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ROLE OF CASPASE 9 ACTIVITY IN APOPTOTIC AND NECROTIC CELL DEATH INDUCED BY NEPHROTOXIC DRUG CISPLATIN

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INTRODUCTION

Acute kidney injury (AKI) has serious health and economic consequences. Drug nephrotoxicity is a common cause of AKI. Nephrotoxic drugs comprise many drug families including platinated chemotherapeutic drugs, such as cisplatin, which limits its therapeutic use and its efficacy. A central aspect of cisplatin's nephrotoxicity is the tubular injury, which results mainly from tubular epithelial cell death. Prevention of tubular injury is an unmet therapeutic goal that would improve the pharmaco-toxicological profile of the drug. In vivo, cisplatin induces necrosis of the proximal tubule cells, and mainly apoptosis of the distal tubule cells. It has been observed that, in cultured cells, cisplatin induces both apoptosis (at low doses) and necrosis (at higher ones), which might indicate that, in vivo, proximal tubule cells might be exposed to or take more cisplatin (or both) than distal cells.

AIMS

The identification of the signalling pathways leading to one form of cell death or another is of critical interest in order to identify suitable pharmacological targets and implement appropriate treatments with a theranostic approach. The aim of this work was to gain further insight into the role of caspase 9 in the apoptosis and necrotic mechanisms underlying cisplatin's cytotoxicity.

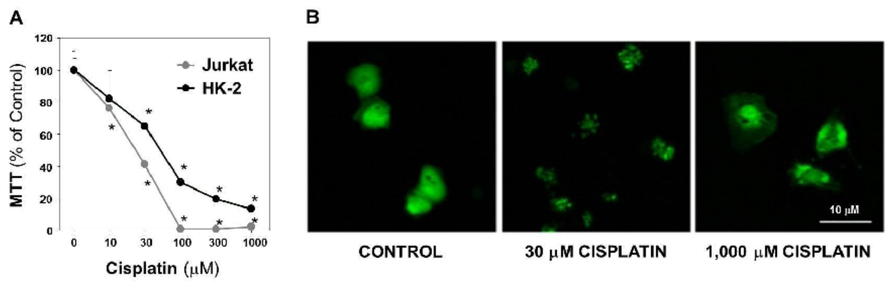

Figure 1. A) Antiproliferative effect of cisplatin and morphological phenotypes induced by cisplatin. MTT-based proliferation/viability, dose-effect prolife of Jurkat and HK2 cellls treated during 24 hours with cisplatin (0-1000 microM). Data represent average ±SD of n=3. * p< 0.05 with respect to 0 microM cisplatin. **B) Representative photographs (n=3) of HK2 cells transiently transfected with EGFP, treated during 20 hours with 0, 30 and 1000 microM cisplatin. C) Representative light microscopy photographs (n=4) of HK2 cells treated with 0, 30, 100 and 300 microM cisplatin during 8 and 18 hours.** White arrows: apoptotic cells; Black arrows : necrotic cells.

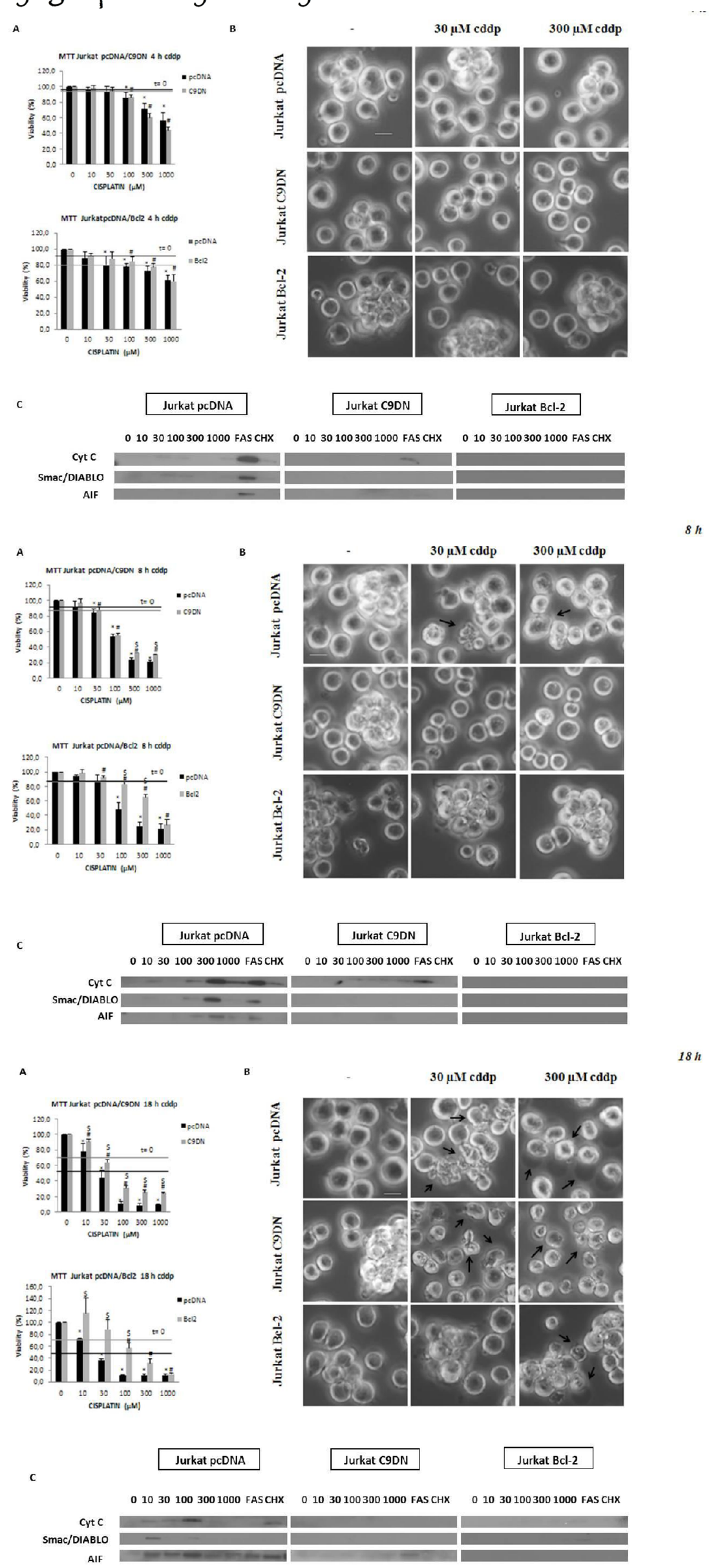

Figure 2.. Study of the cytotoxic mechanisms induced by cisplatin (4, 8 and 18 h of treatment with different concentrations of cisplatin (0-1000 microM). A) Cell viability/proliferation was assessed by the MTT method. Data represent average ± SD of 3 different experiments. * p< 0.05 with respect to 0 μM cisplatin in control group; # p< 0.05 with respect to 0 μM cisplatin in its group, $ p<0.05 with respect to the same concentration of cisplatin in control group. **B) Representative light microscopy photographs (n=3) of Jurkat cells treated with 0, 30, and 300 microM cisplatin during 4, 8 and 18 hours.** Black arrows : different phenotypes of cell death. **C) Biochemical characterization of cell death types induced by cisplatin.** Representative images of western blot analysis (n=3) of cytochrome c, Smac/DIABLO and AIF release.

CONCLUSIONS

Our results indicate that concentrations of cisplatin inducing a necrotic like cell death mode are capable of activating the apoptotic machinery. We demonstrate that caspase 9 activity plays a critical role in the cytotoxicity mechanism induced by cisplatin, accordingly with the increase in the viability and reduction in the expression and activation of pto apoptotic proteins. Moreover, with a view on its clinical utility, this knowledge may allow us to develop drugs that block cisplatin induced apoptosis in the kidney, thus reducing its nephrotoxicity. Furthermore, a reduction in cell necrosis could probably be associated to a lower kidney tissue inflammation, which represents a further renal damage in several models of nephrotoxicity.

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