Nephrotoxicity of Aminoglycosides and Comparisons with Cis-Platinum

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Aminoglycosides in the 70’s ...

- Potent antimicrobials but toxic
  - nephrotoxicity (reversible)
  - ototoxicity (irreversible)

- All very similar biophysical, chemical, microbiological and pharmacokinetic properties, but...
  - Why are there differences in toxicities?
  - Are those differences real?
  - What is/are the mechanism(s)?
  - Can we protect patients?
Aminoglycosides monitoring in the 80’s ...

- avoid high peaks
  ... to reduce toxicity

- get sufficiently high trough levels
  ... to get efficacy

Very small range, isn’t it?

Abott TdX manual, 1986

Abott TdX manual, 1986
Aminoglycosides toxicity incidence is highly variable among patient populations

Patients with nephrotoxic reaction after treatment with gentamicin

- young volunteers  
  Smith et al., 1982
- random hospital populat.  
  Smith et al., 1980
- critically-ill patients  
  Plaut et al., 1979

All those patients were under close monitoring ...
A look in the microscope ... 

P. Maldague, unpublished
Somewhat closer ...
Compare ...
And examine ... 

P. Maldague, unpublished
Gentamicin accumulates in lysosomes of proximal tubular cells

Schmitz et al., J. Biol. Chem. 277:618-622, 2002
Aminoglycoside entry in proximal tubular cells is via brush border binding *

  Silverblatt & Kuehen, Kidney Intern., 1979

binding to
• megalin
  (Moeströp et al., 1995)
• acidic phospholipids
  (Humes et al, 1983)
Mice deficient in megalin do not accumulate gentamicin in kidney

Schmitz et al., J. Biol. Chem. 277:618-622, 2002
Towards a mechanism ...

1. binding to brush border
2. accumulation in lysosomes
Intralysosomal gentamicin causes phospholipidosis

Tulkens, Am. J. Med. 80:105-114, 1986
Intralysosomal gentamicin binds to phospholipids and cause phospholipidosis

Tulkens, Am. J. Med. 80:105-114, 1986
Phospholipidosis is related to the binding of gentamicin to acidic phospholipids and subsequent inhibition of lysosomal phospholipases.

Release of lysophosphatidylcholine (% control)

Drug concentration (µM)

Gentamicin
IC$_{50}$ = 220 µM

Isepamicin
IC$_{50}$ = 100 µM

Adapted from Brasseur et al., 1989

P. Lambricht, 1991
A first global hypothesis...
Gentamicin causes apoptosis at low, therapeutically-relevant dosages


Hematoxylin/eosin


Tunel
Gentamicin-induced apoptosis can be reproduced with cultured kidney and non-kidney cells ...
Is lysosomal rupture causing apoptosis and necrosis?

Maldague et al., 1983

Servais et al., unpublished
APOPTOSIS: main signaling pathways ...

Extrinsic pathway

- Fas
- TNF-α
- Pro-caspase 8
- caspase 8
- DISC
- FADD,

Intrinsic pathway

- Bid, Bax, Bak
- Cytochrome c
- tBid, Bax, Bak
- Bcl-2, Bcl-xl
- Apaf-1
- Pro-caspase 9

Mitochondria

- Caspase 8
- caspase 3 (6-7)
- Pro-caspase 3 (6-7)
- caspase 9
- D
- PARP, lamin
- ICAD,...
Extrinsic pathway

Fas
TNF-α

Pro-caspase 8

caspase 8

Extrinsic pathway

DISC
FADD,

Fas
TNF-α

Pro-caspase 8

caspase 8

Intrinsic pathway

Cytochrome c

Bcl-2, Bcl-xl

Pro- caspase 9

Lysosomes

Lysosomal proteases

Bid

tBid, Bax, Bak

Pro-caspase 3 (6-7)

caspase 3 (6-7)

Nucleus

PARP, lamin
ICAD,...

APOPTOSIS
and aminoglycosides
Making use of this knowledge to protect patients …
### TABLE 2. Main approaches toward reduction of aminoglycoside nephrotoxicity

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Decrease or prevention of drug accumulation by kidneys</td>
<td></td>
</tr>
<tr>
<td>Intracellular sequestration of aminoglycosides</td>
<td>Dextran sulfate (59)</td>
</tr>
<tr>
<td>Polymeric compounds</td>
<td>Inositol hexasulfate (67)</td>
</tr>
<tr>
<td>Acidic drugs</td>
<td></td>
</tr>
<tr>
<td>Piperacillin (44)</td>
<td></td>
</tr>
<tr>
<td>Latamoxef metilacetic [(68)]</td>
<td></td>
</tr>
<tr>
<td>Fosfomycin (33, 54)</td>
<td></td>
</tr>
<tr>
<td>Pyridoxal 5'-phosphate (114)</td>
<td></td>
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<tr>
<td>Competition with or decrease in aminoglycoside binding to brush border membrane</td>
<td></td>
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<tr>
<td>Raising the urine pH</td>
<td></td>
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<tr>
<td>Competitors</td>
<td></td>
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<tr>
<td>Ca^{2+} (diet supplementation [31] or vitamin D-induced hypercalcemia [21])</td>
<td></td>
</tr>
<tr>
<td>Lysozyme (58)</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides (as their own competitors) (39)</td>
<td></td>
</tr>
<tr>
<td>Increase in exocytosis</td>
<td>Furosemide (9)</td>
</tr>
<tr>
<td>II. Prevention or decrease of lysosomal phospholipid inhibition</td>
<td></td>
</tr>
<tr>
<td>N-substitution</td>
<td></td>
</tr>
<tr>
<td>Amikacin (75), isepamicin (133), arbekacin, <em>1-N</em> and 6'-N-pepidic and aminosul derivative of kanamycin A and neomycin (72)</td>
<td></td>
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<tr>
<td>Other substitution</td>
<td></td>
</tr>
<tr>
<td>Fluorinated derivatives</td>
<td></td>
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<tr>
<td>5, 3' or 3' fluoro derivatives of tobramycin, dibekacin, arbekacin, or kanamycin</td>
<td></td>
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<tr>
<td>Disaccharidic aminoglycosides</td>
<td></td>
</tr>
<tr>
<td>Astromycin (forimycin) (73)</td>
<td></td>
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<tr>
<td>Daptomycin (41)</td>
<td></td>
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<tr>
<td>Torsayfline (32)</td>
<td></td>
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<tr>
<td>Coadministration of agent preventing intralysosomal phospholipidosis</td>
<td></td>
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<tr>
<td>Intralysosomal sequestration of aminoglycosides</td>
<td></td>
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<tr>
<td>Increase of membrane negative charge</td>
<td></td>
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<tr>
<td>Other</td>
<td></td>
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<tr>
<td>III. Protection against necrosis and other gross cellular alterations</td>
<td></td>
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<tr>
<td>Antioxidants</td>
<td></td>
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<tr>
<td>Deferoxamine (11)</td>
<td></td>
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<tr>
<td>Methimazole (24)</td>
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<tr>
<td>Sarieto (94)</td>
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<tr>
<td>Vitamin E + selenium, vitamin C (1, 57)</td>
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<tr>
<td>Lower copper feeding (58)</td>
<td></td>
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<tr>
<td>Antioxidant and multifactorial factors</td>
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<tr>
<td>Lipoic acid (107)</td>
<td></td>
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<tr>
<td>IV. Protection against vascular and glomerular effects</td>
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<tr>
<td>Suppression of renin-angiotensin activation</td>
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<tr>
<td>Protection against Ca^{2+} influx</td>
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<tr>
<td>Undefined mechanism</td>
<td></td>
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<td>V. Increase in kidney regeneration capabilities</td>
<td></td>
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<tr>
<td>Unspecific mitogenic effect</td>
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<tr>
<td>Growth factors</td>
<td></td>
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</tbody>
</table>

* References refer to publications dealing with the proposed mechanism; see text for further details on the extent and characterization of the protection.
* Mechanism is assumed on the basis of the substitution made (see reference 83 for a discussion and references to original papers), but it has not actually examined.
Aminoglycoside toxicity is **not** linked to peak ...
Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...

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

this is where patients are in a q8h schedule!!
Phospholipiduria …

**URINARY EXCRETION OF PHOSPHATIDYLINOSITOL**

- Netilmicin TID
- Netilmicin qD
- Amikacin BID
- Amikacin qD

Tulkens et al., 1989
And auditory alterations ...

<table>
<thead>
<tr>
<th></th>
<th>Low tone (0.25-8 kHz)</th>
<th>High tone (10-18 kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amikacin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• q24h</td>
<td>1 (1)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>• q12h</td>
<td>0</td>
<td>6 (6)</td>
</tr>
<tr>
<td><strong>Netilmicin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• q24h</td>
<td>0</td>
<td>3 (7)</td>
</tr>
<tr>
<td>• q8h</td>
<td>2 (3)</td>
<td>8 (9)</td>
</tr>
</tbody>
</table>

* loss of 15dB or more over baseline (max. loss recorded: 30 dB)

Tulkens et al., 1989
Aminoglycoside peak/MIC ratio is predictive of clinical efficacy.

Relationship between the maximal peak level/MIC ratio and the rate of clinical response. Vertical bars represent SE values.

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)
And what bout cis-platin now …

- induces a more chronic type of renal failure, mostly in S3 segment
- with large focal necrosis, cyst formation and marked interstitial proliferation and fibrosis.
- Cis-platin causes intrastrand cross-linking in DNA, blocking all subsequent DNA-dependent activities.
- It also triggers apoptosis.
- Compared to AG, post-necrosis and post-apoptotic regeneration seem largely impaired in cis-platin renal toxicity.
Cisplatin induces widespread tubular necroses and destruction.

5 days after a single 5 mg/kg body weight injection
From: Sheikh-Hamad et al., Arch Toxicol. 2003

7 days after a single 8 mg/kg body weight injection
Courtesy of G. Laurent
Cyst formation and failing regeneration...

21 days after a single 8 mg/kg body weight injection
(BdU-PAP staining)
Courtesy of G. Laurent, 2004
But also extended apoptosis ...

Tunel staining

Sheikh-Hamad et al., Arch Toxicol. 2003 Oct 10 [Epub ahead of print]
through caspase activation …

Sheikh-Hamad et al., Arch Toxicol. 2003 Oct 10 [Epub ahead of print]
Extrinsic pathway

Fas
TNF-α
Pro-caspase 8

APOPTOSIS: Signaling pathway for cis-platin?

FADD,

DISC

caspase 8

caspase 2 → Pro-caspase 3 (6-7) → caspase 3

Golgi

Pro-caspase 3

Nucleus

PARP, lamin

ICAD,...
Towards a mechanism of toxicity …

• platinum is accumulated by renal tissue against a concentration gradient.
• it is thought to produce renal damage because of interaction with renal sulfhydryl (SH) groups and ensuing depletion of SH groups (and the same mechanism may be operating in cochlea)

Dobyan et al., 1980; Weiner et al., 1983; Singh et al., 1988; Ravi et al., 1995; Towsend et al., 2003
Towards a mechanism of toxicity …

• the steroisomer trans-platin is not toxic …

• cisplatin may be activated in the kidney to a toxic metabolite through the same pathway that has been shown to activate the halogenated alkenes.

• Inhibition of cysteine-S-conjugate beta-lyase reduces toxicity in cultured cells

Dobyan et al., 1980; Weiner et al., 1983; Singh et al., 1988; Ravi et al., 1995; Towsend et al., 2003
Proposed pathway based on the metabolism of the glutathione-conjugates of the halogenated-alkenes to nephrotoxins (Anders and Dekant, 1998). This mechanism distinguishes toxicity towards dividing cells (anticancer effect) and quiescent cells (nephrotoxicity). The key and final event is the conversion to a highly reactive thiol by cysteine S-conjugate β-lyase.

Towards a protection by antioxidants...

Effects of antioxidants on cisplatin-induced cytotoxicity in M-1 cells (treated for 2 hr with 0.5 mM cisplatin) and with:

- 30 µM DMTU (dimethylthiourea)
- 50 µM DFO (deferoxamine)
- 10 µM DPPD (diphenyl-p-phenylene-diamine)

and also in animals ...

Figure 1. Effects of buthionine sulfoximine, glutathione and methimazole on cisplatin-induced nephrotoxicity in rats. Each point represents the mean ± s.d. (n = 4). **P < 0.01 compared with cisplatin.

Thanking people for work on aminoglycosides...
and a bit on cis-platin ...

Starting very basically in the early 80’s ...

G. Laurent
M.B. Carlier
R. Brasseur
J.M. Ruysschaert
Aminoglycosides...
*The once-a-day story...*  
with also a lot of histopathology, rat killing and urine collection ...

In the late 80’s ...

S. Ibrahim  
P. Maldague  
L. Giurgea  
F. Renoird  
M.C. Cambier  
G. Laurent  
D. Beauchamp

and

F. Clerckx-Braun (FATC)  
J. Donnez (St Luc)  
M.P. Mingeot  
P. Lambricht  
R. Wagner  
B. Rollmann (CHAM)  
P. Herman (SP-Belg.)  
M.E. De Broe (UZ-UIA)  
G. Verpooten (UZ-UIA)  
A. Giuliano (UZ-UIA)  
B. Kaufman (VUB)  
B. Derde (VUB)
And here is a next generation, and they work(ed) on a lot of things …

M.P. Mingeot
F. Van Bambeke

polyaspartic acid

B.K. Kishore
Z. Kallay

M. El Mouedden
H. Servais

phospholipidosis

apoptosis