Aminoglycoside-induced apoptosis in renal (LLC-PK1) and non-renal (J774 macrophages) cells: Comparison between gentamicin and amikacin.

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Tuesday, April 03, 2007 10:06-10:18 (oral session) presentation O428
Aminoglycoside toxicity ...

From: Tulkens, 1986 Amer. J Med. 80(Suppl 6B);105-114
Morphological changes in rat renal cortex (A) upon treatment with gentamicin at low doses (10 mg/kg; 10 days) and in cultured LCC-PK1 renal cells (B) upon incubation with gentamicin (under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated in A [approx. 10 µg/g]).

Servais et al. In: Toxicology of the Kidney (Target Organ Toxicology Series), 2004, chap. 16, pp 635-685.
Apoptosis is probably induced by disruption of gentamicin-loaded lysosomes

Appearance of acridine orange in LLC-PK1 cells in confocal microscopy.

control  + gentamicin 3h  + gentamicin 4h

Electroporation allows to by-pass lysosomes and increases cell-susceptibility to gentamicin-induced apoptosis.

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<th>incubated</th>
<th>electroporated</th>
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<td>no GEN</td>
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<td>GEN (0.03 mM)</td>
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Staining of nuclei of LLC-PK₁ cells by 4’,6’-diamidine-2’-phenylindole (DAPI).

Aims of the study

• to examine whether the capacity of gentamicin to induce apoptosis is restricted to renal cells

• to compare amikacin to gentamicin in this context, since amikacin is generally considered to be less nephrotoxic than gentamicin and causes less lysosomal phospholipidosis

Materials & Methods

- non-confluent murine J774 macrophages and porcine LLC-PK1 renal cells grown to 80% confluency.
- Enumeration of apoptotic cells after DAPI staining by observers unaware of the experimental conditions, and expressed as percentage of all visible cells. ...
- Cell viability checked by measurement of LDH release
- Electroporation performed on trypsinized LLC-PK1 cells (8 square wave pulses; 800 v/cm; 1 ms)
Apoptosis in incubated J774 macrophages

24 h incubation

⇒ major difference in susceptibility towards amikacin vs. gentamicin

Note: no LDH release
Apoptosis in incubated LLC-PK₁ renal cells

72 h incubation

% apoptotic cells (DAPI [+])

- Control
- GEN 1 mM
- GEN 2 mM
- GEN 3 mM
- AMK 3.16 mM

-major difference in susceptibility towards amikacin vs. gentamicin (which caused concentration-dependent apoptosis)
LDH-release from incubated LLCPK₁ renal cells

72 h incubation

% LDH release

Control  GEN 1mM  GEN 2mM  GEN 3mM  AMK 3mM

⇒ no significant difference in direct contact cytotoxicity
Apoptosis in electroporated LLC-PK1 cells

Note: extracellular concentration are 30-fold lower than for incubated cells

→ major differences between drugs

% of apoptotic cells (DAPI [+])

extracellular concentration (µM) during electroporation

Note: extracellular concentration are 30-fold lower than for incubated cells
Conclusions

- Apoptosis develops in both renal and non-renal cells upon incubation with gentamicin.

- The lack of apoptosis observed with amikacin with both incubated (renal and non-renal) and electroporated (renal) cells supports the concept that this aminoglycoside is intrinsically less toxic than gentamicin.

- These models could be used for the fast screening of new aminoglycosides with respect to potential renal toxicity.