Toxicodynamics…

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William C. Craig Symposium
My early steps to toxicodynamics …


THE UPTAKE AND INTRACELLULAR ACCUMULATION OF AMINOGLYCOSIDE ANTIBIOTICS IN LYSOSOMES OF CULTURED RAT FIBROBLASTS

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Cathepsin D

Glucomamidase

Gentamycin

Density (g/cm³)
My early steps to toxicodynamics ...

Gentamicin-Induced Lysosomal Phospholipidosis in Cultured Rat Fibroblasts
Quantitative Ultrastructural and Biochemical Study

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But are those concentrations PK meaningful?
You said aminoglycoside nephrotoxicity?

• Typing "(gentamicin OR aminoglycoside*) AND nephrotoxicity" on PubMed will yield 1540 papers (among which 229 reviews), with the first one in 1969...
  (gentamicin was introduced in the clinics in 1967...)

• Controversies were immediate since among the 6 first papers, two say opposite things:
  – Falco et al. Nephrotoxicity of aminoglycosides and gentamicin.

• Perhaps the true was:
  – Schultze et al. Possible nephrotoxicity of gentamicin.
What was monitoring aminoglycosides in the early times of gentamicin?

- avoid high peaks... to reduce toxicity
- get sufficiently high trough levels... to get efficacy

Very small range, isn’t it?

From an “Abbott TdX booklet” (1976) to which I contributed... Belgian guidelines kept this until 2000...
Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis **at low doses**...
Aminoglycoside toxicity cascade (1st version)...

From: Tulkens, 1986 Amer. J Med. 80(Suppl 6B);105-114
Aminoglycoside entry in proximal tubular cells is via brush border binding *..."
Aminoglycoside toxicity is not linked to peak (alone)
Aminoglycoside accumulation in the kidney is saturable at clinically meaningful concentrations.*

* Giuliano et al., J. Pharm. Exp. Ther., 1986

This is where patients are in a q8h schedule!!
Néphrotoxicity and schedule of administration …
the first large scale clinical trial

• 141 predominantly elderly patients with severe bacterial infections.
• All patients received once-daily doses of 2 g ceftriaxone, in addition to netilmicin.

"Netilmicin-induced toxicity may be reduced by using once-daily dosing regimens and limiting the duration of treatment."

Is the once-a-day schedule * used?


National survey of extended-interval aminoglycoside dosing (EIAD).
Chuck SK, Raber SR, Rodvold KA, Areff D.

- 500 acute care hospitals in the United States
- EIAD adopted in 3 of every 4 acute care hospitals
  - 4-fold increase since 1993
  - written guidelines for EIAD in 64% of all hospitals
- rationale
  - 87.1% : equal or less toxicity
  - 76.9% : equal efficacy
  - 65.6% : cost-savings
- dose: > 5 mg/Kg
- 47% used extended interval in case of decline in renal function (38% with Hartford nomogram)

* a direct application of PK/TD principles…
Can we further prevent aminoglycoside toxicity?

Protection against Gentamicin-Induced Early Renal Alterations (Phospholipidosis and Increased DNA Synthesis) By Coadministration of Poly-L-Aspartic Acid

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Mechanism of Protection Afforded by Polyaspartic Acid against Gentamicin-Induced Phospholipidosis. II. Comparative in Vitro and in Vivo Studies with Poly-L-Aspartic, Poly-L-Glutamic and Poly-d-Glutamic Acids

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Fig. 2. Activity of sphingomyelinase and phospholipid content of kidney cortex expressed as percentage of control values. Control, animals infused with saline (groups 1a and 1b); polyaspartic acid (pAsp), animals infused with pAsp (250 mg/kg) (groups 3a and 3b); GM, animals infused with GM (100 mg/kg) (groups 2a and 2b); GM + pAsp, animals infused with GM (100 mg/kg) and pAsp (250 mg/kg) (groups 4a and 4b). See table 1 for definition of the experimental protocols and for number of animals in each group.

Fig. 1. Results of a typical experiment showing the binding of gentamicin to polyamionic peptides as a function of pH at 4 mM ionic strength. Results obtained by dialyzing 100 nmol of gentamicin against 150 nmol of acid equivalents of polyamions at 37°C for 3 hrs. The buffers used were HCl-KCl (pH 2-3.5), Na acetate (pH 4-5.4), phosphate (pH 6-7.4), Tris-HCl (pH 6-8.5) and carbonate-bicarbonate (pH 9-10). Bmax occurs between pH 5 to 6 in case of poly-L-Asp (p-L-Asp) and around pH 6 in case of poly-L-Glu (p-L-Glu) and poly-d-Glu (p-D-Glu).
Apoptosis in kidney and renal cells as first sign of toxicity...

Morphological changes in rat renal cortex (A,C,D) 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, B, and C [approx. 10 µg/g;]

Servais et al. In: Toxicology of the Kidney (Target Organ Toxicology Series), 2004, chap. 16, pp 635-685,
Electroporation allows to by-pass lysosomes and increases cell-susceptibility to gentamicin-induced apoptosis in cultured cells.

**Figure 1:** Staining of nuclei of LLC-PK1 cells by 4′,6′-diamidine-2′-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the absence (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (0.03 mM; 13.9 mg/L), and examined 24 h later. In the absence of gentamicin, both electroporated and incubated cells show a diffuse finely reticulated staining characteristic of euchromatin of diploid interphase animal cells. In contrast, cells electroporated or incubated in the presence of gentamicin show typical changes associated with apoptosis, consisting in the condensation and fragmentation of the nuclear material.

Apoptosis in electroporated cells as a means to test for toxicity

FIG. 2. Apoptosis in electroporated cells. Cells were electroporated in the absence (controls) or in the presence of neomycin B, gentamicin, isepamicin, or amikacin and returned to aminoglycoside-free medium, and apoptotic nuclei were enumerated 24 h later. Values are means ± standard deviations (n = 3). Statistical analysis was performed by two-tailed analysis of variance (P < 0.01). All values for neomycin B and gentamicin, except those observed for the largest concentration tested (0.256 mM), are significantly different from those of the controls; isepamicin values observed for 0.192, 0.288, and 0.384 mM concentrations are significantly different from those of controls; amikacin values did not differ from control values. The 0.12 mM concentration corresponds to approximately 74 mg/liter for neomycin B, 56 mg/liter for gentamicin (taking into account the respective contents of the commercial gentamicin in C1, C1a, and C2 components), 68 mg/liter for isepamicin, and 70 mg/liter for amikacin. See the supplemental material for structures of tested compounds.

Could aminoglycoside toxicity be a paradigm?

Interaction of the macrolide azithromycin with phospholipids. I. Inhibition of lysosomal phospholipase A₁ activity

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Mixed-Lipid Storage Disorder Induced in Macrophages and Fibroblasts by Oritavancin (LY333328), a New Glycopeptide Antibiotic with Exceptional Cellular Accumulation

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But old cars can still drive you far …
You are never old when you do good research

Oh, yes Professor Craig!
You are never old when you do good research...

Now I understand PK/PD.