Intracellular mechanisms of apoptosis induced by aminoglycoside antibiotics

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Introduction
What do we expect from an antibiotic?
Aminoglycoside antibiotics: potent and useful...

- Treatment of severe infections caused by Gram negative bacteria (Drusano et al., 2007; Lopez-Novoa et al., 2011)
- Treatment of genetic diseases? (Malik et al., 2010)
- Antiviral? (Houghton et al., 2010)
BUT... toxic!

- Cochlear and vestibular toxicity

- Nephrotoxicity: 10-25% of treatments
  
  - Clinical features:
    - non-oliguric renal failure
    - Slow rise in creatinine

  - Risk factors: duration of the treatment, older age, reduced renal function, hepatic dysfunction, interactions with other drugs
Nephrotoxicity of aminoglycosides

De Broe et al., 1984
Kidney structure
Nephrotoxicity of aminoglycosides

De Broe et al., 1984  Giurgia-Marion et al., 1986
El Mouedden et al., 2000a
Tubular toxicity

- Release of brush border and lysosomal enzymes
- Decreased reabsorption of filtered proteins
- Wasting $K^+$, $Mg^{2+}$, $Ca^{2+}$ and glucose
- Phospholipiduria
Lysosomal alterations induced by aminoglycoside antibiotics

Inhibition of lysosomal phospholipases

From Mingeot-Leclercq, 1999
Lysosomal alterations induced by aminoglycoside antibiotics

Phospholipidosis induced in renal proximal tubular cells of rats treated with low, therapeutically-relevant doses.

From Tulkens, 1986
Induction of apoptosis

Rats treated for 10 days with saline (control), 10 mg/kg of gentamicin and netilmicin, or 40 mg/kg of isepamicin and amikacin

El Mouedden et al., 2000a
Apoptosis

- Active form of cell death
- Embryogenesis
- Maintenance of homeostasis
- Implicated in cancer, neurodegenerative diseases, ...
- Can be induced by exposure of the cell to a toxic substance or drug
Nephrotoxicity of aminoglycosides

De Broe et al., 1984
Giurgia-Marion et al., 1986
El Mouedden et al., 2000a
El Mouedden et al., 2000b
Servais et al., 2005
Servais et al., 2006
Apoptosis induced by gentamicin

Mitochondria
Cytochrome c
Caspase 9
Caspase 3
Bax

Endosome
Lysosome

Golgi
ER

Apoptosis
Aim of the study: How gentamicin induces apoptosis?
Part I

Endosome

Golgi

Lysosome

Mitochondria

Cytochrome c

Caspase 9

Caspase 3

Apoptosis
ENDOSOME

LYSOSOME

MITOCHONDRIA

CYTOCHROME c

CASPASE 9

CASPASE 3

APOPTOSIS

PART II: OTHER AG?
Part III

Apoptosis
Results
Part I

GENTAMICIN-INDUCED LYSOSOMAL MEMBRANE PERMEABILIZATION AND MECHANISM INVOLVED
Does gentamicin induce lysosomal membrane permeabilization?
Follow of LMP by vital imaging with Lucifer Yellow

- pH insensitive, membrane bilayer-impermeant
- Increase of sensitivity of the method by inhibiting the organic anion transporter with probenecid
- MitoTracker red

Scale bars = 20 µM
Gentamicin induces lysosomal membrane permeabilization

What is the underlying mechanism?
Implication of Reactive Oxygen Species ??

DNA

\[ \text{O}_2^{*} \]

\[ \text{H}_2\text{O}_2 \]

\[ \text{\cdotOH} \]

Proteins

Lipids
Role of ROS in GEN-induced lysosomal permeabilization

Priusca et al.
Intracellular localisation of GEN-induced ROS production

GEN-induced ROS production occurs in lysosomes

Scale bars = 5 µM
Implication of Reactive Oxygen Species ??

N-acetylcysteine

O$_2$$^{-}$

H$_2$O$_2$

•OH

Catalase

Lipids

Proteins
Protection afforded by anti-oxidant molecules

Partial protective effect of catalase (1.000 U/ml) and N-acetylcysteine (1 mM) against GEN-induced ROS production and apoptosis induction (nuclear fragmentation)
Implication of iron evaluated with the lysosomal iron chelator deferoxamine (DFO)

DFO partially reduces GEN-induced ROS production and apoptosis.
Gentamicin induces lysosomal ROS production, lysosomal membrane permeabilization and apoptosis

Lysosomal iron chelator and antioxidants afford partial protective effect against these events
Part II

COULD WE GENERALIZE THE POTENTIAL EFFECT OF CYTOSOLIC GENTAMICIN TO OTHER AMINOGLYCOSIDES?
Choice of molecules

- 2 nephrotoxic aminoglycosides: GENTAMICIN and NEOMYCIN B
- 2 less nephrotoxic aminoglycosides: AMIKACIN and ISEPAMICIN
Less nephrotoxic aminoglycosides induce less apoptosis
Importance of apoptosis in aminoglycoside-induced nephrotoxicity
Part III

P53 SIGNALING PATHWAY, PROTEASOME IN GENTAMICIN-INDUCED APOPTOSIS
Is p53 signaling pathway implicated in gentamicin-induced apoptosis?

- p53 is a transcription factor with an important role in apoptosis induction...

Amaral et al., 2010
Is p53 signaling pathway implicated in gentamicin-induced apoptosis?

Pifithrin α, a p53 inhibitor, affords a partial protective effect against gentamicin-induced apoptosis.
A role of proteasome?

- Non-lysosomal degradation of proteins, important role in regulation of many cellular processes.

- Large multi-subunit protein complex comprised of a peptide degrading 20S core cylinder capped at both ends by a 19S regulatory cap.
  - β1: caspase-like activity: cleaves after acidic residues
  - β2: trypsin-like activity: cleaves after basic residues
  - β5: chymotrypsin-like activity: cleaves after hydrophobic residues

Ubiquitin-proteasome system
Inhibition of proteasome by gentamicin?

- Increase in the cell content of pro-apoptotic Bax protein and Ub-Bax after incubation with GEN (Servais et al., 2006)

- Binding of GEN to β9-subunit of proteasome (Horibe et al., 2004)

Does gentamicin inhibit the proteasome?
Effect of gentamicin on proteasome catalytic activities in cellular lysates

A. Chymotrypsin-like activity

B. Trypsin-like activity

C. Caspase-like activity

Fluorescence 460 nm (arbitrary units)

GEN concentration (mM)

0 5 10 15 20 25 30

Fluorescence 460 nm (arbitrary units)

EPX SA
Impact on ubiquitinated proteins degradation
Conclusions
Endosome

Golgi

Lysosome

Fe^{2+}

ROS

Mitochondria

Bax

Cytochrome c

Caspase 9

Caspase 3

Proteasome inhibition?

Apoptosis

p53
Perspectives
Could we generalize the concept of ROS production to other AG?
Involvement of cathepsins release?
Roles of p53, NFκB?

- Endosome
- Lysosome
- Mitochondria
- Cytochrome c
- Caspase 9
- Caspase 3
- Proteasome inhibition?
- Apoptosis

- Golgi
- ER

- Bax

- Fe²⁺
- ROS

- Roles of p53, NFκB?
Nephrotoxicity of aminoglycosides
Thank you for your attention
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