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IMMUNOHISTOCHEMICAL ANALYSIS OF UROTHELIAL TRPV1 IN PATIENTS SUFFERING FROM INTERSTITIAL CYSTITIS WHO UNDERWENT INTRAVESICAL TREATMENT WITH RESINIFERATOXIN

Hypothesis / aims of study

Interstitial cystitis (IC), a syndrome characterized by motor and sensory dysfunction of the lower urinary tract, which is known also with the new term of painful bladder syndrome, represents a diagnostic and therapeutic challenge even to highly skilled physicians. Recently intravesical instillation of resiniferatoxin, an agonist of the vanilloid receptor type 1 (VR₁), has been considered a new strategy to treat patients with IC by targeting the receptors expressed on the terminal ending of sensory nerves. VR₁ a non-selective cation channel also known, according to a new nomenclature, as transient receptor potential vanilloid 1 (TRPV₁), is expressed in a peptide-containing sub-population of primary sensory nerves of the rat and human urinary bladder which are involved in the regulation of micturition reflexes. In humans, the TRPV₁ has been detected in the sensory nerve endings, in some of the cells present in the sub-urothelium, in the smooth muscle cells and recently it was shown the presence of the receptor in the normal human urothelium (1).

The aim of this study is to identify, by immunohistochemistry, the morphological changes which occur in urothelial cells expressing VR₁ in patients with interstitial cystitis before and after a prolonged intravesical instillation of RTX.

Study design, materials and methods

Specimens were obtained from human urinary bladder of 4 patients who presented a diagnosis of IC according to Interstitial Cystitis Data Base Study Group (ICDBSG) criteria by multiple cold cup biopsy. These patients received a prolonged instillation of RTX by an external drug delivery system for 10 days as previously described (2). The specimens were obtained before the intravesical instillation of RTX and at the end of the instillation when the suprapubic catheter was removed.

Series were processed for light microscope immunohistochemistry and for fluorescence microscope immunohistochemistry. Then the sections were incubated for the primary antibody. Three antibodies were used. The rabbit polyclonal antibody raised against capsaicin receptor (vanilloid receptor VR₁, C-terminus, Chemicon International, Temecula, CA, USA), the Vanilloid Receptor, VR₁ (N-15) goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA) and the Vanilloid Receptor, VR₁ (C-15) goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA). All the sections were counterstained with hematoxylin for nuclei labelling. Fluorescence microscope immunohistochemistry was also performed in some series of samples. Hematoxylin-eosin staining was also performed for all the specimens. Two non-blinded histologists reviewed the immunohistochemical preparations.

Results

In the pre-treatment biopsies, hematoxylin-eosin staining confirmed that all the specimens presented a moderate grade of inflammation, characterized by the presence in the urothelium and sub-urothelium of migrating, immune cells and mast-cells.

In the pretreatment samples the urothelium was positive for all the three types of antibodies which we use; it was positive in all the cell types (basal, superficial and club-shaped cells) and the labelling was intracytoplasmatic, often slightly granular. In the sub-urothelium and among muscle bundles, mast cells with intensely stained granules were always detected. A very moderate staining of nerves was also detected in the sub-urothelium. After the prolonged instillation of RTX we found that only the basal cells of urothelium were positive while the labelling was no more present in the superficial and club-shaped cells. Only occasionally the labelling was in sub-urothelial nerves.

Interpretation of results

In the present study we confirmed the presence of VR1, or TRPV1 according the new nomenclature, in the human urinary urothelium. In particular, a VR₁-positivity was seen in the urothelium of patients suffering from IC and it was not different from normal subjects as we showed in other studies. We confirmed the presence of VR1 in the mast-cells and only a very moderate presence of nerves which are positive for VR1. At the end of a prolonged instillation of RTX the immunoistochemical positivity seemed limited to the basal layer of urothelium with the disappearance in the more superficial layers. Our results are puzzling for many reasons. The sample size is very small and the prolonged intravesical instillation of RTX remained at the stage of pilot study.

Concluding message

Further study are mandatory to offer speculations or suggestions about our results and to understand the mechanism of action of intravesical vanilloids when they are used to treat painful bladder syndromes.

References

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