Autophagy and apoptosis in NRK-52E renal cells exposed to cisplatin

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Cisplatin (CisPt) is a well known antineoplastic drug with severe side effects in the kidney, mainly in S3 segment of proximal tubules [1]. CisPt-induced cell damage is linked to multiple pathways that lead to both apoptosis and necrosis [2]. Recently autophagy, a general cell response to stress, has been also detected in mouse kidney treated with CisPt and its modulation regulated apoptosis in transfected renal cells [3].

Autophagy is morphologically associated to autophagosomes that can fuse with lysosomes, thus contributing to maintenance of cellular homeostasis. In mammals LC3, a protein ortholog of ATg8, an autophagy related gene, is converted from a soluble form LC3-I to LC3-II, transiently bound to phosphatidylethanolamine in the autophagosome membrane.

So this in vitro study aimed to detect autophagy and apoptosis in an established rat proximal tubular cell line, NRK-52E, treated for different times with increasing CisPt doses (from 10 up to 200 µM). NRK-52E cells grown in medium devoid of nutrients were used as autophagy positive controls. In these experimental conditions we also considered the production of reactive oxygen species (ROS) and the expression of some cytoprotective chaperones, heat shock proteins (HSPs) and glucose regulated proteins (GRPs) that counteract oxidative damage and apoptosis [4].

Here we evaluated: a) the expression and distribution of LC3, HSP25, HSP60, GRP75, and GRP78 by immunohistochemistry and Western blotting, b) intracellular ROS levels and apoptosis by FACS; c) morphological and ultrastructural features.

LC3 was present as a weak diffuse signal in NRK-52E controls but more evident and punctate at 6h either in CisPt 10 µM or in starved cells (Figure 1A-B). This signal was undetectable at 24-48h or in NRK-52E cells exposed to higher CisPt doses (50-100µM) when mainly apoptosis occurred (Figure 1C).

These data were confirmed by quantitative Western blotting analysis of LC3 I and II forms (Figure 2). Transmission electron microscopy showed autophagic inclusions after 6h of treatment with 10 µM CisPt (Figure 3 A), while condensed nuclear chromatin was visible later, following 50 µM CisPt exposure (Figure 3 B).

ROS levels were undetectable in cells exposed for 6-24h to 10 µM but enhanced in respect to controls at 24 h after CisPt concentrations ≥ 50 µM. Apoptosis and necrosis correlated with this event and significantly increased at 48 h after ≥ 50 µM CisPt. Analyzed at 24h, mainly HSP25 and GRP75 enhanced after 50-100 µM CisPt, while GRP78 increased from 10 up to 100 µM CisPt treatments.

In non transfected NRK-52E cells exposed to CisPt a moderate autophagy occurs only at low doses of the drug and is associated to nuclear and cytoskeleton integrity; however autophagy appear to be independent of the cell stress response activation and apoptosis.

These results confirm the different temporal progression of autophagy in respect to apoptosis, as already proved in a renal transfected *in vitro* system. We hypothesized that this

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in vitro model will be useful to study the mechanism underlying the role of autophagy in response to CisPt toxicity.

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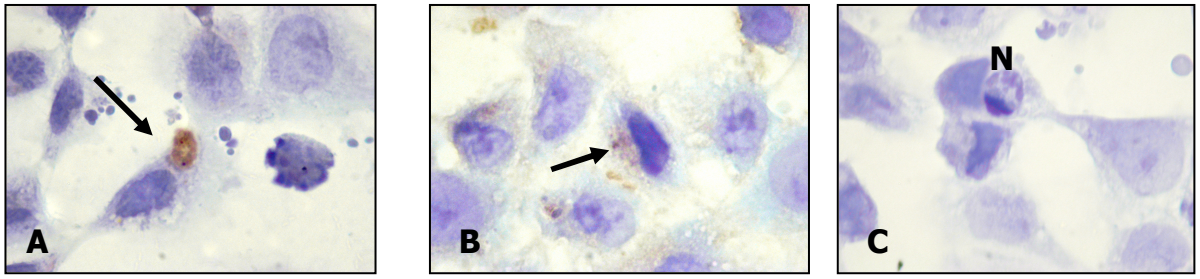

Figure 1 LC3 Immunohistochemistry on NRK52E cells starved (**A**) or exposed to CisPt 10µM for 6h (**B**) or 50µM for 24h (**C**). Punctate signal (→) is evident in the cytoplasm of both starved and 10µM CisPt treated cells, but undetectable after prolonged treatment when apoptosis occurs (**N**, apoptotic nucleus). Original magnification 100X.

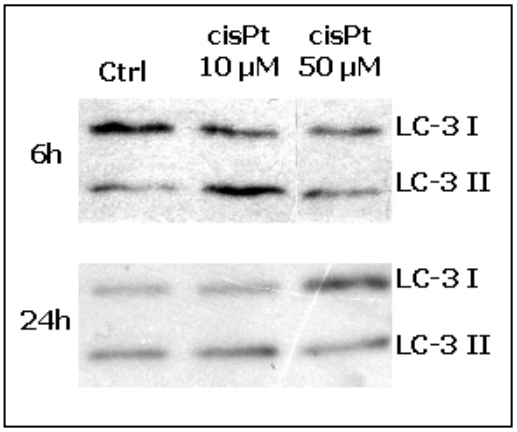

Figure 2. LC3 Immunoblotting on rat renal NRK52E cells. Bands relative to LC3-I form and enhanced LC3-II form only after 10µM CisPt at 6h.

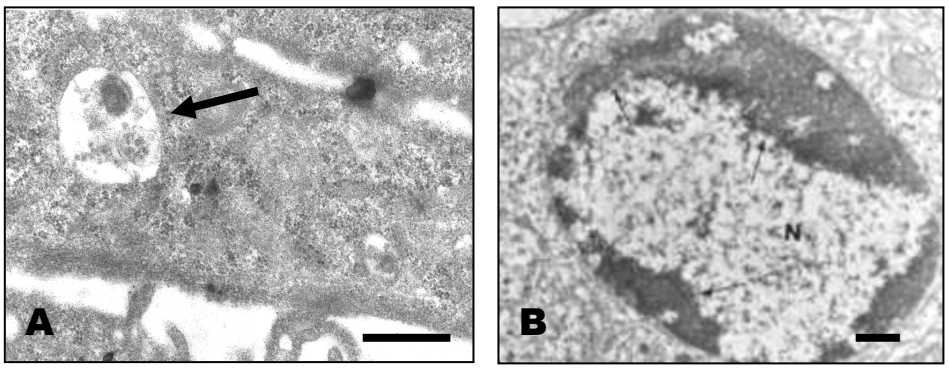

Figure 3. TEM micrographs show autophagic inclusions (→) after 10µM CisPt at 6h (**A**) and an apoptotic nucleus after 50µM CisPt at 24h (**B**). A, bar=0.2µm and B, bar=0.5µm.

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