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Abstract

Purpose: To further know the signaling pathways involved in hypoxia-induced apoptosis in retinal pigmented epithelial (RPE) cells and to improve the understanding of the antioxidant *N*-acetyl-cysteine (NAC) treatment effect.

Methods: We analyzed the expression levels of several apoptosis-related genes by semiquantitative reverse transcriptase–polymerase chain reaction in RPE after 72 h of maintained hypoxia, with or without 10 mM NAC treatment.

Results: Under hypoxic conditions, we detected a higher expression level of *p53* and *CASP8*. Cell treatment with NAC 10 mM prevented this increase. Other apoptosis-related genes such as *bax*, *CASP3*, *CASP4*, *CASP7*, and *fas* did not show an increase in expression levels in hypoxia.

Conclusions: NAC prevents the increased expression levels of *p53* and *CASP8* induced by long-term maintained hypoxia. The supply of antioxidants could be a useful preventive approach in protecting RPE from the effects of chronic oxygen stress, which is of great interest in oxygen stress-related diseases such as age-related macular degeneration and other senescence-associated pathologies.

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Abstract

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Introduction

In elderly people, retinal pigmented epithelial (RPE) may suffer apoptosis when aging is accompanied by the metabolic stress that is linked to hyperoxia or to the "ischemia" caused by the simultaneous deprivation of glucose and oxygen.[1,2] Some antioxidants, such as *N*-acetyl-cysteine (NAC) or Vitamin C, have been shown to delay senescent changes that are linked to free radical attack, which is probably due to improved protection of the mitochondrial membranes and genome against the oxygen radicals that are produced in the respiratory chain.[3–6]

The retina and its pigmented epithelium are constantly exposed to sunlight as well as to high oxygen levels, both of which are potent sources of free radicals. It has been suggested that the cumulative effects of oxidative stress over a lifetime may be the initiating stimulus for age-related macular degeneration (AMD).[1,3,7–9] This multifactorial disease is the most frequent retinal degeneration and the cause of irreversible severe visual loss among the elderly population in industrialized countries.[1,3,10,11] It has been shown that an increase in oxidative stress leads to the apoptotic death of RPE and AMD development.[12–15] The retinal pigmented epithelium is a cell monolayer located between the retinal photoreceptors and the choroidal blood vessels that plays a key role in the mechanical and metabolic support of the photoreceptors.[16]

The results of the previous work of our group showed that hypoxia can cause cell death on RPE through an oxidative-stress-related mechanism, which is in agreement with the concept that apoptosis is a mechanism of RPE cell loss during the early phase of AMD. It was also shown that antioxidants such as NAC protect against hypoxia-induced apoptosis of RPE.[17]

Currently, we have increasing data that suggest antioxidants play a protective role in oxygen-related stress injuries, but the mechanism for this protection is still not well understood.[3] Apoptosis pathways are not only stimulus specific but also cell-type specific.[18] As an oxidative stress model, some groups have studied the effect of H_2O_2 on cultured RPE and its role as an apoptosis inducer.[19,20] It has been shown that, when RPE are incubated with H_2O_2, the

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