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ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS) EXPRESSION IN NEUROGENIC URINARY BLADDERS TREATED WITH INTRAVESICAL RESINIFERATOXIN (RTX)

Aims of Study

Intravesical resiniferatoxin (RTX) has been used as a treatment for neurogenic detrusor overactivity (NDO), and it has been shown that following effective treatment the number of TRPV1 (capsaicin receptor VR1) expressing sensory fibres is reduced. Chronic bladder inflammation and chronic partial bladder outlet obstruction (PBOO) are common findings in patients with suprasacral NDO. Chronic PBOO in animals is associated with a relative increase in bladder mucosa microvessel density that is consistent with angiogenesis. Endothelial nitric oxide synthase (eNOS), a mediator of vasodilation and angiogenesis, has been reported to be increased in bladder biopsies from MS patients, but the reason for that remains unknown. The aim of this study was to investigate eNOS immunoreactivity in bladder biopsies from NDO patients before and after treatment with intravesical RTX, and make comparisons with control material. The distribution of two other vascular markers, the structural vessel marker von Willebrand Factor (vWF) and the angiogenesis marker vascular endothelial growth factor (VEGF), was also studied.

Methods

Flexible cystoscopic bladder biopsies were obtained from 8 control subjects being investigated for asymptomatic microhaematuria and 19 patients with refractory spinal NDO due to multiple sclerosis (13 patients), spinal cord vascular accidents (3 patients), transverse myelitis (2 patients) and tropical spastic paraparesis (1 patient), who were recruited in a prospective, randomized, parallel-group, double-blind, placebo-controlled trial using escalating doses to a maximum of 1µMol RTX. Biopsies were obtained at baseline, at 4 weeks after the first and each subsequent instillation, and at the time of maximum clinical response. Specimens were examined by histology (haematoxylin-eosin staining) and immunohistochemistry, using polyclonal rabbit antibodies for eNOS (Santa Cruz, sc-654; 1:5,000 dilution), vWF (Novacastra Laboratories, NCL-vWFp; 1:10,000 dilution) and VEGF (Santa Cruz, sc-152; 1:5,000 dilution). Sites of antibody attachment were revealed using the ABC (peroxidase) method. Fewer baseline NDO specimens (n=8) were available for vWF and VEGF staining. Computerized image analysis was performed for quantification of immunoreactivity. The Mann-Whitney test was used for statistical analysis.

Results

Fourteen NDO patients received maximum RTX dose; 5/14 responded clinically and the other 9 showed no improvement. Despite RTX adsorption problems to the delivery system, leading to retrospective extrapolation of the actual dose of RTX delivered in each instillation according to the pre-instillation 'incubation' time, there appeared to be no difference in the mean dose of RTX administered in responders as opposed to the non-responders. Clinical and histopathological features were also similar in the two patient groups.

Endothelial NOS immunoreactivity was found in the suburothelium and, less often, in the urothelium, with a distribution indicating localization in small blood vessels at the urothelium-suburothelium junction. vWF immunostaining showed similar localization. There was a trend to higher pre-treatment eNOS values in responders than non-responders to RTX (P=0.059), and a significant reduction in eNOS-immunoreactive vessel staining following successful RTX treatment (P=0.016)(Figure 1). VEGF staining was weaker, but there was a significant increase in pre-treatment biopsies of responders to RTX (P=0.048).

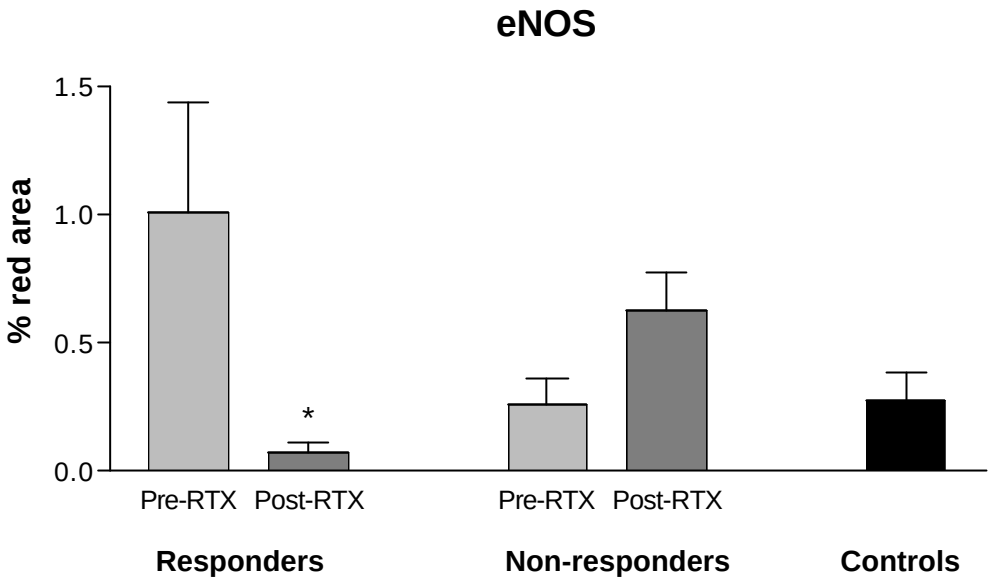

Figure 1 - Endothelial NOS immunoreactivity in responders and non-responders to RTX, before and after treatment, and compared to controls. Post-RTX eNOS expression in responders is reduced (*P = 0.016) in comparison with pre-treatment values, to levels similar to controls. Baseline non-responders values are very similar to those of controls subjects.

Conclusions

The trend for higher eNOS expression in patients with NDO who responded to RTX suggests that increased vasculature or vasodilation in the suburothelium may be necessary for successful intravesical RTX treatment. The appearance of small blood vessels staining for eNOS at the urothelium-suburothelium junction may indicate a functional role for eNOS in NDO. Further studies with larger numbers of patients are required to confirm the changes of bladder vascular markers with RTX treatment, and to examine the mechanisms underlying changes in bladder vasculature in respect with levels of detrusor overactivity.