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

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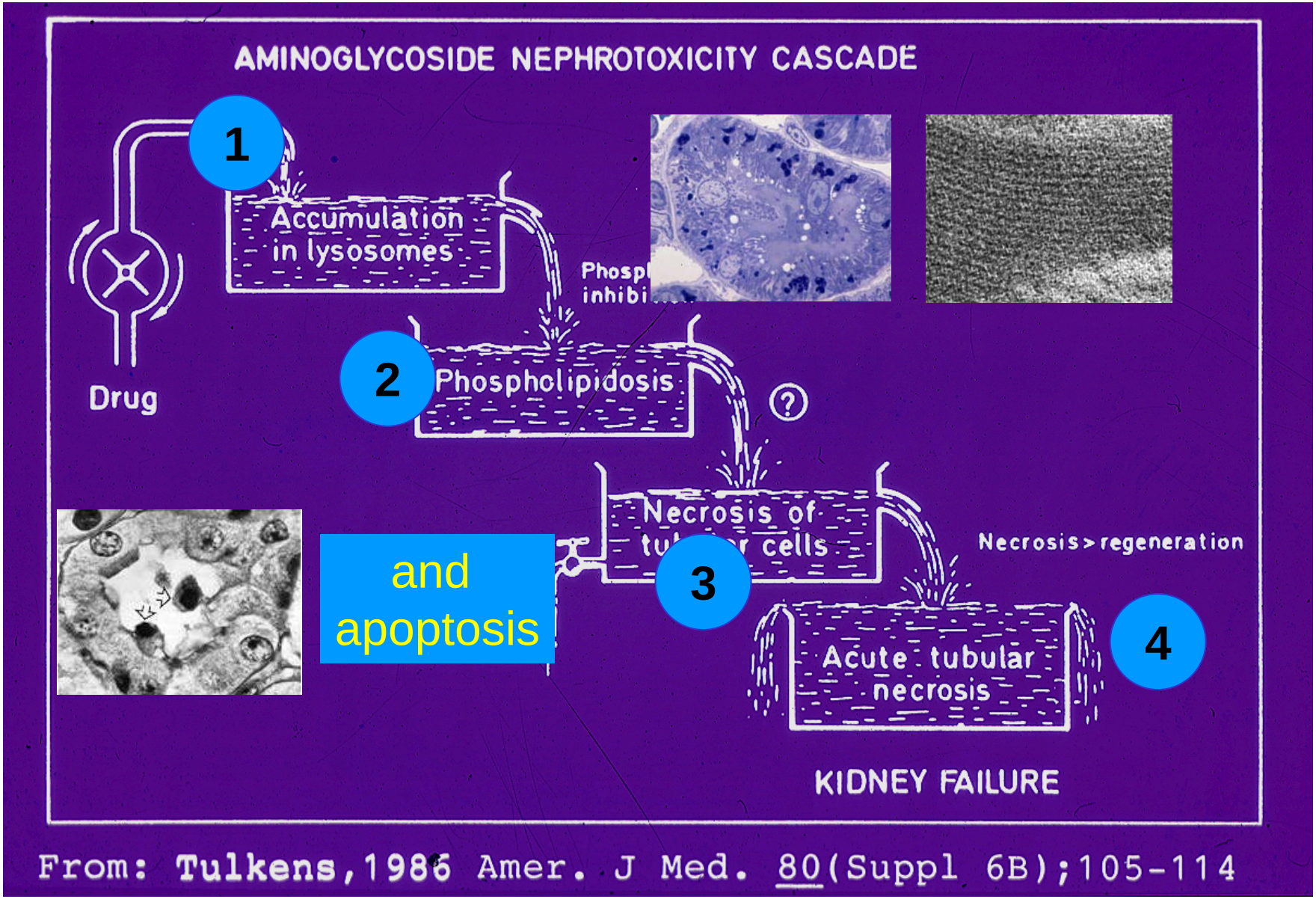
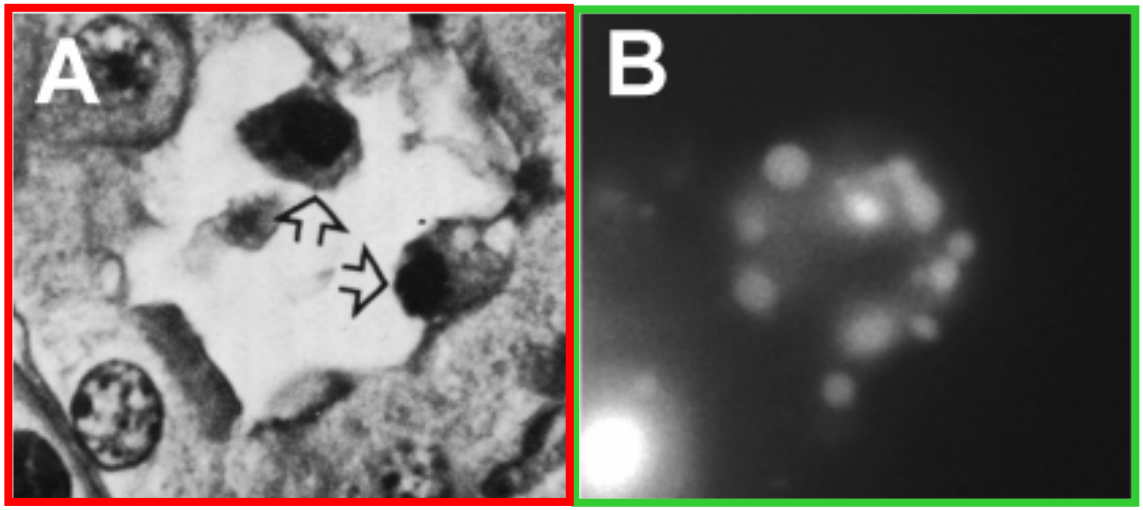
Tuesday, April 03, 2007 10:06-10:18 (oral session) presentation O428

Aminoglycoside toxicity …



Apoptosis in kidney and renal cells …

rat cortex **LLC-PK1 cells**

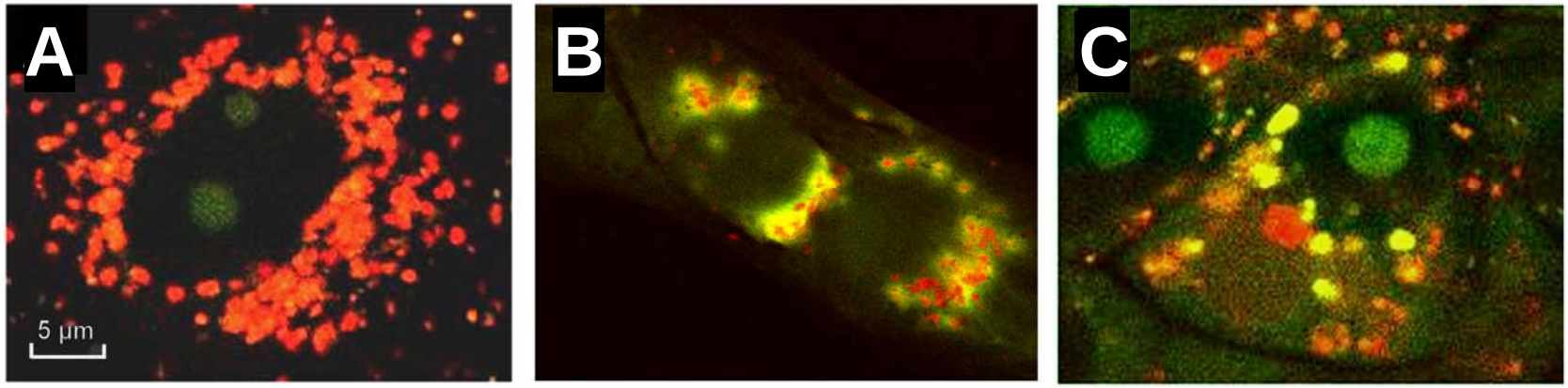


Morphological changes in rat renal cortex (A) upon treatment with gentamicin at low doses (10 mg/kg; 10 days) and in cultured LCC-PK1 renal cells (B) upon incubation with gentamicin (under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated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

Laurent et. al Antimicrob Agents Chemother (1983) 24:586-593.
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

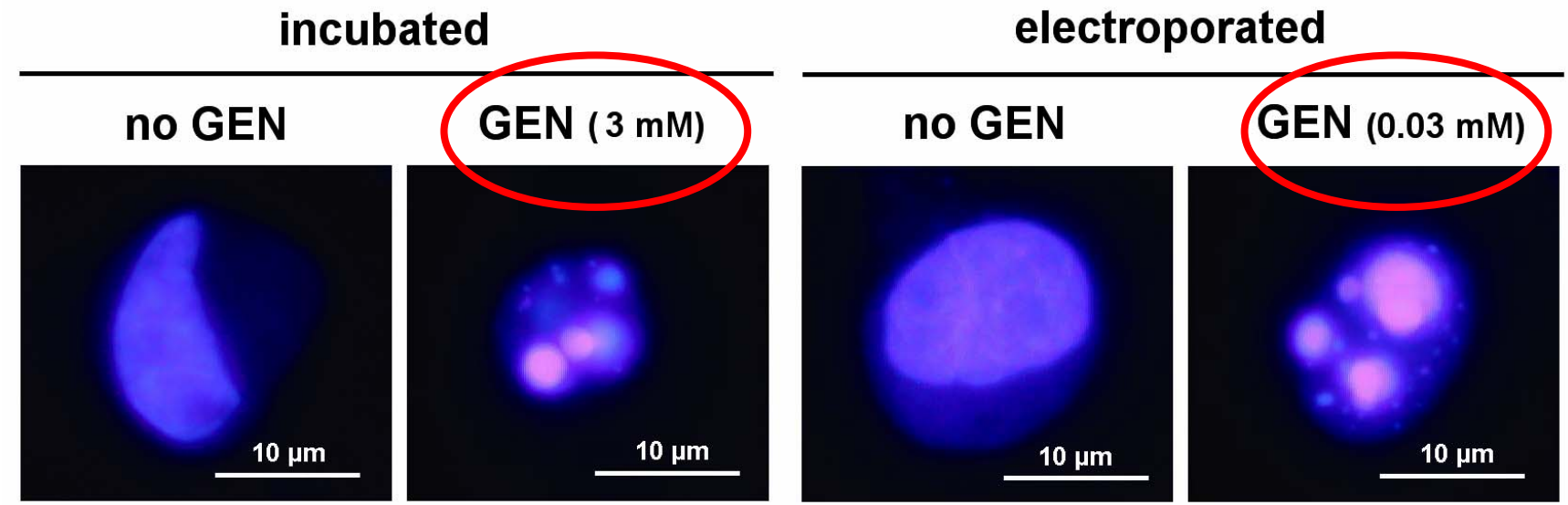
Appearance of acridine orange in LLC-PK1 cells in confocal microscopy.



control **+ gentamicin 3h** **+ gentamicin 4h**

H. Servais et al. / Toxicology and Applied Pharmacology 206 (2005) 321–333

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



Staining of nuclei of LLC-PK$_1$ cells by 4',6'-diamidine-2'-phenylindole (DAPI).

Servais et al., Antimicrob. Agents Chemother. (2006) 50:1213-1221

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

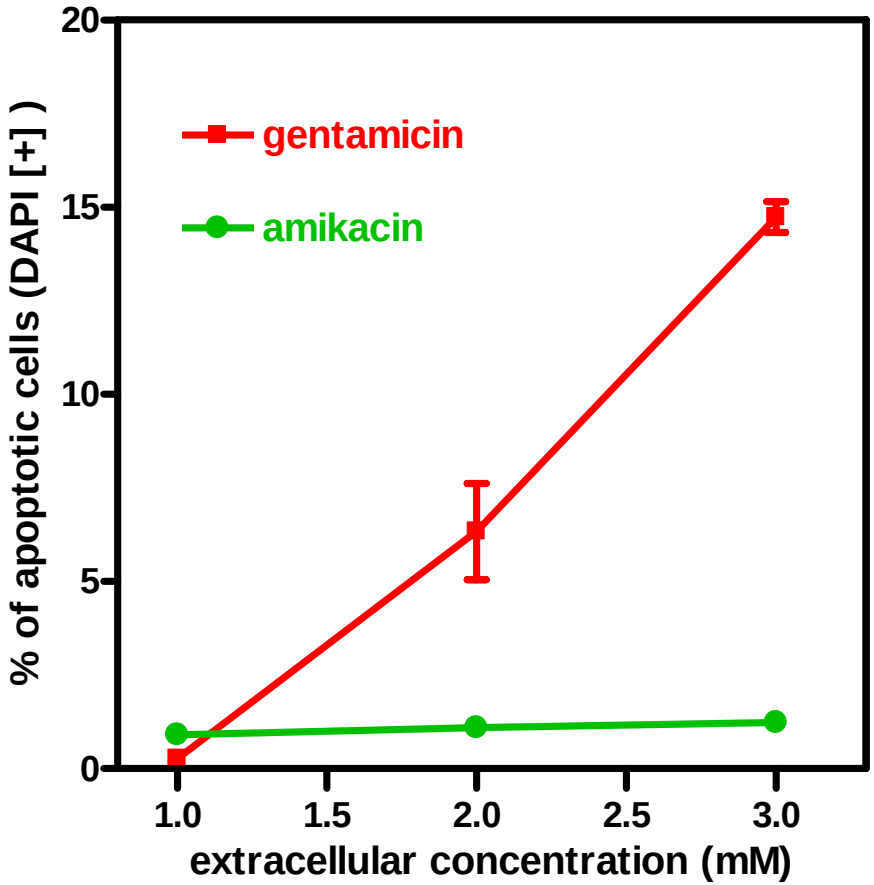
  (Mingeot-Leclercq & Tulkens, Antimicrob Agents Chemother. 1999;43:1003-12).

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)

  (Servais et al., Antimicrob Agents Chemother. 2006;50:1213-21).

Apoptosis in incubated J774 macrophages

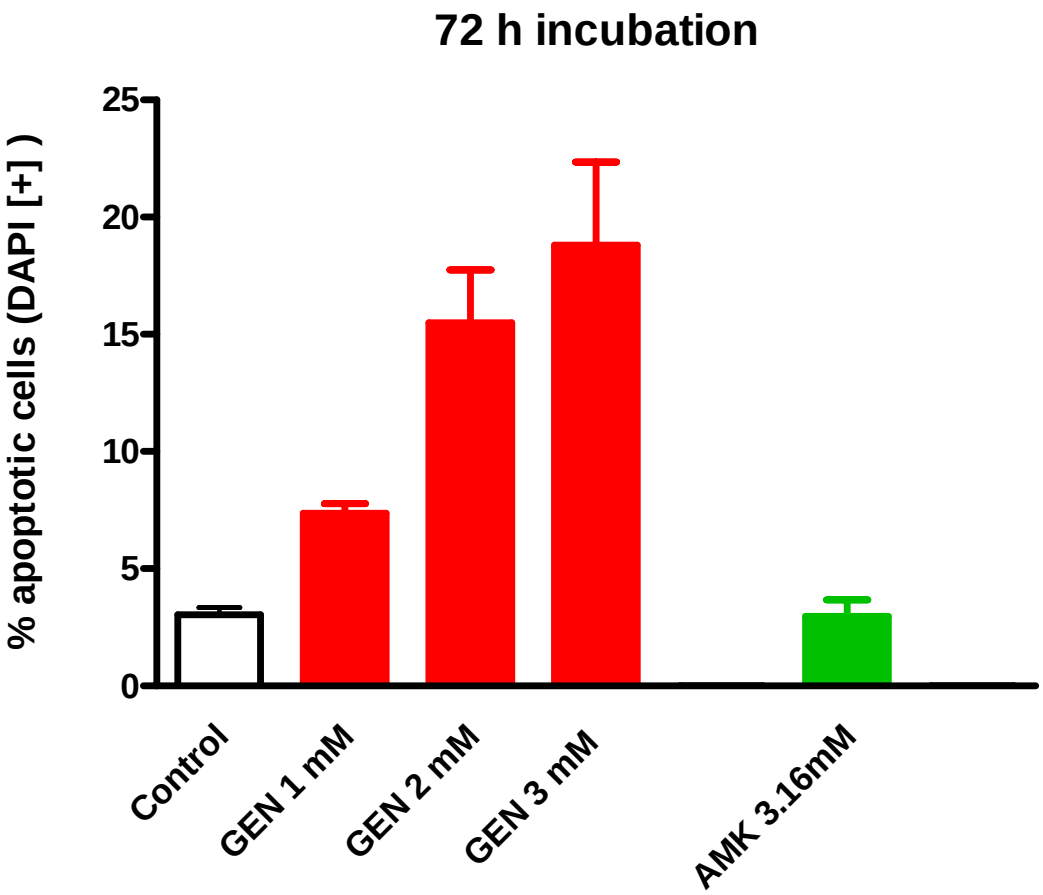


24 h incubation

➔ major difference in susceptibility towards amikacin *vs.* gentamicin

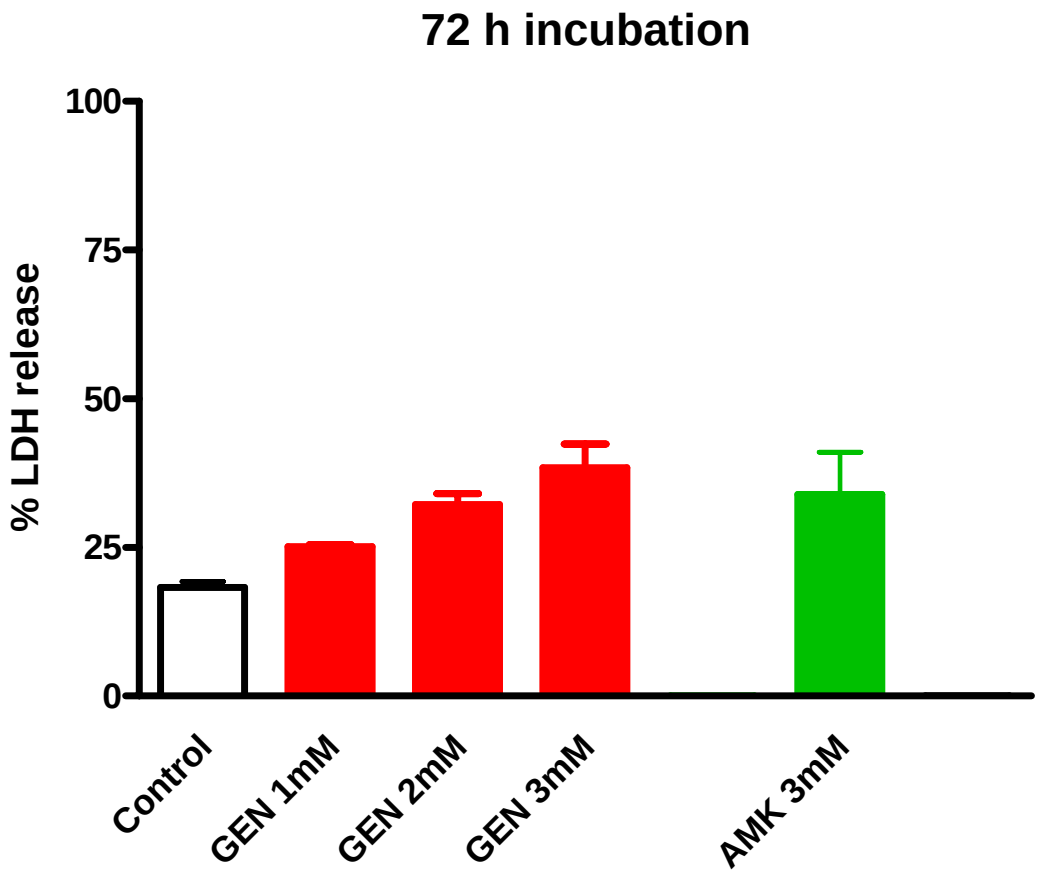
Note: no LDH release

Apoptosis in incubated $LLCPK_1$ renal cells



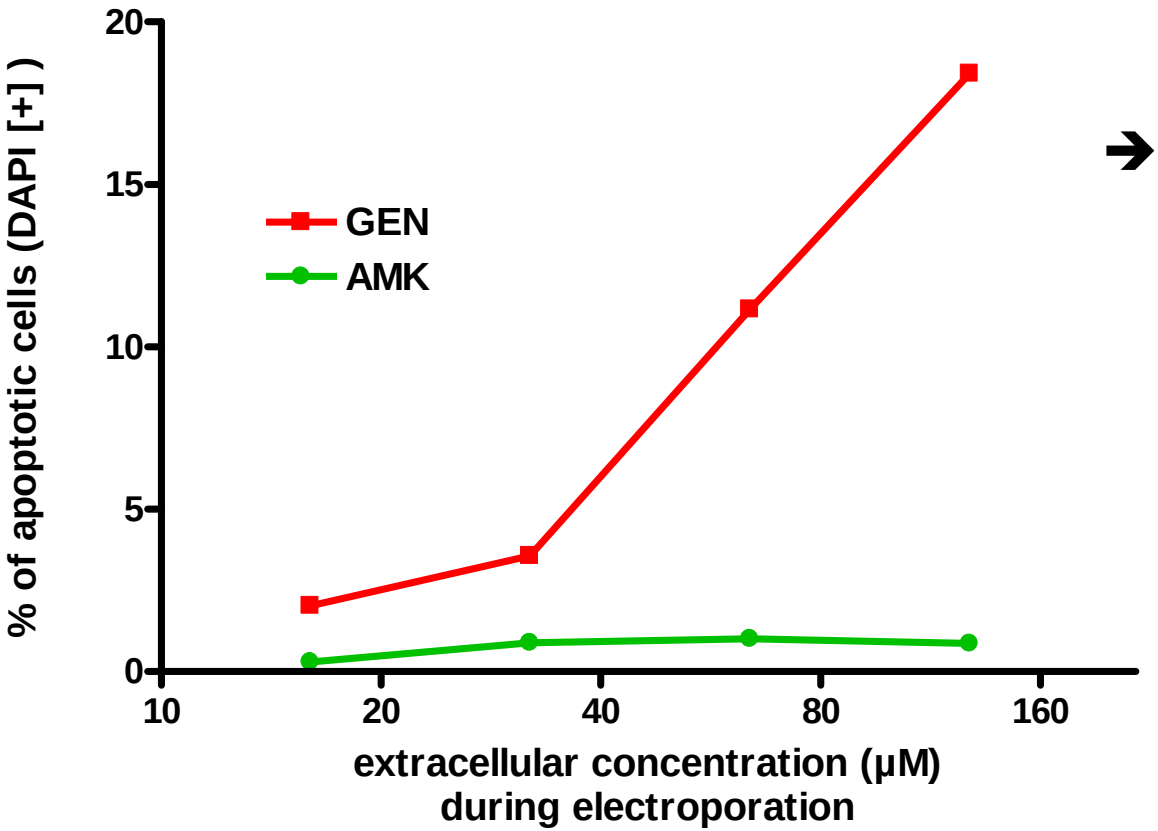
➔ major difference in susceptibility towards amikacin *vs.* gentamicin (which caused concentration-dependent apoptosis)

LDH-release from incubated $LLCPK_1$ renal cells



➔ no significant difference in direct contact cytotoxicity

Apoptosis in electroporated in LLCPK1 cells



➔ major differences between drugs

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