MITOCHONDRIA-TARGETED APPROACHES TO PREVENT GENTAMYCIN TOXICITY

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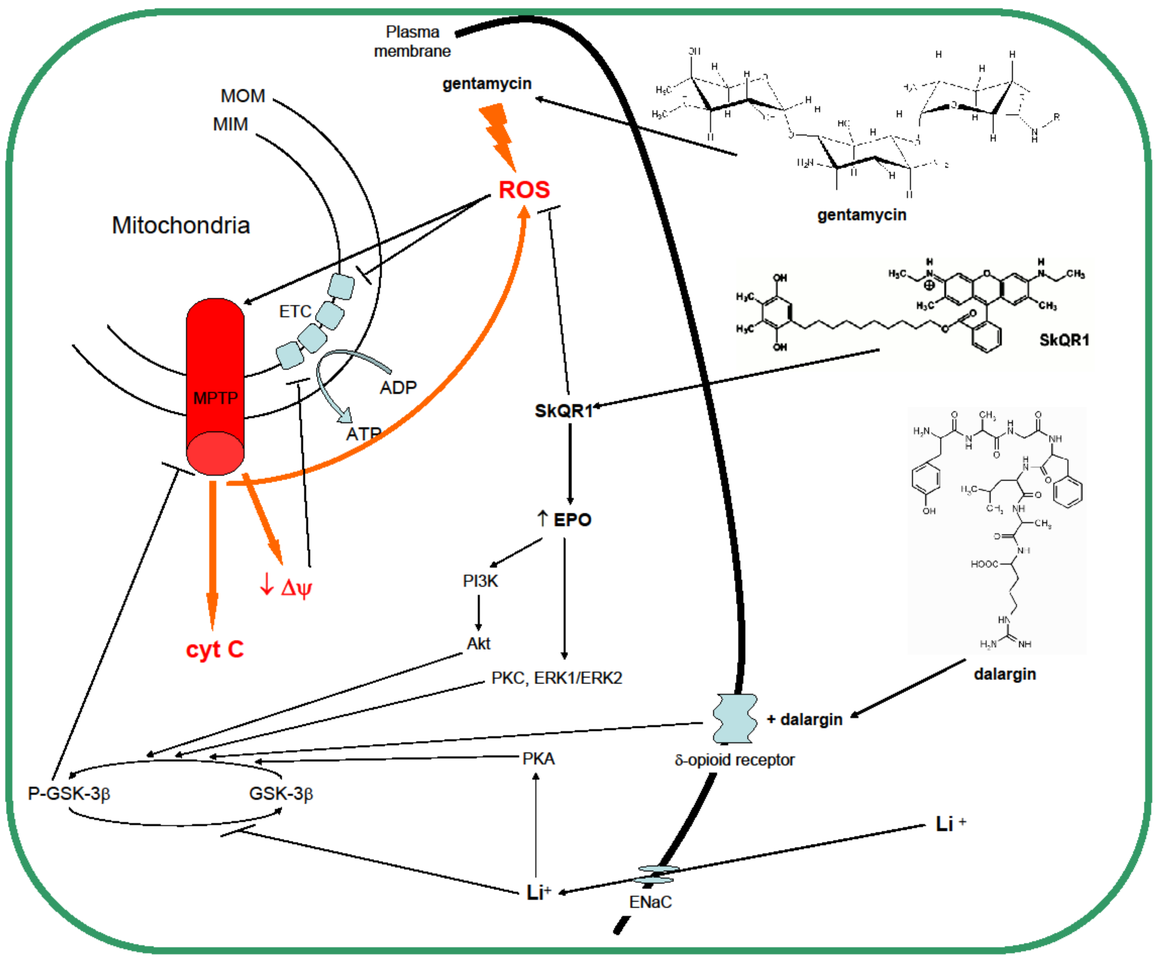
AIMS:

The toxic effects of aminoglycoside antibiotics are realized via stimulating intercellular reactive oxygen species (ROS) overproduction. Oxidative damage of mitochondria results in the opening of mitochondrial permeability transition pore (MPTP). The signaling prevents opening of MPTP are know as preconditioning phenomenon. Preconditioning signaling pathways are converged on glycogen synthase kinase 3β (GSK3β) and results in its inhibition by phosphorylation.

The aim of the study was to investigate whether mitochondria-targeted antioxidant SkQR1 and δ-opyoid receptor agonist (dalargin) and GSK3β inhibitor (LiCl) are able to prevent gentamycin toxicity.



MATERIALS AND METHODS:

Male outbreed rats were treated with gentamycin (i.p. 160 mg/kg/day) for 6 days. On 7th day the blood samples were obtained and concentration of creatinine and blood urea nitrogen (BUN) was measured. Rats were killed and kidneys were excised for measuring of erythropoietin (EPO) level by Western blotting.

Assessment of hearing acuity

The hearing acuity of the animals was assessed using the neurological test based on the Preyer reflex on the 7th, 10th, 13th, and 20th days of experiment. The hearing acuity was assessed semi-quantitatively: score 0 – nearly absolute deafness; score 1 – severe hearing reduction; score 2 – moderate hearing decrease; score 3 – hearing acuity comparable to that of intact animals.

ROS and oxidized proteins determination

Renal cortex tissue slices 100 μm thick were prepared with VibroSlice microtome. The ROS-sensitive fluorescent probe 2,7-DCF-DA dissolved in DMEM/F12 without bicarbonate (final concentration 10 μM) was added to kidney slices and incubated for 15 min at 37^{0}C followed by a wash with DMEM/F12 without bicarbonate. DCF fluorescence was imaged with an LSM510 inverted confocal microscope.

Kidney tissue protein oxidation was assessed using an OxyBlot™ kit (Millipore, USA). The manufacturer's instructions were followed and 20 μg of protein was derivatized for each sample.

Signaling pathways

To study signaling pathways the Western blotting of whole kidney homogenates was prepared. Samples were loaded onto 15% Tris–glycine polyacrylamide gels and after electrophoresis, gels were blotted onto PVDF membranes.

Statistics

All data are presented as mean ± SEM. Comparisons between groups were made using a Student t-test with a P value less than 0.05 taken to indicate statistical significance.

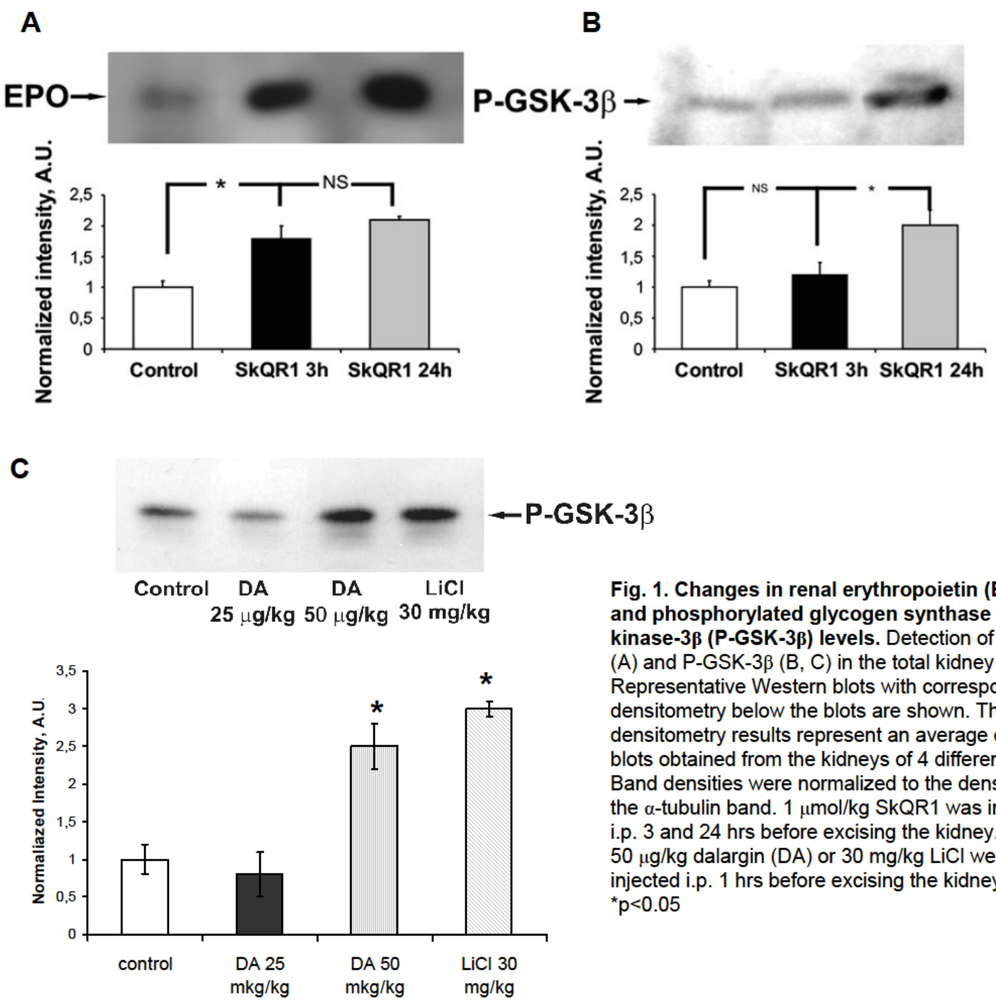


Fig. 1. Changes in renal erythropoietin (EPO) and phosphorylated glycogen synthase kinase-3β (P-GSK-3β) levels. Detection of EPO (A) and P-GSK-3β (B, C) in the total kidney tissue. Representative Western blots with corresponding densitometry below the blots are shown. The densitometry results represent an average over 6 blots obtained from the kidneys of 4 different rats. Band densities were normalized to the density of the α-tubulin band. 1 μmol/kg SkQR1 was injected i.p. 3 and 24 hrs before excising the kidney. 25 or 50 μg/kg dalargin (DA) or 30 mg/kg LiCl were injected i.p. 1 hrs before excising the kidney. *p<0.05

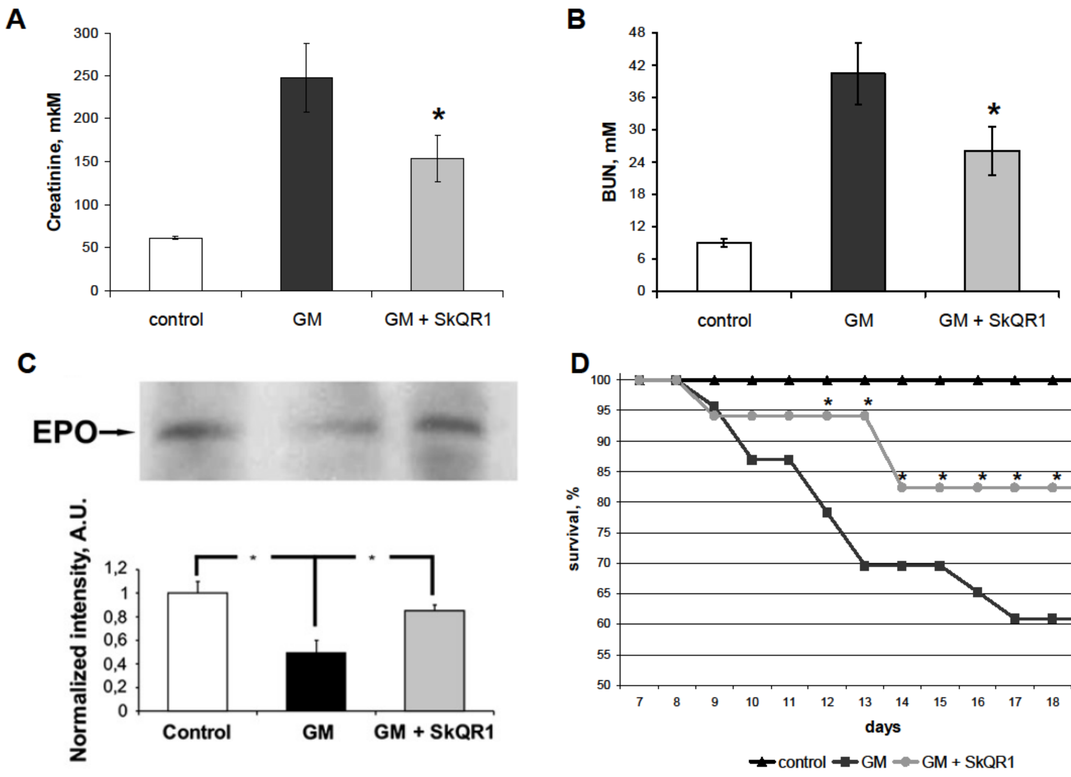


Fig. 2. Indices of kidney dysfunction during gentamycin-induced AKI and the rescue effect of SkQR1. Concentrations of (A) serum creatinine, (B) blood urea nitrogen (BUN), (C) renal erythropoietin content and (D) animal survival after 6-day gentamycin treatment of control, gentamycin-treated (GM) and SkQR1-treated (GM+SkQR1) rats. SkQR1 (100 nmol/kg) injections were made 3 hrs before each GM injection. *p<0.05.

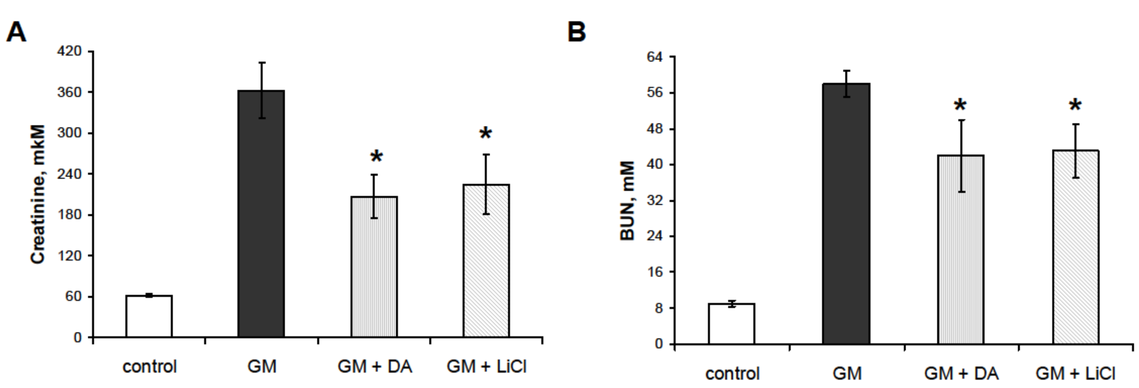


Fig. 3. Indices of kidney dysfunction during gentamycin-induced AKI and the rescue effect of LiCl and dalargin. Concentrations of (A) serum creatinine and (B) blood urea nitrogen (BUN) after 6-day gentamycin treatment in the plasma of control, gentamycin-treated (GM), LiCl-treated (GM+LiCl) and dalargin-treated (GM+DA) rats. LiCl (30mg/kg) or DA (50 μg/kg) injections were made 3 hrs before each GM injection. *p<0.05.

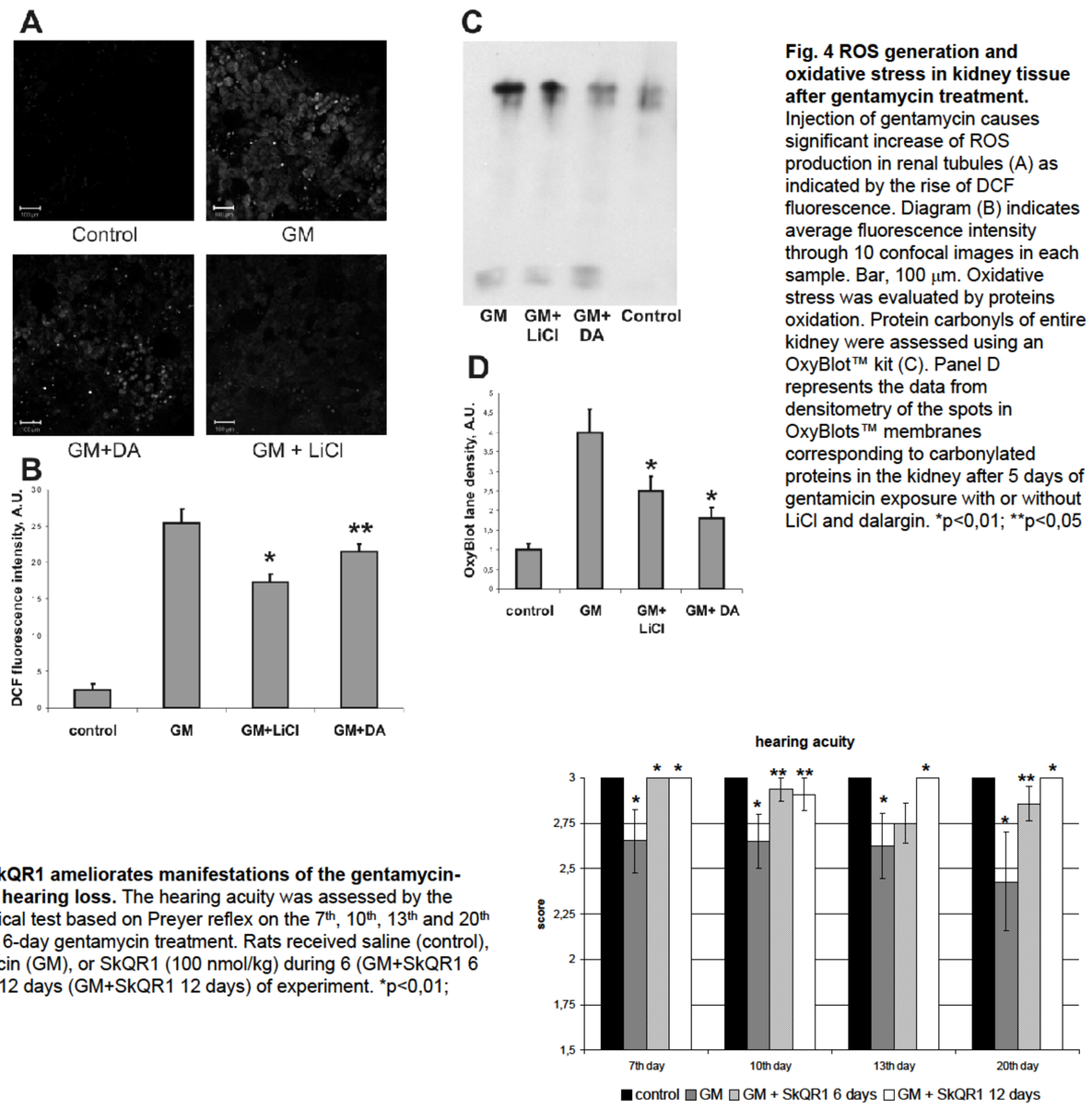


Fig. 4 ROS generation and oxidative stress in kidney tissue after gentamycin treatment. Injection of gentamycin causes significant increase of ROS production in renal tubules (A) as indicated by the rise of DCF fluorescence. Diagram (B) indicates average fluorescence intensity through 10 confocal images in each sample. Bar, 100 μm. Oxidative stress was evaluated by proteins oxidation. Protein carbonyls of entire kidney were assessed using an OxyBlot™ kit (C). Panel D represents the data from densitometry of the spots in OxyBlots™ membranes corresponding to carbonylated proteins in the kidney after 5 days of gentamicin exposure with or without LiCl and dalargin. *p<0,01; **p<0,05

Fig. 5. SkQR1 ameliorates manifestations of the gentamycin-induced hearing loss. The hearing acuity was assessed by the neurological test based on Preyer reflex on the 7th, 10th, 13th and 20th day after 6-day gentamycin treatment. Rats received saline (control), gentamycin (GM), or SkQR1 (100 nmol/kg) during 6 (GM+SkQR1 6 days) or 12 days (GM+SkQR1 12 days) of experiment. *p<0,01; **p<0,05

CONCLUSION:

We conclude that mitochondria-targeted antioxidant SkQR1 effectively prevented nephrotoxicity of gentamycin. Partially this protective effect could be referred to induction of PC signaling. Moreover, different compounds that inhibits GSK-3β thus protecting mitochondria, such as LiCl and dalargin, may serve as promising agents for preventing negative consequences of aminoglycoside therapy

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