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.

RESULTS

1. GEN-induced lysosomal membrane permeabilization

Treatment of cells with 3mM gentamicin induces lysosomal 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 H2O2 (200 μM) treatments. Cells were incubated with H2DCFDA (to detect ROS production) in combination 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 H2DCFDA 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).

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.