RESUMEN

Background: Renal ischemia induces a broad range of cell responses including loss of adhesion and cell death, depending on the cell type and the duration of the ischemic period. Sublethal renal I/R provokes primarily proximal tubular epithelial cell shedding. Using animal models of I/R, loss of epithelial polarity, actin cytoskeleton dynamics alterations and disruption of intercellular junctions have been reported in the proximal tubule. For an appropriate study of the epithelial cell response to I/R, it is important to use *in vitro* systems which closely reproduce the *in vivo* experimental conditions and effects as the one we have established. In the present work, we have used the human proximal tubule epithelial cell line HK2.

The morphology and the correct function of proximal tubular epithelial cells are determined by the structured cytoskeleton, the organized intercellular unions as well as the firm focal contacts with the ECM, where collagen IV is the major component. This anchorage is mediated by the Focal Adhesion Complexes (FAC). Epithelial cell-cell adhesion is established through different structures: tight junctions (TJ), adherens junctions (AJ) and desmosomes.

The ATP and oxygen alterations subsequent to I/R can lead to oxidative stress generation. Indeed, several authors have documented the production of reactive oxygen species (ROS) in animal models of I/R and in chemical hypoxia/ATP depletion *in vitro*

models. Additionally, the administration of antioxidants showed beneficial effects on both *in vivo* and *in vitro* systems.

Calcium is a crucial second messenger capable to activate several intracellular signalling pathways, including PKC signalling. Oxygen deprivation causes an increase in the cytoplasm levels of Ca2+, which leads to damage in many cells.

PKC is a family of proteins which play a key role in the intracellular signalling. It has been divided in three subfamilies: classical PKCs including PKCα; novel PKCs; atypical PKCs including PKCζ. All of them have a catalytic domain which mediate serine-treonine phosphorylation of theirs effectors and a regulatory domain which is activated by DAG and Ca2+ for classical PKCs and DAG for novels PKCs. Atypical PKCs do not respond to any of these stimuli. All PKCs isoforms when activated traslocate to different cell compartments, being the plasma membrane the most common site of traslocation. Regarding to the I/R tubular damage, it has been reported that both isoforms traslocate to the membrane and contribute to this injury, even though their role remains still poorly understood.

Objectives: The main objective of this work is to determine the role of the PKCα and PKCζ isoforms in the mechanisms mediating the tubular damage caused by I/R, using an *in vitro* model of H/R in

HK2 cells. To asses this, we have studied the activity and the regulation of both isoforms during H/R and the effects of PKCα and PKCζ activity in intercellular adhesion and cytoskeleton organization, using specific inhibitors. Additionally, we have determined the localization of both isoforms in human specimens showing acute tubular necrosis (ATN).

Methodology: To achieve these objectives we have determined, *in vitro,* the expression of PKC isoforms by western blot; their traslocation by immunofluorescence and subcelullar fractionation and western blot; the calcium and ROS levels by flow cytometry; the distribution of intercellular adhesion molecules as well as the organization of cytoskeleton components by inmmunofluorescence; the epithelial monolayer integrity by colorimetry; in human samples, the localization of all the proteins was studied by immunohistochemistry.

Conclusions:

1. Several PKC isoforms are expressed in HK2, mainly PKCα and PKCζ, no exhibiting changes in expression during H/R.

2. PKCα is transiently activated during reoxygenation due to Ca^{2}+ increase and ERK1/2 activation and leads to cell contraction, TJ disruption and epithelial monolayer integrity disturbance. In human

samples, PKCα localized in the plasma membrane of damaged tubular cells.

3. PKCζ is also transiently activated during reoxygenation due to ROS generation and causes cytoskeleton components disorganization. In human samples, PKCζ localization in the membrane correlates with injured cells.

4. Our results strongly suggest that PKCα and PKCζ could be identified as prognostic markers of tubular damage after ischemia and could be considered as targets for new and more efficient FRA therapies in future.