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Apoptosis induced by aminoglycosides (AGs) in cultured cells : comparison between gentamicin (GEN) and amikacin (AMK) using incubated and electroporated cells.

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ABSTRACT

Purpose: Multi-resistance in Gram-negative bacteria and difficulties in developing truly novel compounds calls for efforts in improving established antibiotics such as AGs. Models are needed, however for the rapid screening of less nephrotoxic derivatives. Apoptosis develops in proximal tubules of animals receiving low, therapeutically pertinent doses of AGs, with GEN causing more changes than AMK (Antimicrob Agents Chemother 44:665-75, 2000). Apoptosis develops also in renal cells either incubated (Toxicol Appl Pharmacol 206:321-33, 2005) or electroporated (Antimicrob Agents Chemother 50:1213-21, 2006) with GEN. We have examined whether AMK could also be differentiated from GEN in these two models.

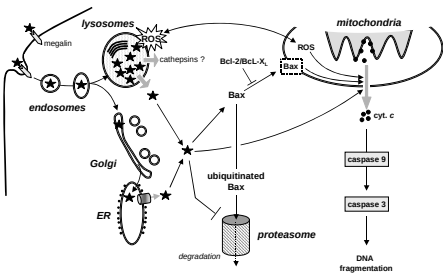
Methods: LLC-PK1 renal cells were (i) incubated with 0-3 mM GEN or 0-9 mM AMK for up to 3 days; or (ii) electroporated with GEN (0.03-1 mM [13.9-463 mg/L]) or AMK (0.09-3 mM [52.7-1758 mg/L]) and examined 24 h later. Apoptosis was quantitated by microscopy after DAPI staining, and confirmed by measuring the increase in caspase-3 activity (using Ac-DEVD-AMC).

Results: Incubated cells: GEN caused a time- and concentration-dependent increase in the percentage of DAPI-positive cells while AMK caused less changes (values at 72 h: GEN, 15.5 ± 3.9 at 2 mM; AMK, 5.6 ± 1.7 at 6 mM; controls: 2.1 ± 0.3). GEN, but not AMK, caused significant increase (up to 277 ± 52 % at 2 mM and 48h) in capase-3 activity. Electroporated cells: gentamicin caused apoptosis for concentrations spanning between 0.064 and 0.25 mM (29.6 and 111.75 mg/L), with a maximum (11.3 ± 1.3 vs. 0.6 ± 0.3 % for controls) at 0.128 mM (59.2 mg/L). AMK was without effect at all concentrations tested.

Conclusion: These models confirm the lower apoptogenic potential of AMK observed *in vivo* in comparison to GEN, and may help in designing and/or screening less nephrotoxic aminoglycosides.

BACKGROUND

- Multi-resistance in Gram-negative bacteria and lack of truly novel compounds in clinical development calls for reappraisal and improvement of formerly established antibiotics. Aminoglycosides offer definite possibilities in this context, but fast and efficient methods are needed to screen for the nephrotoxic potential of new drug candidates.
- The renal toxicity of aminoglycosides results from endocytic uptake by proximal tubular cells and ensuing lysosomal sequestration, which triggers a succession of alterations including lysosomal phospholipidosis, apoptosis and necrosis.
- Gentamicin-induced apoptosis can be reproduced with cultured renal and non-renal cells using large extracellular concentrations,[1] but low concentrations are sufficient if using electroporation to directly deliver gentamicin into the cytosol.[2]
- Amikacin has been consistently shown to cause less phospholipidosis, apoptosis and necrosis than gentamicin in animals treated at therapeutically-relevant doses.[3]



Main mechanisms of renal toxicity of gentamicin with emphasis on apoptosis. The drug is symbolized by a symbolized by a black filled star. From Servais *et al.*, Apoptosis, in press (2007 Oct 30; [Epub ahead of print]) with data from refs 1-5

METHODS

- All methods and products (culture, detection of apoptosis, electroporation) were as described for LLC-PK1 cells. [4]
- Amikacin was systematically compared to gentamicin at 3-fold larger molar concentrations (weight ratio: 2.37) to account for the usual dosage ratio used in the clinics. Gentamicin and amikacin were purchased as Geomycine and Amukin (branded products registered for clinical use in Belgium and complying with the European Pharmacopea), and all concentrations expressed as free base.
- Statistical analyses were made using GraphPad Prism version 4.02) and GraphPad Instat version 3.06 (GraphPad Prism Software, San Diego, CA).

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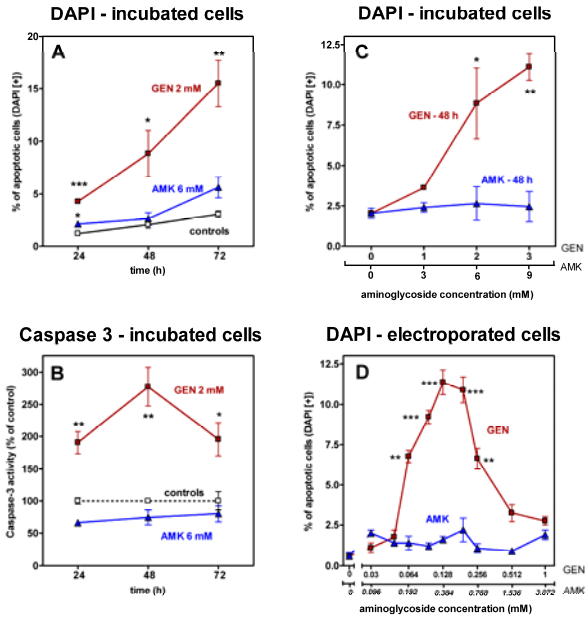
AIM OF THE STUDY

To examine whether GEN and AMK can be differentiated for induction of apoptosis, using a model of proximal tubular cells incubated or electroporated with each of these drugs.

RESULTS

time effect

concentration effect



Panels A and B **: Time course of the development of apoptosis in cells incubated for up to 3 days with 2mM GEN or 6mM AMK. Apoptosis was determined by counting of apoptotic nuclei after DAPI staining (A) or by caspase-3 activity assay with Ac-DEVD-AMC**

Panels C and D **: Concentration effect relationship for the development of apoptosis in cells incubated for 48h (C) or electroporated and reincubated for 24h ((D)) with GEN or AMK. Apoptosis was determined by counting of apoptotic nuclei after DAPI staining**

In incubated cells, GEN caused a time- and concentration-dependent increase in the percentage of apoptotic cells or caspase-3 activity while AMK caused no or less changes even when used at a 3 times higher concentration.

In electroporated cells, GEN induced apoptosis was evidenced for 10 times lower concentrations than in incubated cells, while AMK did not cause significant apoptosis over the whole range of concentrations investigated.

Lactate deshydrogenase release (as an index of necrosis), remained non significantly different from matching controls in all conditions.

DISCUSSION

- Drug-induced apoptosis usually develops at lower dosages than necrosis and organ dysfunction. LLC-PK1, however, take up aminoglycosides only slowly and to a limited extent,[1] making necessary to use of extracellular concentrations largely exceeding what can be observed in vivo to study apoptosis.
- Electroporation makes possible (i) to compare the drugs at more clinically-relevant concentrations; (ii) and to confirm the low apoptogenic potential of amikacin in comparison with gentamicin (see our previous observations[3]).
- This suggests specific interaction(s) of gentamicin with those cellular constituents susceptible to trigger apoptosis.

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