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EXPERIMENTAL STUDY

EVALUATION OF THE EFFECTIVENESS OF A TOPICAL MIXTURE OF BACITRACIN, POLYMYXIN B, CLOTRIMAZOLE AND BETAMETHASONE CREAM IN THE TREATMENT OF EXPERIMENTALLY INDUCED OTITIS EXTERNA IN RATS

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ABSTRACT

Aim: This study aimed to evaluate the clinical, microbiological, and histopathological effects of a topical combination of bacitracin, polymyxin B, clotrimazole, and betamethasone in the treatment of iatrogenic acute otitis externa induced in the external ear canal of rats.

Materials and Methods: A total of 39 adult Wistar albino rats were included. Acute external otitis was created by mechanical trauma to the external auditory canal. The rats were randomly divided into three groups: a control group treated with isotonic saline, a group treated with ciprofloxacin–dexamethasone drops, and a group treated with a combination of bacitracin–polymyxin B ointment, clotrimazole cream, and betamethasone cream. Cultures and histopathological evaluations were performed at multiple time points.

Results: The combination group demonstrated a bacterial eradication rate of 71.4% on day 10, which was significantly higher compared to the control group. Histopathological evaluation showed reduced epithelial thickness and edema in the treatment groups.

Conclusion: The combination therapy may be considered as an alternative treatment for patients with difficulties in regular drop application due to its antibacterial, antifungal, and anti-inflammatory properties, weekly application, and low cost. Planned experimental animal studies or clinical trials involving a larger group are needed to evaluate the efficacy of the treatment.

Keywords: *Bacitracin, Polymyxin B, Clotrimazole, Betamethasone, Otitis externa, Rat model*

Ratlarda Deneysel Olarak Oluşturulan Dış Kulak Yolu İltihabının Tedavisinde Basitrasin, Polimiksin B, Klotrimazol ve Betametazon İçeren Topikal Kremin Etkinliğinin Değerlendirilmesi

ÖZET

Amaç: Rat dış kulak yolunda iatrojenik olarak oluşturulacak akut otitis eksternanın tedavisinde basitrasin, polimiksin B, klotrimazol ve betametazon karışımının klinik, mikrobiyolojik ve histopatolojik olarak değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmada 39 adet erişkin Wistar albino rat kullanıldı. R at dış kulak yolu travmatize edilerek iatrojenik akut otitis eksterna oluşturuldu. Ratlar 13'er adetten oluşan 3 gruba ayrıldı. 1. grup 0,1 ml 7 gün salin verilen kontrol

grubuydu. 2. grup siprofloksasin– deksametazon damla 0,1 ml'den 7 gün uygulanan ratlardan oluşturuldu. 3. grup ise basitrasin merhem, polimiksin B merhem, klotrimazol krem ve betametazon krem karışımı uygulanan gruptu. 1. 4. 7. 10. ve 20. günlerde tüm ratların dış kulak yollarından kültürler alındı. 4. 7. ve 10. günlerde her gruptan 3 rat 20. günde 2. gruptan 4, 1. ve 3. gruplardan 3 rat dekapite edilerek histopatolojik olarak dış kulak yolu epitel ve stroma kalınlığı, ödem, inflamasyon ve fibroblastik aktivite değerlendirildi.

Bulgular: Karışım tedavisi uyguladığımız grupta 10. gün kür oranı %71,4'tü ve kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksekti. Karışım tedavisi uyguladığımız grupta histopatolojik değerlendirmede epitel kalınlığı ve ödem daha düşük saptandı.

Sonuç: Kombinasyon tedavisi antibakteriyel, antifungal ve antiinflamatuar özelliği, haftalık uygulanması ve düşük maliyeti nedeniyle düzenli damla uygulamasında zorluk yaşayan hastalarda alternatif bir tedavi olarak düşünülebilir. Tedavinin etkinliğinin değerlendirilmesi için daha geniş grupta planlanmış deneysel hayvan çalışması ya da klinik çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Basitrasin, Polimiksin B, Klotrimazol, Betametazon, Otitis eksterna, Sıçan modeli*

INTRODUCTION

Acute otitis externa (AOE) is an inflammatory disorder of the external auditory canal characterized by otalgia, otorrhea, pruritus, and canal edema. It is one of the most common otologic conditions encountered in both primary care and otolaryngology practice and may significantly impair quality of life because of severe pain and temporary conductive hearing loss [1-3].

Bacterial pathogens, particularly *Pseudomonas aeruginosa* and *Staphylococcus aureus*, account for most cases of AOE, whereas fungal organisms such as *Aspergillus* and *Candida* species are responsible for a smaller but clinically important proportion of infections, especially following prolonged topical antibiotic therapy or recurrent disease [4-6].

Current evidence-based clinical practice guidelines recommend topical antimicrobial therapy, with or without corticosteroids, as the first-line treatment for uncomplicated AOE because topical administration achieves high local drug concentrations while minimizing systemic adverse effects [1,2,5]. Fluoroquinolone-based preparations, either alone or combined with corticosteroids, are widely prescribed because of their favorable efficacy, safety profile, and low risk of ototoxicity [7,8,9].

Despite generally favorable treatment outcomes, several therapeutic challenges remain, including recurrent disease, inappropriate antibiotic prescribing, antimicrobial stewardship concerns, mixed bacterial–fungal infections, and patient adherence to treatment regimens requiring multiple daily administrations [10-12]. Recent systematic reviews have demonstrated that although topical therapies are highly effective, no single antimicrobial regimen has consistently shown superiority over others, highlighting the need to investigate alternative topical treatment strategies [5,12]. Topical combination therapies that simultaneously target bacterial pathogens, fungal organisms, and inflammation may therefore represent an attractive therapeutic option in selected clinical settings.

Bacitracin provides activity against Gram-positive bacteria, whereas polymyxin B is highly active against Gram-negative organisms, particularly *Pseudomonas aeruginosa*, the predominant pathogen in AOE [4,13]. Clotrimazole provides broad antifungal coverage against *Candida* and *Aspergillus* species,

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while betamethasone reduces inflammation, tissue edema, and pain, potentially improving local healing and patient comfort [6,13]. The combined use of these agents has been described for persistent inflammatory disorders of the external auditory canal; however, experimental evidence supporting this specific combination remains limited [13,14].

To our knowledge, no previous experimental study has evaluated this four-drug topical combination using both microbiological and histopathological outcome measures in an animal model of acute otitis externa. Therefore, the present study aimed to compare a topical mixture of bacitracin, polymyxin B, clotrimazole, and betamethasone with topical ciprofloxacin–dexamethasone and isotonic saline using microbiological and histopathological outcome measures in experimentally induced acute otitis externa in rats.

MATERIALS AND METHODS

Experimental Animals

Thirty-nine adult male Wistar albino rats weighing 250–300 g were included in this experimental study. The animals were housed under standard laboratory conditions at a controlled temperature of 22 ± 2°C with 50–60% relative humidity under a 12-hour light/dark cycle and had free access to standard laboratory chow and water throughout the study period. The study protocol was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (Approval No. 2020/39). All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Experimental Design

Following induction of acute otitis externa, the animals were randomly assigned to one of three treatment groups:

Group 1 (Control): isotonic saline

Group 2: topical ciprofloxacin–dexamethasone

Group 3: topical bacitracin–polymyxin B, clotrimazole, and betamethasone combination

Microbiological cultures and histopathological examinations were performed according to the study protocol on days 4, 7, 10, and 20 after treatment.

Anesthesia

All procedures were performed under general anesthesia induced by intraperitoneal administration of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg). Adequate anesthesia was confirmed before all experimental procedures.

Microbiological Evaluation

Sterile swab specimens were collected from the external auditory canal before treatment and at predetermined follow-up time points. Samples were cultured using standard microbiological techniques, and bacterial growth was recorded as positive or negative according to routine laboratory evaluation.

Histopathological Evaluation

Following euthanasia, temporal bone specimens were harvested and fixed in 10% neutral buffered formalin. After decalcification and routine tissue processing, paraffin-embedded sections were stained with hematoxylin and eosin. Histopathological evaluation included epithelial thickness, inflammatory cell infiltration, and tissue edema. All specimens were evaluated by the same experienced pathologist, who was blinded to the treatment allocation throughout the histopathological assessment.

Statistical Analysis

Statistical analyses were performed using Minitab Statistical Software (Version 20.3; Minitab LLC, State College, PA, USA). Categorical microbiological outcomes were compared using Fisher's exact test because of the relatively small sample sizes. Pairwise comparisons of bacterial eradication rates between groups were performed using the two-proportion test. Histopathological findings were analyzed using appropriate non-parametric statistical methods because of the ordinal nature of the variables. A two-sided p value <0.05 was considered statistically significant.

RESULTS

Microbiological Findings

Acute otitis externa was successfully established in all experimental animals before treatment. Microbiological culture results obtained during the follow-up period are presented in Table 1, and pairwise statistical comparisons are summarized in Table 2. On day 4, both the ciprofloxacin–dexamethasone group and the bacitracin–polymyxin B–clotrimazole–betamethasone combination group demonstrated significantly lower bacterial growth than the control group ($p < 0.05$). No statistically significant difference was observed between the two active treatment groups. Similar findings were obtained on days 7 and 10, with both treatment groups showing significantly greater bacterial eradication than the control group, whereas bacterial eradication rates remained comparable between the active treatment groups. By day 20, bacterial growth was not detected in any of the remaining animals.

Table 1: Culture-based bacterial isolation rates

Groups/Days	1st	4th	7th	10th	20th
Group 1 (Control)	13/13	10/10	9/9	6/6	0/3
Group 2 (Cipro)	13/13	2/10	2/10	1/7	0/4
Group 3 (Mixture)	13/13	3/10	3/10	2/7	0/3

Histopathological Findings

Representative histopathological findings are presented in Figure 1. Histopathological examination demonstrated progressive improvement in epithelial architecture and inflammatory changes in both treatment groups throughout the follow-up period. A significant reduction in epithelial thickness was observed in the ciprofloxacin–dexamethasone group on day 4 and in the combination therapy group on day 7 compared with the control group ($p = 0.05$). No statistically significant differences were detected between the two active treatment groups with respect to epithelial thickness. No statistically significant differences were observed among the groups regarding stromal thickness, edema, inflammatory cell infiltration, or fibroblastic activity at any evaluation time point ($p > 0.05$). Nevertheless, both treatment groups showed a consistent trend toward reduced inflammatory changes and improved tissue architecture compared with the control group.

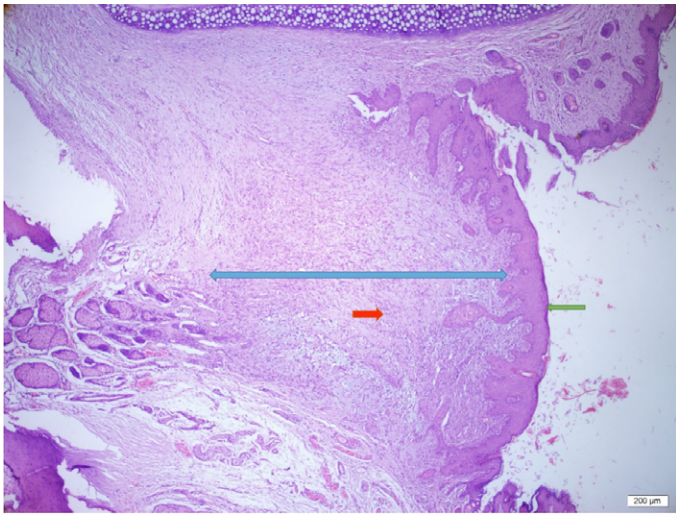

Figure 1.

Overall Findings

Overall, both topical treatment regimens achieved significantly greater microbiological eradication than the control treatment. Histopathological findings supported tissue recovery in both treatment groups, with no significant differences between the ciprofloxacin–dexamethasone and bacitracin–polymyxin B–clotrimazole–betamethasone groups.

Table 2: Pairwise comparison of bacterial growth between treatment groups

Comparison	Day	Difference in proportions	95% CI	Fisher's exact p value
Control vs Ciprofloxacin	4	0.80	0.55–1.00	0.001*
Control vs Combination	4	0.70	0.42–0.98	0.003*
Ciprofloxacin vs Combination	4	-0.10	-0.48–0.28	1.000
Control vs Ciprofloxacin	7	0.80	0.55–1.00	0.001*
Control vs Combination	7	0.70	0.42–0.98	0.003*
Ciprofloxacin vs Combination	7	-0.10	-0.48–0.28	1.000
Control vs Ciprofloxacin	10	0.86	0.60–1.00	0.005*
Control vs Combination	10	0.71	0.38–1.00	0.021*
Ciprofloxacin vs Combination	10	-0.14	-0.57–0.28	1.000

DISCUSSION AND CONCLUSIONS

The present experimental study demonstrated that the bacitracin–polymyxin B–clotrimazole–betamethasone combination achieved microbiological eradication and histopathological improvement comparable to those obtained with topical ciprofloxacin–dexamethasone in an experimental model of acute otitis externa. Both treatment regimens resulted in significantly greater bacterial eradication than the untreated control group, whereas no statistically significant differences were observed between the two active treatment groups. Although significant reductions in epithelial thickness were observed at selected time points, other histopathological parameters were generally comparable between the treatment groups. These findings suggest that the combination preparation exhibited therapeutic activity similar to that of the standard topical antibiotic–steroid regimen under the experimental conditions of this study.

Acute otitis externa is one of the most common infectious diseases encountered in otolaryngology practice and is characterized by diffuse inflammation of the external auditory canal, most frequently caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Current evidence-based guidelines recommend topical antimicrobial therapy, with or without corticosteroids, as the first-line treatment for uncomplicated AOE because topical administration provides high local drug concentrations while minimizing systemic exposure and adverse effects [1,2].

Systemic antibiotics are generally reserved for patients with infection extending beyond the external auditory canal, immunocompromised individuals, or those with specific clinical indications, as their routine use has not been shown to improve outcomes in uncomplicated disease and may contribute to unnecessary antibiotic exposure [1,3,9]. Recent systematic evidence has demonstrated that most currently available topical preparations achieve comparable clinical cure rates, with no consistent superiority of one antimicrobial class over another. Consequently, treatment selection should be based on factors such as tympanic membrane integrity, safety profile, patient adherence, local antimicrobial susceptibility, and cost rather than antibiotic class alone [2,5,6]. These findings support the rationale for evaluating alternative topical formulations with broad antimicrobial and anti-inflammatory activity. In the present study, the bacitracin–polymyxin B–clotrimazole–betamethasone combination demonstrated microbiological efficacy comparable to that of ciprofloxacin–dexamethasone, which is consistent with the current understanding that multiple topical treatment strategies may provide similar therapeutic outcomes when appropriately selected [6-8].

The rationale for investigating the bacitracin–polymyxin B–clotrimazole–betamethasone combination is based on the complementary pharmacological properties of its individual components. Bacitracin primarily targets Gram-positive bacteria by inhibiting bacterial cell wall synthesis, whereas polymyxin B exhibits potent activity against Gram-negative organisms, particularly *Pseudomonas aeruginosa*, the most frequently isolated pathogen in acute otitis externa. Consequently, the combination of these two antibacterial agents provides broad antimicrobial coverage against the predominant bacterial pathogens associated with external auditory canal infections [15,16].

Clotrimazole was incorporated to provide antifungal activity. Although fungal organisms are not the primary cause of uncomplicated acute otitis externa, secondary fungal colonization may occur following disruption of the normal ear canal flora or prolonged topical antimicrobial therapy. Therefore, antifungal coverage may be advantageous in selected clinical settings. In addition, betamethasone contributes by suppressing local inflammation, reducing tissue edema, erythema, and pain, thereby improving the inflammatory environment during healing [2,5]. Based on these complementary mechanisms, this combination has the potential to provide simultaneous antibacterial, antifungal, and anti-inflammatory effects. In the present experimental study, the combination therapy achieved microbiological eradication comparable to that of ciprofloxacin–dexamethasone, supporting the concept that different topical formulations may produce similar therapeutic outcomes when appropriate antimicrobial coverage and inflammation control are achieved.

Our microbiological findings are consistent with previous studies demonstrating the effectiveness of topical antimicrobial therapy in the treatment of acute otitis externa. Randomized clinical trials have shown that topical fluoroquinolone-containing preparations achieve high bacterial eradication rates while providing favorable clinical outcomes. Likewise, recent systematic reviews have concluded that most currently available topical preparations are highly effective, with no consistent evidence supporting the superiority of one antimicrobial class over another (6-8). In the present study, no statistically significant differences were observed between ciprofloxacin–dexamethasone and the bacitracin–polymyxin B–clotrimazole–betamethasone combination with respect to microbiological eradication. These findings are in agreement with current evidence suggesting that successful treatment of uncomplicated acute otitis externa depends primarily on adequate topical antimicrobial activity and control of local inflammation rather than the use of a specific antibiotic class [2,6].

Histopathological evaluation demonstrated progressive improvement in epithelial architecture and inflammatory changes in both treatment groups throughout the study period. Although statistically significant differences were observed only for epithelial thickness at selected time points, both active treatment groups consistently exhibited less inflammatory change than the untreated controls. The absence of significant differences in the remaining histopathological parameters may be related to the limited sample size and the ordinal scoring system used for histological assessment rather than the absence of biological treatment effects. These observations are consistent with the microbiological findings and suggest that successful bacterial eradication was accompanied by gradual resolution of tissue inflammation. Nevertheless, because this was an experimental animal study, the histopathological findings should be interpreted cautiously and cannot be directly extrapolated to clinical practice.

Current treatment strategies increasingly emphasize antimicrobial stewardship and the rational use of topical antibiotics. Recent evidence suggests that appropriate selection of topical therapy should consider not only antimicrobial efficacy but also safety, patient adherence, tympanic membrane status, and the potential impact on antimicrobial resistance [2,3,6].

Strengths of the Study

The present study has several strengths. First, the experimental design included random allocation of animals to treatment groups and blinded histopathological assessment, reducing the potential risk of observer bias. Second, both microbiological and histopathological outcomes were evaluated, allowing comprehensive assessment of treatment efficacy rather than relying solely on bacterial eradication.

Third, the inclusion of an active comparator (ciprofloxacin–dexamethasone), which represents a widely accepted topical treatment for acute otitis externa, strengthened the clinical relevance of the experimental model. These methodological characteristics increase the internal validity of the study and provide a robust basis for comparison between the investigated treatment regimens.

Limitations

Several limitations should also be acknowledged. First, this was an experimental animal study, and therefore the findings cannot be directly extrapolated to human clinical practice. Second, the relatively small sample size within each subgroup may have limited the statistical power to detect subtle histopathological differences. Third, histopathological evaluation was based on semi-quantitative ordinal scoring, which may not fully capture minor tissue changes. In addition, pharmacokinetic properties, local drug concentrations, adverse effects, and long-term outcomes were not investigated. Therefore, although the present findings demonstrate comparable experimental efficacy between the two topical preparations, further well-designed clinical studies are required before this combination can be considered an alternative therapeutic option for patients with acute otitis externa.

Conclusion

In this experimental rat model of acute otitis externa, the bacitracin–polymyxin B–clotrimazole–betamethasone combination demonstrated microbiological and histopathological efficacy comparable to that of topical ciprofloxacin–dexamethasone. These findings provide preliminary experimental evidence supporting the potential therapeutic value of this combination. However, given the experimental nature of the study, further clinical investigations are required to confirm its efficacy and safety before routine clinical application can be recommended.

DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (Approval No. 2020/39).

Conflict of Interest: The authors declare that there is no conflict of interest.

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