Lysosomal Membrane Permeabilization, a Key Event for Apoptosis Induced by Aminoglycoside Antibiotics.

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BACKGROUND AND AIM OF THE STUDY

- Aminoglycoside antibiotics are used to treat life-threatening Gram negative infections. But at therapeutics concentrations, they accumulate in lysosomes and induce apoptosis in kidney proximal tubules *(El Mouedden et al., Antimicrob Agents Chemother. 2000;44:665-75)*. Incubation of renal cells with gentamicin (GEN) leads to successive alteration of the permeability of lysosomes, triggering the mitochondrial pathway, and activation of caspase 3 *(Servais et al., Toxicol Appl Pharmacol. 2005; 206:321-33)*.
- Gentamicin is known to form complexes with iron and arachidonic acid which can be responsible of formation of free radicals *(Priuska et al., Inorganica Chim.Acta 1998; 273 : 85-91)*

The aim of this study is to examine ROS production as a possible cause of gentamicin-induced lysosomal permeabilization and apoptosis, and the implication of iron in these events.

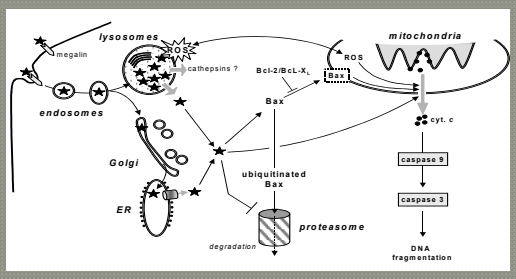
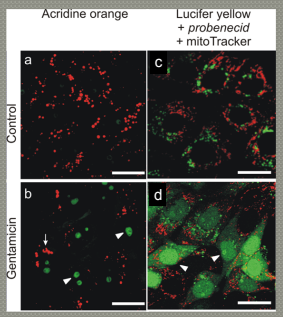


Fig.1 : Main mechanisms of renal toxicity of gentamicin with emphasis on apoptosis. The drug is symbolized by a black filled star. From Servais *et al.*, Apoptosis 2008; 13:11-32

RESULTS

1. GEN-induced lysosomal membrane permeabilization



Treatment of cells with 3mM gentamicin induces lysomomal membrane permeabilization

Fig.2 : Lysosomal membrane permeabilization upon GEN treatment. Cells were loaded with acridine orange (a,b) or lucifer yellow, probenecid and MitoTracker (c,d).

2. GEN-induced ROS production in lysosomes

GEN-induced ROS production is mainly localized in lysosomes.

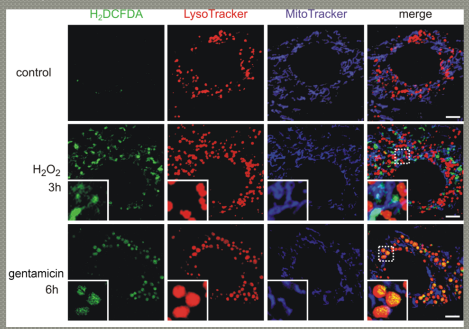
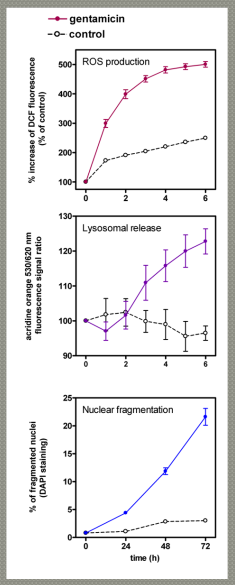


Fig.3 : Intracellular localization of ROS production upon gentamicin (2 mM) and H_2O_2 (200 µM) treatments. Cells were incubated with H$_2$DCFDA (to detect ROS production) in combinaison with LysoTracker (to detect lysosomes) and MitoTracker (to evidence mitochondria).

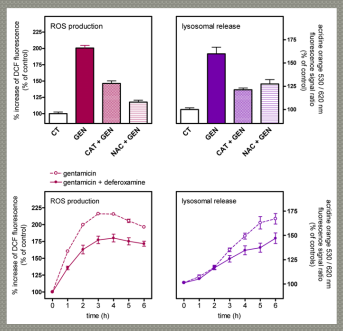
3. GEN-induced ROS-production, lysosomal permeabilization and apoptosis



Incubation of cells with GEN 2mM leads to an increase in ROS production, lysosomal permeabilization and apoptosis.

Fig.4 : ROS production as the fluorescence intensity of H$_2$DCFDA at 530 nm (upper panel), release of acridine orange monitored by the fluorescence intensity ratio at 530/620 nm (middle panel), and apoptosis quantified as the % of fragmented nuclei counted after DAPI staining (lower panel).

4. Partial protective effect of catalase (CAT), N-acetylcysteine (NAC), and iron chelator deferoxamine (DFO) on lysosomal permeabilization and ROS production induced by GEN



Treatment with anti-oxidants or the iron chelator deferoxamine partly decreases GEN-induced ROS production and lysosomal permeabilization.

Fig.5 : Effect of catalase (1,000U/mL), N-acetylcysteine (1mM) and deferoxamine (10µM) on ROS production (left) and lysosomal permeabilization (right) induced by GEN (2 mM).

METHODS

- LLC-PK1 cells were cultivated as described in Servais et al. (2005) and incubated with GEN 2 or 3 mM. They were preincubated and incubated with N-acetylcysteine (0,1 - 10mM) or catalase (100-1000 U/ml) to evaluate the role of oxidative stress, and with the iron chelator deferoxamine (10 µM) to assess implication of iron.
- Apoptosis was quantified by DAPI staining. Lysosomal permeabilization was assessed by following the relocation from lysosomes to the cytosol of the membrane impermeant dye Lucifer Yellow or of the weak base acridine orange by fluorimetry and confocal microscopy. ROS production was evaluated using the intracellular probe 2', 7'-dichlorodihydrofluorescein diacetate (H$_2$DCFDA) that converts to a highly fluorescent compound (2', 7'-dichlorofluorescein) upon oxidation.

CONCLUSIONS

- ROS are produced in lysosomes of cultured LLC-PK1 cells incubated with gentamicin, and can lead to lysosomal permeabilization and apoptosis.
- These effects can be prevented by preincubation with antioxidants or deferoxamine.
- Our data further point to lysosomal iron as a key actor in triggering the renal cell toxicity induced by gentamicin, an aminoglycoside antibiotic.

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