**Bufalin-Induced Epithelial-Mesenchymal Transition in High Passage (P>80) LLC-PK1 Cells In Vitro.**

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Renal aging is associated with reduced renal filtration rates and promotes epithelial-mesenchymal transition (EMT), resulting in renal fibrosis. Bufalin, an endogenous cardiotonic steroid that binds to Na/K-ATPase, induces changes consistent with EMT in LLC-PK1 cells. Interestingly, this phenomenon occurs more significantly in cells with higher passages (P>80), suggesting increased cellular sensitivity to bufalin due to in vitro aging. Our objective was to evaluate the molecular and morphological features of the transition which these cells were submitted. In vitro aged LLC-PK1 cells were investigated using immunofluorescence of cellular adhesion molecules and EMT markers after 48 h incubation with 20 nM bufalin. Statistical significance was determined by Student’s t-test (p<0.05), and the data are expressed as means ± SEM. The results revealed significant changes in bufalin-treated cells. Specifically, we observed a reduction in the intensity of pan-cadherin. Although there were no significant differences in β-catenin and α-tubulin, there was a marked decrease in E-cadherin staining. When analyzing the proteins occludin, claudin-1, and ZO-2, bufalin treatment reduced the intensity of fluorescence, but there was no difference in ZO-1 labeling. Additionally, the transcription factor ZEB1, which is involved in EMT, showed a reduction in fluorescence intensity. Our results suggest that LLC-PK1 cells with passages greater than 80 are more sensitive to bufalin-induced some EMT-like process. These findings provide a solid foundation for understanding the effects of aging and sensitivity for endogenous cardiotonic steroids, as well as the association with the pathophysiology of renal injury.

Keywords: Na/K-ATPase, Bufalin, Epithelial mesenchymal transition, Renal disease

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