

## THE EFFECTS OF INTRAVESICAL RESINIFERATOXIN ON SUBUROTHELIAL NERVE DENSITY IN PATIENTS WITH NEUROGENIC DETRUSOR OVERACTIVITY

### Aims of Study

Somatic and autonomic neural pathways integrated in the CNS regulate urine storage and voiding. When spinal cord lesions disrupt these pathways, an aberrant segmental sacral reflex emerges, the afferent limb of which is comprised of small unmyelinated C-fibres that originate in the suburothelium. This pathological reflex is thought to be the underlying cause of "spinal" neurogenic detrusor overactivity (NDO).

We are currently evaluating the role of resiniferatoxin (RTX) in the treatment of refractory "spinal" NDO. RTX is an ultra-potent capsaicin analogue purified from the dried latex of the succulent plant *Euphorbia Resinifera*. The vanillyl group of RTX facilitates the action of the vanilloid receptor (VR-1), a non-selective cation channel expressed on suburothelial C-fibres. Intravesical therapy with a single dose of RTX results in early acute excitatory effects ("sensitisation") followed by inhibition of neuropeptide release and neuronal terminal field degeneration ("desensitisation") (Avelino et al., 2001).

In patients with refractory NDO, it has been shown that intravesical therapy with vanilloid compounds such as RTX or capsaicin significantly decreases lower urinary tract symptoms for a period of weeks or months, depending on factors such as dose and disease progression. It is thought that the eventual recurrence of symptoms is related to C-fibre regeneration. We have previously shown that the mean suburothelial nerve density decreases in patients who respond to capsaicin. Our experimental aim is to measure suburothelial nerve density in flexible cystoscopic biopsies in controls and patients with NDO before and after intravesical RTX or placebo.

### Methods

The study met with local ethics committee approval and study participants gave informed consent. Flexible cystoscopic biopsies were taken from the postero-lateral wall of the bladder 2cm above the ureteric orifice in 8 normal control subjects and 18 patients before intravesical RTX or placebo as part of a placebo controlled multi-centre trial. 11 patients had multiple sclerosis, 6 had spinal cord injury and 1 had tropical spastic paraparesis. Following treatment with RTX, biopsies were taken 4 weeks following treatment as per protocol or at a time where the clinical response was continuing. Multiple 10 $\mu$ M frozen sections were stained with H&E for routine histological examination and with anti bodies to the pan-neuronal marker PGP 9.5 for nerve density analysis.

The mean nerve density for each biopsy was determined by counting the number of nerves per mm<sup>2</sup> in 3 sections per biopsy using a graticule with a 10 x 10 grid. In order to eliminate bias, the observer was blinded to the nature of the control tissue (control, pre RTX and post RTX or placebo) and the clinical response.

### Results

The mean nerve density in pre RTX biopsies in patients with NDO was significantly higher when compared to normal controls [326.5 per mm<sup>3</sup> (SD 194.1, SE mean 64.9) and 69.9 per mm<sup>3</sup> (SD 87.5, SE mean 29.2) respectively, p=0.009 paired samples t-test]. Biopsies were taken 4 weeks following treatment as per protocol or at a time where maximum clinical response was maintained. In those who responded clinically to RTX therapy (n= 5) the density of PGP 9.5 staining nerves in the suburothelium decreased by an average of 229.1 (SD 143.86) to 22.9 (SD 21.27) per mm<sup>3</sup> following various doses of RTX (p=0.033, Table 1). In contrast, nerve densities in non-responders (n= 8) increased by a mean of 217.5% following treatment (table 2).

Table 1. Changes in nerve density in patients responding clinically to RTX

Patient study #	Clinical response to RTX	Nerve Density Pre RTX	Estim. dose RTX	Time (weeks) from RTX to biopsy	Nerve Density Post RTX
13	Complete	68.03	0.7µmol	10	1.36
14	Complete	449.03	0.4µmol	40	31.19
16	Complete	266.67	0.2µmol	6	34.82
01	Partial	148.3	0.4µmol	4	47.62
05	Partial	213.66	0.55µmol	7	0
Mean(med) Density		<b>229.138 (213.66)</b>			<b>22.998 (31.19)</b>

Table 2. Changes in nerve density in patients not responding to RTX

Patient study #	Nerve Density Pre RTX	Nerve Density Post RTX	% Change
04	43.19	331.93	+ 668.5
08	383.52	602.82	+ 57.2
10	57.91	172.79	+198.4
12	53.51	491.13	+ 817.8
15	176.18	312.35	+ 77.3
18	574.11	429.03	- 25.3
19	567.59	518.05	- 8.7
20	397.54	220.38	- 44.6
		MEAN = + 217.5%	

### **Conclusions**

We have shown that in patients with spinal NDO, the suburothelial nerve density is higher than in normal controls, this result compliments previous studies. Intravesical RTX decreased the number of immunoreactive suburothelial nerves in patients who responded to treatment. We believe that these results support further trials of RTX for patients with refractory NDO.