INHERITED RETINAL DEGENERATION AND ITS TREATMENT

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NORPA-PLCβ4 GENE EXPRESSION IN THE RAT BRAIN AND RETINA

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Purpose : The NorpA gene leading to a retinal degeneration when mutated is predominantly expressed in the retina of Drosophila melanogaster and the expression of the mammalian homologous gene has recently been observed in bovine cones.

Methods: Taking advantage of the known rat PLCβ4 nucleotidic sequence we have designated a complementary antisense oligonucleotidic probe ^{35}S radioactively labelled. We have determined by in situ hybridization the PLCβ4 gene expression pattern on rat brain and retinal tissue sections.

Results: We found that the gene is strongly expressed in Purkinje cells of the cerebellum, thalamic nuclei especially the median geniculate bodies, habenula and substantia nigra pars compacta. The gene is also expressed in the superior colliculi, mammillary nuclei, layers II, III, V and VI of the neocortex and mitral cells of the olfactive bulbes. No signal is observed in the hippocampus or the striatum. On retinal sections we could not detect a signal in our first set of experiments. However we expect a signal in a more extensive study that we have recently performed.

Conclusions: This study demonstrates for the first time the PLCβ4 gene expression pattern in the rat brain .

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BCL-2 AND P53 COULD BE INVOLVED IN RETINAL DEGENERATION IN RD MICE

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Purpose To study the expression of bcl-2 and p53 genes in the retinas of rd mice and to analyse the role of these proteins in the pathophysiology of programmed cell death.
Methods Eyes of rd/rd mice ages 1 to 6 weeks were analysed by TUNEL (TdT-mediated dUTP-biotin Nick End Labeling) method. Immunohistochemistry was performed using anti-bcl-2 and anti-p53 monoclonal antibodies at different ages.
Results Histological and immunohistochemical showed programmed cell death in the retinas of rd/rd mice. No staining was observed with monoclonal antibodies suggesting another pathway of PCD in this animal model of hereditary retinal degeneration.
Conclusion PCD seems to be the final pathway of retinal degeneration in rd mice. Other factors might regulate epistatically neuroretinal loss in thes animals.

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Fatty Acid Composition in Red Blood Cells Membrane Phospholipids in Retinitis Pigmentosa.

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In this study we report the level of more significant fatty acid methyl esters in red blood cells membrane phospholipids in various genetic form of Retinitis Pigmentosa and relative controls. Lipids from erythrocytes membrane were estracted with chloroform - methanol (2:1 V/V) two titres and washed one time by a modified method of Falch et al. The lipid extracts were resolved into lipid classes by two dimensional thin -layer chromatography using KG silica gel plates as described by Chae and Anderson. The silica gel containing the individual lipid classes was scraped into a glass tube and methyl esters of fatty acids were analysed by a Varian 3400 gas - liquid chromatograph.
Our data show significant lower mean level of Docosahexanoic acid (DHA) in Retinitis Pigmentosa patients compared to control subjects ($P < 0,005$ by Student's t Test).
The reported changes in the metabolism of these fatty acids of phopholipidis membranes of erythrocytes suggest a biochemical abnormalities which at present remain mysterious.

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AN ADENOVIRUS VECTOR FOR GENE TRANSFER OF A REPORTER GENE INTO THE RETINA

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Purpose.
RCS rats are affected by an autosomal recessive retinal degeneration. bFGF slows the rate of photoreceptor degeneration in RCS rats. The ultimate goal of our trial is to perform direct therapeutic injections of recombinant adenoviral particles into the subretinal space of RCS rats. We will also assess the potential therapeutic effect of transplantation of retinal pigment epithelial (RPE) cells expressing recombinant bFGF *in situ* in RCS rats.
Methods.
We investigated the feasability of subretinal injections in new-born rats using a replication-deficient adenoviral vector expressing the β-galactosidase gene under the control of the RSV (Rous Sarcoma virus) promotor, and containing a nuclear localization signal. We also investigated the possibility of infecting bovine RPE cells by the same adenoviral vector.
Results.
Our preliminary results suggest the feasability of infecting ocular bulbi in rat pups P0; Further studies are undertaken for analyzing the cellular pattern of β-galactosidase gene expression in the retina. RPE cells showed an almost 100% rate of infection;We are presently evaluating the possibility of a long term β-galactosidase expression in such RPE cell cultures.
Conclusions.
These results encourage us to use adenoviral vectors for transfering bFGF in the retina of RCS rats 1) in vivo directly into the subretinal space, and 2) in vitro in RPE cells for retinal transplantation.

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