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DO CANNABINOID AGONISTS USED FOR THE TREATMENT OF REFRACTORY LOWER URINARY TRACT SYMPTOMS IN PATIENTS WITH NEUROGENIC DETRUSOR OVERACTIVITY HAVE AN EFFECT ON BLADDER AFFERENT PATHWAYS?

Hypothesis / aims of study

Recent clinical trials suggest a beneficial effect of cannabis-based medicinal extracts (CBMEs) on urgency incontinence, daytime frequency and nocturia associated with neurogenic bladder dysfunction in patients with multiple sclerosis (MS). In a multicentre randomised, controlled trial using sublingual spray of extracts containing delta-9-tetrahydrocannabinol (Δ 9-THC) and cannabidiol (CBD) in a 1:1 ratio, the active treatment group showed significantly better improvements in daytime frequency, nocturia and Patient Global Impression of Change compared to placebo.

The action of CBMEs is exerted via the cannabinoid receptors CB1 and CB2. CB1 had been identified in both the central and peripheral nervous system and related to vasodilation and reduced motility, while CB2 mostly outside the CNS associated with anti-inflammatory, antinociceptive and immunosuppressive actions. Recently, both receptors were identified in the human bladder, more so in the urothelium than the detrusor muscle. CB2 receptors were localised in sensory nerve fibres expressing the vanilloid receptor TRPV1, as well as in cholinergic fibres. However, their expression in bladder dysfunction has not been studied to date. Finally, activation interactions between CB1 and TRPV1 by endocannabinoids are known to exist.

We hypothesised that cannabinoid receptors are differentially expressed in neurogenic overactive bladders compared to non-overactive bladders. We also hypothesised that cannabinoid agonists used for the treatment of neurogenic OAB have a local effect on bladder afferent pathways. To explore these hypotheses, we examined the expression of CB1 and CB2 receptors in bladder biopsies from MS patients with intractable OAB symptoms before and after treatment with either placebo or a sublingual endocannabinoid modulator comprising THC and CBD in a 1:1 ratio, and in comparison with control biopsies. We also examined the expression of TRPV1 and muscarinic receptors type 1, 2 and 3 (M1, M2, M3) in the same biopsies.

Study design, materials and methods

Bladder biopsies were obtained from 20 MS patients via flexible cystoscope before and at 8 weeks after treatment with either Sativex (THC:CBD 1:1 ratio, GW Pharmaceuticals) or placebo. All MS patients suffered from urgency incontinence refractory to treatment with antimuscarinics, had urodynamically proven detrusor overactivity (DO) and were not performing clean intermittent self-catheterisations. Control, cold-cup bladder biopsies were obtained from 20 patients undergoing transurethral bladder tumour resection or prostatectomy. All specimens were taken from macroscopically normal parts of the bladder approximately 2cm above and lateral to the ureteric orifices. Control patients had to be free of OAB symptoms having scored 0-1 in the urgency question of the IPSS questionnaire. All biopsies were obtained following written informed consent and approval by the local Research Ethics Committees and Hospitals' Scientific Boards of the two involved Institutions.

RNA extraction: Tissue was snap-frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted from specimens using TRIzol[®] Reagent (Invitrogen). Contaminating DNA was removed by treatment with RNase-free DNase (Fermentas Life Sciences) at 37°C for 30min.

Reverse transcription and real-time PCR: The reverse transcription was done on 0.8-1.0 μ g total RNA by SuperScript[™] First-Strand Synthesis System (Invitrogen) using 50ng random hexamers/reaction. No RT control reactions corresponding to each sample RNA were included. Expression levels for CB1, CB2, TRPV1, M1, M2 & M3 transcripts were determined by real-time PCR using human gene-specific primers and probes. All primers and probe sets were from Applied Biosystems (Assay-on-Demand Gene Expression Products). All real-time PCR reactions were carried out starting with cDNA equivalent to ~30ng of total RNA. Reactions were pre-incubated at 50°C for 2 min and at 95°C for 10 min and then subjected to 40 cycles of amplification at 95°C for 15 sec and 60°C for 1 min, for denaturing and annealing extension, respectively. Comparative Δ Ct run and analysis method was used in all cases and all experiments were performed in duplicate. Expression levels of transcripts in all cases were normalized by the use of β -actin as internal standard.

Statistics. The unpaired t-test was used for baseline versus control comparisons, and the paired t-test for intra-group comparisons. All values are expressed as mean \pm SEM. P values less than 0.05 were considered significant. Biopsy results were associated with bladder diary and urodynamic data.

Results

DO biopsies versus controls. Increased CB2 expression was found in DO biopsies (0.78 ± 0.16 v 0.21 ± 0.11 , $p=0.0059$). By contrast, CB1 (0.88 ± 0.14 v 1.22 ± 0.02 , $p=0.052$) and M1 expression (0.69 ± 0.14 v 1.15 ± 0.07 , $p=0.009$) was found to be higher in controls. No differences between DO and controls were noted in TRPV1, M2 and M3 expression ($p=0.89$, $p=0.91$ and $p=0.26$ respectively).

Pre- versus post-treatment. CB2 expression was reduced after active treatment (0.68 ± 0.23 v 0.28 ± 0.19 , $p=0.19$) but remained unchanged after placebo ($p=0.97$). By contrast, CB1 and M1 receptors expression was left unchanged after active treatment ($p=0.89$ and $p=0.91$, respectively) but increases were noted in those who received placebo treatment (0.84 ± 0.20 v 1.31 ± 0.02 , $p=0.07$ for CB1, and 0.64 ± 0.22 v 1.19 ± 0.14 , $p=0.03$ for M1). No changes were noted in TRPV1, M2 and M3 expression after either active or placebo treatment.

Clinical findings – urodynamics. Urodynamic parameters were unchanged after either active or placebo treatment (volume at first desire to void: p=0.89 and 0.39 respectively, volume at normal desire to void: p=0.49 and 0.09 respectively, maximum cystometric capacity: p=0.22 and 0.85 respectively).

Bladder diary data: Both the active and the placebo treatments achieved significant reductions in incontinence episodes (p=0.005 and p=0.016 respectively), with a trend for a superior improvement after active treatment compared to placebo (p=0.08). Only active treatment produced significant improvements in the number of urgency episodes (p=0.019), daytime frequency (p=0.002), nocturia (p=0.019) and Patient Global Impression of Change (p=0.0002).

Interpretation of results

Cannabinoid receptors appear to be differentially expressed in neurogenic overactive bladders compared to controls. The increase in CB2 could be due to the frequent presence of chronic inflammation in neurogenic bladders, but also associated with OAB symptoms considering the localisation of CB2 in sensory nerve fibres. In this respect, post-treatment decrease could be associated with symptomatic improvement. CB1 and M1 receptor expression showed parallel changes in neurogenic bladders before and after treatment, suggesting possible common regulatory mechanisms. Results should be cautiously interpreted as they may not represent respective receptor protein changes.

Concluding message

Cannabinoid receptors appear to be differentially expressed in neurogenic overactive bladders compared to controls. Urothelial/suburothelial CB2 receptors may be important in the local effect of oral cannabinoid agonists on bladder function, as their changes followed the changes in patients' symptoms.

<i>Specify source of funding or grant</i>	GW Pharmaceuticals FP7-2007 INComb (Combating incontinence, from basic science to clinical practice) Unit for Study of Urological Diseases, Aristotle University of Thessaloniki
<i>Is this a clinical trial?</i>	Yes
<i>Is this study registered in a public clinical trials registry?</i>	No
<i>Is this a Randomised Controlled Trial (RCT)?</i>	Yes
<i>What were the subjects in the study?</i>	HUMAN
<i>Was this study approved by an ethics committee?</i>	Yes
<i>Specify Name of Ethics Committee</i>	Joint UCL/UCLH Research Ethics Committee, London, UK Bioethics Committee, Aristotle University of Thessaloniki, Greece
<i>Was the Declaration of Helsinki followed?</i>	Yes
<i>Was informed consent obtained from the patients?</i>	Yes