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

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Hypothesis / aims of study
Interstitial cystitis (IC), a syndrome characterized by motor and sensory dysfunction of the lower urinary tract, which is know 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 (VR_1), 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 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 $TRPV_1$ 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 showed 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 VR_1 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 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 instilation of RTX and at the end of the instillation when the sovrapubic 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 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 was positive while the labelling was nor more present in the superficial and club-shaped cells. Only occasionally the labeling was in sub-urothelial nerves.