Involvement of mitochondrial pathway and Bax in gentamicin (GEN)-induced apoptosis in renal LLC-PK1 cells.

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AIM OF THE STUDY:

To analyze the involvement of the mitochondrial pathway, focusing on the role of pro-apoptotic Bax protein and on the release of cytochrome c.

To study the kinetic relationship between mitochondria and caspase-3 activity.

Background: GEN induces apoptosis in the proximal tubule epithelium of rats treated at low, therapeutically relevant doses. This effect has been reproduced on LLC-PK1 renal cells and is correlated with cell GEN content. Lysosomal permeabilization appears as a primary event driven as from 2 h following exposure to GEN. We have now examined the potential role of mitochondrial pathway (apoptosis expression and translocation, release of cytochrome c).

Methods: Cells were treated with GEN 2 mM or similar concentrations to those observed in proximal tubular cells of animals receiving 10 mg/kg GEN for 10 days. Bax and cytochrome c were detected by western blot and their respective translocations assessed by cell fractionation. Caspase 3 was assayed with Ac-DEVD-AFC.

Results (1): Increase of Bax protein level in cell lysates

From 8 hours of incubation with gentamicin, a significant increase of Bax protein level is observed in cell lysates of treated cells as quantified by densitometric analysis. This increase does not result from an increase of Bax expression as shown by RT-PCR analysis.

Results (2): Translocation of Bax from supernatant to the granule fraction

An increase of cytochrome c is detected in the supernatant but also in the nuclear fraction after 12 hours of treatment with gentamicin.

Results (3): Cytochrome c release from mitochondria

An increase of cytochrome c is detected by immunoperoxidase and western blot analysis, in cytolysates obtained by sono-homogenization of control and treated cells with 3 mM of gentamicin.

Results (4): Caspase-3 activity

Caspase-3 activity is significantly increased from 24 hours of incubation with gentamicin. We observe a decrease of this activity after 48-72 hours.

Conclusions: Gentamicin induces an increase in the amount of the pro-apoptotic protein Bax associated with its translocation from the cytosol to the mitochondria. This is followed by the release of cytochrome c from mitochondria. The data suggest that mitochondrial activation is involved in gentamicin-induced apoptosis.

INTRODUCTION:

Gentamicin has been shown to induce apoptosis in the LLC-PK1 cell line and several perturbations have been associated with this process, namely (i) a lysosomal destabilization observed after 2 hours of treatment; lysosomes are the major site of accumulation of this antibiotic (ii) a decrease of mitochondrial potential (after 10 hours) and (iii) nuclear fragmentation.

Mitochondria play a central role in apoptosis which is tightly regulated by the Bcl-2 family of proteins. Some are anti-apoptotic (such as Bcl-2 and Bcl-xL) whereas others are pro-apoptotic (such as Bak and Bax). Activation of the proapoptotic Bax appears to involve its subcellular translocation and dimerization.

Thus, in viable cells a substantial portion of Bax is found either in the cytosol or loosely attached to membranes. Following a death stimulus, cytosolic Bax translocates to the mitochondria where it becomes an integral mitochondrial protein and can trigger the opening of specialised pores with release of intermembrane mitochondria pro-apoptotic proteins such as cytochrome c. Cytochrome c can activate caspase-9 and further caspase-3 through the formation of the "apoptosome".

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