Background: Retinitis pigmentosa (RP) is a group of retinal degenerative diseases that in many cases are caused by mutation in a rod specific protein, but the cones also degenerate. Several mechanisms have been suggested to explain the spread of degeneration from rods to cones including (i) toxic chemicals secreted by the dying rods and (ii) lack of cone-survival factor that is synthesized by rods.

Research Hypothesis: Rod-cone gap junctions allow the flow of small chemicals between the two cells, and thus serve to mediate the spread of apoptosis from dying rods to healthy cones.

Aims: (1) Induce local photoreceptor degeneration in a normal rat retina. (2) Study the spread of degeneration from dying photoreceptors to healthy ones.

Methods: Two types of adeno-associated viral vector (AAVs) were constructed; one containing only GFP as reporter gene and the other containing also P23H opsin gene. (Albino rats were injected subretinally in one eye with one of the above viral vectors. The electroretinogram (ERG) was recorded monthly to assess deterioration of retinal function. Rats were sacrificed at different time intervals after the injection (one to nine months). TUNEL was used to identify cells undergoing apoptosis. Immunocytochemistry for Glial Fibrillary Acidic Protein (GFAP) expression in retinal Muller cells was used as a cell marker for retinal stress.

Results: The ERG responses in pAAV-P23H-IRES-hrGFP injected eyes deteriorated gradually during 9 months of follow-up, while no significant reduction was seen in the ERG responses of eyes injected subretinally with the pAAV-IREs-hrGFP (control). TUNEL assay showed positive staining from three months post injection at the pAAV-P23H-IRES-hrGFP injected eyes that appeared in regions further away from the injected region. There was no positive staining in the control infected eyes. The retina from the pAAV-P23H-IRES-hrGFP infected eyes showed extended staining of GFAP far beyond the infected area, comparing to control infected eyes.

Discussion: The ERG reflects the summation of light-induced electrical activity of the entire retina. Therefore, a small lesion in the retina does not affect it. ERG reduction in the rats injected subretinally with the viral vector containing the P23H opsin gene, started at 3 months after injection and continued to decrease gradually with time. This functional evidence of spread of degeneration was supported by photoreceptor apoptosis in regions, which were not transfected initially and could not be explained by diffusion of the viral vector since no GFP fluorescence could be detected in these regions.

Conclusions: We succeeded to create a rat model for studying the spread of degeneration from affected photoreceptors to non-affected ones. This model can serve as a valuable tool to study in vivo the pathogenesis of retinal diseases, and to test experimental therapies that are designed to block spread of degeneration from dying cells to healthy ones.

Key words: Retinitis Pigmentosa, rat, gap junctions, rods, cones, apoptosis, electroretinogram, GFAP
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Publications associated with the project: None yet