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Low dose combination steroids control autoimmune mouse hearing loss

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ABSTRACT

The severe side effects of glucocorticoids prevent long term management of hearing loss. Alternative steroid treatments that minimize or eliminate these effects would significantly benefit therapeutic control of hearing disorders. A steroid treatment study of autoimmune mouse hearing loss was conducted to determine the efficacy of combining aldosterone and prednisolone at low doses. An assessment also was made of low dose fludrocortisone, a synthetic mineralocorticoid that also has a slight glucocorticoid effect. MRL/MpJ-*Fas*^{lpr} mice were tested for baseline ABR thresholds at 3 months of age and then treated with aldosterone (3.0 μg/kg) or prednisolone (1.0 mg/kg) to determine the lowest effective dose of each. Other mice were given the two steroids in combination at doses of Pred 0.5 mg + Aldo 1.5 μg; Pred 1.0 mg + Aldo 3.0 μg; or Pred 1.5 mg + Aldo 5.0 μg. Mice were retested with ABR at 1 and 2 months to determine the efficacy of the different steroid treatments in controlling hearing loss. Another series of mice were given the synthetic mineralocorticoid fludrocortisone at low (2.8 μg/kg) or high (10 μg/kg) doses and retested at monthly intervals for 3 months. Autoimmune mouse hearing loss developed in untreated controls. This threshold elevation was not prevented by prednisolone at 1 mg/kg or by aldosterone at 3 μg/kg when each was given alone. However, the two steroids combined at these doses effectively controlled hearing loss. The fludrocortisone treatments also were effective at low doses in preventing or reversing the autoimmune mouse hearing loss. This efficacy of combined steroids at low doses suggests the potential for reducing the side effects of glucocorticoids in the therapeutic control of hearing disorders.

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1. Introduction

Because hearing loss is often suspected to result from immune-mediated or inflammatory processes (Dornhoffer et al., 1997), glucocorticoids (prednisone, prednisolone, and dexamethasone) are frequently used to treat such hearing disorders as sudden or rapidly progressive hearing loss (Alexiou et al., 2001; Rauch, 2004; Aoki et al., 2006), Meniere's disease (Barrs, 2004), and autoimmune inner ear disease (AIED) (Loveman et al., 2004; Niparko et al., 2005). However, in spite of the glucocorticoids' effectiveness, their severe side effects prevent long term management of such inner ear dysfunction (Nadel, 1996; Sismanis et al., 1997; Trune et al., 2007). Alternative steroid treatments that minimize or eliminate these side effects would have significant benefit in the therapeutic control of hearing disorders.

This laboratory has been studying the steroid control of hearing loss in the MRL/MpJ-*Fas*^{lpr} autoimmune mouse model to better understand these steroid-responsive mechanisms in the ear. Autoimmune mouse hearing loss progresses with systemic disease development, pathology is limited to the stria vascularis, and treatment with the mineralocorticoid aldosterone is as effective as prednisolone in reversing or preventing hearing loss (Trune and Kempton, 2001). The mineralocorticoid receptor-mediated function (ion homeostasis) of glucocorticoids was determined to be as important for hearing control as their glucocorticoid receptor-mediated functions (anti-inflammation and immunosuppression) (Trune et al., 2006; Trune and Kempton, 2009). The autoimmune mouse loses strial endothelial cell tight junction integrity due to immune complexes (Trune, 1997), which causes a drop in the endocochlear potential and hearing loss (Lin and Trune, 1997; Ruckenstein et al., 1999). Thus, restoration of strial ion homeostatic processes by mineralocorticoid receptor-mediated processes appears critical for hearing recovery, in spite of the initiating cause being systemic autoimmune disease. This parallels current hypotheses that some human AIED is due to circulating inflammatory factors (inflammatory cells, autoantibodies) that disrupt cochlear vasculature (Harris and Ryan, 1995; Barna and Hughes, 1997; Garcia-Berrocal and Ramirez-Camacho, 2002; Mathews and Kumar, 2003; Yehudai et al., 2006).

Given this relevance of the mineralocorticoid function of glucocorticoids for recovery of AIED, it is possible that both mineralocorticoid and glucocorticoid steroids could be employed in combination. If their effects are complementary or additive, combining them at lower doses may still provide therapeutic control of hearing loss while reducing glucocorticoid side effects. Therefore, the present study was conducted to determine if the mineralocorticoid aldosterone and the glucocorticoid prednisolone could be combined at low doses to effectively treat hearing loss in the autoimmune mouse model. If reduced levels of each are needed when combined, it would provide important preliminary

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information regarding optional treatments to better control the detrimental side effects during long term glucocorticoid therapy.

Fludrocortisone acetate (Florinef® acetate) is a synthetic mineralocorticoid given for chronic adrenocortical insufficiency (Addison disease) when low maintenance levels are needed over long periods of time. This drug also was tested to determine if it could suppress hearing loss in this mouse model. If effective, it would offer an alternative therapy in which the mineralocorticoid receptor-mediated functions can be obtained with minimal glucocorticoid side effects.

2. Materials and methods

The drugs employed in the present study were the synthetic glucocorticoid prednisolone, the natural mineralocorticoid aldosterone, and the synthetic mineralocorticoid fludrocortisone. Their immune suppressive and sodium transport potencies relative to the natural glucocorticoid cortisol are shown in Table 1 (Schimmer and Parker, 2006). Prednisolone has a predominantly glucocorticoid effect, but has a strong binding affinity for the mineralocorticoid receptor, thus able to mediate both receptor-mediated functions. Aldosterone is a mineralocorticoid with virtually no binding affinity for the glucocorticoid receptor for immune suppression. Fludrocortisone is a synthetic mineralocorticoid with a relatively less glucocorticoid effect, somewhat the opposite of prednisolone. All animal studies were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee.

2.1. Prednisolone–aldosterone combinations

Previous studies of MRL/MpJ-*Fas*^{lpr} autoimmune mice have shown that hearing loss was controlled with a prednisolone dose of 5 mg/kg or an aldosterone dose of 5 μg/kg (Trune and Kempton, 2001). These doses are at the approximate therapeutic levels given to patients (Table 1). However, the minimum dose of each that is capable of controlling the mouse hearing loss has not been determined. Furthermore, the efficacy of the two steroids in combination has never been explored. Therefore, a dose response study was employed to assess whether the two steroids in combination would be more effective than their equivalent doses alone.

2.2. Minimum effective dose

MRL/MpJ-*Fas*^{lpr} autoimmune mice normally develop systemic autoimmune disease and hearing loss at 3–4 months of age and approximately 25–30% die of disease by 5 months of age. Therefore, 3-month old mice were tested for baseline ABR thresholds at 4, 8, 16, and 32 kHz (Mitchell et al., 1999). Mice were then treated with steroids in their drinking water for 2 months according to our previous protocols (Trune and Kempton, 2001; Trune et al., 2006). They were given either aldosterone (3 μg/kg, N = 8) or prednisolone (1.0 mg/kg; N = 7) to determine if these doses were effective individually. Untreated mice (water only, N = 42) served as controls. Mice were retested after 1 and 2 months of treatment to determine any change in ABR thresholds.

2.3. Combination doses

Following the individual steroid treatments, another series of mice was given the two steroids in various combination doses. Following baseline ABR testing, mice were given:

Prednisolone 0.5 mg/kg + Aldosterone 1.5 μg/kg (N = 24)
Prednisolone 1.0 mg/kg + Aldosterone 3.0 μg/kg (N = 22)
Prednisolone 1.5 mg/kg + Aldosterone 5.0 μg/kg (N = 22)

The mice were retested after 1 and 2 months to determine the efficacy of each steroid combination treatment in preventing progression of hearing loss. An analysis of variance (ANOVA) was conducted of the threshold shift across all 4 test frequencies for each treatment group. Posthoc tests (Bonferroni) were conducted if the treatment effects were significant ($P<0.05$) at a particular frequency.

2.4. Fludrocortisone acetate

Fludrocortisone acetate is a synthetic mineralocorticoid given for chronic adrenocortical insufficiency (Addison disease) when low maintenance levels are needed over long periods of time. It has a significant mineralocorticoid effect, but a smaller glucocorticoid effect (Table 1), making it functionally a combination drug with low dose glucocorticoid impact. The prescribed dose for humans is 0.1–0.2 mg per day, which equates to a dosage range of 1.5–3.0 μg/kg/day for a 70 kg person. Therefore, mice were treated for 3 months with either 3.0 μg/kg (N = 17) or 10.0 μg/kg (N = 42) to test the drug effectiveness at controlling progression of the hearing loss. Untreated mice (water, N = 46) served as controls. Hearing thresholds were tested at the end of each month to compare to baseline measures. Thresholds shifts among the groups were compared with the ANOVA.

3. Results

3.1. Untreated controls

Without any steroid treatment (water controls), hearing thresholds continue to rise as systemic disease progresses. One month after treatment began, untreated mice showed slight elevations at 8 and 32 kHz (Fig. 1). Thresholds at these frequencies continued to rise and at 2 months paired *t*-test of baseline and 2 month thresholds showed a significant elevation of thresholds at 4 and 32 kHz ($P<0.05$). This pattern of predominantly high frequency threshold shift is typical for the autoimmune mice.

3.2. Prednisolone and aldosterone minimum effective doses

The objective of the lower doses selected in the present study was to find the dose of each steroid that was ineffective at controlling hearing loss. Neither aldosterone at 3 g/kg nor prednisolone at 1.0 mg/kg preõvented the progression of hearing loss (Fig. 1). Mice treated with each steroid at these doses showed continued elevation of thresholds at 1 and 2 months. By 2 months these thresholds were significantly elevated from baseline at all four frequencies for both steroids (paired *t*-test; $p<0.05$),

Table 1
Potency equivalence of corticosteroids relative to natural glucocorticoid cortisol.

Corticosteroid	Daily adrenal production[#]	Anti-inflammation potency	Sodium retention potency	Therapeutic dose (/day)	Effective dose range (70 kg Person)
Cortisol	10 mg	1	1		
Prednisolone		4	0.8	5–80 mg	0.1–1.2 mg/kg
Aldosterone	125 μg	0	3000	150–300 μg	2.1–4.3 μg/kg
Fludrocortisone		10	125	50–200 μg	0.7–3.0 μg/kg

[#] Adrenals produce 0.15 mg/kg/day of cortisol and 1.8 μg/kg/day of aldosterone.

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