CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
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Project Number
S1499

Project Title
Is Gentamicin-Induced Apoptosis in HEI-OC1 Auditory Cells Mediated by LIGHT and LTBR?

Abstract

Objectives/Goals
Gentamicin is an antibiotic used to treat a wide variety of bacterial infections. It causes hearing loss by inducing apoptosis, "programmed cell death," in inner ear sensory cells. An important step of the complex sequence of apoptosis involves caspase-3, one of the members of a family of intracellular cysteine proteases. Upon activation, caspase 3 emits 405 nm wavelength light. The amount of light released indicates the number of cells dying and can be measured with a microplate reader. Although the damaging, irreversible side effects of gentamicin are known, the mechanisms of gentamicin-induced apoptosis are not. The object of my experiment was to investigate the pathway by which gentamicin induces apoptosis in inner ear sensory cells. More specifically it was designed to ascertain whether or not this pathway involves LIGHT and LTBR (receptor). To test my hypothesis that LIGHT and LTBR mediate the pathway, I used a decoy receptor (DCR3) in hopes of inhibiting the binding of LIGHT to LTBR.

Methods/Materials
The experiment was conducted with HEI-OC1 Auditory Cells, a cell line previously developed from the inner ear sensory cells of mice. These cells are sensitive to the proapoptotic activity of ototoxic drugs and thus they provide an ideal in vitro model system for ototoxicity screening. Three groups of these cells were grown and later incubated, one with gentamicin, one with gentamicin and DcR3, and the third with nothing (control). Then a Colorimetric CaspACE Assay was performed. Absorbance at 405 nm was measured using a computer-controlled microplate reader with DeltaSoft 3 ELISA software.

Results
As measured by the microplate reader and as seen by the caspase-3 activity, there was approximately the same amount of apoptosis of cells incubated only with gentamicin and of cells incubated with gentamicin and the decoy receptor, DcR3. The decoy receptor had no substantial effect on the amount of cell death.

Conclusions/Discussion
The results of this experiment suggests that the LTBR signaling pathway is not the major apoptotic pathway involved. Either the LTBR signaling pathway takes no part in the ototoxic side effect of gentamicin or it is working simultaneously with one of the other pathways that mediate gentamicin-induced apoptosis and is the less predominant pathway. This is a significant step toward discovering the exact mechanisms that control gentamicin-induced apoptosis.

Summary Statement
My project is an attempt to learn more about the pathway by which the antibiotic gentamicin induces apoptosis in auditory cells and to determine if this pathway is mediated by LIGHT and LTBR.

Help Received
I used lab equipment at the House Ear Institue under the direction of Federico Kalinec, Ph.D., and Gilda Kalinec, Ph.D., who also provided the cell line. My mother frequently drove me to the lab.