EFFECTS OF CILASTATIN ON GENTAMICIN-INDUCED RENAL DAMAGE. IN VITRO AND IN VIVO EVIDENCE

Jado JC[1], Humanes B[1], Lopez-Parra V[1], Camaño S[1], Lara JM[2], Cercenado E[3], Tejedor A[1], Lazaro A[1]

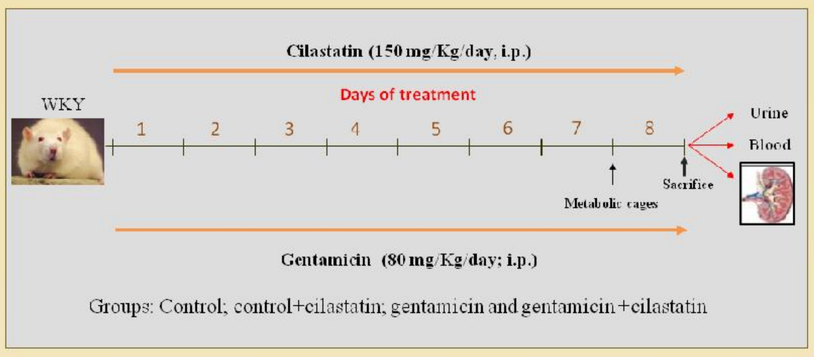
1 Laboratory of Renal Physiopathology, Department of Nephrology, 2 Department of Pathology, 3 Department of Microbiology; IiSGM, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Contact: alazaro10@gmail.com

INTRODUCTION

- Gentamicin (aminoglycoside antibiotic) is widely used in the hospital setting due to its efficacy in the treatment of severe gram-negative bacterial infections.
- However, its clinical usefulness is sometimes complicated by the development of acute nephrotoxicity (range between 15-30%) which is characterized by tubular injury.
- Cilastatin, a specific inhibitor of renal enzyme dehydropeptidase I has proved to be protective against other types of renal toxicity induced by drugs such as cisplatin.

MATERIAL AND METHODS

- ***In vitro* experiments,** were done on porcine primary proximal tubule epithelial cells (PTECs) cultures.
  Apoptosis was evaluated by oligonucleosomes formation (ELISA kit), cellular morphology (optical microscopy) and cell detachment (flow cytometry).
- ***In vivo* experiments,**



- Measure of serum creatine, BUN and proteinuria levels,
- Hematoxylin/Eosin staining,
- Immunohistochemistry,
- Western Blot,
- TUNEL,
- Fluorescence polarization immunoassay with TDx FLx® Analyzer.

AIM

To examine the potential therapeutic beneficts of cilastatin as nephroprotector on gentamicin-induced renal injury.

RESULTS

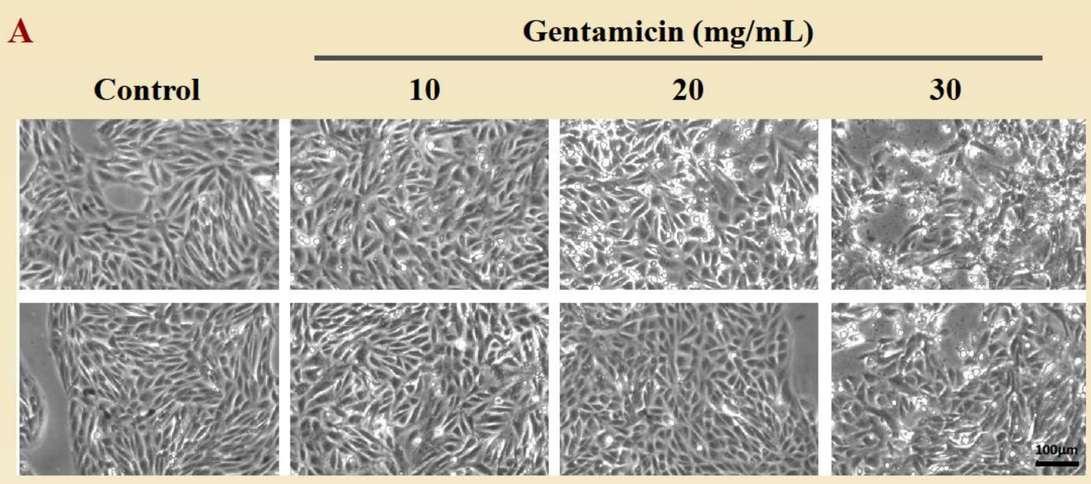


FIGURE 1. Effects of cilastatin in PTECs morphology and apoptosis during treatment with gentamicin. PTECs were cultured in the presence of gentamicin 10, 20 and 30 mg/mL and cilastatin (200 µg/mL) for 24 hours. **A)** Phase-contrast microscopic photographs are shown (original magnification 40X). **B)** The effect of cilastatin on gentamicin-induced detachment of PTECs was measured by flow cytometry and determined by counting the number of cells in an equal volume of buffer. **C)** Oligonucleosomal DNA fragmentation was quantified in the cell-soluble fraction and detected with an enzyme-linked immunosorbent assay kit. Data are represented as the mean S.E.M. of at least three separate experiments. *p< 0,0001 vs. control and control + cilastatin; †p< 0.02 vs. same date without cilastatin.

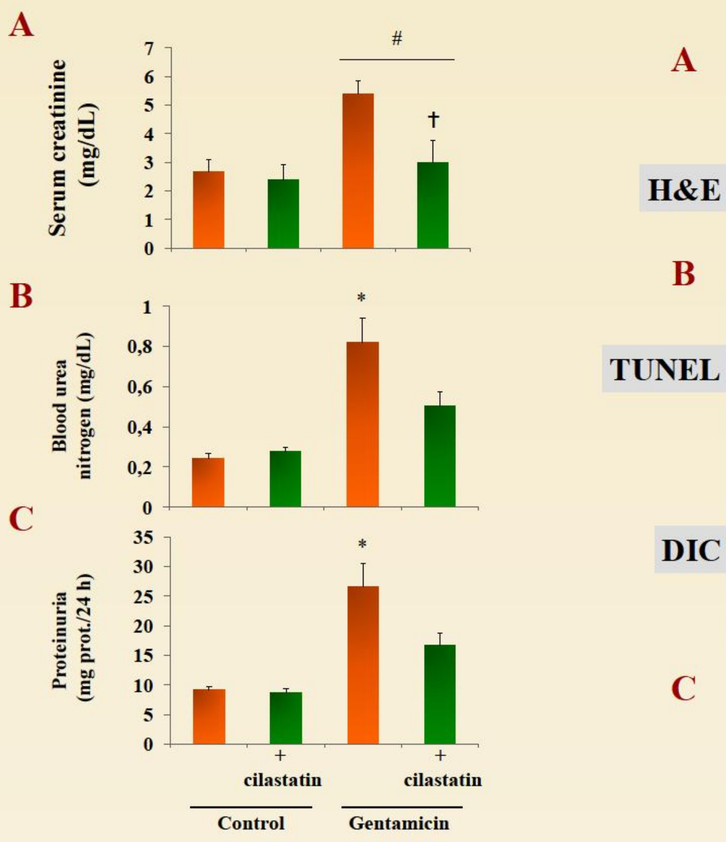
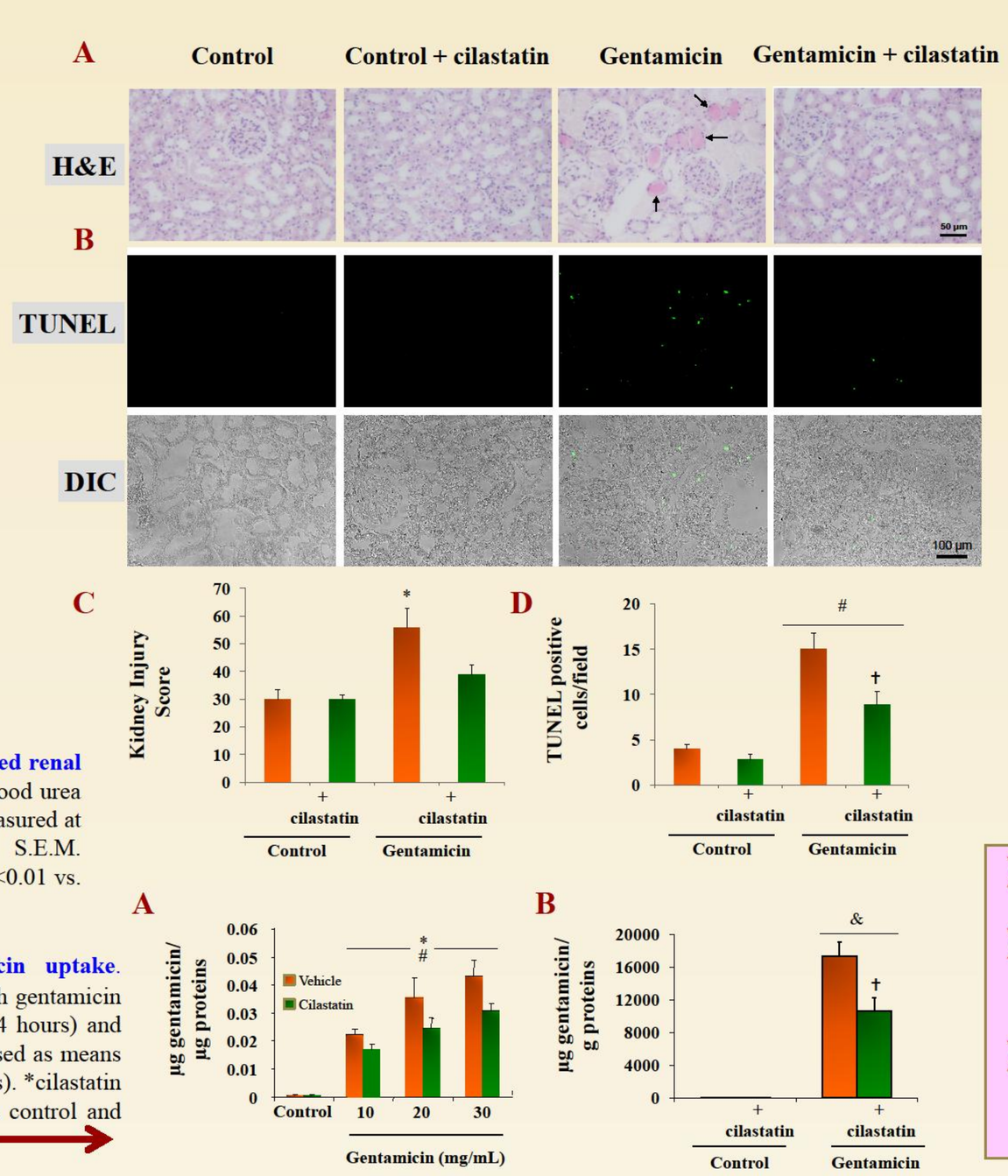


FIGURE 2. Effects of cilastatin on gentamicin-induced renal toxicity, as determined by: **A.)** serum creatinine; **B.)** blood urea nitrogen and **C.)** proteinuria. These parameters were measured at the end of the treatment. Values are expressed as mean S.E.M. (n=6-8), #p<0.01 vs. control and control+cilastatin; †p<0.01 vs. gentamicin; *p<0.002 vs. other groups.



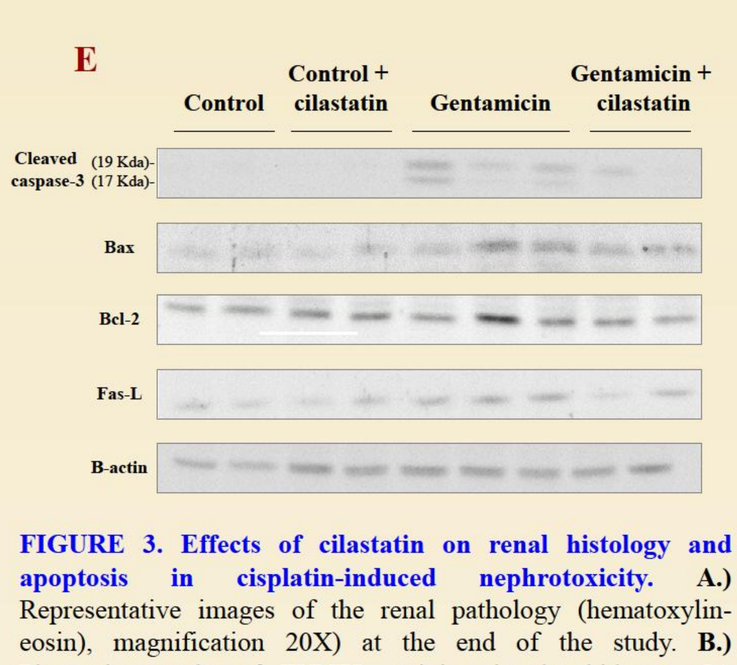


FIGURE 3. Effects of cilastatin on renal histology and apoptosis in cisplatin-induced nephrotoxicity. A.) Representative images of the renal pathology (hematoxylin-eosin), magnification 20X) at the end of the study. **B.)** Photomicrographs of TUNEL staining in the kidneys. **C.)** Semiquantitative renal injury score. **D.)** Quantitative analysis of TUNEL-positive cells. **E.)** Renal western blots of active-caspase 3, Bax, Bcl-2 and Fas-L. Results are expressed as as mean S.E.M. (n=6-8), #p<0.05 vs. control and control+cilastatin; †p<0.05 vs. gentamicin; *p<0.005 vs. other groups.

FIGURE 4. Effects of cilastatin on gentamicin uptake. Intracellular accumulation in **A.)** PTECs (treated with gentamicin in presence or absence of cilastatin200 µg/ml for 24 hours) and **B.)** kidney tissue of the animals. Values were expressed as means S.E.M. (n= 4 different experiments, and 6-8 animals). *cilastatin effect p< 0.05; # dose effect p<0.05; &p<0.005 vs. control and control+cilastatin; †p<0.001 vs. gentamicin.

CONCLUSIONS

- Cilastatin has a protective effect on gentamicin-induced nephrotoxicity by preventing apoptosis.
- The mechanism of the beneficial effect could be attributed at least in part, to a decrease in gentamicin accumulation by the cells.
- Cilastatin might represent a novel strategy in the prevention of gentamicin-induced acute kidney injury.

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