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Research Article

# LYSOSTAPHIN AS AN ALTERNATE THERAPY IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) INDUCED ENDOPHTHALMITIS: AN EXPERIMENTAL STUDY

**N.ASKARI, SHAMIM AHMAD, ABHISHEK, ABDUL WARIS, MOUSUMI MALAKAR**

**Faculty, A.M.U. Inst. of Ophthalmology, VR Surgeon, MS (Ophthal), FICS (USA), FICO (UK), FRCS (Glasg), FRCS (Edin) ,4/143-G, Shibli Bagh, Hamdard Nagar A, Aligarh 202002 (U.P) India. Email: waris_eye@yahoo.co.in**

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**ABSTRACT**

Objective: To assess the susceptibility of MRSA to lysostaphin *in vitro*, effectiveness of intravitreal injection of lysostaphin for MRSA-mediated endophthalmitis, and evaluation of its toxicity on retina by histopathological examination.

Methods: We designed an experimental study in 17 rabbits, All rabbits were divided into 3 groups (A, B and C). Group A included 12 rabbits which were further subdivided into 4 subgroups of 3 rabbits each. The left eyes in group A and C were used as controls and right eyes were used as experimental eyes and the test drug lysostaphin was injected in these eye.

Results: In group A lysostaphin was injected 8 hrs PI, there was a significant difference in the reduction of CFU counts with p value ≤0.001. In Group B the 4 eyes were treated with different concentrations of lysostaphin, all the eyes were found to be sterile except one eye, there was a significant reduction (p≤ 0.001). In group C we used the combination of ceftazidime and vancomycin (control group) and lysostaphin (experimental group). We found a significant difference between two regimens (p=0.001).

Conclusion: In conclusion Intravitreal lysostaphin is an effective treatment for MRSA endophthalmitis in a rabbit model in both early and the late phases of endophthalmitis. None of the eyes treated with lysostaphin showed any sign of toxicity on histology. In light of the above study, intravitreal lysostaphin is safe and effective novel strategy against MRSA endophthalmitis.

**Keywords:** MRSA (methicillin resistant staphylococcal aureus), PI (post infection), CFU(colony forming units)

## INTRODUCTION

Endophthalmitis is a devastating complication of intraocular surgery and penetrating ocular trauma. The organism, staphylococcus aureus is one of the most commonly isolated organism in clinical cases of post-operative bacterial endophthalmitis and in majority of reported cases ranks second to coagulase negative staphylococcus epidermidis. The emergence of multiple antibiotic-resistant variants in the treatment of staphylococcal infections has been a serious problem in medicine. The increasing evidence of S. aureus resistance to even newer generation of various antibiotics has been well documented for the MRSA [1].

Lysostaphin has been proven as a therapeutic agent for the treatment of experimental S. aureus infections [2-7]. Lysostaphin has not yet been approved as a therapy for staphylococcus endophthalmitis. In the present study there is an assessment of the susceptibility of MRSA to lysostaphin *in vitro*, effectiveness of intravitreal injection of lysostaphin for MRSA-mediated endophthalmitis, and evaluation of its toxicity on retina by histopathological examination.

## METHODS AND MATERIALS

After obtaining the ethical clearance present study was conducted on 34 eyes of 17 rabbits from the animal house of JNMC, and the study was conducted in the Institute of ophthalmology, AMU, Aligarh.

Equipment used: (1) laminar flow bench- using a dynamic balance machine the blowers and motor assembly are statically balanced to provide adequate airflow over the entire surface of HEPA (high efficiency particular air) filter. (2) spectrophotometer.

Lysostaphin (Sigma Aldrich, Germany) comes in 5mg vial. It 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, shipped in dry ice & stored at a temperature of 20$^{o}$C.

Culture used: nutrient agar, nutrient broth, human blood agar, mannitol salt agar saline solution 0.85%. Drug used: lysostaphin, ceftazidime, vancomycin hydrochloride, basal salt solution. MRSA strains Mu 50 was employed in the present study. These bacterial strains were kindly provided by Prof. Keiichi Hiramatsu and his team of Prof. Teruyo Ito, Juntendo University, Tokyo, Japan.

All rabbits were divided into 3 groups (A, B and C). Group A included a total of 12 rabbits which were further subdivided into 4 subgroups of 3 rabbits each. 2 rabbits were studied in group B while group C contained only 3 rabbits. The left eyes (LE) in group A and C were used as controls and right eyes (RE) were used as experimental eyes and the test drug lysostaphin was injected in these eyes.

Inoculation of MRSA in rabbits were done by i.m injection of ketamine (22.5mg/kg).

Endophthalmitis was induced by the MRSA strain Mu 50 and the drug lysostaphin was tested against it for early and late endophthalmitis models. We present the result and conclusions of this experiment and a comparison of lysostaphin with the standard therapy of ceftazidime with vancomycin against MRSA endophthalmitis.

The data was analysed by unpaired t-test, p-value of <0.05 was considered significant.

## RESULTS AND DISCUSSION

### GROUP A

The rabbits in this group were further subdivided in 4 groups on the basis of quantity of MRSA inoculated and duration between inoculation and the starting of experiment.

### Subgroup A1

The eyes of experimental group was sterile but those treated with placebo showed a mean CFU of 26x10$^{4}$± 52915.03, whereas log units of mean was 5.3984 ± 0.0843 (p value 0.001).

**Table 1: It shows Antibacterial effect of lysostaphin on MRSA induced endophthalmitis in subgroup A1**

| S. No. | Exp animal | Exp eye CFU/ml | Exp eye Log | Control eye CFU/ml | Control eye Log |
|---|---|---|---|---|---|
| 1 | Rabbit 1 | 0 | 0 | $20x10^4$ | 5.3010 |
| 2 | Rabbit 2 | 0 | 0 | $28x10^4$ | 5.4471 |
| 3 | Rabbit 3 | 0 | 0 | $30x10^4$ | 5.4471 |

Using unpaired t-test, p value 0.001

(Exp-experimental)

**Subgroup A2**

The mean CFU 39.6667±68.7047 (mean log CFU 0.6918±1.1983) but those treated with placebo showed a mean CFU of $36x10^5$±929157, where as in log units of mean was 6.5553±0.1068 (p value ≤ 0.001, highly significant)

**Table 2: Shows Efficacy of lysostaphin in late experimental endophthalmitis caused by test strain MRSA Mu50:**

| S. No. | Exp animal | Exp eye CFU/ml | Exp eye Log | Control eye CFU/ml | Control eye Log |
|---|---|---|---|---|---|
| 1 | Rabbit 4 | 0 | 0 | $34x10^4$ | 6.5314 |
| 2 | Rabbit 5 | 119 | 2.0755 | $29x10^4$ | 6.4624 |
| 3 | Rabbit 6 | 0 | 0 | $47x10^4$ | 6.6721 |

(p value 0.007)

**Subgroup A3**

The eyes in experimental group did not show any growth but those treated with placebo showed a mean CFU of $17.6667x10^6$±$3.0550x10^6$, where as in log units the mean was 7.2429± 0.0738 (p value <0.05).

**Table 3: Shows effect of lysostaphin on experimental endophthalmitis caused by 200CFU/ml of MRSA Mu 50 strain**

| S. No. | Exp animal | Exp eye CFU/ml | Exp eye Log | Control eye CFU/ml | Control eye Log |
|---|---|---|---|---|---|
| 1 | Rabbit 7 | 0 | 0 | $15x10^4$ | 7.1761 |
| 2 | Rabbit 8 | 0 | 0 | $17x10^4$ | 7.2304 |
| 3 | Rabbit 9 | 0 | 0 | $21x10^4$ | 7.3222 |

(p value 0.0001)

**Subgroup A4**

One eye in experimental group was unsterile and the mean CFU count was 145.3333± 251.7247 (mean log 0.8798±1.5239). In the control group mean CFU was $70.6667x10^6$± $12.4230x10^6$,in log units the mean was 7.2429±0.0738. The significance on student t-test p value ≤0.001.

**GROUP B**

Out of the 4 eyes in which different concentration (0.001mg/ml, 0.005mg/ml, 0.01mg/ml, 0.05 mg/ml) of lysostaphin was given the eye in which 0.001 mg/ml of lysostaphin was injected was unsterile and rest eyes were sterile yielding no bacteria.

**Table 4: Effect of lysostaphin on late experimental endophthalmitis caused by 200CFU/ml of MRSA Mu 50 strain:**

| S. No. | Exp animal | Exp eye CFU/ml | Exp eye Log | Control eye CFU/ml | Control eye Log |
|---|---|---|---|---|---|
| 1 | Rabbit 10 | 0 | 0 | $63x10^4$ | 7.1761 |
| 2 | Rabbit 11 | 436 | 2.6395 | $85x10^4$ | 7.2304 |
| 3 | Rabbit 12 | 0 | 0 | $64x10^4$ | 7.3222 |

(p value <0.05 )

**GROUP C**

Amongst the 3 eyes in which lysostaphin was injected two eyes were sterile (mean 1.3333±2.3094) and the log CFU count was 0.2007±0.3476 but none of the eyes in control group (ceftazidime and vancomycin) were sterile. They had mean CFU/ml counts of 366.6667±97.3516, log CFU/ml counts were 2.5542±0.1139. p value was <0.05 which was significant.

**Table 5: Shows the comparative efficacy of lysostaphin with ceftazidime and vancomycin therapy in the treatment of MRSA induced endophthalmitis**

| S. No. | Exp animal | Exp eye CFU/ml | Exp eye Log | Control eye CFU/ml | Control eye Log |
|---|---|---|---|---|---|
| 1 | Rabbit 1 | 0 | 0 | 280 | 2.4472 |
| 2 | Rabbit 2 | 4 | 0.6020 | 348 | 2.5415 |
| 3 | Rabbit 3 | 0 | 0 | 472 | 2.6739 |

(On student t-test p value was 0.003)

Histopathological changes that occurred in the ocular tissue were mainly due to acute inflammation.

At present the treatment accounted for MRSA is vancomycin but the emergence of resistance to vancomycin has further complicated the issues in clinical medicine including ophthalmology [8]. The increasing evidence of S. aureus resistance to various antibiotics has been well documented for MRSA [1]. The resistance of S. aureus for lysozyme has been reported [9].

Various new drugs have been tested against MRSA out of them lysostaphin has shown promising results. The potential use of lysostaphin-lysozyme combination for topical therapy of staphylococcal infections resistant to other antibiotics has been demonstrated by Cisani *et al* 1982[10].

In view of the promising therapeutic antibacterial efficacy of lysostaphin in various staphylococcal infections including eye infections like eye blinding endophthalmitis the present study was undertaken on rabbit models after producing endophthalmitis. Lysostaphin therapy at either early (8 hrs PI) or late (24 hrs PI) period using a single injection of 0.1 mg/ml was evaluated.

In group A lysostaphin was injected 8 hrs PI, there was a significant difference in the reduction of CFU counts with p value ≤0.001. Dajics et al, 2001 in their study described that eyes injected with 50 CFU of S. aureus and treated at 8 hrs PI with lysostaphin demonstrated a ≥6 log unit reduction in CFU relative to the untreated control. This difference could be attributed to the virulence of the organism.

In the eyes injected with 50 CFU of S. aureus and treated with lysostaphin at 24 hrs and 48 hrs PI showed the reduction in the mean log CFU units/ml of treated as compared to untreated eyes was 5.8635 which was approximately equal to the difference as found by Dajics et al, 2001. When the data obtained after injecting 200 CFU/ml and injecting lysostaphin 8 hrs PI was analysed, there was a log reduction of 7.2429 in the treated eyes as compared to the control eyes. This findings was in coordination of findings by Dajics et al [11].

In Group B, four eyes in this group were treated with different concentrations (0.001mg/ml, 0.005mg/ml, 0.01mg/ml, 0.05 mg/ml) of lysostaphin, all the eyes were found to be sterile except one eye which was treated with 0.001 mg/ml of lysostaphin. However, there was a significant reduction (p≤ 0.001).

In group C we used the combination of ceftazidime and vancomycin (control group) against MRSA as a comparative group of lysostaphin (experimental group). We found a significant difference between two regimens (p=0.001).

On comparing the antibacterial efficacy of lysostaphin administration at 8 hrs or 24 hrs PI with 50 CFU/ml and 200 CFU/ml of MRSA Mu50 in group A we found that 5 out of 6 eyes were sterile in 50 CFU/ml group and 4 out of 6 eyes were sterile in 200 CFU/ml group.

On the basis of observations made in present study, following conclusions were drawn:

Intravitreal lysostaphin is an effective treatment for MRSA endophthalmitis in a rabbit model in both early and the late phases of endophthalmitis. Efficacy of lysostaphin is more than standard antibiotic regimen of ceftazidime and vancomycin in MRSA endophthalmitis.

None of the eyes treated with lysostaphin showed any sign of toxicity on histology.

In light of the above study, intravitreal lysostaphin is safe and effective novel strategy against MRSA endophthalmitis. However its broad clinical application wait for standardization of drug formulation either alone or in combination with other antibiotics and they should to be validated on a larger experimental studies and clinical trials before considering its therapeutic uses in humans.

**REFERENCES**

1. Long TE *et al*. Recent progress toward the clinical development of new anti MRSA antibiotics. IDrugs; 2003 Apr;351-59.
2. Zygmunt WA, Tavormina PA. Lysostaphin: model for a enzymatic approach to infectious disease. Prog drug Res1972;16:309-333.
3. Climo M, Ehlert K, Archer G. Mechanism and suppression of lysostaphin –resistance in oxacillin resistant S.aureus. antimicrob agents chemother 2001;45:1431-1437.
4. Climo MW, Patron RL, Goldstein BP, Archer GL. Lysostaphin treatment of experimental MRSA Aortic valve endocarditis. Antimicrob agents chemother 1998;42:1355-1360.
5. Patron RL, Climo MW, Goldstein BP, Archer GL. Lysostaphin treatment of experimental aortic valve endocarditis caused by a S. aureus. Antimicrob agents chemotherapy 1999;43: 1754-1755.
6. Dajcs JJ, *et al.* Lysostaphin treatment of MRSA keratitis in rabbit. Invest ophthalmol Vis Sci 2000;41:1432-1437.
7. Kokai-kun JF, Walsh SM, Chanturiya T, Mond JJ. Lysostaphin cream eradicates S.aureus nasal colonization in a cotton rat model. Antimicrob Agents chemother 2003;47:1589-1597.
8. Hiramatsu K *et al.* Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeously resistant to vancomycin. Lancet 1997;350: 1670-73.
9. Bera A, Herbert S, Jakob A, Vollmer W, Gotz F. Why are pathogenic staphylococci so lysozyme resistant? Mol Microbial 2005; 55: 778-787.
10. Cisani G, Varaldo PE, Grazi G, Soro O. High level potential of lysostaphin anti-staphylococcal activity by lysozyme.Anti-microbial agents chemother1982;21: 531-535.
11. Dajcs JJ, Thibodeaux BA, Hume EB, Zheng X, O'Callaghan RJ. Lysostaphin is effective in treating MRSA endophthalmitis in the rabbit. Curr Eye Res 2001;22: 451-457.