Steroid Control of Acute Middle Ear Inflammation in a Mouse Model

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Objective: To investigate steroids for their potential for therapeutic approaches to control otitis media. Glucocorticoids and mineralocorticoids have differential effects on inflammation and fluid absorption, but little is known of their control of middle and inner ear manifestations of acute otitis media.

Design: Both glucocorticoid (prednisolone and dexamethasone) and mineralocorticoid (aldosterone and fludrocortisone) steroids were investigated for their ability to reduce inflammatory symptoms in a mouse otitis media model.

Setting: Academic medical center.

Subjects: Acute inflammation was induced by trans-tympanic injection of heat killed Streptococcus pneumoniae to 100 BALB/c mice.

Interventions: Twenty mice in each experimental group (prednisolone, dexamethasone, aldosterone, and fludrocortisone) were given a steroid in their drinking water the day before inoculation, and these treatments were continued until the mice were killed for histologic examination. Twenty control mice were treated with water only.

Main Outcome Measures: Histologic measure of inflammation: middle ear fluid, inflammatory cell number, and tympanic membrane thickness.

Results: Histologic middle ear morphometrics showed significant steroid effects at both 3 and 5 days in reduction of fluid area, cell number, and tympanic membrane thickness.

Conclusions: Glucocorticoids were most effective in controlling inflammation. Interestingly, the mineralocorticoids were also effective in reducing the inflammatory response at 5 days, suggesting that their fluid transport function helped clear disease. Thus, steroid control of middle ear disease may be useful in alleviating symptoms faster and reducing the risk to the inner ear.


Otitis media (OM) is one of the most prevalent inflammatory diseases in the pediatric population. The only effective primary treatment for acute OM (AOM) is antibiotics. However, a consequence of antibiotic therapy is bacterial death and release of bacterial inflammatory products (lipopolysaccharide, peptidoglycan, and DNA), which can exacerbate and prolong inflammation in the middle ear (ME). Alleviation of symptoms can be accomplished by oral anti-inflammatory agents, oral antibiotics, or the infection can resolve on its own up to 70% of the time. Recent efforts to withhold the use of antibiotics for this disease entity have been put forward to decrease the number of antibiotic prescriptions written every year for this common disorder. Decreasing the use of antibiotics should decrease bacterial resistance to antibiotics, an emerging problem in many parts of the world. Therefore, new strategies are needed for alleviating the pain and hearing loss that accompany AOM.

Inflammation results from the innate immune response to bacteria present in the ME. Thus, strategies to decrease fluid and inflammation would be potentially beneficial in the treatment of AOM. The lack of therapeutic options beyond antibiotics or tympanostomy tubes has led to considerable animal research efforts to better understand the mechanisms of AOM and develop new strategies for its prevention or treatment.

Glucocorticoids lessen inflammation by suppressing the immune response, but they also bind to the mineralocorticoid receptor to enhance Na⁺ (sodium) transport and fluid absorption. Mineralocorticoids, such as aldosterone, only act on fluid homeostasis by increasing production of Na⁺, K⁺ (potassium)–adenosine triphosphatase and the epithelial sodium channel. It has been reported in both in vivo and in vitro studies that the ME epithelium is involved in ac-
tive fluid transport via the epithelial sodium channel. This fluid homeostasis function of the ME epithelium is likely to have a major role in removing fluid that accumulates during inflammation such as seen with acute and chronic OM. If the fluid were controlled, hearing would be restored and pain from inflammation would be alleviated.

The possibility that both glucocorticoids and mineralocorticoids could affect ME inflammation in OM was investigated using a previously developed animal model of AOM. The goal of the study was to differentiate which steroid roles, fluid homeostasis (mineralocorticoids) or immune suppression (glucocorticoids), would experimentally provide the control of ME fluid and cellular infiltration in this model of AOM.

METHODS

BALB/c mice (N = 100) were examined with the otomicroscope to establish absence of infection in the external ear or ME. They were then bilaterally inoculated transtympanically with 3.5 µL of heat-killed Streptococcus pneumoniae (10^9 organisms per milliliter) and their MEs examined after 3 days or 5 days of treatment. Control mice (n = 20) received no steroid treatment and 10 were killed at each time point. The remaining mice (n = 80) were given steroid treatments that began the day before inoculation and continued until they were killed. Oral steroid treatments were given in the drinking water per our previous protocol and consisted of either prednisolone (5 mg/kg/d), fludrocortisone (10 µg/kg/d), or aldosterone (15 µg/kg/d). Animals drank the medication-containing water ad libitum. Dexamethasone was given as a subcutaneous injection daily (0.75 mg/kg/d). Ten mice were examined at each time point for each treatment.

The animals were killed at either the 3- or 5-day treatment end point by an overdose of anesthetic (ketamine and xylazine), fixative was intracardially perfused (1.5% glutaraldehyde–3% paraformaldehyde in 0.1M phosphate buffer), and the dissected skulls were immersed in fixative overnight. The middle and inner ears were left intact and connected to each other by the skull base so both ears were processed together for histologic analysis and sectioning. Tissues were microwave decalcified in EDTA, embedded in glycol methacrylate plastic, sectioned in the horizontal plane at 5 µm, serially mounted on glass slides, stained with basic fuchsin and methylene blue, and coverslipped. One ear from a 5-day aldosterone-treated mouse was not available for evaluation, leaving 19 ears in this group for analysis.

Middle ears were assessed at a ×10 magnification of a standard ME section (level of the stapedial artery) for fluid area, number of inflammatory cells, and round window membrane (TM) thickness. These parameters were previously determined to best assess acute inflammation, with significant inflammation first occurring at day 3, increasing at day 5, and resolving by day 7. Therefore, measurements were made at days 3 and 5 because these post-inoculation times would provide the maximum inflammation for the demonstration of any potential steroid effects.

The fluid area was measured with a calibrated micrometer grid in the eyepiece and the cells within the fluid area were counted. Tympanic membrane thickness was measured with a micrometer scale within the opposite eyepiece. Three sections were measured, and the 3 individual measurements for each ME parameter were averaged to derive a mean value for each parameter for each ear. Statistical analyses (analysis of variance [ANOVA]; SPSS Inc, Chicago, Illinois) were then performed on data from the treatment groups and controls to determine if steroid treatments suppressed ME inflammation.

RESULTS

HISTOPATHOLOGIC ANALYSIS

Control (bacteria only) ears often had considerable inflammation of the ME at both days 3 and 5 on histologic analysis. Features of inflammation commonly seen were accumulation of inflammatory exudate, hypertrophy of the ME mucosal epithelium, and thickening of the round window membrane and TM (Figure 1A-C). Extensive ME fluid and inflammatory cells were present as well. The inflammatory cells were primarily neutrophils (acute phase inflammatory cells). Often, fluid filled the entire ME space. By contrast, steroid-treated ears had less inflammation (Figure 1D). These MEs were often clear of fluid and inflammatory cells, and the mucosal epithelium was seldom hypertrophied. Many ears appeared normal.

An assessment was made of the number of ears with any degree of inflammation, such as fluid or inflammatory cells. Occasionally, an ear will not manifest an inflammatory response in spite of bacterial inoculation. This could be due to the inoculate draining before an innate immune response is initiated, a particular animal being more resistant to bacterial stimulation, or slower inflammation development. Therefore, the total number of ears showing inflammation at the 2 ages was evaluated to determine if the steroid treatments altered the normal pathologic progression. Of the 40 inoculated ears in the control group, 4 showed MEs completely free of inflammation (Table). In comparison, glucocorticoid-treated ears showed a significantly higher incidence of inflammation-free ears. The prednisolone-treated mice had 10 MEs without symptoms, while 11 dexamethasone-treated ears were clear. Results from χ^2 analyses showed that both of these were statistically significantly better than controls (Table). However, mineralocorticoid-treated ears were no different than the bacterially controls (P > .05), suggesting that these steroids did not reduce the overall incidence of inflammation.

INFLAMMATION MEASURES

To determine if there were quantitative differences in the extent of inflammation, ANOVA was conducted on the measures of fluid area, number of inflammatory cells, and TM thickness. Control mice without steroid treatment showed the normal progression of inflammation measures that paralleled the qualitative changes described herein. The mean fluid area was 1902 µm^2 at 3 days, increasing to 3158 µm^2 by day 5, while the number of inflammatory cells within the fluid increased proportionately from 106.5 to 167.9 (Figure 2). The steroid treatments served to reduce this inflammation. The ANOVA results showed significant overall group differences with regard to steroid effects at both 3 and 5 days (Figure 2). This significant steroid effect was an overall reduction in all histologic measures at both 3 and 5 days, with the exception of fluid area at day 3 (Figure 2).

All animal procedures were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University, Portland. Institutional guidelines regarding animal experimentation were followed.
Post hoc comparisons showed that the steroids had an impact as early as day 3 on the number of cells and TM thickness (Figure 2). Prednisolone and dexamethasone both appeared to reduce cell number, but the post hoc test results showed statistical significance only between the prednisolone and fludrocortisone results. The thickness of the TM is measured away from the injection site to reflect changes due to the bacterial stimulation and not due to needle trauma. Both glucocorticoid-treated groups were significantly different from the controls for TM thickness at day 3 (Figure 2). The reduction in TM thickness measured in the mineralocorticoid-treated mice was not different from controls, despite a lower mean value.

By day 5, all of the steroid treatments had some impact when compared with controls (Figure 2). Overall treatment effects were seen with regard to fluid area, with mean values being reduced in all steroid-treated groups.

Table. $\chi^2$ Analysis of Inflammation-Free Ears at 3 and 5 Days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Ears</th>
<th>$\chi^2$ Value $^a$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Clear 4, Inflamed 36</td>
<td>10.00</td>
<td>.002</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Clear 10, Inflamed 30</td>
<td>13.61</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Clear 11, Inflamed 29</td>
<td>0.028</td>
<td>.87</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>Clear 4, Inflamed 35</td>
<td>0.278</td>
<td>.60</td>
</tr>
<tr>
<td>Fludrocortisone</td>
<td>Clear 5, Inflamed 35</td>
<td>0.028</td>
<td>.87</td>
</tr>
</tbody>
</table>

$^a$The $\chi^2$ values were derived from the steroid treatment groups compared with controls.
Findings from the post hoc tests showed that all steroid results were not different from each other; however, for the dexamethasone-treated mice, the mean fluid area was statistically significantly less compared with the control mice. The number of inflammatory cells was significantly reduced in all steroid groups compared with controls, generally showing only one-third of the cells seen in control mice. The TM healing that occurs between days 3 and 5 is reflected by the reduction in TM thickness during this period. There was an overall group effect for the steroids, and they did not differ from each other at day 5. However, the dexamethasone-treated mice were statistically significantly different from the controls.

Thus, the results of the steroid treatments were generally similar in reducing ME inflammation during the observation period. Post hoc comparisons generally showed that 1 or both of the glucocorticoids were better at reducing inflammation than the mineralocorticoids. However, all steroids were effective in reducing the number of inflammatory cells by day 5, suggesting that some aspects of inflammation were sensitive to both steroid groups.

While systemic steroids have been used extensively for ear diseases such as sudden sensorineural hearing loss and in earlier trials for the treatment of OM with effusion,12-13 to our knowledge, animal research on the use of various steroids for OM has not been conducted. Specifically, an investigation of the use of steroids to clear the ME fluid that accompanies OM has not been explored. Middle ear fluid present during OM causes conductive hearing loss by virtue of decreasing the transmission of sound through the ME. Therefore, earlier resolution of this fluid would alleviate troubling sequelae of OM such as pain and decreased hearing. Currently, no good immediate therapy exists to clear fluid with the exception of myringotomy or tympanostomy tube placement. While clearance of ME fluid has long been ascribed to the function of the eustachian tube, there is now evidence that the ME epithelium plays a major role in fluid absorption, while the role of the eustachian tube is more to allow gas pressure equilibration.5 Other laboratories have reported that fluid regulation in the ME is via the Na+ channel–dependant process in the ME mucosal epithelium. The use of mineralocorticoids offers some appeal, since the use of such an agent would avoid the systemic morbid sequelae of glucocorticoids.

The glucocorticoids, prednisolone and dexamethasone, were the most effective in our study in reducing ME inflammation in response to bacterial challenge to the ME at both 3 and 5 days. Presumably, this is due to the strong immunosuppressive and anti-inflammatory action of these steroids. Interestingly, prednisolone also has a modest mineralocorticoid effect, one-fifth that of the glucocorticoid effect. One could speculate that the mineralocorticoid action of prednisolone could also be acting in a positive way to decrease ME fluid. Aldosterone is basically a pure mineralocorticoid (10 000:1 mineralocorticoid vs glucocorticoid action), and fludrocortisone has a much stronger mineralocorticoid than glucocorticoid effect (12.5:1 mineralocorticoid vs glucocorticoid action).14 Interestingly, mineralocorticoids were also found to reduce ME inflammatory response to bacterial injections, but only at the 5-day end point. Thus, while the glucocorticoid agents were effective in decreasing fluid area and cell count in the ME, the mineralocorticoids were also moderately effective at...
the low therapeutic doses used in this study. This lends support to the concept that sodium transport of ME fluid may be an important function in clearing fluid and thus in controlling inflammation in the ME. Also, the mineralocorticoids may have been more effective at the 5-day end point because their actions (clearance of fluid) would be expected to take longer than the stronger pure immune suppressive glucocorticoids. While we did not see a significant effect on fluid area at the 3-day end point for the mineralocorticoids compared with the glucocorticoids, nor an overall effect, both steroid groups were equally effective at day 5. At the 5-day end point, dexamethasone was the only treatment significantly better at decreasing fluid area compared with the other steroids, but the overall effect was essentially equal in the remaining steroid groups. This would indicate that fluid area was affected by both types of steroids and affected equally well by the mineralocorticoids.

The immune-suppressive actions of glucocorticoids were most effective in reducing the severity of the ME innate immune response. Nevertheless, mineralocorticoids were also moderately effective in reducing the inflammatory response at 5 days. These studies offer insight into the potential steroid control of middle and inner ear disease during AOM. Thus, steroid control of ME disease may be useful in alleviating symptoms faster and reducing risk to the inner ear. Further investigations are ongoing into the relative efficacy of steroids and antibiotics for the suppression of inflammatory response to OM in the mouse model.

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Author Contributions: Dr MacArthur had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MacArthur and Trune. Acquisition of data: MacArthur and Kempton. Analysis and interpretation of data: MacArthur, DeGagne, Kempton, and Trune. Drafting of the manuscript: MacArthur and Trune. Critical revision of the manuscript for important intellectual content: MacArthur, DeGagne, Kempton, and Trune.


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REFERENCES