

Original Article

INFLUENCE OF TOPICAL RETAPAMULIN (1%) OINTMENT, SOFINOX- RD CREAM, SOFINOX CREAM (2%), ZINC FUSIDATE CREAM (2%) AND ZINC FUSIDATE OINTMENT (2%) ON NASAL MUCOSAL SURFACE SAFETY IN NEW ZEALAND ALBINO RABBITS

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Received: 27 Oct 2014 Revised and Accepted: 22 Nov 2014

ABSTRACT

Objective: To investigate the comparative safety of Retapamulin 1% ointment, Sodium fusidate 0.25%, Sofinox 2% cream, Zinc fusidate 2% cream and Zinc fusidate 2% ointment on nasal mucosal surface in rabbits.

Methods: In the experiment a total of 30 adult New Zealand albino rabbits of either sex were used. The rabbits were divided into five groups of six rabbits each. A thin layer of the drug was applied in the right nostril and left nostril was kept as control (no treatment) to their corresponding treatment group. Nasal safety of these drugs was assessed with the help of a symptom scoring system and photographic observations.

Results: There was significant decrease of nasal rubbing (Rhinocnesmus) behaviors in experimental animals of Sofinox RD cream ($p < 0.001$), Sofinox 2% cream ($p < 0.001$), Zinc fusidate cream ($p < 0.001$) and Zinc fusidate ointment ($p < 0.001$) treated groups when compared with Retapamulin 1% ointment treated group. More redness was observed in nasal mucosal surface of both nostrils (Right nostril- Retapamulin 1% ointment, Left nostril- Control) of Retapamulin group animals in comparison with Sofinox RD cream, Sofinox 2% cream, Zinc fusidate 2% cream and Zinc fusidate 2% ointment group animals.

Conclusion: In the present study, the comparative safety of test drugs on nasal mucosal surface of rabbits were found as- Zinc fusidate ointment > Zinc fusidate cream > Sofinox RD cream ~ Sofinox 2% cream > Retapamulin 1% ointment. Further, clinical evaluation has to be performed to precisely define the safety of Zinc fusidate ointment, Zinc fusidate cream, Sofinox RD cream and Sofinox 2% cream on nasal mucosal surface of human subjects.

Keywords: Nasal safety, Retapamulin, Zinc fusidate, Sofinox.

INTRODUCTION

The nasal mucosa is a major site of entry of infection and nasal disease may precede skin disease by several years [1]. The ecological niches of *Staphylococcus aureus* strains are the anterior nares [2]. Elegant studies have shown that the nares are the most consistent area from which this organism can be isolated [3]. Moreover, when the nares are treated topically to eliminate nasal carriage, in most cases the organism also disappears from other areas of the body [4, 5]. Fusidic acid acts as a bacterial protein synthesis inhibitor by preventing the turnover of elongation factor G (EF-G) from the ribosome. The global problem of advancing antimicrobial resistance has led to a renewed interest in its use recently. Fusidic acid is primarily active in vitro against various strains of staphylococci including Methicillin susceptible and resistant variety of *S. aureus*, heterogenous and non-heterogenous vancomycin intermediate *S. aureus*, and most coagulase negative staphylococci, clostridia species are susceptible. *Neisseria*, *Moraxella* and *Legionella pneumophila* are susceptible gram negative bacteria to fusidic acid. Topical use of fusidic acid and retapamulin is well established [6-10]. The steroid like structure of fusidic acid confers good skin penetration and does not possess the unwanted side effects of steroids. Zinc is necessary for the functioning of more than 300 different enzymes and plays a vital role in an enormous number of biological processes. Zinc is a cofactor for the antioxidant enzyme superoxide dismutase (SOD) and is in a number of enzymatic reactions involved in carbohydrate and protein metabolism. Its immune-enhancing activities include regulation of T lymphocytes, CD4, natural killer cells, and interleukin II.

Retapamulin is a member of a newly developed antibacterial class of antibiotics for treatment in humans referred to as pleuromutilins,

which selectively inhibit the elongation phase of bacterial protein synthesis at a unique site on the prokaryotic ribosome. It has in vitro activity against both *S. aureus* and *S. pyogenes*, including isolates resistant to established agents such as β -lactams, macrolides, quinolones, fusidic acid and mupirocin.

Hence the present study was planned to investigate the comparative safety of Altargo 1% ointment (Retapamulin; GSK, UK), Sofinox RD cream (Sodium fusidate 0.25%; Apex Laboratories Private Ltd., India), Sofinox 2% cream (Sodium fusidate 2%; Apex Laboratories Private Ltd., India), Zinc fusidate 2% cream (Apex Laboratories Private Ltd., India) and Zinc fusidate 2% ointment (Apex Laboratories Private Ltd., India) on nasal mucosal surface in rabbits.

MATERIALS AND METHODS

Animals: 30 adult New Zealand albino rabbits of either sex were housed individually in polypropylene cages, maintained under standard conditions with temperature (22–24° C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rabbits had continuous access to norm caloric diet and to tap water. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/87/2013) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Drugs: Altargo 1% ointment (Retapamulin 1%; GSK, UK), Sofinox RD cream, Sofinox 2% cream, Zinc fusidate (FXZ) cream and Zinc

fusidate (FXZ) ointment were obtained from Apex Laboratories Private Limited, Chennai (India).

Experimental procedure

In the experiment a total of 30 adult New Zealand albino rabbits of either sex were used. The rabbits were divided into five groups of six rabbits each. Before the experiment, the animals were placed in an observation cage for about 10 min for acclimatization. A thin layer of drug (25-30 mg) was applied in the right nostril and left nostril was kept as control (no treatment) to their corresponding treatment group.

Treatment was done twice daily for 10 days as follows- **Group I:** Retapamulin 1% ointment **Group II:** Sofinox RD cream **Group III:** Sofinox 2% cream **Group IV:** Zinc fusidate (FXZ) cream **Group V:** Zinc fusidate (FXZ) ointment

Investigational indices

Symptoms score within 30 min after the nasal application of respective drugs, rabbits in all the five groups were observed for symptoms like nasal rubbing, sneezing and nasal discharge and scored. The following quantitative scoring method of totaling itemized scores was adopted to analyze all indices.

Table 1: The symptom scoring system used for analyzing the safety of drug on nasal mucosal surface

Score	Symptoms		
	Rhinocnesmus	Sneezing	Watery nasal discharge
0	No symptoms	No symptoms	No symptoms
1	Gently nasal rubbing once or twice	1-3 times	Flowing to anterior nares
2	Acutely rubbing nasal face	4-10 times	Overstepping anterior nares
3	Medially rubbing nasal face	≥11 times	Covering whole cheeks

Photographic observation for all the rabbits, individual photographs of nasal mucosal surface (both nostrils) was taken daily before the 1st time application of drug from day 2nd day till 10th day (Since, the treatment plan was suggested as twice daily for 10 days).

Data analysis

Using SPSS 20.0, normally distributed (uniform) data were expressed as Mean ±SEM and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. Whereas non-uniform data were expressed as Median (Quartile1, Quartile 3) and analyzed by non-parametric test- two independent samples followed by Mann-Whitney U test. P value less than 0.05 was considered as statistically significant.

RESULTS

Effect on rhinocnesmus (nasal rubbing), sneezing and watery nasal discharge

On 10th day, symptom scores for nasal rubbing (Rhinocnesmus), sneezing and watery nasal discharge were evaluated for 30 minutes after application of test drugs to the respective treatment groups (Table 1). There was significant decrease of nasal rubbing (Rhinocnesmus) behaviors in experimental animals of Sofinox RD cream, Sofinox 2% cream, Zinc fusidate cream and Zinc fusidate ointment treated groups when compared with Retapamulin 1% ointment treated group. The order of significant increase in nasal rubbing behavior was found as Retapamulin 1% ointment > Sofinox RD cream = Sofinox 2% cream > Zinc fusidate cream > Zinc fusidate ointment. There was no significant change in nasal rubbing behaviors among Sofinox RD cream and Sofinox 2% cream treated groups (Table 2). There were no significant changes in symptom scores for sneezing and watery nasal discharge among all the treatment groups.

Table 2: Symptom score for rhinocnesmus (Up to 30 minutes after topical application on 10th day)

Groups (n=6)	Dose	Mean ±SEM
I	Retapamulin 1% Ointment	21.66±0.42
II	Sofinox RD Cream	9.00±0.25***a,d,e
III	Sofinox 2% Cream	9.83±0.47***a,d,e
IV	Zinc Fusidate Cream	4.66±0.49***a,b,c,e
V	Zinc Fusidate Ointment	1.33±0.21***a,b,c,d,e

*** $p < 0.001$ (Significant)^a compared to Retapamulin 1% ointment, ^b compared to Sofinox RD cream, ^c compared to Sofinox 2% cream, ^d compared to Zinc Fusidate cream, ^e compared to Zinc Fusidate ointment.

Photographic evaluation

There was more redness observed in nasal mucosal surface of both nostrils (Right nostril- Retapamulin 1% ointment, Left nostril- Control) of Retapamulin group animals in comparison with Sofinox RD cream, Sofinox 2% cream, Zinc fusidate 2% cream and Zinc fusidate 2% ointment group animals.

There was not much more difference for symptoms like redness of nasal mucosal surface among all other groups except Retapamulin group. Watery nasal discharge from both nostrils of 1-2 rabbits (especially right nostril- drug treated) was found among Sofinox RD cream and Sofinox 2% cream groups (fig. 1-5).



Fig. 1: Effect of retapamulin 1% ointment (Photographs of both nostrils of rabbits from day 2nd - day 10th)



Fig. 2: Effect of Sofinox RD cream Photographs of both nostrils of rabbits from day 2nd-day 10th)



Fig. 3: Effect of Sofinox 2% cream (Photographs of both nostrils of rabbits from day 2nd - day 10th)



Fig. 4: Effect of Zinc fusidate cream (Photographs of both nostrils of rabbits from day 2nd - day 10th)



Fig. 5: Effect of Zinc fusidate ointment (Photographs of both nostrils of rabbits from day 2nd - day 10th)

DISCUSSION

In the present study, the comparative safety of test drugs on nasal mucosal surface of rabbits was found as- Zinc fusidate ointment > Zinc fusidate cream > Sofinox RD cream ~ Sofinox 2% cream > Retapamulin 1% ointment. Retapamulin has caused significant nasal irritation which supports the warning of its prohibited use on nasal mucosa, given by the manufacturer of Altrago 1% ointment (Retapamulin 1% ointment, GlaxoSmithKline; UK) [11]. Ointments are oily and contain very little water. They are messy, greasy, and difficult to wash off. Ointments are usually better than creams at delivering active ingredients into the skin. A given concentration of a drug is more potent in an ointment than in a cream. Ointments are less irritating than creams. Several bacterial microorganisms can infect the nasal mucosal surface, but the most common agents are *S. aureus* and group A (*S. pyogenes*) streptococci [12, 13]. The management of nasal infections lends itself to more direct or topical therapy for a number of reasons, including the ability to achieve high local drug concentrations at the site of infection, the low incidence of systemic side effects due to low or no absorption, the ability to combine several agents to empirically treat a range of potential cutaneous pathogens, cost-effectiveness, patient compliance and the potential to limit anti-microbial resistance selection among other bacteria in the body compared with oral or parenteral antimicrobials [14]. Many drugs have limited efficacy because of sub-optimal pharmacokinetics and advances in drug delivery are needed to improve the pharmacokinetics of such drug. Recent advances in the field of biomaterials and their medical applications indicate the significance and potential of various microbial polysaccharides in the development of novel classes of medical materials [15]. Advances in drug delivery can improve the pharmacokinetics of promising drugs for many diseases [16]. Fusidic acid based formulations can be promising candidates for various types of nasal infections associated with inflammation in which conventional preparations have shown less efficacy.

CONCLUSION

The present study revealed the safety order of test drugs on nasal mucosal surface of rabbits as- Zinc fusidate ointment > Zinc fusidate cream > Sofinox RD cream ~ Sofinox 2% cream > Retapamulin 1% ointment. Further, clinical evaluation has to be performed to precisely define the safety of Zinc fusidate ointment, Zinc fusidate cream, Sofinox RD cream and Sofinox 2% cream on nasal mucosal surface of human subjects. "This information would eventually complement our findings, opening the way to sustain nasal infection conditions in human population".

CONFLICT OF INTERESTS

Declared None

REFERENCES

- Melo Naves M, Gomes Patrocinio L, Patrocinio JA, Naves Mota FM, Diniz de Souza A, Negrão Fleury R, et al. Contribution of nasal biopsy to leprosy diagnosis. *Am J Rhinol Allergy* 2009;23:177e80.
- Kluytmans J, Belkum AV, Verbrugh H. Nasal carriage of staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;10(3):505-20.
- Williams REO. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev* 1963;27:56-71.
- Parras F, Guerrero MC, Bouza E, Blaazquez MJS, Moreno M, Cruz M, et al. Comparative study of mupirocin and oral cotrimoxazole plus topical fusidic acid in eradication of nasal carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1995;39:175-9.
- Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, et al. Elimination of coincident *S. aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann Int Med* 1991;114:101-6.
- Spelman D. Fusidic acid in skin and soft tissue infections. *Int J Antimicrob Agents* 1999;12 Suppl 2:S59-66.
- Free A, Roth E, Dalessandro M, Hiram J, Scangarella N, Shawar R, et al. Retapamulin ointment twice daily for 5 days vs oral cephalexin twice daily for 10 days for empiric treatment of secondarily infected traumatic lesions of the skin. *Skin Med* 2006;5(5):224-32.
- Yang LP, Keam SJ. Retapamulin: a review of its use in the management of impetigo and other uncomplicated superficial skin infections. *Drugs* 2008;68(6):855-73.
- Parish LC, Jorizzo JL, Breton JJ, Hirman JW, Scangarella NE, Shawar RM, et al. Topical retapamulin ointment (1%, wt/wt) twice daily for 5 days versus oral cephalexin twice daily for 10 days in the treatment of secondarily infected dermatitis: results of a randomized controlled trial. *J Am Acad Dermatol* 2006;55(6):1003-13.
- Tomayko JF, Li G, Breton JJ, Scangarella ON, Dalessandro M, Martin M. The safety and efficacy of topical retapamulin ointment versus placebo ointment in the treatment of secondarily infected traumatic lesions: a randomized, double-blind superiority study. *Adv Skin Wound Care* 2013;26(3):113-21.
- https://www.gsk.com.au/resources.ashx/prescriptionmedicin/esproductschilddatadownloads/2924/File/1A97CADB6600BFDCC1F583723B0F33B/Altargo_CMI_AU_002_Approved.pdf
- Templer SJ, Brito MO. Bacterial skin and soft tissue infections. *Hospital Physician* 2009;26:9-16.
- Vincent Ki, Rotstein C. Bacterial skin and soft tissue infections in adults: a review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. *Can J Infect Dis Med Microbiol* 2008;19(2):173-84.
- Pangilinan R, Tice A, Tillotson G. Topical antibiotic treatment for uncomplicated skin and skin structure infections: review of the literature. *Expert Rev Anti Infect Ther* 2009;7(8):957-65.
- Czaja W, Krystynowicz A, Bieleckia S, Brown RM. Microbial cellulose-the natural power to heal wounds. *Biomaterial* 2006;27:145-51.
- Satish MC, Adiga S, D'Souza A, Nayak V, Bharti Chogtu B, Bairy KL. Effect of betamethasone valerate with biopolymer on skin inflammatory models in albino mice and wistar rats. *Int J Pharm Sci Rev Res* 2010;4(2):173-5.