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PATIENTS WITH INCREASED EXPRESSION OF TRPV1 IN BLADDER BENEFIT FROM RESINIFERATOXIN INTRAVESICAL INSTILLATIONS FOR REFRACTORY DETRUSOR OVERACTIVITY

Hypothesis / aims of study
Intravesical treatment with vanilloids was previously thought to act as selective neurotoxins for afferent nerve desensitization and had a therapeutic effect in patients with neurogenic detrusor overactivity (NDO) due to spinal cord injury. The binding of this agent to vanilloid receptor (TRPV1) on bladder sensory fibers suppresses the micturition reflex at low volume and could successfully abolish or reduce DO refractory to anticholinergic treatment. Recent studies have demonstrated that vanilloid receptors TRPV1 participate in normal bladder function are essential for normal mechanically evoked purinergic signaling by the urothelium and are involved in adenosine triphosphate release. In conditions of NDO or idiopathic DO (IDO), there is up-regulation of unmyelinated nerve fibers expressing vanilloid receptors. Although clinical evidences have shown that resiniferatoxin has therapeutic effects on IDO as well as NDO, it is unable to predict which patients will benefit from intravesical vanilloid therapy. Real-time quantitative PCR method is a sensitive and accurate method with a large dynamic range to measure gene expression levels. Quantitative reverse transcription-polymerase chain reaction (quantitative RT-PCR) methods was used to measure the expression levels of transient receptor potential vanilloid 1(TRPV1) mRNA in bladders from patient with IDO and control group. From TRPV1 mRNA expression levels and the clinical outcome of intravesical RTX treatment, the correlation of intravesical resiniferatoxin (RTX) therapeutic efficiency and TRPV1 mRNA expression in IDO patients was further evaluated.

Study design, materials and methods
Twenty-eight patients with NDO or IDO refractory to anticholinergics were enrolled and treated with four weekly intravesical instillations of 10 nM resiniferatoxin. Bladder mucosa were biopsied at baseline in all patients. The quantitative analysis of TRPV1 expressions in bladder mucosa was performed and compared between the responders and nonresponders. A group of bladder biopsies from non-bladder conditions was also used as normal control. Total RNA was extracted from each sample and aliquot of RNA was used for individual quantitative reverse transcription-polymerase chain reaction (quantitative RT-PCR). Relative standard curve method was used for quantification of expression. The expression level of TRPV1 mRNA was normalized by glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and 18S rRNA mRNA. TRPV1, GAPDH and 18S rRNA mRNA were measured using pre-developed TaqMan assay reagents MGB probe according to the manufacturer's protocol (Applied Biosystems). All experiments were performed in duplicate. Differences in normalized TRPV1 mRNA levels between IDO and controls were assessed using an independent Student *t*-test. All the calculations were implemented using SPSS for windows version 10.0.

Results
A total of 28 patients with IDO were enrolled in a trial of multiple intravesical 10 nM RTX treatment . At 3 months after completing the treatment, 14 patients (50%) had an excellent or improved result in urgency and incontinence episode (responders) while the other 50% failed the treatment (non- responders). Among these patients, bladder biopsies were available for TRPV1 mRNA analysis in 18, 7 of them responded clinically and 11 were non-responders. After normalized with reference genes, transcript levels correlated significantly with the RTX therapeutic effect (p=0.004) with elevated TRPV1 mRNA expression measured in RTX responders (n=7, TRPV1/GAPDH median value= 1.50, range from 0.89 to 2.78) compared with non-responders (n=11, TRPV1/GAPDH median value= 0.74, range from 0.34 to 1.32). In addition, RTX responders also had higher TRPV1 expression levels compared with the control group (n=5, TRPV1/GAPDH median value=0.87, range from 0.62 to 1.04) although it was not statistically significant (p=0.067). In the non-responders, the TRPV1 transcript levels were not different significantly from the control (p= 0.367).
Interpretation of results
Although intravesical resiniferatoxin treatment is theoretically effective in the treatment of DO, successful therapeutic results are not obtained in all patients. The vanilloid receptors on the sensory fibers in the bladder become over-expressed in DO might be the key for the successful treatment with resiniferatoxin. The decrease of TRPV1 immunoreactive nerve fibers in responders to resiniferatoxin had been shown in neurogenic DO, however, the baseline nerve fiber values were similar in responders and nonresponders. This study has shown that the mean expression of TRPV1 was similar between the nonresponders and normal control. The expression of TRPV1 in the responders was significantly higher than in the nonresponders, suggesting an over-expression of TRPV1 in NDO or IDO could be the cause for their clinical symptoms. The other 50% of patients with DO who did not respond to intravesical resiniferatoxin might have intrinsic neurogenic disorders other than over-expression of TRPV1 and should be treated with other neurotoxins.

Concluding message
DO can occur in myogenic or neurogenic conditions that many sensory receptors and transmitters are involved in the mechanisms, including TRPV1 in the bladder. Successful intravesical resiniferatoxin treatment depends on the existence of over-expression of TRPV1 in the bladder mucosa and submucosa. Although bladder biopsy is not a suitable procedure as a predictor for responder of resiniferatoxin, the result of this study has demonstrated the underlying mechanism for a successful intravesical resiniferatoxin in patients with DO.

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HUMAN SUBJECTS: This study was approved by the IRB of Tzu Chi genernal hospital and followed the Declaration of Helsinki Informed consent was obtained from the patients.