ROLE OF THE FAST CALCIUM WAVE IN CELL PROLIFERATION AND APOPTOSIS DURING WOUND HEALING OF BOVINE CORNEAL ENDOTHELIAL CELLS IN CULTURE

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Introduction. Cell migration and proliferation are two of the most important processes during wound closure. It has been suggested that apoptosis may also play a role in the mechanisms of wound healing. The fast calcium wave (FCW), triggered immediately after a wound is produced, has been proposed to be involved in determining healing responses in epithelia.

Objectives. To determine the effects of the reversible inhibition of the FCW on the healing velocity, proliferation rate and apoptosis in healing bovine corneal endothelial (BCE) cells in culture.

Materials and Method. FCW was reversibly inhibited by incubation with cyclopiazonic acid and EGTA. Apoptosis and proliferation rate were assessed by active-caspase-3 immunofluorescence and bromodeoxyuridine incorporation respectively.

Results. Our results show that the reversible FCW inhibition produces only a slight decrease in the velocity of healing and about a 30% decrease in proliferation. An important finding of this study is that caspase-dependent apoptosis occurs during the healing process. We found evidence that this programmed cell death has at least two stages. The first stage occurs during the initial 6 hours of healing and is independent of the migrated distance. The second stage starts at about 6 hours, continues at least until 24 hours after wounding, and has a linear dependence with the migrated distance. The most remarkable finding of this study is that the reversible inhibition of the FCW determines a 100% increase in the apoptotic index. Moreover, our results show that preincubation of an unwounded BCE monolayer with ATP, a treatment that mimics the cytosolic calcium increase of the FCW, protects the monolayer from an apoptotic stimulus.

Conclusions. We conclude that caspase-dependent apoptosis takes place during wound healing in BCE cells in culture and suggest that one of the main roles of the FCW is to inhibit an excessive apoptotic response.

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