

ACTUALIZACIÓN EN INVESTIGACIÓN DE RETINA

SIRCOVA, 2010

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**M^a Dolores Pinazo Durán
Amparo Navea Tejerina
Manuel Díaz Llopis
Bernardo Salóm Vayá**

Therapeutic effects of anti-apoptotic and antioxidant agents for the treatment of retinitis pigmentosa disease

Laura Fernández-Sánchez (1), Pedro Lax (1), Gema Esquiva (1), Isabel Pinilla (2), José Martín-Nieto (1), Nicolás Cuenca (1)

(1) Departamento de Fisiología, Genética y Microbiología. Universidad de Alicante. (2) Hospital Clínico Universitario Lozano Blesa, Zaragoza. Instituto Aragonés de Ciencias de la Salud.
e-mail: laura.fs@ua.es

Supported by: MEC (BFU2009-07793/BF1), MSyC RETICS RD07/0062/0012, FUNDALUCE, ONCE, Fundación Mutua Madrileña.

PREMIO SIRCOVA 2010 a la mejor Comunicación Oral

INTRODUCTION

Retinitis pigmentosa (RP) is a heterogeneous group of neurodegenerative diseases causing gradual vision loss and eventually blindness due to the loss of photoreceptor cells by apoptosis. Patients with this disease exhibit migration of pigment epithelial cells into the retina and a remodelling of retinal vessels to form vascular complexes, which correlate with degeneration of photoreceptors. A known animal model of autosomal dominant RP is the transgenic P23H rat, which carries a mutation in the *Rho* gene resulting in the substitution of histidine for proline at position 23 of rhodopsin. The mutant protein is unable to fold correctly in the endoplasmic reticulum, being highly prone to forming high molecular weight aggregates and triggering photoreceptor apoptosis. This model shares a lot of features with RP disease in humans. Bile from bear has been used in Asian medicine for several millennia to treat visual disorders. Recently, Western medicine is trying to find out the properties of a main constituent of bear bile, the so-called tauroursodeoxycholic acid (TUDCA). This compound has been proven neuroprotective in animal models of apoptosis-related disorders, such as Huntington, Alzheimer and Parkinson diseases and acute stroke (Amaral et al., 2009). Safranal is an organic compound isolated from saffron (dried *Crocus sativus* stigmas) which has been largely used in traditional medicine for its anti-apoptotic and anti-carcinogenic properties. In addition, recent studies indicate that safranal could have antioxidant effects. Prefeeding of rats with saffron provides protection against photoreceptor death induced by exposure to continuous bright light (Maccarone et al., 2008). The aim of this study was to evaluate the effect of TUDCA and safranal on the degenerating retina of transgenic P23H rats.

MATERIAL AND METHODS

Homozygous P23H line 3 rats were used in this study. Animals were injected i.p. with TUDCA (500 mg/kg) once a week or with safranal (400 mg/kg) twice a week, from postnatal (P) day 20 to P120 (4 months old). Retinal function was then assessed by electroretinogram (ERG) as described (Barhoum et al., 2008). Briefly, rats were dark-adapted overnight and, after anaesthesia with a mixture of ketamine and xylazine, the scotopic ERG response was recorded simultaneously from both eyes. Then the rats were light-adapted for 20 min to evaluate the photopic ERG response. Animals were thereafter sacrificed in a CO₂ gas chamber and eyes were processed according to published procedures (Martínez-Navarrete et al., 2007). Briefly, eyes were enucleated, fixed in 4% paraformaldehyde and subjected to sucrose cryoprotection. Cryostat vertical sections of retinas were subjected to single and double labellings with antibodies to specific retinal neuronal and glial markers, in order to evaluate the number of photoreceptor rows and synaptic connectivity. Whole-mount retinas were also obtained and processed for immunohistochemistry as described (Cuenca et al., 2003) to evaluate astrocyte cells and their relationship with vessels. Images were obtained by means of immunofluorescence confocal microscopy. NADPH-diaphorase histochemistry was performed according to previous studies (Haverkamp et al., 2000) to assess retinal vascular changes. The vascular plexus was drawn using a camera lucida and quantified using the NIH ImageJ software.

RESULTS

Our ERG studies demonstrated that the two treatments were separately able to improve the amplitudes of a- and b-waves under both scotopic and photopic conditions, being those higher in safranal- or TUDCA-treated animals as compared to untreated animals. The amplitude of the b-wave was better preserved in safranal- than in TUDCA-treated animals. In the normal rat retina there are ca. 12 rows of photoreceptor nuclei, whereas in the 4-month-old transgenic rat the outer nuclear layer (ONL) became reduced to 2 rows of photoreceptors. Our results showed that both treatments prevented the loss of photoreceptors, resulting in a total of 5-6 rows remaining in the central retina at P120 (Fig. 1). Both treatments were also capable of maintaining the normal morphology of cone photoreceptors and their axon terminals. The P23H rat model is characterized by a loss of synaptic connectivity at the outer plexiform layer (OPL) level. Double

immunostaining for synaptic ribbons (bassoon), and photoreceptor post-synaptic cell markers (calbindin and PKC) revealed a preservation of synaptic contacts in the OPL upon injection of either TUDCA or safranal. There are three vascular plexuses in the rat retina. The superficial plexus is mainly arterial-venous and is located in the ganglion cell layer. The deep plexus corresponds to the capillary network and is located at the OPL level. The intermediate plexus connects both, superficial and deep plexuses. Vascular changes have been related to retinal diseases (Wang et al., 2000). We found the deep vascular plexus reduced in the P23H rat at 4 months. However, treatment with TUDCA or safranal was able to prevent its disruption. Vascular alterations in retinal diseases are associated with changes in the number and morphology of astrocyte cells (Ramírez et al., 2010). We found that TUDCA treatment prevented the astrocyte loss occurring in the P23H rat retina. On the other hand, several studies indicate that chronic microglial activation is associated with several neurodegenerative diseases, including retinal dystrophies (Langmann, 2007). We evaluated the number of microglial cells in both treated and untreated subjects. Our results showed that only the safranal treatment was capable to prevent the increase in the number of microglial cells compared to untreated P23H animals.

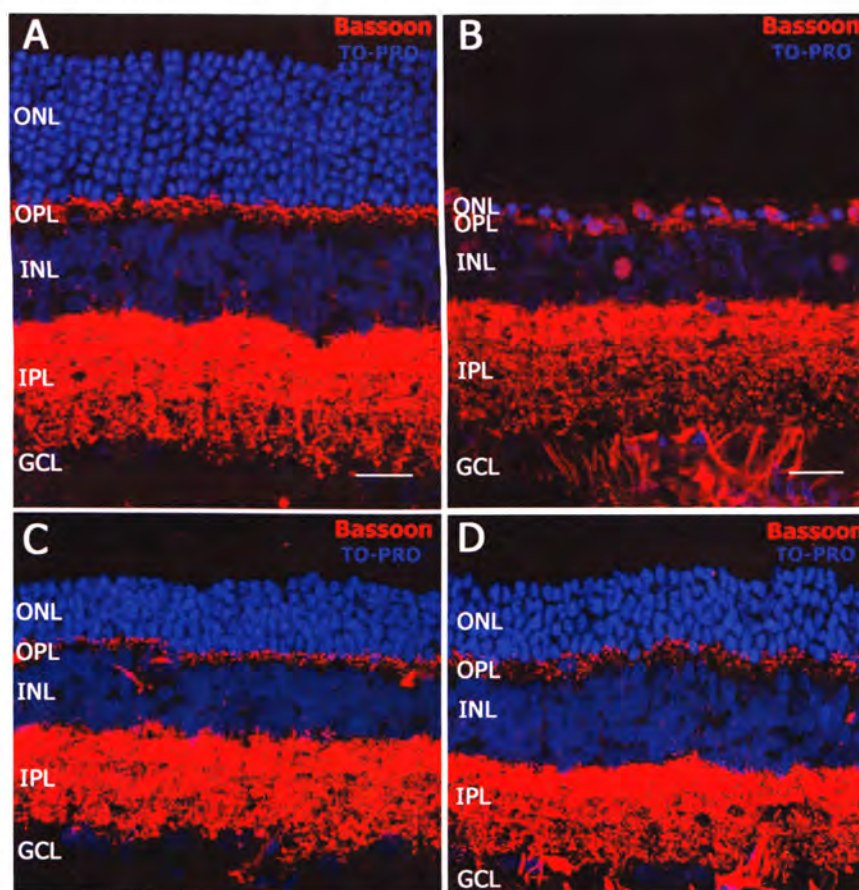


Figure 1. Cryostat sections of Sprague-Dawley and P23H rat retinas immunostained with antibodies against bassoon (red) at P120. Nuclei were stained with TO-PRO-3 (blue). (A) Section of control Sprague-Dawley retina. (B) The loss of photoreceptor cells is evident in the 4-month-old P23H retina. Retinal degenerative changes are reversed in the P23H rat retina upon treatment with TUDCA (X) or safranal (Δ); see text. ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: Ganglion cell layer. Scale bar, 20 μm.

DISCUSSION

Neuroprotective effects of TUDCA have been described in two animal models of retinal degeneration so far: the *rd10* mouse and the light-induced retinal degeneration mouse (Boatright et al., 2006; Phillips et al., 2008), but not in any RP rat model. The present study demonstrates that the systemic administration of TUDCA or safranal through P120 to the homozygous P23H transgenic rat is effective in sustaining photoreceptor cell survival and function. Both treatments were equally effective in preventing the decrease of a- and b-wave amplitudes in the ERG response, with better results for safranal than for TUDCA in the case of the b-wave. Synaptic connectivity in the OPL was preserved as well upon both treatments. During photoreceptor death the level of oxygen in the retina becomes markedly elevated,

increasing free radicals and activating apoptotic pathways in retinal neurons (Stone et al., 1999). The antioxidant activity of safranal (Assimopoulou et al., 2005) could account for its preventive effects on retinal degeneration. The decrease of microglial cell numbers in safranal-treated animals is in agreement with this idea. It has been described that TUDCA is able to modulate several proteins involved in apoptotic pathways in different neurodegenerative diseases at the nuclear and mitochondrial levels (Ramalho et al., 2007). This could explain why TUDCA is also capable to prevent retinal degeneration in the P23H rat model. In this context, we have previously found upon immunostaining for different mitochondrial markers that TUDCA treatment prevents the loss of mitochondria in photoreceptors. In P23H rats a reduction of the deep capillary plexus occurs without changes in the superficial plexus, although a loss of astrocytes takes place in the latter. TUDCA treatment was able to prevent astroglial degeneration and to maintain the normal morphology of astrocytes and their relationship with vessels. We also found in this study that TUDCA and safranal treatments were able to counteract the capillary network disruption, probably as an indirect result of preventing photoreceptor cell death in the ONL.

CONCLUSIONS

This work suggests that the neuroprotective effects of TUDCA and safranal could be useful for the future treatment of retinitis pigmentosa. Further studies are necessary to uncover the precise molecular mechanisms of action of these compounds.

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