Unit of Renal and Cardiovascular Pathophysiology
Department of Physiology and Pharmacology. University of Salamanca. Spain.

[1]Unidad de Fisiopatología Renal y Cardiovascular, Instituto "Reina Sofía" de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Salamanca, Spain.
[2] Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain.
[3] Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain.

INHIBITION OF OXIDATIVE STRESS DOES NOT PREVENT RENAL TUBULAR CYTOTOXICITY INDUCED BY CISPLATIN.

Sandra M. Sancho-Martínez[1,2], Laura Prieto García[1,2], **José M. López-Novoa**[1,2], Francisco J. López-Hernández FJ[1,2,3]

INTRODUCTION

Drug nephrotoxicity is a serious health and economic problem affecting approximately one out of four among the 100 most used drugs in intensive care units. Cisplatin-induced acute kidney injury is characterized mostly by proximal and distal tubular damage. The death phenotype depends on the concentration of cisplatin to which cells are exposed. Necrosis appears as a response to high concentrations, whereas apoptosis to low concentration of the drug. Oxidative stress has been shown to be implicated in cisplatin cytotoxicity and nephrotoxicity both in vitro and in vivo. However, neither the role of oxidative stress in the different forms of cell death induced by increasing concentrations of cisplatin nor the subcellular sources of cisplatin-induced oxidative stress has been assessed under these distinct phenotypical and biochemical scenarios.

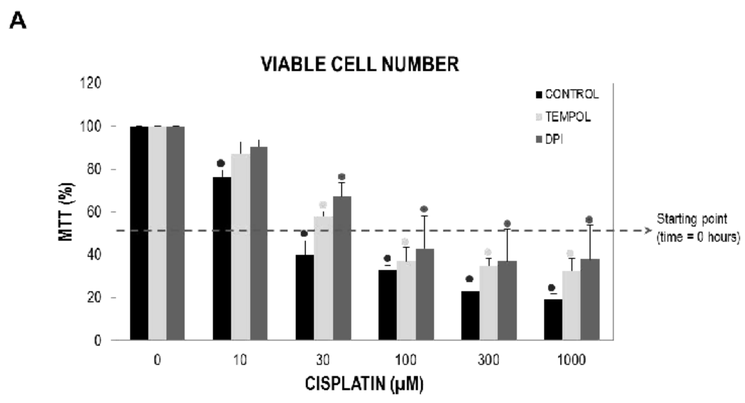

Figure 1. Cisplatin had a concentration-dependent cytotoxic effect (A) MTT-based proliferation/viability, dose-effect profile of HK2 treated during 18 hours with vehicle, as control (-), 0.03 mM 4-hydroxy-TEMPO or 3 µM DPI, in the present of 0-1,000 µM of cisplatin. Data represent average ± SD of n=3. ***p<0.05 with respect to the same concentration of cisplatin in control group. (B) Representative light microscopy photographs (n=3) of HK2 cells treated with 0 (control), 10, 30, 100, 300 and 1,000 µM cisplatin during 18 hours. White arrows: apoptotic cells; black arrows: necrotic cells.

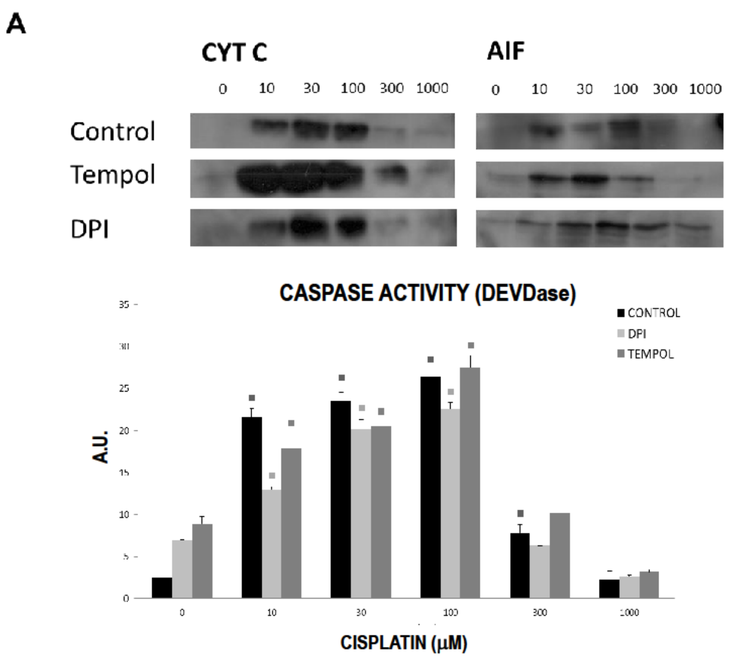

Figure 2. Biochemical characterization of cell death types induced by cisplatin. HK2 treated during 18 hours with vehicle, as control (-), 0.03 mM 4-hydroxy-TEMPO or 3 µM DPI, in the present of 0-1,000 µM of cisplatin. (A) Representative images of western blot analysis of cytochrome c and AIF release. (B) DEVDase (caspase) activity measured in cells extracts. Data represent average ± SD of n=3. *p<0.05 with respect to 0 µM cisplatin in its group, **p<0.05 with respect to the same concentration of cisplatin in control group.

AIMS

We aimed to test the ability of a NADPH oxidase inhibitor (DPI), and the SOD mimetic tempol to prevent oxidative stress in order to identify molecular targets to minimize nephrotoxicity. Necrosis is the result of exposure of epithelial cells to high concentrations of the drug , whereas apoptosis is induced by lower concentrations. For this purpose we treated a human proximal tubule cell line (HK2) with cisplatin (0-1000 µM) in the presence or absence of 0.03 mM 4-hydroxy-TEMPO (tempol) and 3 µM DPI. Biochemical and phenotypic characteristics evoked by pro-apoptotic and pro-necrotic concentrations of cisplatin were studied in the presence and absence of the antioxidants .

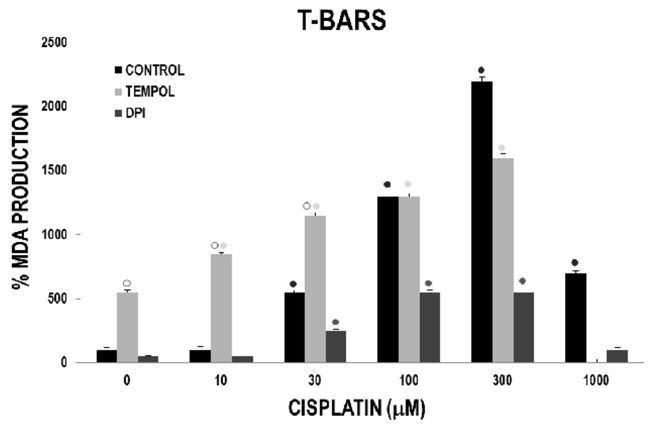

Figure 3 Effect of cisplatin on lipid peroxidation. HK2 treated during 18 hours with vehicle, as control (-), 0.03 mM 4- hydroxy-TEMPO or 3 µM DPI in the present of 0-1,000 µM of cisplatin. Data represent average ± SD of 3 different experiments. °p<0.05 with respect to 0 µM cisplatin in control group, **p<0.05 with respect to the same concentration of cisplatin in control group.

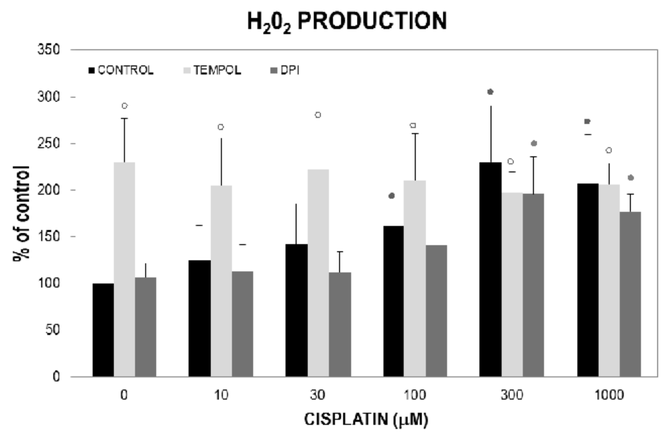

Figure 4 Effect of cisplatin on H2O2 production. HK2 treated during 2 hours with vehicle, as control (-), 0.03 mM 4-hydroxy-TEMPO or 3 µM DPI, in the present of 0-1,000 µM of cisplatin. Data represent average ± SD of n=3. °p<0.05 with respect to 0 µM cisplatin in its group, *p<0.05 with respect to the same concentration of cisplatin in control group .

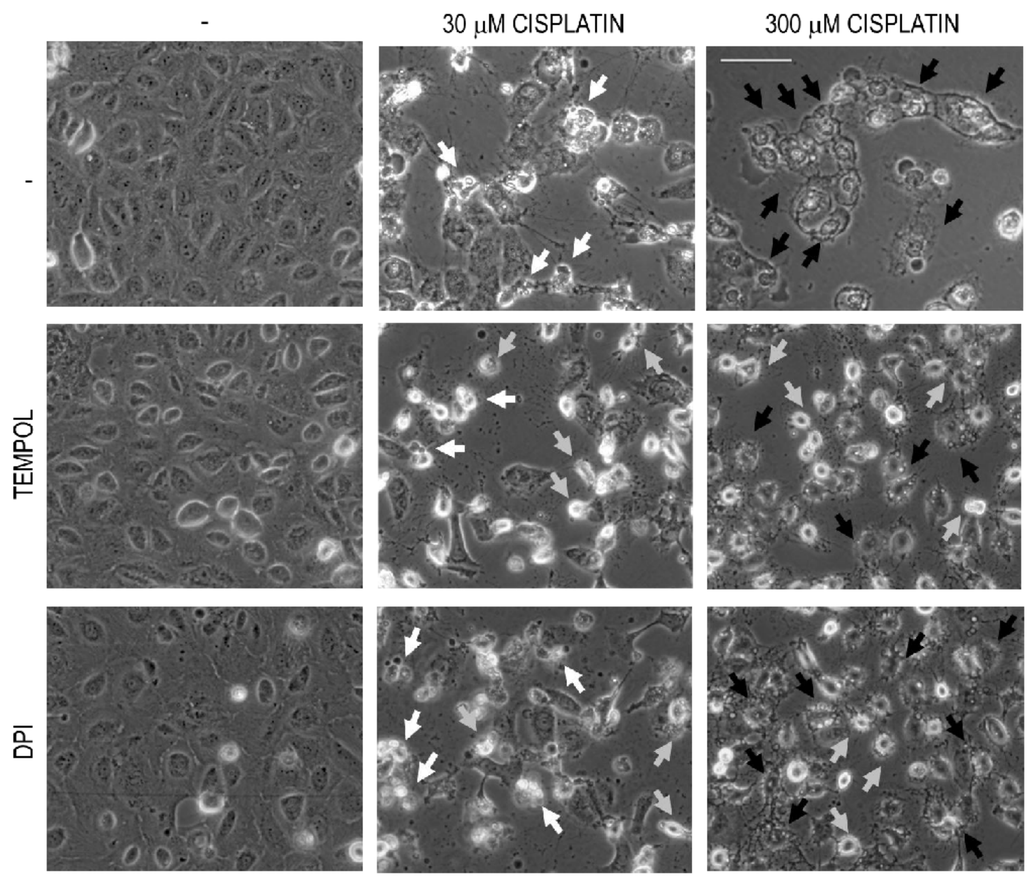

Figure 5. Morphological phenotypes induced by cisplatin. Representative light microscopy photographs (n=3) of HK2 cells treated during 18 hours with vehicle, as control (-), 0.03 mM 4-hydroxy-TEMPO or 3 µM DPI in the present of 0, 30 and 300 µM of cisplatin. White arrows: apoptotic cells; black arrows: necrotic cells; grey narrows: non-apoptotic cell death.

CONCLUSIONS

Our data show that most of the oxidative stress induced by cisplatin is originated from mitochondrial NADPH oxidase activity, as lipid peroxidation and H2O2 production is clearly reduced by the NADPH oxidase inhibitor DPI. However, inhibition of oxidative stress does not prevent the cytotoxicity induced by cisplatin in renal tubular cells. These results suggest that oxidative stress is not the only important mechanism behind cisplatin cytotoxicity; and that oxidative stress has more relevance in the death by apoptosis than in that caused by necrosis.

50ERA-EDTA
20--MP
AKI - experimental models
Jose M. Lopez-Novoa
DOI: 10.3252/pso.eu.50era.2013
Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 18-21, 2013
PosterSessionOnline.com