Retina Australia Grant Report

Title: Neuroprotective effect of SAHA in Retinitis Pigmentosa. Do time and frequency matter?

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Retinitis pigmentosa (RP) is a family of inheritable retinopathies characterized by a progressive loss of rod photoreceptors followed by loss of the cone photoreceptors. As there are currently no treatment options for RP, the goal of this proposal was to investigate the neuroprotective effect of an FDA-approved histone deacetylase (HDAC) inhibitor, SAHA, in preventing photoreceptor degeneration in different mouse models of RP. Neuroprotective pharmacological therapies provide a gene-independent therapy that may preserve photoreceptors and extend the window of sufficient useful vision. Epigenetic neuroprotection is a novel growing area of pharmacological therapies that has shown promise in recent years, especially with HDAC inhibition. This study set out to evaluate the neuroprotective efficacy of pan-HDAC inhibitor SAHA in vivo, adding to the literature of HDAC inhibitors therapeutic effects in RP models, and it was hypothesised that SAHA would provide temporary neuroprotection to photoreceptors. We hypothesized that intravitreal (IVT) delivery of SAHA will increase photoreceptor survival and outer nuclear layer preservation in two unique mouse models of RP. Our treatment approaches investigated the efficacy and safety of this treatment strategy which could benefit overall photoreceptor or cone-specific survival.

Aim 1: Evaluate photoreceptor neuroprotection after a single delivery of SAHA in two unique RP mouse models:

This aim tested the effect of a single IVT dose of SAHA on photoreceptor survival in two RP mouse models, rd1 and P23H when. Since RP mouse models can have a rapidly degenerating retina, optomotor testing was ideal for the assessment of any quantitative difference between treatment and control groups due to its high sensitivity in both scotopic and photopic conditions. Optomotor testing allows for the assessment of mouse visual function by measuring the optomotor response in mice, a compensatory head reflex, to a rotating, black-and-white striped stimulus. These stimuli can be presented at different rotating frequencies, luminosities and in a sequential or step-wise manner to see if mice can react through unrestrained head and body movements.

Two different doses of SAHA were injected IVT in rd1 mice groups, 1µM and 10µM, followed by optomoter assessment at 4 days (PN16, n=4-10 animals) and 12 days (PN24, n=4-10 animals) post-treatment. At PN16, scotopic optomoter testing showed no significant difference of either doses of SAHA-treatment when compared to either un-injected or sham-injected rd1 mice. Scotopic vision was completely absent in un-injected and sham-injected rd1 mice, consistent with extent of primary rod degeneration at PN16 in the rd1 mouse model. SAHA's inability to rescue rod photoreceptors is most likely due to sufficient degeneration already occurring by this stage, illustrated by the difference between WT and rd1 mice, indicating that a one off treatment with SAHA was insufficient to significantly delay or halt cell death due to disease.

The photopic acuity paradigm displayed a wider range of results with daylight vision in rd1 mouse lines being significantly reduced compared to WT mice, but still present and measurable at PN16 in some animals. Due to the fast degeneration seen in the rd1 model, some animals already have no measurable photopic response (non-responders) even at P16. No significant statistical difference was observed between un-injected, sham-injected or both doses of SAHA-treated groups. However, our data showed a non-significant trend increase in both doses in the SAHA-injected group, especially when non-responders were excluded from the analysis. This result indicates that SAHA could be beneficial to cones if delivered early enough, but more treated animals at an earlier age are needed to confirm this effect. Furthermore, electroretinogram (ERG) testing of SAHA-treated rd1 mice at both doses at PN16 showed a similar lack of response as the un-injected or sham-treated rd1 mice, suggesting that the ERG test is not a sensitive enough test to pick up small therapeutic effects as the optomoter.

By PN24, rod photoreceptors are almost completely absent from the rd1 retina, and this is reflected by an absence of scotopic contrast sensitivity and photopic acuity responses in all rd1 groups regardless of SAHA dosage. WT mice still retained highly sensitive and stable scotopic vision, as well as improved photopic acuity vision from PN16. All photopic acuity vision had already been lost in rd1 mice groups by PN24, and SAHA

administration was unable to rescue photopic vision. This is most likely a result of significant secondary cone degeneration once most rods have degenerated, resulting in heavily reduced photopic acuity vision between PN16 and PN24, and not enough viable cones to receive and benefit from SAHA treatment.

As part of this aim, we also optimised time points for SAHA neuroprotection on a newly introduced mouse line to our laboratory, the RhoP23H/+ model of autosomal dominant RP. This heterozygote mouse model has slower onset and progression of retinal degeneration compared to the rd1 model. Assessment of scotopic contrast sensitivity displayed no significant differences between WT, un-injected and 1µM SAHA-injected RhoP23H/+ mice at 4 days post-injection (PN16) and 23 days post-injection (PN35). SAHA administration did not cause any improvement in scotopic vision at either time points, most likely as sufficient rod degeneration has not occurred at this age. Time constraints in this study prevented testing of later timepoints when degeneration is more evident and SAHA might show a protective effect. We have however, completed a time course of disease from PN12 to PN180 in the RhoP23H/+ mouse which includes ERG, optomoter, cone counts and outer nuclear layer thickness. We have now defined PN60 as the ideal timepoint to evaluate treatment efficacy as both scotopic and photopic visual assessments are reduced, but not absent, at this time. Morphological analysis showed that at PN60 there are sufficient rod and cone photoreceptors left suggesting a slower degeneration profile compared to the rd1 mouse.

Aim 2: Investigate the effectiveness and safety achieved by IVT administrations of SAHA:

To reinforce the results found in optomotor testing, histological analysis was conducted to measure the outer nuclear layer (ONL) thickness comparing several retinal regions from each of the treatment groups, and to assess the immunoreactivity of the retina to IVT injections of SAHA. Remarkably, photoreceptor rescue was observed in central retinal regions with a slightly thicker ONL in both SAHA-treated groups compared to un-injected rd1 mice. However, only in the CI retina, the 1uM SAHA-treated rd1 ONL was significantly thicker than both un-injected and sham-injected groups. These results suggest the potential photoreceptor rescue via the neuroprotective effects of SAHA in the central retina.

Further assessment of the safety profile and therapeutic potential of SAHA was undertaken through GFAP staining, allowing activated Müller glia to be visualised. Müller glia are an important retinal component providing metabolic support to photoreceptors and retinal structure. Müller glia activation can be measured qualitatively through comparing the upregulation of GFAP. At PN16, rd1 groups had a visibly higher level of Müller glia activation compared to WT, with un-injected, sham-injected and 10µM SAHA-injected groups all showed similar levels of GFAP upregulation. Interestingly, 1µM SAHA-injected had lesser activation of Müller glia compared to other rd1 controls. Thus, lower levels of GFAP expression may be due to the lesser degeneration in the ONL from SAHA neuroprotection, reinforcing its ONL quantification results. By PN24, rod generation is almost complete and the peak of secondary cone loss has been reached resulting in prevalent retinal degeneration. Retinal histology showed most rd1 retinal sections had only one row of photoreceptors in the ONL, explaining the high level of GFAP upregulation from substantial retinal degeneration and subsequent loss of ONL structure. Although there may still be signs of neuroprotection, it is hypothesized that rd1 retinal degeneration has mostly overcome SAHA neuroprotective effect at this stage.

Aim 3: Understand the gene expression pathways modulated by SAHA treatment by whole-genome transcriptomic analysis:

Due to the time constraints of the study, gene expression studies (e.g. next generation sequencing) were unable to be completed. We have however, been able to collect and extract RNA from rd1 and RhoP23H/+ SAHA-treated retinas and they have been submitted to WA Genomics for gene expression experiments. We are currently waiting for the results and analysis. Future studies should prioritise this focus to ensure that neuroprotective changes to the photoreceptor layer are due to the molecular and the pharmacological effect of SAHA. Furthermore, we are also continuing with this project this year conducting more testing of SAHA on the RhoP23H/+ mice at later timepoints. Alternative delivery options, such as nanoparticles, are also being explored to improve SAHA delivery and sustained effect in the retina.