Aminoglycosides: a new look at old but probably faithfull antibiotics *

* if you can use them properly …

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What do I do (and where do I do it)?

- Teaching of Pharmacology and Pharmacotherapy
- Post-graduate training on Drug Development
- Launching of Clinical Pharmacy in Europe
- Web-based courses on anti-infective Pharmacology
- 15 graduating students, doctoral fellows and post-graduate fellows working on anti-infective therapy (laboratory and clinical applications)
- Toxicity, medicinal chemistry, and improved schedules of aminoglycosides
- novel beta-lactams, and continuous infusion
- fluoroquinolones efflux and PK/PD
- Novel glycopeptides and derivatives thereof and models of intracellular infection

www.facm.ucl.ac.be

- Editorial board of AAC
- Member of the General Committee of EUCAST (for ISC)
- Member of the Belgian Antibiotic Policy Coordination Committee
- Founder and Past President of the International Society of Antiinfective Pharmacology (ISAP)

www.isap.org

A partial view of our University Clinic (900 beds) and the Education and Research buildings (5,000 students), in the outskirts of Brussels, Belgium
Streptomycin: the first aminoglycoside

- discovered by par Waksman at Rutgers University in New Jersey in 1943
- broad spectrum including Gram (+) and Gram (-), and *Mycobacterium tuberculosis*
- highly bactericidal
- but gave rapidly rise to resistance (ribosomal alteration [target modification])
- well know for its ototoxic pontential (more for dihydrostreptomycine), but largely due to its use for prolonged treatments
- rarely used nowadays except for tuberculosis (2d or 3d line), tularemia, plague, and, sometimes, endocarditis
Streptomycin was the first antibiotic to be discovered by systematic screening

From the point of view of human benefit, never was a Nobel prize so justifiably awarded as was the award to Selman Waksman for the discovery of streptomycin and other antibiotics produced from *Streptomyces spp.* Waksman and his talented team (many of whom went on to make important antibiotic discoveries in their own right) developed the concept of systematic screening of microbial culture products for biological activity, a technology which has provided the foundation of the antibiotic industry, and for this alone his name should rank high in any pantheon of microbiology.

J. Davies: In Praise of Antibiotics, ASM News
Main clinically usable aminoglycosides in the 80's...

flanked with 2 aminosugars

build up around a 4,6 substituted 2-deoxystreptamine

Aminoglycoside  R₁  R₂  R₃  R₄  R₅  R₆'  R₇  R₈  R₉  R₁₀
Kanamycin A   OH  OH  OH  H  NH₂  H  CH₃OH  OH  H  H
Kanamycin B   NH₂  OH  OH  H  NH₂  H  CH₃OH  OH  H  H
Kanamycin C   NH₂  OH  OH  H  OH  H  CH₃OH  OH  H  H
Amikacin      OH  OH  OH  H  NH₂  COR  CH₃OH  OH  H  H
Tobramycin    NH₂  H  OH  H  NH₂  H  CH₃OH  OH  H  H
Diberakin      NH₂  H  H  H  NH₂  H  CH₃OH  OH  H  H
Arbekacin      NH₂  H  H  H  NH₂  COR  CH₃OH  CH₃  H  H
Gentamicin C₁  NH₂  H  H  CH₃  NHCH₃  H  H  CH₃  OH  CH₃
Gentamicin C₂  NH₂  H  H  H  NH₂  H  CH₃  OH  CH₃
Gentamicin C₃  NH₂  H  H  CH₃  NH₂  H  CH₃  OH  CH₃
Gentamicin C₄  NH₂  H  H  H  NH₂  H  CH₃  OH  CH₃
Gentamicin C₅  OH  OH  OH  H  NH₂  COR  CH₃  OH  CH₃
Sisomicin     --  --  --  --  --  H  H  CH₃  OH  CH₃
Netilmicin    --  --  --  --  --  --  CR''  H  CH₃  OH  CH₃

* R = CHOCHOCH₂NH₂; R' = CHO(CH₂)₂NH₂; R'' = CH₂CH₃
(a) = primed sugar for sisomicin and netilmicin
What were the advantages of aminoglycosides as seen in the mid 80's?

- **Microbiology**
  - wide spectrum, but especially active against Gram (-) organisms including "difficult" ones (*P. aeruginosa*, *Serratía*, etc…)
  - concentration-dependent bactericidal activity (related to peak) with prolonged post-antibiotic effect ...
  - low propensity to cause resistance (and possibility to rotate among derivatives with distinct resistance patterns)
  - synergy with cell-wall acting agents with no cross-resistance ...

- **Pharmacokinetics:**
  - no metabolism, few drug interactions, rapid elimination (except kidney) ...
  - linear pharmacokinetics and predictable blood levels
  - several fast methods for monitoring

- **Pharmaceutics:**
  - excellent shelf stability
  - cheap to make …

Aminoglycosides in the 80’s: Questions raised ...

- Can they be really be used without fearing resistance?
- What is the real risk (and liabilities) of toxicity?
  - nephrotoxicity (reversible ...)
  - ototoxicity (irreversible !)
- All seem to have quite similar biophysical, chemical, microbiological and pharmacokinetic properties, but...
  - are they (some and real) differences in toxicities that may suggest the preferential use of one over the others (beyond differences in susceptibility to resistance mechanisms)?
  - can we further dissociate activity and toxicity?
  - what is/are the mechanism(s) of these adverse effects?
  - can we protect patients?
Activity
(and resistance)

...
Aminoglycosides: mode of action (the classical view)...

Figure 46–2. Effects of aminoglycosides on protein synthesis.

A. Aminoglycoside (represented by closed circles) binds to the 30 S ribosomal subunit and interferes with initiation of protein synthesis by fixing the 30 S–50 S ribosomal complex at the start codon (AUG) of mRNA. As 30 S–50 S complexes downstream complete translation of mRNA and detach, the abnormal initiation complexes, so-called streptomycin monosomes, accumulate, blocking further translation of message. Aminoglycoside binding to the 30 S subunit also causes misreading of mRNA, leading to B. premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein, or C. incorporation of incorrect amino acids (indicated by the “X”), resulting in the production of abnormal or nonfunctional proteins.
Recent views on the mode of action of aminoglycosides

- **Resting stage**: The pairing of mRNA-tRNA is correct.
- **In the presence of an aminoglycoside**: The binding of the aminoglycoside causes a protrusion of A1492 without need for energy. The pairing will always be considered as correct. The code is "confirmed" but the actual pairing may be incorrect (wrong tRNA).

How and why were the main aminoglycosides used in the 90's (and still now) developed?

- reasonable activity against Gram (-) organisms resistant to SM
- moderate toxicity

kanamycin A

largest commercial success since its launch in 1965!!
The "gentamicin"...
Emergence of resistance through enzymatic inactivation (drug modification)

kanamycin B

2'-acetylation
3'-phosphorylation
4'-adenylation
6'-acylation
3-acetylation
2''-adenylation
2''-phosphorylation

kanamycin A

gentamicin C1 : R_1 = CH_3; R_2 = CH_3

gentamicin C1a: R_1 = H; R_2 = H

gentamicin C2: R_1 = CH_3; R_2 = H

Aminoglycosides
Groton, CT, 12 Apr. 2006
• more active than kanamycine A
• but more toxic …

A partial response

• less toxic
• resistant to 3'phosphotransferases (rares)
• more active against *Pseudomonas*
→ large clinical success (1975-1995)

• more toxic plus toxique
• resistant to 3' phospho- et 4' adnylyl transferases (rares)
• no advantage for *Pseudomonas*
• weak towards *Serratia*
→ no success outside Japan (1975-1995)
A more fundamental response ...

- activity largely maintained raisonnable
- decreased toxicity (but disputed)
- resistance to enzymes acting on 2'' and 3 (frequents) et naturally insensitive to those acting on 2' (frequents)

➔ large clinical success from 1985…
Some less used 1-N substituted aminoglycosides ...

- gentamicine C1a: $R_1 = H$; $R_2 = H$
- gentamicine B
- isepamicin
- netilmicin

- reasonable activity
- resistant to some enzymes (less than amikacin)
- toxicity largely controversial
  - variable success (1985…)
- activity = amikacin
- toxicity $\leq$ amikacin
- resistant to inactivation $\geq$ amikacin
  - good clinical success in Japan only
And still another less used 1-N substituted aminoglycoside ...

- activity > amikacin
- toxicity ? (controversies)
- resistant to inactivation ≥ amikacin and to the bifunctional enzymes

⇒ used sparingly in Japan
The situation in the mid-90's

- **gentamicin**: faces the largest rate of resistance but still remain active in a large number of situations
- **tobramycin**: becomes mostly reserved for *P. aeruginosa* infections (because of lower MIC's), although it is said to be less toxic than gentamicin;
- **amikacin**: becomes widely used (active against resistance strains; probably less toxic [although this is hotly debated]; and … good marketing…); *isepamicin (which is slightly superior to amikacin)* remains confined to Japan
- **arbekacin**: (HABA derivative of dibekacin) acquires a special niche in Japan because of an unanticipated activity against methicillin-resistant S. aureus (active against the bifunctional enzyme)
- the non "4,6 disubstituted 2-deoxystreptamine" aminoglycosides ("non classical") are almost not used in human medicine but have niches in veterinary medicine and/or are used for resistance diagnostic and research purposes
Some non-classical aminoglycosides ...

4,5-DISUBSTITUTED DEOXYSTREPTAMINE

Aminoglycoside | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$
--- | --- | --- | --- | --- | ---
Neomycin B | H | NH$_2$ | OH | X | H
Paromomycin I | H | OH | OH | X | H
Lividomycin A | H | OH | H | X | Manoside
Ribostamycin | H | NH$_2$ | OH | H |
Baltrocin B | Y | NH$_2$ | OH | H |

$X = \begin{array}{c}
\text{NH}_2 \\
\text{H}_2\text{N} \\
\text{O} \\
\text{NH}_2 \\
\text{OH} \\
\end{array}$

$Y = \begin{array}{c}
\text{OH} \\
\text{C} \\
\text{O} \\
\text{NH}_2 \\
\end{array}$

$B \quad C$

$R = \begin{array}{c}
\text{H} \\
\text{NH}_2 \\
\text{CH}_2\text{OH} \\
\text{HNC}_3 \text{H} \\
\text{H}_3\text{N} \\
\text{NH}_2 \\
\end{array}$

Dactimycin | NH-CH=NH

Apramycin
Resistance from the mid-90's to now …

- enzyme-mediated resistance remains the main mechanism and gets highly complex by the simultaneous presence of distinct enzymes, and the occurrence of bifunctional ones…

- efflux has been described in *P. aeruginosa* and explains the relatively low activities observed against this species

- a new mechanism of ribosomal methylation (arm) has been described that causes resistance to all 2,4 disubstituted deoxystreptamine-containing aminoglycosides and to fortimicin (but not to paromomycin and similar derivatives). It is plasmid-mediated and could, therefore, spread easily…
Enzyme-mediated resistance in the late 90's...

FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (phenotypic classification; each phenotype comprises several distinct gene products [denoted by lowercase letters after the roman numeral in the text]); at least one enzyme is bifunctional and affects both positions 2' (O-phosphorylation) and 6' (N-acetylation). The main clinically used aminoglycosides on which these enzymes act are as follows: amikacin (A), dibekacin (Dbk), commercial gentamicin (G) (see text), gentamicin B (GbM), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S), and tobramycin (T) (see text for discussion of arbekacin, sagamicin, and dactinomycin). The drug abbreviations which appear in parentheses are those for which resistance was detectable in vitro even though clinical resistance was not conferred. Based on the data of Shaw et al. (89).
Efflux...

Contribution of the MexX-MexY-OprM Efflux System to Intrinsic Resistance in Pseudomonas aeruginosa

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To test the possibility that MexX-MexY, a new set of efflux system components, is associated with OprM and contributes to intrinsic resistance in Pseudomonas aeruginosa, we constructed a series of isogenic mutants lacking mexXY and/or mexAB and/or oprM from a laboratory strain PAO1, and examined their susceptibilities to ofloxacin, tetracycline, erythromycin, gentamicin, and streptomycin. Loss of either MexXY or OprM from the MexAB-deficient mutant increased susceptibility to all agents tested, whereas loss of MexXY from the MexAB-OprM-deficient mutant caused no change in susceptibility. Introduction of an OprM expression plasmid decreased the susceptibility of the mexAB-oprM-deficient-mexXY-maintaining mutant, yet caused no change in the susceptibility of a mexAB-oprM- and mexXY-deficient double mutant. Immunoblot analysis using anti-MexX polyclonal rabbit serum generated against synthetic oligopeptides detected expression of MexX in the PAO1 cells grown in medium containing tetracycline, erythromycin, or gentamicin, although expression of MexX was undetectable in the cells incubated in medium without any agent. These results suggest that MexXY induced by these agents is functionally associated with spontaneously expressed OprM and contributes to the intrinsic resistance to these agents.
Efflux…

Observed in

- *S. aureus* (MdeA [MFS])
- *E. coli* (MdfA, SetA [MFS]; AcrD [RND])
- *S. maltophilia* (SmeE [RND])
- *P. aeruginosa* (MexXY [RND]; constitutively expressed but may be overproduced in resistant strains)

Responsible for

- low intrinsic susceptibility ... (intrinsic resistance)
- adaptative resistance (post-exposure effects)
- cross resistance to most 4,6 disubstituted-2-deoxystreptamine containing aminoglycosides (previously considered as permability mutants)

Plasmid-Mediated High-Level Resistance to Aminoglycosides in *Enterobacteriaceae* Due to 16S rRNA Methylation

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A self-transferable plasmid of ca. 80 kb, pIP1204, conferred multiple-antibiotic resistance to *Klebsiella pneumoniae* BM4536, which was isolated from a urinary tract infection. Resistance to β-lactams was due to the *blaTEM*1 and *blaCTX-M* genes, resistance to trimethoprim was due to the *dhfrII* gene, resistance to sulfonamides was due to the *sulf* gene, resistance to streptomycin-spectinomycin was due to the *ant3*9 gene, and resistance to nearly all remaining aminoglycosides was due to the *aac3-II* gene and a new gene designated *armA* (aminoglycoside resistance methylase). The cloning of *armA* into a plasmid in *Escherichia coli* conferred to the new host high-level resistance to 4,6-disubstituted deoxystreptamines and fortimicin. The deduced sequence of ArmA displayed from 37 to 47% similarity to those of 16S rRNA m7G methyltransferases from various actinomycetes, which confer resistance to aminoglycoside-producing strains. However, the low guanine-plus-cytosine content of *armA* (30%) does not favor an actinomycete origin for the gene. It therefore appears that posttranscriptional modification of 16S rRNA can confer high-level broad-range resistance to aminoglycosides in gram-negative human pathogens.
armA resistance ... and other methylases ...

- *armA* originally in Klebsiella pneumoniae together with the *bla*(TEM1) and *bla*(CTX-M) genes
  - act by methylation of the 16S RNA (target modification)
  - affects all aminoglycosides except streptomycin
  - difficult to detect specifically in clinical microbiology laboratories unless including a fortimicin susceptibility test (non classical aminoglycoside)
  - may be more widespread than originally thought and could spread fast because it is carried on a conjugative plasmid flanked by putative transposable elements

- but several other plasmid-mediated 16S rRNA methylases identified in pathogenic *Enterobacteriaceae* (*RmtC, RmtB, and RmtA*).

⇒ The acceleration of aminoglycoside resistance among Gram (-) bacilli by plasmid-mediated 16S rRNA methylases may become an actual clinical hazard in the near future ...
Toxicity …

This is where disputes come into the picture…
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
Aminoglycosides toxicity incidence is highly variable among patient populations

Patients with nephrotoxic reaction after treatment with gentamicin

- young volunteers
- random hospital populat.
- critically-ill patients

Smith et al., 1980
Plaut et al., 1979
Smith et al., 1982

All those patients were under close monitoring...
Why do we see such a variation?

**PROVEN, CLINICALLY RELEVANT RISK FACTORS IN AMINOGLYCOSIDE NEPHROTOXICITY***

**Patient-related**
- Age
- Large initial creatinine clearance
- Impaired renal function (if dose not adjusted)
- Liver disease
- Critically ill state and shock
- High tissue accumulation

**Treatment-related**
- High peak levels**
- Sustained elevated levels***
- Total dose
- Duration of treatment
- Co-administration of other potentially nephrotoxic drugs (vancomycin, cephaloridine and perhaps cefalothin, but not other beta-lactams, amphotericin, cisplatin)
- Co-administration of loop diuretics and volume-depleting agents

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* Based partly on Refs. 9 and 55 and various reports on animal studies.
** For the schedule of administration considered. Thus, patients treated once a day may have much higher peak levels than patients treated three times a day, without signs of toxicity. Determination of standards for peak levels in the once-a-day regimen have, however, not yet been determined.
*** Usually determined 8 h after last administration; sustained levels usually related to inadequate elimination, tissue storage and/or too frequent dosing and are therefore highly indicative of potential toxicity.

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*Toxicology Letters, 46 (1989) 107–123*
High doses in animals cause precipitous renal nevrosis, tubular dysfunction, and renal failure associated with regeneration.

Fig. 1. Renal changes in Fischer 344 rats after gentamicin (40 mg/kg per day in two injections per day). From Ref. 13.

But low doses allow to observe a clear succession of events …
A look in the microscope in a rat treated with low doses …
(10mg/kg)
Somewhat closer in the control …
Compare …
And examine …
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 *...

binding to
• megalin
  (Moeströp et al., 1995)
• acidic phospholipids
  (Humes et al., 1983)

  Silverblatt & Kuehen, Kidney Intern., 1979
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.

*Adapted from Brasseur et al., 1989*

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

Hematoxylin/eosin


Tunel

What is the mechanism of gentamicin–induced apoptosis and its relation to necrosis in kidney cortex?

FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-like structures, most commonly referred to as myeloid bodies); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses (C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. The figure is adapted from reference 76 and is based on the typical descriptions given in references 38, 40, 71, 76, 77, 127, and 138.
Gentamicin-induced apoptosis can be reproduced with cultured kidney and non-kidney cells …

El Mouedden et al., Toxicol. Sci., 56:229-239, 2000
APOPTOSIS: main signaling pathways ...

**Extrinsic pathway**

Fas  
TNF-α  
Pro-caspase 8  
caspase 8  
Pro-caspase 8 (6-7)  
caspase 3 (6-7)  
PARP, lamin  
ICAD,...

**Intrinsic pathway**

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

Mitochondria

Aminoglycosides  Groton, CT, 12 Apr. 2006
APOPTOSIS and aminoglycosides

Extrinsic pathway

Fas
TNF-α
Pro-caspase 8

FADD,

Pro-caspase 8

caspase 8

FADD

DISC

Intrinsic pathway

Mitochondria

Cytochrome c

Bcl-2, Bcl-xl

Pro-caspase 9

Pro-caspase 3 (6-7)

Caspase 3 (6-7)

Lysosome

Lysosomal proteases

tBid, Bax, Bak

Bid

Nucleus

PARP, lamin
ICAD,...
Could lysosomal rupture cause apoptosis and necrosis?
Are lysosomes disrupted by gentamicin?

Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 μg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 μM, 3 h).

What if you by-pass lysosomes?

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.

Bypassing lysosomes in cultured cells ...
Towards a renewed hypothesis …

• gentamicin enters proximal tubular cells by megalin- and acid phospholipids mediated pinocytosis and ends up in lysosomes

• a minor part escapes lysosomes either by membrane destabilization (our hypothesis) or by retrograde transport (Molitoris' hypothesis) to reach the cytososol and the mitochondria … where it induces apoptosis and other toxic disturbances…

• you could prevent toxicity either
  – by impairing the pinocytic uptake of aminoglycosides, or making an aminoglycoside that does not bind to megalin…
    ➔ block or avoid step one …
  – developing an that does not destabilize lysosomes and/or does not cause apoptosis …
    ➔ block step 2 and/or its consequences…
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>
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## Table 2. Main approaches toward reduction of aminoglycoside nephrotoxicity

<table>
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<tr>
<th>Mechanism</th>
<th>Compound</th>
</tr>
</thead>
</table>
| I. Decrease or prevention of drug accumulation by kidneys | Dextran sulfate (59)  
Polyanionic compounds  
Inositol hexaniurate (67)  
Acidic drugs  
Piperacillin (44)  
Lamotrigine-morolactam (68)  
Fosfomycin (33, 54)  
Pyridoxal-5’-phosphate (114)  
Competition with or decrease in aminoglycoside binding to brush border membrane | Bicarbonate (19, 20)  
Compeitors  
Ca²⁺ (diet supplementation [51] or vitamin D-induced hypercalcaemia [21])  
Lysine (81)  
Aminoglycosides (as their own competitors) (30)  
Increase in exotoxicity | Heroxacin (9)  
II. Prevention or decrease of lysosomal phospholipase inhibition  
Derivatives with lesser intrinsic binding*  
N-substitution | Amikacin (75), isepamicin (133), arbekacin,* 1-N- and 6-N-peptidic and aminoglycosidic derivative of kanamycin A and neamine (72)  
Other substitution  
6’-substituted kanamycin B (88)  
Fluorinated derivatives*  
5, 3’ or 3’ fluoro derivatives of tobramycin, dibekacin, arbekacin, or kanamycin  
Disaccharide aminoglycosides | Astromicin (fortimicin) (73)  
Dactinomycin (2-N-formacyl-astromicin) (53, 73)  
Co-administration of agent preventing intralysosomal phospholipidosis  
Intralysosomal sequestration of aminoglycosides  
Increase of membrane negative change  
Other | Polyaspartic acid (55, 62)  
Daptomycin (41)  
Tobramycin (32)  
III. Protection against necrosis and other gross cellular alterations | Deferoxamine (11)  
Mercaptoproto (24)  
Sodium (84)  
Vitamin E + selenium, vitamin C (1, 57)  
Lower copper feeding (58)  
Anioxidant and multifactorial factors | Lipoic acid (107)  
IV. Protection against vascular and glomerular effects  
Suppression of renin-angiotensin activation  
Protection against Ca²⁺ influx  
 Undefined mechanism | Dexethionine and saline drinking (45)  
Ca²⁺-channel blockers (97)  
Platelet activation antagonists (104)  
V. Increase in kidney regeneration capabilities  
Unspecific mitogenic effect  
Growth factors | Ulinastatin (92)  
Fibroblast growth factor 2 (78)  
Heparin-binding epidermal growth factor (106)  
* References refer to publications dealing with the proposed mechanism; see text for further details on the extent and characterization of the protection.  
* See reference 83 for structures  
* 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
Phospholipiduria …

URINARY EXCRETION OF PHOSPHATIDYLINOSITOL

nmol/g creatinine (Thousands)

Tulkens et al., 1989
And auditory alterations …

no. of patients [over 20 in each group] with lesions* and total no. of frequencies affected

<table>
<thead>
<tr>
<th></th>
<th>low tone (0.25-8 kHz)</th>
<th>high tone (10-18 kHz)</th>
</tr>
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<tbody>
<tr>
<td>amikacin</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>netilmicin</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.

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)
Conclusions

- aminoglycosides remain, even in 2005, potent and useful drugs against Gram (-) organisms if
  - appropriate resistance surveillance is in place
  - accepting that they need to be administered by intravenous route
  - toxicity is minimized by using a once-daily (extended interval) schedule and taking the known risk factors in due consideration...

- it could be possible to design/screen for new aminoglycosides with reduced toxicity based on our present knowledge of its mechanisms

- medicinal chemistry is needed to find new ways to avoid resistance (drug inactivation and target mutation...); additional screening may be needed to avoid efflux ...

- new aminoglycosides made along these lines could be important drugs in the future because of the demise of many other classes towards Gram (-) organisms (β-lactams, fluoroquinolones, ...)
Why not?
It all started only a few years ago …
It all started only a few years ago …

But, what about him (her ?)