Is there any role of epithelial to mesenchymal transition İn the pathogenesis of contrast nephropathy?

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Aim:

Contrast medium-induced nephropathy is one of the major complications of intravenous contrast medium use. But its pathogenesis is unclear. Epithelial mesenchymal transition (EMT) is defined as the transformation of the primer epithelial cells to mesenchymal cells. EMT in tubular cells might cause tubulointerstitial damage. In this study, we investigated whether or not EMT has a role in radiocontrast induced nephropathy. Radiocontrast medium might be triggering reversible EMT via SGK 1. We investigated the effect of different concentrations of the contrast agent iopromide on HK 2 cell culture by measuring the level of SGK1, Snail1,CTGF and COL1A1.

Method:

We conducted a scratch assay and qPCR. HK-2 cells were cultured in the petri dishes/flasks and starved with serum-free medium. The 40, 20, and 10 mg/mL doses of iopromide were administrated to cells. The scratches were photographed immediately and again at the 20th hour. The levels of gene expression of SGK1, SNAIL1, CTGF, and COL1A1 were measured using the Real-Time qPCR system at the end of the 24th hour.

Result:

Iopromide caused the breaking of intercellular connections, the disappearance of the cobblestone appearance of cells, and the migration of cells at the 20th hour in the scratch assay. It also increased the expression of SGK1, SNAIL1, CTGF, and COL1A1 genes.

Discusion:

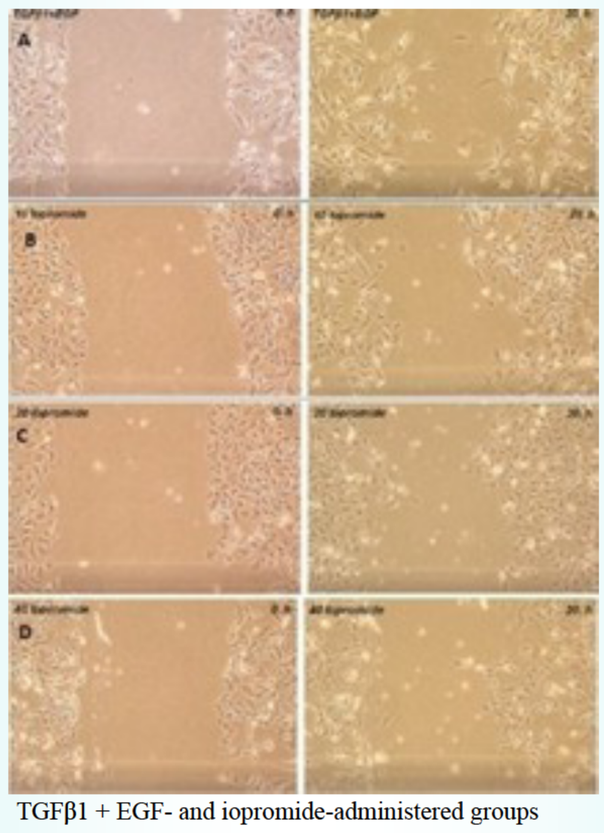
In our study, we used TGF-ß as positive control and scratch assay in HK2 cell culture. With this method, we induced EMT formation in cells. We measured SGK1, CTGF, Snail and Col1a1 in different concentrations of CM. The increased blood viscosity observed as a result of contrast agent intake, increased osmotic load in the distal tubules and impaired tubuloglomerular “feedback” mechanism contribute to the development of hypoxia. Contrast agent also has direct cytotoxic effects on the tubular cells of the kidney[1]. SGK1 may be induced as a result of high osmolality in tubular epithelial cells.

We detected EMT formation via scratch assay test in our study. EMT formation was also observed in the TGFβ1-EGF-added media as positive control. We observed similar changes in all doses of iopromide. Iopromide has led to EMT formation in the HK2 media. We detected in our tubular epithelial cell culture model that the genes that have a role in EMT were increased by the induction of the contrast agent. No similar study has been performed on the pathophysiology of contrast agent up to this date. Contrast agent may lead to reversible EMT in tubular epithelial cells. 40, 20, and 10 mg/mL of iopromide in serum-free DMEM/F12 were calculated to be 442 mOsm/kgH2O, 371 mOsm/kgH2O, and 354 mOsm/kgH2O, respectively in our study. CM may be leading to the expression of SGK1 via probable hyperosmolar effect and/or cell damage[2]. In our study, we used TGF-ß as positive control and scratch assay in HK2 cell culture. With this method, we induced EMT formation in cells. We observed increased CTGF, both in the study group and in the positive control group in our study. CM expresses CTGF in HK 2 cells. This may be an indicator of the appearance of EMT. SNAIL1 plays a key role in the regulation of transcriptional repressor EMT. In a study performed on the biopsy materials of patients with kidney transplantation, SNAIL1 was found to be closely related to the fibrogenic, EMT-like response of the tubular epithelium in human renal grafts, which was predictive of graft function loss[3] . In our study, CM increased the expression of SNAIL 1 in the HK2 cell culture.

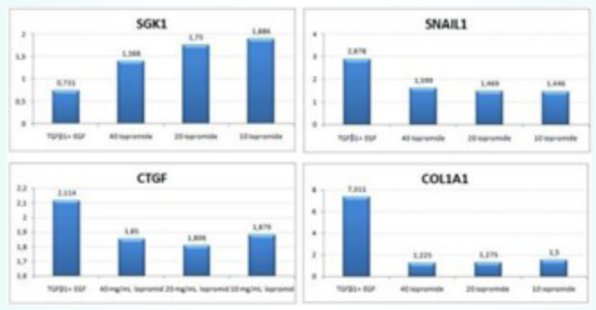
Increased collagen 1 and 3 production is observed in fibrosis. Increased COL1A1 and COL3A1 gene expressions were shown in the epithelial cells following EMT induction in the kidney[4]. we observed increased COL1A1. Although an increase was observed in all doses of iopromide, the most significant increase was observed in the lowest iopromid dose (10 mg/mL). Lower expression of COL1A1 has been demonstrated in epithelial cells than fibroblasts following 7 days of TGFβ1 stimulation Our study was terminated in 24 hours. The COL1A1 level may be observed to be higher if it were continued longer.

Conclusion:

In this study, we investigated whether or not EMT has a role in CIN. Until now, there has been no study of these pathways of EMT in CIN pathogenesis. Hyperosmolarity caused by the Radiocontrast medium might be triggering reversible EMT via SGK 1. We investigated the effect of different concentrations of the contrast agent iopromide on HK 2 cell culture by measuring the level of SGK1, SNAIL1,CTGF and COL1A1.



TGFβ1 + EGF- and iopromide-administered groups



TGFβ1-EGF increased the expression of SNAIL1, CTGF, and collagen type I, alpha 1 (COL1A1) genes

References

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