DOI 10.18699/SBB-2022-645

Both melatonin and mitochondrial antioxidant SkQ1 had different effect on the retinal glutamate / GABA in healthy Wistar and senescence-accelerated OXYS rats

Telegina D.V.*, Fursova An.Zh., Kolosova N.G.
Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
** telegina@bionet.nsc.ru*

Key words: glutamate, GABA, retina, aging, retinopathy, melatonin, SkQ1, OXYS rats

Motivation and Aim: Glutamate and ©-aminobutyric acid (GABA) are the most abundant amino acids in the retina and a proper balance between neural excitation and inhibition is crucial for retinal physiological functions. An imbalance between them may cause a series of pathological changes in the retina involved in the pathogenesis of many neurodegenerative diseases such as age-related macular degeneration (AMD). In the present study, we explored the role of alterations in the glutamatergic and GABAergic systems in the retina during aging and progression of AMD using mitochondria-targeted antioxidant SkQ1 and melatonin as therapeutic agents with previously proven retinoprotective properties.
Methods: To conduct such studies, we used senescence-accelerated OXYS rats – a unique model of premature aging whose manifestation is early development of retinopathy similar to AMD. To assess the influence of oral melatonin or SkQ1 administration (from age 12 to 18 months) on aging and progression of the AMD-like pathology, we randomly assigned 12-month-old male Wistar and OXYS rats to one of six groups (8 rats per group). Two groups (Wistar and OXYS rats, $n = 8$ in each group) consumed a diet supplemented with SkQ1 (250 nmol/[kg body weight]) and two other groups (Wistar and OXYS rats, $n = 8$ in each group) consumed a diet supplemented with 0.04 mg/[kg body weight] of melatonin (Melaxen; Unipharm, NY, USA). The supplements were provided on dried bread slices between ages 12 and 18 months every day. Each rat in these four groups received either melatonin or SkQ1 with dried bread individually daily at 8 p.m. Twelve-month-old OXYS and Wistar rats ($n = 8$ each strain) that ingested dried bread without a drug served as controls (two more groups).
All the rats were examined by an ophthalmologist two times: before supplementation at the age of 12 months, and after of the treatment at age 18 months. All the rats underwent funduscopy with a Heine BETA 200 TL Direct Ophthalmoscope (Heine, Herrsching, Germany) after dilatation with 1 % tropicamide. The protein level of glutamate/GABA system were analyzed by Western blotting and immunohistochemistry.
Results: We did not detect any signs of retinopathy at the first inspection and during repeated inspections of the retina in control Wistar rats and Wistar rats treated with either SkQ1 or melatonin. There were no differences in the average severity of retinopathy among the groups of OXYS rats during the first examination at the age of 12 months. Pairwise comparisons of the eye condition at the age of 12 and 18 months showed that clinical sings of retinopathy progressed in untreated OXYS rats, whereas melatonin attenuated the rate of progression and SkQ1 completely prevented the progression. As a consequence (one-way ANOVA, Newman–Keuls test), the average severity of

retinopathy in OXYS rats treated with either SkQ1 ($F1,92 = 32.3$, $p < 0.0000$) or melatonin ($F1,92 = 6.4$, $p < 0.01$) was significantly lower than that in control groups.
Supplementation with either melatonin or SkQ1 had no effect on protein amounts of either glutaminase or glutamine synthetase in OXYS rats. In the retina of Wistar rats, supplementation with SkQ1 or melatonin raised the protein level of glutaminase and reduced that of glutamine synthetase ($p < 0.05$). Furthermore, supplementation with melatonin or SkQ1 diminished the GLAST protein level in Wistar rats ($p < 0.05$) and had no significant effect in OXYS rats. Treatment with melatonin had no influence on NMDAR1 and GluA1 protein levels in both Wistar and OXYS rats. There was a marginally significant decrease in the GluA1 level in Wistar rats after melatonin supplementation ($p = 0.08$). Treatment with SkQ1 significantly lowered retinal NMDAR1 expression only in OXYS rats ($p < 0.05$).
Supplementation with melatonin and SkQ1 had no impact on GABA-T and GAT1 protein levels in the retina of the two strains. We observed that SkQ1 raised the level of GAD67 in the retina of Wistar rats but not OXYS rats. By contrast, treatment with melatonin increased the GAD67 protein level in OXYS rats and had no effect in Wistar rats ($p < 0.05$). Supplementation with either melatonin or SkQ1 had no influence on the GABAAR1 protein level in the OXYS retina. Nonetheless, each drug lowered the GABAAR1 protein level in the Wistar retina ($p < 0.05$). Similar data were obtained by immunostaining of the retina from untreated Wistar and OXYS rats at various ages. Both GAD67 and GABA-T were detectable in the ganglion cell layer, inner and outer plexiform layers, and in individual cells of the inner nuclear layer. Nevertheless, fluorescence intensity of GAD67 was higher in the ganglion cell layer and inner plexiform layer, while fluorescence intensity of GABA-T was greater in the outer plexiform layer. GAT1 and GABAAR1 were found to be predominantly expressed in the ganglion cell layer, inner plexiform cell layer, and in individual cells of the inner nuclear layer.
Conclusion: Here we confirmed the ability of the supplementation with SkQ1 to completely suppress the progression of retinopathy in OXYS rats already having pronounced clinical signs of AMD and for the first time report the ability of melatonin to suppress the progression of the clinical signs of the disease. In spite of delaying the development of AMD-like retinopathy, supplementation with melatonin or SkQ1 only moderately affected the glutamate/GABA system in OXYS rats. In contrast, supplementation with melatonin and SkQ1 (separately) caused significant alterations of the glutamate/GABA system in Wistar rats. We observed decreasing protein levels of glutamine synthetase and GLAST and an increasing level of glutaminase. These data are suggestive of alterations in the rapid clearance and recycling of glutamate in neurons and glial cells. We found that prolonged use of these drugs can worsen the condition of the retina during normal aging by contributing to gliosis and to an excessive glutamate level in the synaptic gap in the aged retina without a pathology.
Acknowledgements: This research was funded by Russian Science Foundation grant No. 21-75-00029.