Gentamicin (GEN) causes apoptosis at low concentrations in LLC-PK1 cells subjected to electroporation (EP).

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ABSTRACT

INTRODUCTION

Aminoglycosides antibiotics cause acute renal failure in patients, associated with histological and functional signs of proximal tubules toxicity. The underlying molecular mechanisms remain, however, poorly defined (Gilbert, 2005).

Aminoglycosides enter proximal tubular cells by pinocytosis from the luminal pole and accumulate in lysosomes where they cause a conspicuous phospholipidosis (Tulkens, 1986). Besides lysosomal changes, however, proximal tubules of animals treated with gentamicin also show clear signs of apoptosis (El Mouedden et al., 2000a). This can be reproduced in vitro with both renal and non-renal cells, which, however, must be exposed to large concentrations of gentamicin because of inefficient drug uptake (El Mouedden et al., 2000b).

Using LLC-PK1 cells, we observed that gentamicin causes an early permeabilization of lysosomes before activation of the mitochondrial pathway of apoptosis can be detected (Servais et al., 2005). This raised the question as to whether the lysosomal sequestration of gentamicin would not actually protect cells from its apoptogenic effect.

AIM OF THE STUDY

- To assess if the cytotoxic delivery of GEN by electroporation makes cells more susceptible to develop apoptosis than if incubated with the drug.
- To analyze if the pro-apoptotic Bax protein, present in the cytosol and acting upstream the mitochondrial pathway, is involved GEN-induced apoptosis.

RESULTS

Development of apoptosis and necrosis as a function of GEN concentration in electroporated vs. incubated cells

Involvement of Bax in GEN-induced apoptosis after electroporation or incubation.

CONCLUSIONS

- GEN induces apoptosis at much lower extracellular concentrations (about 100 times lower; 30µM) in electroporated cells compared to cells incubated with this antibiotic.
- Induction of apoptosis by GEN (after electroporation as well as after incubation with GEN) involves the pro-apoptotic Bax protein. This increase of Bax is associated with an increase in its ubiquitinated forms, suggesting a decrease of its degradation by the proteasome.
- Lysosomal sequestration could actually not trigger cell toxicity, but rather protect cells from the apoptotic effects of GEN.

REFERENCES


