Treatment with a Cannabinoid Receptor 2 Agonist Decreases Severity of Established Cystitis

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Purpose: We investigated whether treatment with the selective cannabinoid receptor 2 agonist GP1a would ameliorate the severity of experimental cystitis. We determined the association of referred hyperalgesia and increased urinary frequency after establishing cystitis in mice by intravesical instillation of acrolein.

Materials and Methods: Cystitis was induced by intravesical instillation of acrolein in female C57BL/6NH mice. Mice were treated with GP1a (10 mg/kg intraperitoneally) or vehicle 3.5, 22 and 30 hours after instillation of acrolein. Mice were tested for mechanical sensitivity of hind paws. Short-term voluntary voiding was assessed by quantifying urine spots of freely moving mice. Bladders were collected, weighed and processed for immunohistochemical, histological and immunoblotting analysis.

Results: At 48 hours after acrolein instillation the bladder of all mice showed histological evidence of inflammation. The severity of edema and increase in bladder weight were inhibited in cannabinoid receptor 2 agonist treated animals ($p < 0.05$). Neither cystitis nor treatment with GP1a or AM630 (selective cannabinoid receptor 2 antagonist) plus GP1a appeared to alter cannabinoid receptor 2-like immunoreactivity abundance in urothelium. Mechanical sensitivity was significantly increased after acrolein and the increase was attenuated in cannabinoid receptor 2 agonist treated mice ($p < 0.05$). The number of small diameter urine spots was significantly increased after acrolein and treatment with GP1a attenuated this increase ($p < 0.05$). GP1a effects were prevented by AM630.

Conclusions: Treatment with a selective cannabinoid receptor 2 agonist decreased severity of established acrolein induced cystitis and inhibited bladder inflammation associated increased referred mechanical sensitivity and increased bladder urinary frequency. Our data indicate that cannabinoid receptor 2 is a potential therapeutic target for treatment of painful inflammatory bladder diseases.

Key Words: urinary bladder; cystitis, interstitial; cannabinoid receptor agonists; N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-1,4-dihydro-6-methylindeno(1,2-c)pyrazole-3-carboxamide; mice

Abbreviations and Acronyms

AM630 = 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone

CB = cannabinoid receptor

GP1a = N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-1,4-dihydro-6-methylindeno[1,2-c]pyrazole-3-carboxamide

IC/PBS = interstitial cystitis/painful bladder syndrome

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CANNABINOIDS have anti-inflammatory effects. Study design has consistently entailed cannabinoid administration before initiating inflammation.[1,2] The effects of cannabinoids are mediated primarily by CB1 and CB2, which are coupled to inhibitory G proteins.[1–3] Intravesical administration of a selective CB1 agonist inhibited bladder afferent nerve sensitization induced

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by bladder inflammation.[4] However, Dinis et al reported that treatment with a selective CB1 antagonist did not affect enhanced reflex reactivity associated with bladder inflammation.[5] On the other hand, CB2 is present in the bladder of various species, including humans, monkeys and rodents,[6–9] particularly in urothelial cells.[6–8] Treatment with a selective CB2 agonist increased micturition interval and volume in normal rats[9] and improved bladder function in rats after partial urethral obstruction.[10] These studies support a role of CB2 in regulating bladder function under physiological and pathophysiological conditions.

IC/PBS is a painful chronic disorder characterized by increased frequency, urgency and bladder pain.[11] The etiology and pathogenesis of IC/PBS remain unknown and no treatment or combination of treatments has been consistently effective in alleviating symptoms in patients with IC/PBS.[11] We previously reported that pretreatment with the CB2 agonist GP1a decreased the severity of acute cystitis induced by acrolein and associated referred hyperalgesia.[12] To further explore the potential of CB2 as a therapeutic target for inflammatory bladder disease we extended our investigation to examine whether treatment with the CB2 agonist GP1a would ameliorate the severity of experimental cystitis and associated referred hyperalgesia after establishing cystitis by intravesical instillation of acrolein. Such studies may have greater clinical relevance since patients typically seek medical treatment of established disease rather than prevention of impending disorders.

MATERIALS AND METHODS

Study Design

Ten to 12-week-old female C57BL/6NH mice were obtained from Harlan®. Experiments were done in accordance with National Institutes of Health Guidelines. All protocols were reviewed and approved by the University of Wisconsin animal care and use committee.

Mice were anesthetized with Avertin® (250 mg/kg) injected intraperitoneally. Cystitis was induced by intravesical instillation of acrolein (0.5 mM and 150 μl total volume, Ultra Scientific, Kingstown, Rhode Island) via a PE10 urethral catheter (BD™) with an inner and outer diameter of 0.28 and 0.61 mm, respectively. Acrolein remained in the bladder for 40 minutes. Control mice received an equivalent volume of intravesical saline (0.9%) instead of acrolein.

The selective CB2 agonist GP1a and antagonist AM630 (Tocris, Bristol, United Kingdom) were dissolved in ethanol as stock solutions and diluted in saline to desired concentrations. GP1a (10 mg/kg) or vehicle was given intraperitoneally 3.5, 22 and 30 hours after acrolein instillation. AM630 (10 mg/kg) or vehicle was given 10 minutes before GP1a injection by subcutaneous injection.

At 48 hours after acrolein or saline instillation the mice were deeply anesthetized with pentobarbital (50 mg/kg intraperitoneally) and perfused with saline through a cannula inserted in the left ventricle. The bladders were removed and weighed. Bladder weight in mg was normalized to body weight in gm. The bladders were then divided into 2 parts. The caudal part, including the neck region, was fixed and tissue sections were made for morphological and immunohistochemical analysis. The urothelium/suburothelium of the remainder of the bladders was mechanically separated from the detrusor and stored at −80C until analysis.[12]

Histological Analysis and Immunohistochemistry

Histological analysis was performed after hematoxylin and eosin staining. Acrolein induced cystitis was characterized primarily by edema in the submucosal region. An edema score was determined, including 0—no evident edema, 1— mild, 2—moderate or 3—severe edema.[13] The increased bladder weight correlated closely with the severity of edema and changes in bladder weight reliably reflect cystitis severity in this model. Therefore, we also measured bladder weight and used these values for semiquantitative analysis of cystitis severity.[13] In contrast to cystitis induced by cyclophosphamide/acrolein in rats, infiltration of inflammatory cells is mild and varies considerably in the same treatment group in mice (unpublished data). Also, urothelial ulceration was not consistently observed with the dose of acrolein used, which produced cystitis of moderate severity.

For immunohistochemistry bladder tissue sections were blocked with 10% normal goat serum. A specific CB2 antibody (1:500, Cayman Chemical, Ann Arbor, Michigan) was applied and tissue sections were incubated in a humid chamber overnight at 4C. Staining was revealed using secondary goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (1:1,000, Sigma®). Slides were rinsed and mounted with antifade solution (Vector Laboratories, Burlingame, California). Slides were examined with a Nikon™ E600 microscope and digital images were captured. CB2 antibody specificity is well described. This antibody reveals CB2 expression in various tissues in wild-type but not in CB2 knockout mice.[14]

Semiquantitative Immunoblot Analysis

The urothelium/suburothelium was homogenized. Protein samples were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to nitrocellulose membranes. Membranes were blocked and incubated at 4C overnight with CB2 antibody (1:1,000, Cayman Chemical). Membranes were incubated with secondary antibody conjugated to horseradish peroxidase. Signals were revealed using a chemiluminescent detection reagent (Amersham™). CB2 abundance was estimated by optical density measurement with ImageJ (http://rsb.info.nih.gov/ij/). Values were normalized to the loading control, as determined by glyceraldehyde 3-phosphate dehydrogenase (1:5,000, Abcam®) abundance in the same sample.[13]

Peripheral Nociception Testing

Hind paw mechanical sensitivity was assessed using von Frey monofilaments and the up-down method.[13] Mice were placed individually in a Plexiglas® chamber with