Nephrology Dialysis Transplantation 31 (Supplement 1): i366–i376, 2016
doi:10.1093/ndt/gfw183.26

RENAL PATHOLOGY EXPERIMENTAL AND CLINICAL - 2

MP094 EVEROLIMUS-INDUCED EPITHELIAL TO MESENCHYMAL TRANSITION IN BRONCHIAL AND PULMONARY CELLS: WHEN THE DOSAGE DOES MATTER IN TRANSPLANTATION

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Introduction and Aims: Several studies have reported an high rate of pulmonary fibrosis-associated adverse effects (including Bronchiolitis obliterans organizing pneumonia) in patients treated with mTOR inhibitors, immunosuppressants widely used in renal transplant patients. It has been suggested that epithelial to mesenchymal transition (EMT) in airway cells may determine this condition. However, at the moment, the exact biological machinery involved is not completely clarified.

Methods: To assess this research objective, we performed a translational study. First we analyzed the *in vivo* pulmonary pro-fibrotic potential of Everolimus (EVE) by computing a pulmonary fibrosis index score (PFIS), obtained by the combination of several computerized tomography, hemogasanalytic and spirometric parameters, in 13 renal transplant patients in EVE maintenance immunosuppressive treatment and 13 patients treated with Tacrolimus (Advagraf, ADV). All patients included were asymptomatic and they did not take medication for pulmonary diseases. Subsequently, in an *in vitro* study we assessed whether EVE at low- (5, 10 nM) or high-dosage (100 nM) or Tacrolimus at different doses (5 nM, 500 nM e 5 μM) were able to induce EMT in Human type II pneumocyte-derived (A549), normal (NuLI-1) and homozygous for the delta F508 mutation causing cystic fibrosis (CUFI-1) bronchial epithelial cell lines.
Results: Biomolecular experiments demonstrated that high doses of EVE (100 nM) up-regulated EMT markers in all cell lines at both gene- and protein levels with a significant AKT-phosphorylation. In the *in vivo* part of the study, we found that, although asymptomatic, the pulmonary fibrosis index was higher in EVE-treated patients compared to those treated with ADV (mean±SD 2.58±1.83 versus 1.21±1.25, p value 0.03). This effect was positively correlated to the trough levels (TL) in EVE-treated patients (R2=0.35).
Conclusions: All together, our data suggested that only elevated doses of EVE may induce pulmonary fibrosis and that this effect could be mediated by EMT in pneumocyte cells. Additionally, they suggest that clinicians should employ, whether possible, low dosages of mTOR-Is in renal transplant recipients, screening periodically pulmonary function.