Effect of Corticosteroid-Antibiotic Agents on Granulation Tissue in a Murine Model

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Objective: To compare the effects of 3 commonly used ototopical corticosteroid-antibiotic agents, currently available for use in the treatment of inflammatory conditions of the external and middle ear, on granulation tissue in an established murine model of wound healing.

Subjects: Twelve C57/BL6J mice.

Design: Eight-millimeter wounds, created bilaterally on the dorsum of the mice, were treated with combinations of 0.3% ciprofloxacin and 0.1% dexamethasone (Cipro-Dex), 0.3% tobramycin and 0.1% dexamethasone (Tobra-Dex), 0.2% ciprofloxacin hydrochloride and 1% hydrocortisone (Cipro HC), or phosphate-buffered saline (n=6 each) for 3 days (days 4-6) and then harvested on day 7. Wound sections were stained with hematoxylin-eosin, Gomori trichrome, and CD31. Extracellular matrix deposition was graded from 1-4, and neovascularization was assessed by counting the number of endothelial-lined vessel lumens per high-power field (HPF).

Results: The mean ± SEM grade of extracellular matrix deposition was lower in CiproDex- (1.7±0.2) and TobraDex- (2.0±0.2) but not Cipro HC– (2.9±0.3) treated wounds compared with control wounds (2.9±0.2) (P<.01). The mean ± SEM number of vessel lumens per HPF was lower in CiproDex- (0.9±0.2 lumens/HPF), TobraDex- (1.5±0.3 lumens/HPF) and Cipro HC– (0.9±0.3 lumens/HPF) treated wounds compared with controls (3.3±0.5 lumens/HPF) (P<.01).

Conclusions: All 3 ototopical corticosteroid-antibiotic agents studied were equally effective at reducing neovascularization, although dexamethasone-based products were more effective at reducing extracellular matrix deposition. The results of this study suggest that ototopical agents containing dexamethasone may be more effective for the treatment of granulation tissue resulting from external and middle ear inflammatory conditions.


Granulation tissue formation is part of the normal wound-healing response to injury. It can occur in the middle ear in response to pathogens that infect the mucosa or as a reaction to foreign bodies, most commonly tympanostomy tubes (TTs). A recent study1 reported a 5% incidence of granulation tissue and a 26% incidence of otorrhea in patients with TTs.

Many topical antibiotic agents are commonly used by pediatricians and otolaryngologists for the treatment of granulation tissue and otorrhea, as well as other inflammatory conditions of the external and middle ear. Many of the available topical medications used in the ear are aminoglycoside-based and are approved by the US Food and Drug Administration for ophthalmologic use only because of the potential risk of ototoxic effects. Animal studies have demonstrated the potential for damage to the cochlea from aminoglycoside agents exposed to the round window membrane.2 Although concern about the use of aminoglycoside-containing drops in humans has been repeatedly raised in the otolaryngology literature,3 other studies4,5 have failed to demonstrate ototoxic effects in patients with TTs. The theoretical risk, however, has led to the development of a number of US Food and Drug Administration–approved, quinolone-based ototopical preparations. Moreover, a recent consensus panel3 on the role of potentially ototoxic antibiotics discouraged the off-label use of aminoglycoside-based agents when use of quinolone-containing agents is feasible.

A number of currently available ototopical preparations contain an antimicrobial in combination with either hydrocortisone or dexamethasone corticosteroid agents. The effect of corticosteroids on inflammatory conditions is well known, and recent studies6-8 have suggested a potential role for these agents in the treatment...
of inflammatory otologic conditions. Although a number of studies have reported on the efficacy of various topical antimicrobial agents, none has compared the efficacy of commonly used ototopical corticosteroid-antibiotic agents (OCAAs) for the treatment of granulation tissue and otorrhea.

The objective of this study was to compare the effects of 2 US Food and Drug Administration–approved and 1 commonly used, nonapproved OCAAs on granulation tissue in an established murine model of wound healing. We hypothesized that all of the study OCAAs would demonstrate similar efficacy in their ability to inhibit granulation tissue formation.

WOUND PREPARATION

Twelve C57/BL6j mice (Charles River Laboratories, Inc, Wilmington, Mass) aged 6 to 8 weeks were anesthetized with methoxylurane inhalation (0.5 mL titrated) and shaved, and their skin was prepared with povidone-iodine (Betadine; Purdue Pharma LP, Stamford, Conn). Eight-millimeter wounds were created bilaterally on the dorsum of each animal with the use of an 8-mm dermal punch biopsy forceps (Miltex Instrument Co Inc, Bethpage, NY), leaving the panniculus carnosus muscle intact. A transparent, sterile dressing was placed over the wound bed with the use of a 1-mL syringe and a 30.5-gauge needle. A transparent, sterile dressing was then applied circumferentially around the trunk of the animal (Tegaderm; 3M Health Care, St Paul, Minn). Wounds were allowed to granulate for 3 days before intervention. The animals were then divided into 4 groups, and wounds were treated with a preparation of either 0.3% ciprofloxacin with 0.1% dexamethasone (CiproDex; Alcon Laboratories, Inc, Fort Worth, Tex [trade name licensed by Alcon Laboratories, Inc, from Bayer Ag, Leverkusen, Germany]), 0.3% tobramycin with 0.1% dexamethasone (TobraDex; Alcon Laboratories, Inc), 0.2% ciprofloxacin hydrochloride with 1% hydrocortisone (Cipro HC; Alcon Laboratories, Inc), or phosphate-buffered saline (PBS) (n=6 wounds per group). One hundred microliters of each preparation was applied once daily for 3 consecutive days by injection into the wound bed with the use of a 1-mL syringe and a 30.5-gauge needle. A transparent, sterile dressing was then placed over the wound to prevent the preparation from “rolling off” of the granulation tissue bed. Animals were euthanized by carbon dioxide inhalation followed by cervical dislocation, and all wounds were harvested on day 7 in preparation for tissue sectioning.

HISTOLOGIC ANALYSIS

Wounds were harvested and fixed overnight in 10% neutral buffered formalin at 4°C, mechanically processed, and embedded in paraffin.

Serial 5-μm sections from the paraffin-embedded wounds were obtained using a microtome (RM 2035; Leica Microsystems, Heidelberg, Germany), collected on electrostatic microscope slides (Superfrost Plus; Fisher Scientific, Pittsburgh, Pa), and stained with hematoxylin-eosin or Gomori trichrome stain. The sections were then mounted on electrostatic microscope slides (Superfrost Plus; Fisher Scientific, Pittsburgh, Pa), collected on electrostatic microscope slides (Superfrost Plus; Fisher Scientific, Pittsburgh, Pa), and stained with hematoxylin-eosin or Gomori trichrome stain. Morphological analysis of the wounds demonstrated a significantly lower density of ECM and inflammatory cells in the central zone of the CiproDex- and TobraDex-treated groups compared with that of the Cipro HC–treated group and control wounds (Figure 1).

The mean ± SEM grade of ECM deposition was lower in CiproDex- (1.7±0.2) and TobraDex- (2.0±0.2) treated wounds, but not in Cipro HC–treated wounds (2.9±0.3) compared with controls (2.9±0.2) (P<.01) (Figure 2 and Figure 3).

The mean ± SEM number of vessel lumens per HPF was lower in CiproDex- (0.9±0.2 lumens/HPF), TobraDex- (1.5±0.3 lumens/HPF) and Cipro HC–(0.9±0.3 lumens/HPF) treated wounds compared with controls (3.3±0.5 lumens/HPF) (P<.01) (Figure 4 and Figure 5).

IMMUNOHISTOCHEMICAL ANALYSIS

Paraaffin-embedded sections were dehydrated, rehydrated with distilled water, immersed in tissue-unmasking fluid (pH 6.2; Signet Laboratories, Dedham, Mass) to reactivate hidden or masked epitopes, and then placed in a laboratory microwave oven (Ted Pella, Inc, Redding, Calif) for 5 minutes on high power to facilitate antigen retrieval. Slides were washed with distilled water and transferred to PBS. Samples were blocked with the use of normal 10% goat serum in PBS (30 minutes at room temperature). Sequential slides were then incubated in rat anti-mouse PECAM (CD31) monoclonal antibody (Pharmingen, San Diego, Calif) (1:20 dilution) with normal 10% rabbit serum for 30 minutes at room temperature and then overnight at 4°C. The slides were washed with PBS, and endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide (30 minutes at room temperature). Slides were rinsed with distilled water and PBS and then incubated with biotinylated species-specific IgG (Vector Laboratories, Burlingame, Calif) (1:200 dilution, 30 minutes at room temperature). The slides were washed with PBS, and avidin-biotin complex (Vector Laboratories) was added. The slides were rinsed in PBS, developed with chromagen 3,3’-diaminobenzidine tetrahydrochloride (Sigma-Aldrich Corp, St Louis, Mo), and lightly stained with hematoxylin. PECAM (platelet endothelial cell adhesion molecule)–positive vessel lumens were counted in 10 high-power fields (HPF) per wound.

DATA ANALYSIS

In each wound, the point of the initial injury was located, and the density of extracellular matrix (ECM) and cellular infiltration was noted in the region adjacent to the injury (transition zone) and in the center of the granulation tissue bed (central zone). Slides were then coded and ECM density (percentage of field stained with Omori trichrome) was graded from 1-4 (where 1 indicates <25%; 2, 26%-50%; 3, 51%-75%; and 4, >75%). Neovascularization was assessed by counting the number of endothelial-lined vessel lumens per HPF. Each value was expressed with the standard error of the mean (SEM). Statistical analysis was performed by using an analysis of variance, and a 2-tailed t test was used to identify differences between the individual groups. P<.05 defined statistical significance. All animal protocols used during this research were approved by the Institutional Animal Care and Use Committee at The Children’s Hospital of Philadelphia, Philadelphia, Pa, in accordance with regulations established by the National Institutes of Health, Bethesda, Md.

RESULTS

Morphological analysis of the wounds demonstrated a significantly lower density of ECM and inflammatory cells in the central zone of the CiproDex- and TobraDex-treated groups compared with that of the Cipro HC–treated group and control wounds (Figure 1).

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COMMENT

Wound healing is the dynamic progression that occurs in response to tissue injury. This process is classically di-
vided into 3 overlapping phases: inflammation, proliferation, and tissue remodeling. Supported by the elaboration of various mediators, including interleukin 1, transforming growth factor β, and tumor necrosis factor α, among others, granulation tissue begins to appear in the wound bed during the proliferative phase of wound healing and is characterized by new capillary formation and fibroblast hyperplasia. Extracellular matrix composed of fibrin, fibronectin, hyaluronic acid, and collagen are deposited by proliferating fibroblasts and are necessary for the support of cell migration. Gradually, collagen becomes the predominant component of the healing wound, which becomes a progressively acellular scar of increasing tensile strength.

The effects of systemic corticosteroids on the production of granulation tissue are well known. Corticosteroids suppress leukocyte migration to the wound, inhibit fibroblast replication, and function by interfering with the production or action of various mediators, including interleukin 1 and tumor necrosis factor α. Corticosteroids alter the quantity, structure, and function of collagen by inhibiting its synthesis and posttranslational processing with the use of various enzymes, including propyl hydroxylase, lysyl oxidase, and collagenase. These agents also interfere with the production of other ECM components, including hyaluronic acid and fibronectin, and have been found to indirectly inhibit angiogenesis by reducing lactate levels, which are necessary for the production of angiogenic factors.

Few studies have looked at the effects of corticosteroids on middle ear inflammation. In an animal model of chronic otitis media, Alper et al demonstrated that the inclusion of dexamethasone with tobramycin accelerated the resolution of otorrhea compared with tobramycin alone. In a randomized, placebo-controlled study, Ruohola et al reported that children with otorrhea and TTs who were treated with oral prednisolone had a significantly shorter course of otorrhea compared with control subjects. Shinkwin et al reported a significantly lower rate of postoperative otorrhea after TT insertion in patients treated with a combination of gentamycin sulfate and hydrocortisone compared with controls. In a recent prospective study, Roland et al demonstrated that a combination of ciprofloxacin and dexamethasone was superior to ofloxacin in the treatment of granulation tissue in children with TTs.
Commercial OCAAs are available with combinations of either 0.1% dexamethasone or 1% hydrocortisone as the corticosteroid agent. The administration instructions for each combination are similar, and thus, comparable quantities are administered in clinical practice despite differences in the concentrations of the various agents.

By administering equal doses of each medication to the granulating wound bed, we effectively gave a 10-fold-higher concentration of hydrocortisone (10 mg/mL) compared with dexamethasone (1 mg/mL). Dexamethasone, however, has a potency that is 20 to 30 times greater than that of hydrocortisone. Thus, despite the higher dose of administered hydrocortisone, wounds treated with dexamethasone were subject to a 2- to 3-fold-higher potency of corticosteroid agent compared with those treated with hydrocortisone. This may partially explain why the 2 dexamethasone-containing OCAAs studied (TobraDex and CiproDex) were superior to the one containing hydrocortisone (Cipro HC) in their ability to reduce ECM deposition.

Although not measured in our study, a second reason for the observed superiority of the dexamethasone-based OCAAs may be related to its systemic absorption. A recent study of the pharmacokinetics of topical Cipro-Dex otic suspension demonstrated observable blood levels of dexamethasone that peaked within 2 hours after administration of the drug in pediatric and adolescent patients. The maximal concentration of the drug, however, was 8.8-fold lower than that found after a 0.5-mg
oral dose of dexamethasone. The package insert for Cipro HC\textsuperscript{16} reports that the predicted maximal concentration of hydrocortisone is within the range of the endogenous counterpart, and therefore cannot be differentiated from the endogenous cortisol.

Despite evidence that the dexamethasone-containing agents were superior in their ability to reduce the ECM component of granulation tissue, all 3 corticosteroid-containing agents were equally effective at reducing neovascularization. The reason for this is unclear, although we speculate that, compared with ECM deposition, angiogenesis may be more sensitive to the effects of corticosteroids, necessitating smaller concentrations to produce a maximal reduction in blood vessel proliferation.

Although a well-accepted model of wound healing was used in our protocol, there are several limitations to this study. In humans, granulation tissue is often associated with a significant infectious component or foreign-body reaction. In this study, the granulation tissue bed was prepared and maintained to be “sterile.” Therefore, the effect of infection or presence of a foreign body cannot be deduced from the present study.

The study protocol was established to evaluate the effect of clinically available preparations. Although the antibiotic component of OCAAs is not generally thought to have a direct effect on uninfected granulation tissue, we cannot be certain of this fact from the present study, given that we did not evaluate the individual components of the preparations separately.
All 3 OCAAs studied were equally effective at reducing neovascularization, although the dexamethasone-based products were more effective at reducing ECM deposition. The results of this study suggest that ototopical agents containing dexamethasone may be more effective for the treatment of granulation tissue resulting from external and middle ear inflammatory conditions.

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