ABSTRACT

Retinal degenerations are the main cause of hereditary blindness mainly due to the loss of photoreceptors, cones and rods. Retinitis pigmentosa (RP) is a common form of retinal degeneration that constitutes the largest genetic cause of blindness in the developed world. Genetic mutations are responsible of rod death. However, the death of cones seems to be due to metabolic changes caused by rod degeneration as hyperoxia (oxidative and nitrosative stress), secretion of different factors (cytokines, chemokines) by rods and other cells of the environment, etc. The death of cones causes the loss of central vision in RP.

The main objective of this thesis is to examine the role of oxidative stress and inflammation in photoreceptors cell death in RP. For that, first, antioxidant response, the presence of oxidative/nitrosative stress markers and the content of cytokines were analysed in aqueous humor and peripheral blood in patients with RP that could be involved in the progression or delay of the disease. Second, we developed an *ex vivo* model of retinal degeneration by performing organotypic cultures of porcine retina exposed to Zaprinast, a phosphodiesterase (PDE) inhibitor, simulating a typical mutation of RP. Last, we evaluated the effect of TNFα inhibition with antibodies anti-TNFα on progression of retinal degeneration in the *ex vivo* model of porcine retina and in an *in vivo* model of RP, *rd10* mouse.

Results obtained in this study show that patients with RP have a deficient antioxidant response in the eye and elevated levels of some markers of oxidative/nitrosative stress in peripheral blood. At ocular level, these patients have less total antioxidant capacity including less activity of the superoxide dismutase (SOD) 3 enzyme. At peripheral level, they also have less SOD3 activity in addition to an increase of lipid peroxidation indicators and an activation of the nitric oxide/cyclic GMP pathway. Patients with better antioxidant response showed better visual function suggesting a role of oxidative stress on the progression of the disease. On the other side, an increase of IL-6 and TNFα content was observed in the aqueous humor of these patients confirming the results obtained by other authors and suggesting that in RP there is a sustained chronic inflammation.

The experimental *ex vivo* model of retinal degeneration reproduces some of the alterations described in murine models and in patients with RP. In this model, the retinal degeneration observed after PDE6 enzyme inhibition with Zaprinast, was accompanied by oxidative stress and inflammation. In this model, TNFα inhibition with the antibody Infliximab, reduces retinal degeneration, Müller cell activation and normalizes total antioxidant capacity although it does not reduce the content of oxidative stress markers.

In *rd10* mouse, treatment with Adalimumab, a monoclonal antibody against TNFα, reduces photoreceptor cell death and reactive gliosis at postnatal day 18. The inhibition of TNFα

reduces poly(ADP) ribose polymerase (PARP) activity, an enzyme involved in the process of cell death, prevents overexpression of TNFα and leukemia inhibitory factor (LIF) cytokines and improves antioxidant response.

Ultimately, these results confirm the hypothesis that alterations in the antioxidant system and inflammatory processes at ocular level are involved in the pathogenesis of RP in patients. The *ex vivo* model of retinal degeneration in porcine retina can be a useful alternative model for the searching for therapeutics targets and for testing diverse drugs that prevent or delay the death of photoreceptors. Our results suggest that in RP, TNFα plays an important role in the death of retinal cells by activating different death pathways. The design of strategies that promote its blockade can be promising therapies in patients with RP.