Synergistic antipruritic effects of gamma aminobutyric acid A and B agonists in a mouse model of atopic dermatitis

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Background: Despite recent insights into the pathophysiology of acute and chronic itch, chronic itch remains an often intractable condition. Among major contributors to chronic itch is dysfunction of spinal cord gamma aminobutyric acidergic (GABAergic) inhibitory controls.
Objectives: We sought to test the hypothesis that selective GABA agonists as well as cell transplant-derived GABA are antipruritic against acute itch and in a transgenic mouse model of atopic dermatitis produced by overexpression of the T_H2 cell-associated cytokine, IL-31 (IL-31Tg mice).
Methods: We injected wild-type and IL-31Tg mice with combinations of GABA-A (muscimol) or GABA-B (baclofen) receptor agonists 15 to 20 minutes prior to injection of various pruritogens (histamine, chloroquine, or endothelin-1) and recorded spontaneous scratching before and after drug administration. We also tested the antipruritic properties of intraspinal transplantation of precursors of GABAergic interneurons in the IL-31Tg mice.
Results: Systemic muscimol or baclofen are antipruritic against both histamine-dependent and -independent pruritogens, but the therapeutic window using either ligand alone was very small. In contrast, combined subthreshold doses of baclofen and muscimol produced a significant synergistic antipruritic effect, with no sedation. Finally, transplant-mediated long-term enhancement of GABAergic signaling not only reduced spontaneous scratching in the IL-31Tg mice but also dramatically resolved the associated skin lesions.
Conclusions: Although additional research is clearly needed, existing approved GABA agonists should be considered in the management of chronic itch, notably atopic dermatitis. (J Allergy Clin Immunol 2017;140:454-64.)

Key words: *Atopic dermatitis, baclofen, chronic itch, GABA, GABAergic progenitor cell transplants, muscimol, pruritogens*

Atopic dermatitis (AD), an inflammatory, relapsing chronic pruritic skin disease, is an often intractable form of chronic itch that negatively impacts the quality of life of millions of patients.[1] Unfortunately, because chronic itch conditions have very different etiologies, most treatments have poor outcomes and are accompanied by unacceptable adverse side effects, notably sedation.[2] Clearly, a better understanding of the pathophysiology of these chronic itch conditions is critical to designing successful therapeutic strategies.

Studies of the etiology of chronic itch[3] generally focus on changes in skin and immune dysfunction. However, there is now considerable evidence for a contribution of primary afferent pruritoceptors that transmit itch messages to spinal cord and brainstem circuits engaged by and that regulate these messages.[4] Of particular interest are studies demonstrating commonalities in the mechanisms underlying nerve injury-induced neuropathic pain and itch and the possibility that comparable approaches may be appropriate for their management.[5]

Although there is evidence for specificity in the transmission of itch and pain messages at the level of the primary afferent nociceptor and pruritoceptor,[6,7] both pain and itch are under spinal cord inhibitory interneuron-mediated control. For example, loss of spinal cord gamma aminobutyric acid (GABA) or glycinergic function is a major contributor to the spontaneous pain and hypersensitivity that develops following nerve injury.[8-10] Moreover, persistent scratching, a manifestation of chronic itch, occurs in the *Bhlhb5* mutant mouse, in which there is dramatic loss of dorsal horn GABAergic inhibitory interneurons.[11] Ablation of glycinergic interneurons also induces excessive scratching and pain.[12] And in a model of dry skin–induced scratching in the mouse, GABA and glycine receptor antagonists can block scratching-induced inhibition of firing in superficial dorsal horn neurons.[13] Finally, in patients, acute withdrawal of intrathecal baclofen, a GABA-B receptor agonist, can induce pruritus.[14]

Given the evidence for a potential contribution of GABA agonists in the management of pruritus, it is surprising that there are no studies that assessed their utility in preclinical or clinical conditions. Here, we demonstrate that both GABA-A and

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Abbreviations used
AD: Atopic dermatitis
CQ: Chloroquine
ED_{50}: Median effective dose
GABA: Gamma aminobutyric acid
GAD: Glutamic acid decarboxylase
GFP: Green fluorescent protein
GRP: Gastrin-releasing peptide
GRPR: Gastrin-releasing peptide receptor
IL-31Tg: IL-31 overexpressing transgenic mouse
ip: Intraperitoneal
MGE: Medial ganglionic eminence
NGST: Normal goat serum in PBS with 0.3% Triton
PPTA: Preprotachykinin-A
TRPV1: Transient receptor potential cation channel subfamily V member 1

GABA-B agonists are not only effective in models of acute itch, but we also show that systemic administration of very low doses of these agonists has synergistic antipruritic effects in IL-31 overexpressing transgenic mouse, a model of AD[15] that is refractory to antihistamines[1,16] and thus particularly difficult to manage. Most importantly, the antipruritic synergy could be produced without concomitant sedation. Finally, we show that sustaining high levels of GABA inhibition can be achieved using intraspinal transplantation of cortical GABAergic interneuron precursor cells. The transplants not only attenuated spontaneous scratching but also dramatically reduced skin lesions in the IL-31 overexpressing transgenic mouse (IL-31Tg) mice.

METHODS

Animals

Male C57BL6/J mice purchased from Jackson Laboratories (Bar Harbor, Maine) were used for all experiments unless otherwise stated. IL-31 transgenic mice were a generous gift from ZymoGenetics/Bristol-Myers Squibb (Seattle, Wash). The IL-31Tg mice were generated as previously described.[15] All experiments were approved by the University of California San Francisco Institutional Animal Care and Use Committee and conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals.*

Pharmacology

Based on our previous studies,[7] we used a minimum of 4 to 5 wild-type C57BL6 male mice per group in acute itch studies. For chronic itch studies, because scratching levels vary among IL-31Tg mice, to decrease variability, we only used mice with skin lesions and who exhibited >100 scratching bouts (over 30 minutes) at the nape of the neck. Mice received intraperitoneal (ip) injections of baclofen (1-4.0 mg/kg in saline) or muscimol (0.3-2.0 mg/kg), and motor coordination/sedation was subsequently evaluated using the rotarod test. Only nonsedating doses were subsequently used for behavioral analyses (≤2.0 mg/kg for baclofen and ≤1.25 mg/kg for muscimol). For acute itch studies, we administered the following pruritogens subcutaneously into the nape of the neck: histamine (500 μg/100 μL), endothelin-1 (25 ng/100 μL) and chloroquine (100 or 200 μg/100 μL). To quantify scratching behavior, mice were habituated, individually, in plexiglass cylinders for 1 hour. Mice were then injected with baclofen or muscimol (ip) and 15 to 20 minutes later with the pruritogen. Behavior (scratching) was monitored by video recording over the next 30 minutes.

To assess the antipruritic effects of baclofen and muscimol in IL-31Tg mice, we injected these mice with baclofen (2.0 mg/kg, ip) or muscimol (1.25 mg/kg, ip) and recorded the scratching behavior for up to 1 hour (muscimol) or 6 hours (baclofen). To assess the synergistic effects of the baclofen-muscimol combinations, we injected IL-31Tg mice with a subthreshold dose of baclofen (1.0 mg/kg; ip) 20 minutes prior to a subthreshold dose of muscimol (1.0 mg/kg; ip) and recorded scratching bouts for the next 60 minutes. Motor performance of the IL-31Tg mice injected with baclofen, muscimol, or the combination was also evaluated with the rotarod test. In all behavioral analyses, the investigator scoring the behavior was blind to treatment and codes were only broken after all scoring was completed.

Statistical analyses

Behavioral and anatomical data are expressed as means ± SEMs, where n represents the number of mice. Raw data obtained in the course of the study were analyzed with a 2-way ANOVA followed by a Bonferroni *post hoc* test. Asterisks (*) indicate statistically significant differences between groups: $*P < .05$; $**P < .01$; $***P < .001$.

Cell transplantation

Medial ganglionic eminence (MGE) cells were dissected and transplanted into the spinal cord of IL-31Tg mice, as previously described.[17] Briefly, green fluorescent protein (GFP)-expressing cells from the MGE were harvested from E13.5 embryos, manually dissociated and resuspended in culture medium. One group of animals received MGE cells (MGE group) and one group received medium only (control group). After hemilaminectomy of the C4 to C8 vertebra, we transplanted cells (50,000) unilaterally over 2 segments of the cervical spinal cord. Photographs of the lesions were taken before and once a week for 4 weeks after transplantation.

Immunohistochemistry

Mice were perfused with 10 ml of PBS followed by 30 ml of ice-cold 10% formalin. Spinal cord and lumbar dorsal root ganglia were dissected, postfixed 3 to 4 hours at 4°C, and cryoprotected overnight in phosphate-buffered 30% sucrose. Frozen cryostat sections of spinal cord and dorsal root ganglia were cut at 25 or 14 μm, respectively. After 1 hour of incubation in 10% normal goat serum in PBS with 0.3% Triton (NGST), the sections were incubated overnight in primary antibody diluted in 1% NGST. The following day, the sections were washed 3 times with PBS, and then incubated 1 hour in secondary antibody (Alexa-488 or Alexa-594, diluted 1:1000 in 1% NGST). After washing 3 times in PBS, sections were mounted and coverslipped with Fluoromount G. Sections were viewed with a Nikon Eclipse fluorescence microscope (Tokyo, Japan) and images were collected with a Zeiss confocal microscope (Oberkochen, Germany). Brightness and contrast were adjusted using Adobe Photoshop, version 6.0 (San Jose, Calif).

Antibodies

We used the following antibodies: rabbit anti-GFP (1:2000; Molecular Probe, Eugene, Ore), rabbit anti-Fos (1:4000; Oncogene Research Products, Cambridge, Mass), chicken anti-GFP (1:2000; Abcam, Cambridge, United Kingdom), rabbit anti-GABA (1:2000; Sigma, St Louis, Mo), mouse antiparvalbumin (1:2000; Sigma), and rabbit antineuropeptide Y (1:2000; gift from J. Allen).

Counts of Fos^+ neurons

Labeled cell bodies were counted from digitized images by an experimenter who was blind to treatment. The percentage of Fos^+ cells was determined by counting all Fos^+ cell bodies in the dorsal horn of 10 spinal cord sections. Both ipsilateral and contralateral sides were counted in 3 mice per experimental group. To calculate the percentage of Fos^+ neurons, we divided the number of Fos^+ neurons ipsilateral to the transplant by the number of Fos^+ neurons on the contralateral side and multiplied by 100. Values are presented as means ± SEMs. Statistical significance was assessed by Student *t* test. $P < .05$ was considered significant and is indicated with an asterisk (*).

Skin histology and scoring

Skin biopsies were collected from transplanted and control IL-31Tg mice 4 weeks posttransplant. Skin samples were processed as previously