学 位 論 文 の 要 旨

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学 位 論 文 名　Carteolol Hydrochloride Reduces Visible Light-induced Retinal Damage *In Vivo* and BSO/glutamate-induced Oxidative Stress *In Vitro*

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論 文 内 容 の 要 旨

INTRODUCTION

The harmful effects of excessive visible white light exposure on the mammalian retina, which is one of the factors of age-related macular degeneration (AMD) occlusion and progression have been demonstrated by various studies. Light-induced retinal damage is considered to be due to photo-oxidative stress, and reactive oxygen species (ROS) are produced by light exposure in the retina. ROS evoke photoreceptor degeneration, which can be prevented, or slowed, by various antioxidants. Carteolol hydrochloride (carteolol; 5-(3-tert-butylamino-2-hydroxy) propoxy-3, 4-dihydrocarbostyril hydrochloride) has non-selective β-adrenoceptor inhibitory activity and is commonly used as an intraocular hypotensive agent. We showed earlier that carteolol possessed hydroxyl radical (•OH) scavenging ability, which can protect the cornea from UV-induced oxidative stress, and also had the ability to scavenge the superoxide anion radical (O_2^-). Topical carteolol should reach retina and choroid through the cornea and the sclera followed by diffusion, and through the conjunctiva via the posterior ocular circulation like other topical beta-blockers. Thus, the purpose of this study was to determine whether carteolol eye drops has protective effects against the light-induced oxidative stress in retina.

MATERIALS AND METHODS

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University. To evaluate the ability of carteolol to protect the retina physiologically and morphologically against excessive visible light, dark-adapted pigmented rats were pre-treated with topical carteolol ophthalmic solution or saline and then exposed to visible light. The effects on electroretinogram (ERG), morphology, oxidative stress, and expression of mRNAs in the retinas were determined. To analyze the cytoprotective effects of carteolol, the L-buthionine-(S,R)-sulfoximine (BSO)/glutamate-induced oxidative stress in 661W cells, a murine photoreceptor cell line, was evaluated by cell death assays, production of ROS, and activation of caspase. Additionally, we also examined the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the lipid peroxidation in porcine retinal homogenates with carteolol.

RESULTS AND DISCUSSION

In vivo studies showed that exposure to light caused a decrease in the amplitudes of ERGs and the outer nuclear layer (ONL) thickness and an increase of the 8-hydroxy-2'-deoxyguanosine (8-OHdG)-positive cells in the ONL. These changes were significantly reduced by pre-treatment with carteolol. Carteolol also significantly up-regulated the mRNA levels of thioredoxin 1 (TRX1) and glutathione peroxidase 1 (GPX1) compared to saline-treated group. Moreover, carteolol and timolol, another β-adrenoceptor antagonist, significantly inhibited BSO/glutamate-induced cell death and reduced caspase-3/7 activity and ROS production *in vitro*. However, carteolol didn't have direct DPPH radical scavenging ability, and didn't reduce the production of thiobarbituric acid reactive substance (TBARS) significantly. These findings indicated that carteolol has physiologically and morphologically protective effects against photo-oxidative retinal damage, and the neuroprotective function would have resulted from multiple indirect effects such as enhancing the antioxidative potential by inducing endogenous antioxidative proteins and decreasing the intracellular ROS production but not from direct radical scavenging ability. There are many common ocular diseases (AMD; dry eye syndrome; corneal and conjunctive diseases; cataract; glaucoma; retinitis pigmentosa; diabetic retinopathy, autoimmune and inflammatory uveitis) associated with oxidative stress. Our findings suggest that carteolol might suppress the development and aggravation of these diseases.

CONCLUSION

Carteolol application alleviated the retinal damage caused by light-exposure *in vivo*, and photoreceptor cell death and apoptosis stimulated by BSO/glutamate-induced oxidative stress

in vitro. The neuroprotective effects could have resulted from the multiple effects such as enhancing the antioxidative potential by inducing TRX1 and GPX1 and decreasing the intracellular ROS production through β-adrenoceptors.