The Dual ET_AR/AT_1R Blocker Sparsentan Prevents Noise-Induced Hearing Loss in Alport Mice

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Introduction

Most emergent therapies for Alport syndrome (AS) address the renal disease but not the inner ear pathology frequently associated with the disease. Endothelin1-mediated activation of the endothelin type A receptor (ETAR) on strial marginal cells results in strial pathology in the coichlea, and activation on glomerular mesangial cellsa congributes to glomerulosclerosis.1,2 Currently, angiotensin converting enzyme inhibitors (ACEi) or angiotensin II type 1 receptor (AT1R) blockers are the standard of care for patients with with AS; however, these drugs have not been shown to improve hearing. Previously we thave reported that sparsentan (SP), a dual ETAR/AT_1R antagonist, is nephroprotective in an AS mouse model, and extends life-span.[3,4] Therefore this report shows that SP, but not Losartan (LS) prevents noise-induced hearing loss in the AS mouse model, and details the changes in underlying pathology that may contribute to this difference

Objective

To assess the effect of dual ET_AR/AT_1R inhibition with sparsentan and monoblockade of AT_1R with losartan on noise-induced hearing loss and cochlear pathology in the wild-type (WT) or COL4A3-/- autosomal Alport mice .

Methods

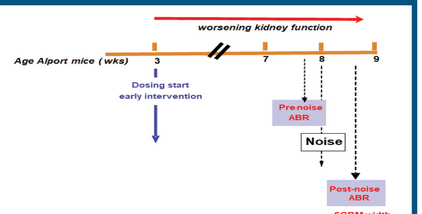


Figure 1. Study design in Alport mice

Study design and Sample Collection

Alport (KO) and WT 192Sv littermates were treated with vehicle (V) or SP (120 mg/kg) by oral gavage from 3-8.75 weeks (wks) of age or with LS (10 mg/kg) by daily oral gavage from 3-4 wks of age and in drinking water from 4-8.75 wks of age. As illuatrated in Fig 1

- Hearing was assessed in WT and KO mice treated with V, SP, or LS between 7.5 -8 wks (n=5-7/grp) using the auditory brainstem response (ABR), the mice were then exposed to a 10-hour noise stress(106 dB SPL, 10kHz OBN) and 5 days post-noise underwent a second ABR analysis, at 8.5 wks of age.
- Cochlea were excised after the final ABR test and the stria vascularis examined using transmission electron microscopy (TEM).
- WT or Alport mice (n=10-13/grp) were treated with V or SP underwent measurement of the endocochlear potential (EP) at 8.75 weeks of age
- Cochlea were obtainedat 7 wks of age from additional WT or Alport mic treated with . V,SP or LS for imunofluorescence microscopy.

Data Collection

- Hearing ability was assessed via the ABR technique using frequency-specific tone pips
- The EP was measured in the basal turn of the right cochlea using a 150 KCl-filled glass pipette coupled to a DC amplifier/electrometer. The stable (1 min) DC voltage was referenced to a Ag/AgCl gground electrode in the flank tissue.
- The thickness of strial capillary basement membranes (SCBM) was measured in TEM digital images (JEOL 1200 EX II, Hammatsu ORCA CCD) taken at 40,000x.
- Accumulation of the extracellular matrix protein, laminin α_2 in the SCBM was determined in frozen mid-modiolar cochlear sections incubated with an anti-laminin α_2 antibody and visualized using a Leica confocal-imaging system.

Data analysis

- Hearing loss was calculated by subtracting the ABR hearing threshold for pre-noise from that of post-noise. Comparison of active dose to the Alport vehicle used t-tests.
- Comparison of the SCBM thickness measures as well as the EP values among groups used one way ANOVA and theTukeys multiple-comparison post-hoc test.
- For all statistical analyses, significance was set at p<0.05.

Results

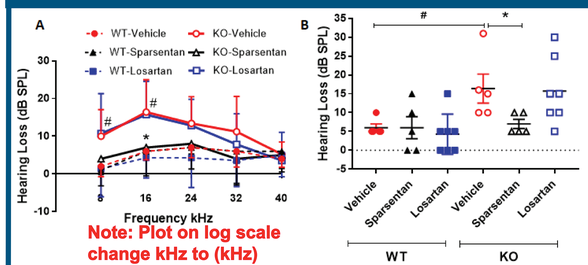


Figure 5 A. Sparsentan (open black triange) but not losartan (open blue square) prevents noise-induced hearing loss in Alport mice. Data are presented as mean ± SD (n=5). #P<0.05 KO-Vehicle vs WT-Vehicle. *P<0.05 KO-Vehicle vs KO-SP or KO-LS.
Figure 5 B. The individual ABR thresholds are depicted for each group at 16 kHz. Note the variability in the KO-Losartan is similar to the threshold spread common in the untreated Alport mice.

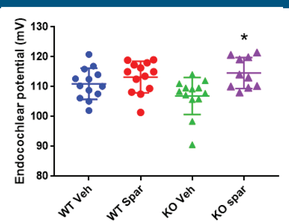
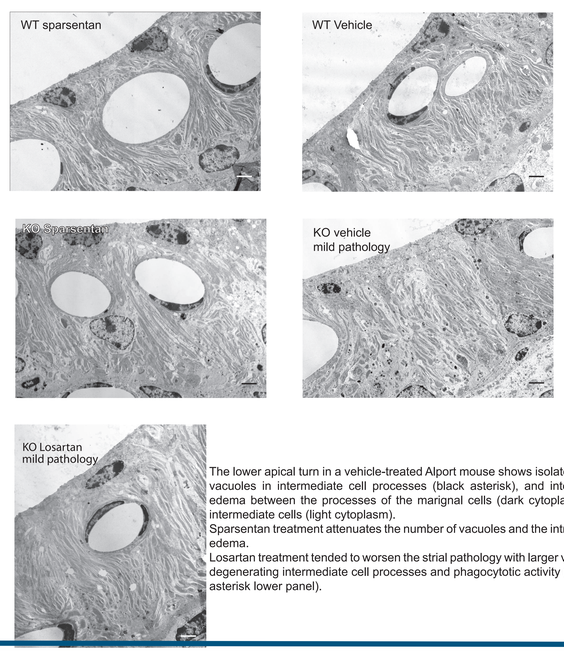
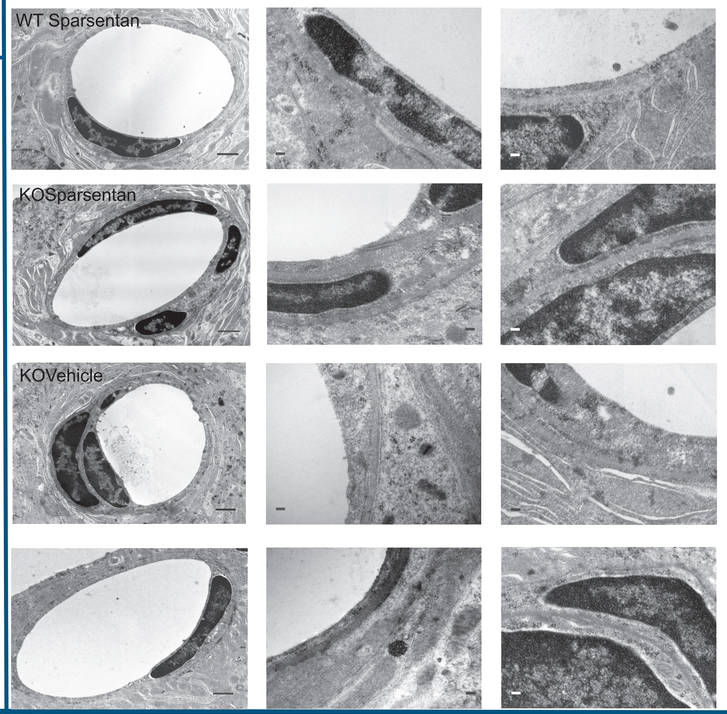


Fig 6. Sparsentan significantly increases the endocochlear potential in Alport mice



The lower apical turn in a vehicle-treated Alport mouse shows isolated lucent vacuoles in intermediate cell processes (black asterisk), and intercellular edema between the processes of the marignal cells (dark cytoplasm) and intermediate cells (light cytoplasm).
Sparsentan treatment attenuates the number of vacuoles and the intracellular edema.
Losartan treatment tended to worsen the strial pathology with larger vacuoles, degenerating intermediate cell processes and phagocytotic activity (white asterisk lower panel).



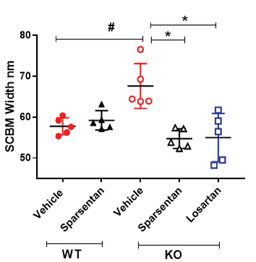


Figure 2. Sparsentan and losartan attenutate SCBM thickening in Alport mice

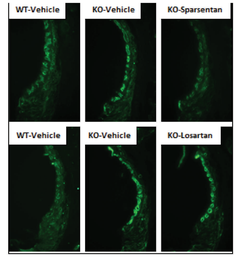


Figure 4. Sparsentan but not losartan prevents laminan2 accumulation in stria of Alport mice

Conclusions

- Sparsentan attenuated inner ear pathology and noise-induced hearing loss, indicating that sparsentan is capable of delaying the structural and functional auditory changes in Alport mice.
  - Sparsentan but not losartan protects Alport mice from noise-induced hearing loss.
    Sparsentan and losartan both prevent SCBM thickening, but this is not translated into a functional hearing improvement for losartan.
  - Sparsentan but not losartan tends to ameliorate the increase in ECM protein laminin 2 and improve stria pathology.
  - Sparsentan increases endocochlear potential in Alport mice.

Results of the current studies in Alport mice with sparsentan in conjunction with previous results on renal pathology present a novel therapeutic approach to reducing both hearing and renal injury in patients with Alport syndrome, if translated to the clinic, may

References

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Disclosures

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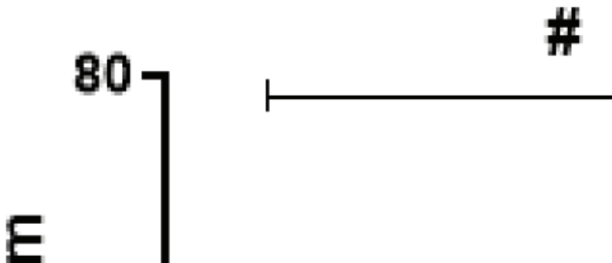
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