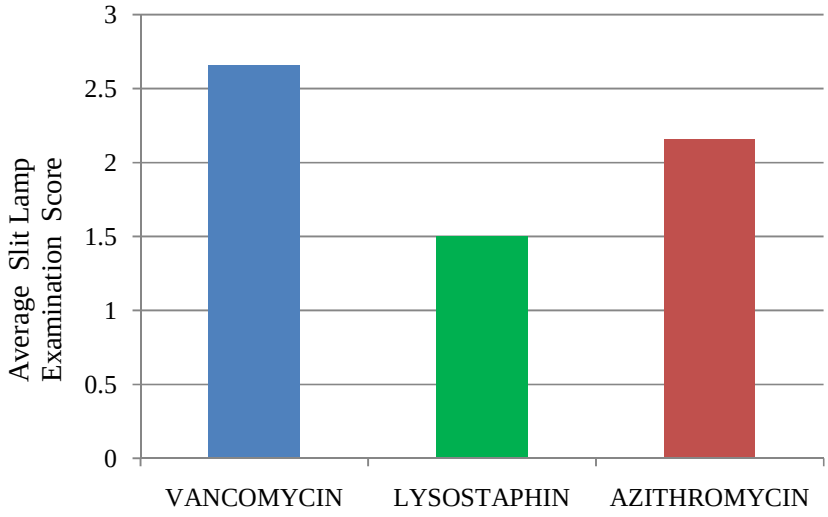

Figure 6:Flare- Slit Lamp Examination Scoring after 10 hours post injection with 100 colonyforming units of bacterial test strain Methicillin-Resistant *Staphylococcus aureus*Mu-50.

Microbiological evaluation

Lysostaphin lowered mean log colony forming units/cornea in experimental eye to 0.6666 ± 1.0327 which was statistically highly significant (p-value 0.008) as compared to vancomycin (mean log colony forming units/Cornea of 2.4761 ± 0.2157) group and azithromycin (mean log colony forming units/Cornea of 2.5131 ± 0.1834) group with p-value 0.008. Vancomycin and azithromycin treated eyes were having insignificant difference amongst them with p-value being 0.798 as shown in Table 3.None of the eyes treated with vancomycin and azithromycin were sterile whereas four out of six eyes became sterile in lysostaphin treated group.

Table 3:Antibacterial effect of Antibiotics on Methicillin-Resistant *Staphylococcus aureus* induced keratitis

S.NO.	Experimental Animal	Colony Forming Units Obtained					
		Vancomycin		**Lysostaphin**		**Azithromycin**	
		Colony forming units/ Cornea	**Log colony forming units/ Cornea**	**Colony forming units/ Cornea**	**Log colony forming units/ Cornea**	**Colony forming units/ Cornea**	**Log colony forming units/ Cornea**
1.	RABBIT 1	200	2.3010	0	0.0000	300	2.4771
2.	RABBIT 2	300	2.4771	0	0.0000	200	2.3010
3.	RABBIT 3	200	2.3010	0	0.0000	500	2.6989
4.	RABBIT 4	500	2.6989	100	2.0000	200	2.3010

5.	RABBIT 5	600	2.7781	0	0.0000	400	2.6020
6.	RABBIT 6	200	2.3010	100	2.0000	500	2.6989

Discussion

Bacterial keratitis, because of its high morbidity and potential complications, is amongst one ofthe most vision threatening infectious ocular pathologies. The treatment of keratitis continues to pose challenge to the ophthalmologists and the issue gets further complicated if the infection is caused by multidrug resistant superbug like methicillin-resistant *Staphylococcus aureus*(MRSA).Lysostaphin has been shown to be a potent antibacterial therapy for treatment of many infections e.g. keratitis [15] and endophthalmitis[16-18] produced by experimental MRSA infection in rabbits.Lysostaphin appears to be more effective than any other drug tested in the treatment of experimental *S. aureus* keratitis[19, 20]Keeping in the view the potential uses of lysostaphin, experimental models of MRSA keratitis were developed in the present study to further evaluate the efficacy of lysostaphin therapy relative to vancomycinand azithromycin. Lysostaphin seemed to have penetrated the intact corneal epithelium and lowered mean log CFU/cornea in experimental eyes which was statistically highly significant as compared to vancomycin and azithromycin. Our findings were in agreement with observations made bystudies done elsewhere, but the minor difference could be attributed to the virulence of the organism employed[15].In the present investigation lysostaphin could sterilize 66% of treated corneas as compared with vancomycin and azithromycin group where none of the corneas were sterile. This was again comparable to the findings of Dajc*set al*, (2000) who demonstrated that lysostaphin sterilized as much as 75% of treated cornea as compared with untreated group[15]. Lysostaphin rapidly lyses the MRSA in both static and log phase, leading to a drastic decrease in the log CFU counts[21]. However, vancomycin is a slow-acting antibiotic that has significant side effects like conjunctival inflammation and corneal edema[22, 23]. Topical use of azithromycin has been studied in the treatment of bacterial or trachomatous conjunctivitis [24]. But its antibacterial use in MRSAkeratitis along with its comparison with lysostaphin and vancomycin has not been well studied before.

Conclusion

On the basis of observations made in the present study topical lysostaphin is an effective novel strategy against methicillin- resistant *Staphylococcus aureu* skeratitis in rabbit models as compared to other antibiotics studied viz. vancomycin and azithromycin. However, its broad clinical applications needs standardization of drug formulation either alone or in combination with other antibiotics and they have to be validated in larger experimental studies and clinical trials before considering them safe for therapeutic uses in humans.

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