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Studio *in vitro* dei meccanismi di nefrotossicità del cloruro di mercurio

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Summary
«In vitro *study of the mechanisms of mercury chloride nephrotoxicity*». Objectives: *Mercury (Hg), one of the most diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states, each of which with unique characteristics of target organ specificity. Exposure to Hg vapour and to organic mercurials specifically affects the CNS, while the kidney is the target organ for inorganic Hg compounds. Despite the increasing number of studies, the molecular bases of the nephrotoxic potential of Hg has not, up to now, been clarified, even if there is evidence suggesting that the ability of the metal to interact with proteins (thiol groups) or to generate oxygen radicals may play a major role. Within this context, the aim of the present study was to investigate,* in vitro, *the mechanism(s) of the early nephrotoxic potential of mercury chloride ($HgCl_2$), one of the most diffused and biologically active mercury (Hg^{2+}) compounds. For this purpose, two kidney-derived* in vitro *systems (the MDCK and the LLC-PK1 cell lines) were tested for their sensitivity to the salt, and MDCK was chosen as the most suitable* in vitro *model for our study. As possible biological markers of the organ-specific toxicity of the metal we analysed: i) critical biochemical parameters related to oxidative stress conditions (effect of Hg^{2+} on the anti-oxidant status of the cell), and ii) gap-junctional function (GJIC).* **Methods:** *Classical toxicity tests (MTT and NR) were used for assessing the sensitivity (IC50) of LLP-CK1 and MDCK cell lines to the mercuric salt. Complete solubilisation of the salt in the culture media was verified by inductively coupled plasma mass spectrometry (ICP-MS). The influence of the metal on cell growth rate and viability were evaluated by conventional proliferation assays. For the following mechanistic studies, cells were exposed for different time periods (4 to 72 hours) to non-cytotoxic (0.1-50 μM) $HgCl_2$ concentrations. The biochemical analysis of the pro-oxidant properties of the mercuric compound was performed by the measurement of anti-oxidant cellular defences against H_2O_2 [catalase (Cat), glutathione peroxidase (Gpx), and total glutathione (GSH)]. The influence of the metal on the GJIC capacity of MDCK cells was assessed by the "microinjection/dye-coupling" assay.* **Results:** *Among the two kidney-derived* in vitro *systems, MDCK cell line was the most specifically sensitive to the toxic effect of $HgCl_2$: it was, consequently,*

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chosen as a "tubular cell model" for the following experimental steps. Tested for various time periods at increasing concentrations, the $HgCl_2$ *effect on MDCK cell proliferation and viability was found to be time- and dose-related. For concentrations* $\leq 50\ \mu M$*,* $HgCl_2$ *inhibits MDCK cell growth rate, being this effect significant (>50% in respect to untreated controls) from the 24th from the beginning of the treatment, while, for concentrations* $>50\ \mu M$*, the metal causes cell death. Concerning the influence of* $HgCl_2$ *on MDCK anti-oxidant defences, the most interesting results were obtained by analysing the influence of the mercury salt on the GSH cell content and Gpx activity. Both were, in fact, significantly affected by the presence of the mercury ion.* $HgCl_2$ *also induced a rapid, dose- and time-related inhibitory effect on the GJIC capacity of the cells.* **Conclusions:** *Even if further investigations are needed to better clarify the possible causal relationship between our findings, they indicate that: a) MDCK cells represent a suitable* in vitro *model for the study of Hg nephrotoxicity; b) GJIC function is, among those considered in our study, one of the most sensitive biological endpoints for investigating the mechanism(s) of* Hg^{2+} *specific toxicity.*