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INCREASED SUBUROTHELIAL NERVE FIBER AND PURINERGIC P2X3 RECEPTOR EXPRESSIONS IN PATIENTS WITH IDIOPATHIC DETRUSOR OVERACTIVITY AND THEIR RELATIONSHIP WITH BOTULINUM TOXIN A THERAPEUTIC OUTCOME

Hypothesis / aims of study

Recent investigations have linked detrusor overactivity (DO) with dysregulation of neurogenic and purinergic signaling, which produces sensory- or motor-activated incontinence. Increases in expressions of neurotrophic factors such as NGF and BDNF have been implicated in the pathogenesis of overactive bladder (OAB) syndrome in many reports. Bladder distension causes release of ATP from the urothelium, and that ATP can activate P2X3 receptors on suburothelial afferent nerve terminals to evoke a neural activation. Accumulated evidence suggests that non-cholinergic activation via purinergic receptors may occur in disease states. Intravesical onabotulinumtoxin A (BoNT-A) injection is a promising treatment for DO. It has been demonstrated that in addition to its inhibitory effect on presynaptic release of acetylcholine, BoNT/A may decrease the expressions of sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers. In this study we investigated suburothelial nerve fibers and purinergic P2X3 receptor expression levels in the bladder wall of idiopathic detrusor overactivity (IDO) patients, and their relationship with the therapeutic outcome of intravesical BoNT/A injection.

Study design, materials and methods

Suburothelial tissues of patients with urodynamically and clinically proven IDO (n=18) were taken before intravesical 100 U BoNT/A injection, and 10 bladder tissues from patients with asymptomatic microscopic hematuria were obtained as controls. Nerve fiber and purinergic P2X3 receptor expression levels were measured by western blot assay. Antibodies recognizing P2X3 receptor or Protein Gene Product 9.5 (PGP9.5, a pan-neuronal marker) were used in the study. Successful BoNT/A treatment was classified as significant improvement in sensory effects with urgency severity score reductions of ≥2 and/or motor effects with cystometric bladder capacity increases of ≥25%. Patients with a successful therapeutic effect lasting more than 3 months were considered as responders; otherwise they were considered as non-responders.

Results

From the western blot assay, after normalization with the internal control GAPDH, the expression levels of P2X3 receptor or pan-neuronal marker PGP9.5 were significantly increased in the suburothelial tissues of the IDO patients (n=18) as compared with the controls (n=10) (p values 0.003 and 0.04, respectively) (Fig. 1, Table 1). According to the duration of the BoNT/A therapeutic effect in the IDO patients, there were 13 responders and 5 non-responders. The responders had higher PGP9.5 (p=0.03) and P2X3 (p=0.01) expression levels in the IDO patients than in the controls. However, only a significant increase in P2X3 (p=0.013) but not in PGP9.5 expression (p=0.371) was observed in the non-responders (Table 2). When analyzing the relationship between PGP9.5 and P2X3 expression, PGP9.5 increase was significantly assocaited with P2X3 elevation (p=0.018) in the responders, but there was no association between PGP9.5 and P2X3 expression in the non-responders (p=0.93) (Fig. 2).

Interpretation of results

Previous studies have found that PGP9.5- and P2X3-expressing nerve fibers were increased in bladder mucosa of patients with neurogenic detrusor overactivity (NDO). Our study demonstrated that the IDO patients had significantly higher urinary NGF, suburothelial PGP9.5 and purinergic P2X3 receptor expression levels than the controls. This study showed that the number of nerve fibers was also increased in the bladder mucosa of IDO patients, as seen in NDO. In addition, non-cholinergic via purinergic P2X3 receptor activation may cause sensory dysfunction and could be involved in the pathogeneses of IDO. Our study also found that the IDO patients who had a longer BoNT/A therapeutic effect the PGP9.5 levels were synergistically increased with P2X3 receptor elevation. The results imply that IDO patients with both neurogenic and purogenic signaling disorder have a longer BoNT/A therapeutic effect.

Concluding message

Elevation of nerve fiber numbers and purinergic P2X3 receptor expression in bladder mucosa of IDO patients implies that both neurogenic and purogenic signaling disorder might be involved in the pathogenesis of IDO. Patients with synergistic increases in nerve fiber numbers and P2X3 receptor expression will have a longer BoNT/A therapeutic duration.

Table 1 Expression of PGP9.5, P2X3 and urinary NGF levels in patients with IDO and controls

	IDO (n=18)	Control (n=10)	*P value*
gender	F:9 M:9	F:10	
age	67.17±3.09	53.60±3.04	*0.01***
PGP9.5	0.53±0.05	0.37±0.05	*0.04**
P2X3	1.15±0.06	0.74±0.10	*0.003***
Urinary NGF/Cr	0.55±0.20	0.04±0.03	*0.024**

Table 2. Comparison of suburothelial PGP9.5, and P2X3 and in IDO patients with different BoNT/A therapeutic effects

	Responder (n=13)	Non-responder (n=5)	Control (n=10)
PGP9.5	0.54±0.06*	0.49±0.27	0.37±0.05
P2X3	1.08±0.05*	1.32±0.16*	0.74±0.10

*: p value <0.05 as compare with control

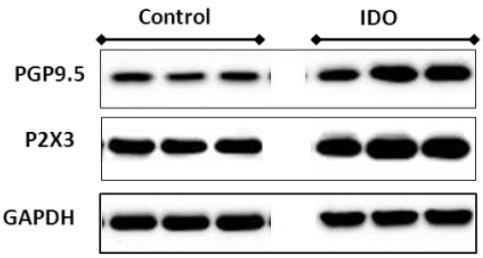

figure 1. Western blot of PGP9.5 and P2X3 protein expression

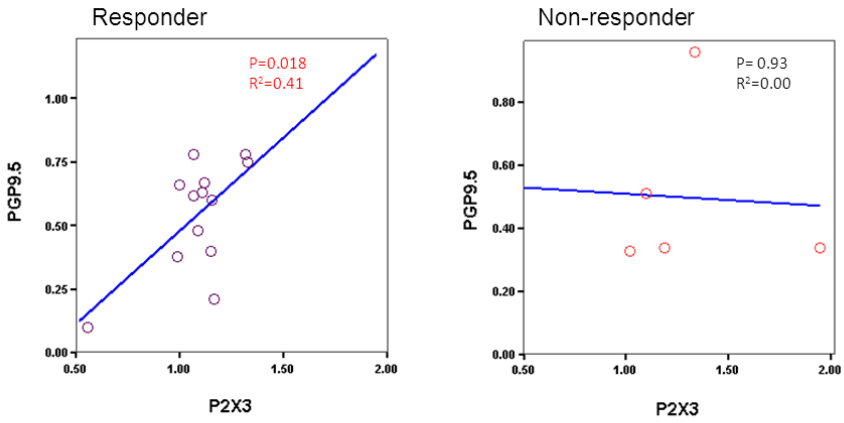

figure 2. Correlation of PGP9.5 and P2X3 expressions in patients with different BoNT/A therapeutic effects

Disclosures
Funding: None **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Research Ethics Committee of Buddhist Tzu Chi General Hospital, Hualien, Taiwan **Helsinki:** Yes **Informed Consent:** Yes