

ANTIPROLIFERATIVE FACTOR (APF) DECREASES OCCLUDIN AND ZO-1 PRODUCTION AND INCREASES PARACELLULAR PERMEABILITY IN BLADDER EPITHELIAL CELL MONOLAYERS

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INTRODUCTION AND OBJECTIVE: Several lines of evidence suggest that the bladder epithelial barrier may be compromised in interstitial cystitis (IC). Antiproliferative factor (APF) is a small glycoprotein made specifically by bladder epithelial cells from IC patients that induces changes in expression of certain cell proteins and profoundly inhibits cell growth. We determined whether APF also affects cell production of two tight junction proteins (occludin and ZO-1) and paracellular permeability in bladder epithelial cell monolayers shown to express tight junctions containing these two proteins.

METHODS: Normal bladder epithelial cell monolayers were treated with HPLC-purified APF at concentrations found in IC patient urine specimens, or similar quantities of a mock APF preparation. The amounts of occludin, ZO-1, E-cadherin, and beta actin proteins were determined by Western blot and immunofluorescence assay. Paracellular permeability was determined by measuring flux of ¹⁴C-mannitol and ³H-inulin between confluent monolayers on Transwell culture plates.

RESULTS: APF specifically and significantly decreased production of occludin and ZO-1 proteins by Western blot, while increasing E-cadherin production and causing no change in beta actin production when normalized to total cell protein content. APF also resulted in attenuation of tight junctions by IFA as compared to mock APF. Within only 2 hours of treatment, APF significantly increased paracellular permeability of both ¹⁴C-mannitol ($13.2 \pm 2.3\%$ vs. $3.2 \pm 1.7\%$, $p = 0.039$) and ³H-inulin ($6.3 \pm 0.5\%$ vs. $2.5 \pm 0.9\%$, $p = 0.035$), in normal bladder epithelial cell monolayers as compared to mock APF.

CONCLUSIONS: APF significantly inhibits bladder epithelial cell production of tight junction proteins and rapidly increases paracellular permeability *in vitro*, and may therefore contribute to the leakiness of the bladder epithelial barrier seen in IC.

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