Methods for Preclinical Assessment of Antipruritic Agents and Itch Mechanisms Independent of Mast-Cell Histamine

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Itch is a sensation that provokes a desire to scratch. Mast-cell histamine was thought to be a key itch mediator. However, histamine and mast-cell degranulation were reported not to elicit scratching in animals. It was difficult to investigate the pathophysiology of itching and to evaluate the antipruritic efficacy of chemical agents in the early 1990s. We showed that hind-paw scratching and biting were elicited by stimulation with pruritogenic agents in mice. Those results demonstrated for the first time that cutaneous itching could be evaluated behaviorally in animals. We established various animal models of pathological itch of the skin (dry skin, mosquito allergy, surfactant-induced pruritus, and herpes zoster) and mucous membranes (pollen allergy). Mast-cell histamine did not play a key role in itching in any animal model examined except for the pollen allergy model. Histamine is not an exclusive itch mediator of mast cells; trypsin and leukotriene B4 released from mast cells also act as itch mediators. Epidermal keratinocytes release several itch mediators, such as leukotriene B4, sphingosylphosphorylcholine, thromboxane A2, nociceptin, nitric oxide, and histamine, which may play important roles in pathological itching. Appropriate animal models of pathological itching are needed for pharmacological evaluation of the antipruritic efficacy of chemical agents.

Key words pruritus; animal model; scratching; biting; keratinocyte; itch mediator

1. INTRODUCTION

The literature on the peripheral and neuronal mechanisms of itching, while still limited in scope, is increasing each year. Animal studies in the past decade have revealed new endogenous itch mediators and shed light on the underlying mechanisms of pathological itching.1–3) Two decades ago, Greaves wrote, “Itching remains an orphan symptom, frustrating to patients and their physicians and sadly neglected by neurophysiologist and pharmacologists.”4) At that time, the methodology for the study of itching in animals was lacking; therefore, there were no animal models of physiological and pathological itch. Mast-cell histamine is a classical endogenous itch mediator, and itch induces a desire to scratch in humans.5) Cutaneous stimulation was shown to elicit hind-paw scratching (scratching reflex) in spinal dogs as early as the 1900s.6) However, in 1965, studies showed that intradermal injections of histamine and the mast-cell degranulator compound 48/80 did not elicit scratching in dogs, although they induced erythema and wheal.7) An intradermal injection of histamine was also reported not to elicit scratching in mice in 1974.8) To compound the issue further, a 1987 study claimed that the “scratching” behavior observed in arthritic rats was due to underlying chronic pain.9) Electrophysiological studies showed that cutaneous stimulation with cowhage spicules, which elicit itching in humans,5) activated C-polymodal nociceptors and myelinated mechanoreceptors in cats.10 11) Histamine was shown to activate most of the C-polymodal nociceptors responsive to bradykinin (an inflammatory mediator that causes pain in humans)12) in a skin-nerve preparation from rats.13) Thus, it was difficult to investigate the pathophysiology of itching and to evaluate the antipruritic efficacy of chemical compounds in the early 1990s. Therefore, we investigated itch-related responses in mice and animal models of pathological itch. This review describes our studies on the development of methods for the preclinical assessment of antipruritic agents and the mechanisms of pruritus, independent of mast-cell histamine.

2. ITCH-RELATED RESPONSES IN ANIMALS

2.1. Scratching Behaviors As mentioned previously, it was difficult to evaluate the efficacy of antipruritic agents in animal experiments in the early 1990s. Since itch induces a desire to scratch in humans, observing scratching behaviors is central to the study of itch in animals. Since compound 48/80 was shown to produce itching via mast-cell degranulation in humans,14) we first examined its effects. An injection of compound 48/80 into the rostral backs of mice elicited hind-paw scratching.15) Substance P was also shown to cause itching via mast-cell degranulation in humans,16) and we found that this peptide could also elicit hind-paw scratching in mice.17) Interestingly, histamine did not cause hind-paw scratching in mice,18) suggesting that mast-cell histamine is not the final common itch mediator. As mentioned previously, “scratching” behavior was claimed to be a sign of chronic pain.19) Therefore, we examined whether algesiogenic agents would elicit scratching in mice and found that injections of the algesiogenic agents capsaicin and formaldehyde into the rostral back did not elicit hind-paw scratching.15) We were thus able to demonstrate for the first time that cutaneous itching could be evaluated behaviorally in animals. Since then, the literature on animal studies of the peripheral and neuronal mechanisms of itch has been gradually increasing (Fig. 1), in most of which hind-paw scratching was used as an index of itching.

Since histamine is a classical itch mediator and H1 hista-
mine receptor antagonists are generally used to treat pruritus, results from itch experiments in which histamine-insensitive mice are used may not be applicable to humans. In our first itch experiment, histamine did not elicit scratching in ddY mice; it also did not elicit scratching in Sprague-Dawley rats (unpublished observation). Therefore, we searched for a strain of mice that would show scratch responses to histamine injection. Serotonin, on the other hand, elicited hind-paw scratching in ddY mice and therefore was used as the positive control. An intradermal injection of serotonin (100 nmol/site) induced hind-paw scratching in all strains of mice tested. In contrast, histamine (100 nmol/site) induced hind-paw scratching only in the ICR strain of mice, and other mice tested were insensitive to histamine (Fig. 2). Therefore, we generally used ICR mice in our animal studies of itch.

### 2.2. Scratching Behaviors and Opioid Receptors

Itch evokes scratching in animals as well as in humans, but scratching is not a behavioral response exclusive to pruritogenetic stimulation. For example, humans and some monkeys scratch their heads with hands as an emotional response, and rodents scratch their flanks with their hind paws for grooming. Therefore, the behavioral distinction between itch-related scratching and non-itch-related scratching is not easy, and we needed to develop new means of verifying when scratching is an itch response. We directed our attention to the effects of opioid antagonists on itching in humans. Opioid antagonists were reported to inhibit experimentally induced itching in healthy individuals and pruritus and scratching in patients with dermatoses and systemic disorders. We found that opioid antagonists suppressed hind-paw scratching induced by pruritogens and associated with allergy and chronic dermatitis in mice. To date, many studies have shown that opioid antagonists inhibit hind-paw scratching in mouse models of pathological itch. Thus, opioid antagonists may be useful to verify that scratching is an itch-related response.

The opioid antagonist naloxone and the µ-opioid receptor antagonist naltrexone inhibit hind-paw scratching induced by intradermal injections of pruritogens in healthy mice. In healthy humans, naloxone increases itch thresholds to histamine. In contrast, µ-opioid receptor agonists elicit facial scratching following intracisternal injection and body scratch-
ing following intrathecal injection, suggesting that both spinal cord and brain are sites of the pruritic side effect of opioid analgesics. However, opioid antagonists may act on the lower brainstem rather than on the spinal dorsal horn and the periphery to inhibit itch signaling. These findings, taken together, suggest that μ-opioid receptors are involved in the transmission or positive modulation of itch signals in the brain under normal conditions.

Opioid peptides have been suggested to be involved in the pruritus of cholestasis. Opioid antagonists reduce scratching activity and pruritus perception in patients with pruritus of cholestasis. However, opioid antagonists cause opioid withdrawal-like side effects in these patients. μ-Opioid receptor binding is decreased in several brain regions in rats that have undergone bile duct resection. Total opioid activity and methionine-enkephalin, an opioid peptide, increase in plasma from bile duct-resected rats. In addition, a micro-injection of plasma from patients with pruritus of cholestasis into the medullary dorsal horn induces facial scratching in healthy monkeys, but plasma from nonpruritic cholestatic patients does not. Thus, the idea that an increase in opioid peptidergic activity in the brain causes cholestatic pruritus is seductive and plausible. However, the release of total opioid peptide from brain regions such as the striatum does not differ between bile duct-resected and sham-operated rats. On the other hand, the expression of preproenkephalin mRNA has been shown to increase in the liver of bile duct-resected rats. It was proposed that peripheral neuroinflammation is a cause of cholestatic pruritus. Thus, there has been no report on pathological pruritus that is attributed to the increased activity of the opioid peptidergic systems in the central nervous system.

2.3. Biting Behaviors Similar to pain information, itch information from the periphery may be modulated in the dorsal horn, and an understanding of the mechanisms of modulation is crucial for the development of centrally acting antipruritic agents. When pruritogen stimuli are applied to the rostral back, sensory information enters the dorsal horn at the higher thoracic and lower cervical levels. It is difficult to administer agents locally to these regions in freely moving rodents. In contrast, intrathecal administration to the lumbar dorsal horn through a lumbar puncture is feasible in freely moving rodents. The lumbar dorsal horn receives sensory information from the hindlimbs and caudal portions of the body. In some studies, licking and biting behaviors were interpreted as a single category of (nociceptive) response. In addition, abdomen scratching and caudally directed biting/licking behaviors induced by the intrathecal injection of substance P were reported to be inhibited by the systemic administration of morphine, and these behaviors were suggested to be nociceptive responses. Therefore, the behavioral distinction of itch responses from pain responses was indispensable to the investigation of the modulation mechanisms of itch signaling in the spinal dorsal horn. We injected serotonin, a pruritogen, into the hind paws of mice and recorded biting and licking behaviors separately. Although serotonin injection induced biting and licking of the injection site, only the biting was suppressed by opioid and serotonin antagonists. On the basis of those results, we hypothesized that biting of the regions that the rodents cannot scratch with their hind paws is an itch-associated behavioral response. In murine models of pathological pruritus, caudally directed biting was shown to be inhibited by opioid antagonists, supporting the idea that caudally directed biting is an itch-associated behavior.

Using biting behavior as an index of itch response, we found that the descending noradrenergic system regulates itch transmission in the spinal cord mediated through the α2 and α4-adrenoceptors. The α4-adrenoceptor agonist clonidine and the serotonin-noradrenaline reuptake inhibitor milnacipran inhibited pruritogen-induced biting through their action on the spinal cord. Under normal conditions, the descending serotonergic system does not seem to exert tonic inhibition of itch transmission.

2.4. Pruriceptive Activity of Primary Sensory Nerves Observation of the activity of sensory nerves may provide important information on how pruritus is induced, and whether antipruritic agents exert their effects peripherally or centrally. We compared the responses of cutaneous nerves in ICR and ddY mice to intradermal injections of histamine and serotonin. The activity of cutaneous nerves was as low as 0.1 impulse/s without stimulation. In ICR mice, both histamine and serotonin markedly increased the activity of primary afferents, the time-courses of which were similar to those of scratching behaviors. In ddY mice, histamine did not increase the activity of primary afferents or hind-paw scratching, although serotonin increased both. Those findings suggested that histamine does not elicit scratching in ddY mice because of the lack of functional expression of histamine receptors in the cutaneous nerves.

NC mice develop severe dermatitis when they are housed for long periods under pathogen-uncontrolled conditions. The frequencies of spontaneous scratching of the rostral part of the body and biting of the caudal part of the body were found to be positively correlated with the spontaneous activities of the cutaneous nerves innervating the corresponding cutaneous areas. This suggests that spontaneous scratching and biting are primarily due to pruriceptive inputs. Systemic administration of naltrexone inhibits spontaneous scratching and biting behaviors without affecting the increased activity of the cutaneous nerves, suggesting that the μ-opioid receptor antagonist inhibits itching by acting on the central nervous system. On the other hand, a single topical application of E6005, a phosphodiesterase 4 inhibitor, decreases both spontaneous scratching and the activity of the cutaneous nerves, suggesting that E6005 inhibits itching by acting primarily in the periphery.

In ICR mice, acute cutaneous allergy increased hind-paw scratching and cutaneous nerve activity, the time courses of which were similar. Terfenadine, a non-sedative H1 histamine receptor antagonist, inhibited neither the increased scratching nor nerve activity. In contrast, azelastine, a less-sedative H1 histamine receptor antagonist, suppressed both increased scratching and nerve activity. The inhibitory effects of azelastine were shown to be due, at least in part, to its direct inhibitory action on the primary sensory neurons. Observation of sensory nerve activity may therefore provide important information on whether antipruritic agents exert their effects peripherally or centrally.

2.5. Pruriceptive Activity of Dorsal Horn Neurons Spinothalamic tract neurons that responded to cutaneous
stimulation with histamine and cowhage were identified in the superficial dorsal horn of cats and primates. We identified dorsal horn neurons that were mechanosensitive and responded to acute cutaneous allergy in ICR mice. Acute allergy-responsive neurons were found in the superficial layers but not in the deep layers of the dorsal horn and constituted only about 10% of mechanosensitive neurons, half of which responded to noxious heat stimulation. The pruriceptive responses of superficial dorsal horn neurons were shown to be suppressed by cutaneous noxious stimuli including scratching in primates and mice.

The main pruritogenic component of cowhage is mucunain, which acts on proteinase-activated receptors (PAR) 2 and 4. In humans, cowhage and histamine activate distinctly different C-fibers, and cowhage-induced itching may be signaled by capsaicin-sensitive and histamine-insensitive C-fibers. In the primate dorsal horn, 25% of the spinothalamic tract neurons tested responded to histamine, 15% responded to cowhage, and none responded to both. Consistent with those findings, we observed that cutaneous stimulation with histamine or a PAR2 agonist (and induction of acute cutaneous allergy) induced Fos expression in different regions in the superficial dorsal horn in ICR mice; Fos-positive cells were mainly distributed in the inner aspect of lamina II after histamine stimulation and lamina I and the outer aspect of lamina II after stimulation with the PAR2 agonist or induction of acute cutaneous allergy. However, in the superficial dorsal horn of ICR mice, most of the histamine-responsive neurons responded to PAR2 and noxious stimulation, and most PAR2-responsive neurons responded to histamine and noxious stimulation. It is difficult to differentiate electrophysiologically between the dorsal horn neurons responsible for histamine-independent itching and the dorsal horn neurons responsible for histamine-mediated itching in mice.

3. PRURITUS INDEPENDENT OF MAST-CELL HISTAMINE

3.1. Substance P Substance P is one of the most potent pruritogenic endogenous peptides. It releases histamine from cutaneous mast cells, which is due to a direct activation of G proteins rather than a receptor-mediated process. Itching induced by intradermal substance P was shown to be inhibited by local pretreatment with compound 48/80 and systemic pretreatment with H1 histamine receptor antagonists in humans. Taken together, these findings suggest an important role for mast-cell histamine in substance P-induced itching. However, paradoxically, we also observed that intradermal substance P elicited hind-paw scratching in histamine-insensitive ddY mice. These discrepancies let us to investigate the mechanisms of substance P-induced itch in histamine-responsive ICR mice. Although an intradermal injection of substance P released histamine from the skin (Fig. 3, unpublished observation), hind-paw scratching induced by substance P was not inhibited by chlorpheniramine, an H1 histamine receptor antagonist, at a dose that suppressed histamine-induced scratching (Fig. 3). Local pretreatment with compound 48/80 markedly inhibited substance P-induced scratching, but this inhibition was observed in mast cell-deficient mice and substance P elicited hind-paw scratching in these mice as well. These findings suggested that the inhibition of itching by compound 48/80 pretreatment does not simply indicate the involvement of mast cells. Substance P-induced scratching in ICR mice was inhibited by NK1 tachykinin receptor antagonists. In contrast, NK1 agonists, but not NK2 and NK3 agonists, elicited hind-paw scratching in these mice. Therefore, our findings suggest that mast-cell histamine is not a primary factor in substance P-induced itching in mice.
Substance P-induced scratching allowed the preclinical assessment of compounds for antihistamine-resistant pruritus for the first time. Toray Industries, Inc. (Kamakura, Japan) was developing the κ-opioid receptor agonist TRK-820 (later named nalfurafine) as an analgesic at that time, but our research on itch changed the developmental target of TRK-820 from pain to antihistamine-resistant pruritus. TRK-820 was shown to inhibit scratching induced by intradermal substance P and intracerebral morphine in mice.70,71 It was also shown to be effective against severe pruritus in hemodialysis patients.72 Nalfurafine has been approved in Japan since 2009 for the treatment of uremic pruritus in hemodialysis patients.

3.2. Mast-Cell Itch Mediators In addition to histamine, many proteinases (endopeptidases) have long been known to elicit itching when administered to the human skin.73 It was shown in 1955 that an intraepidermal injection of the proteinase papain elicited itching without causing wheal-and-flare reactions, although these reactions were induced after intradermal injection.73 In the early 1970s, it was reported that itching induced by trypsin and chymase was mediated by histamine released from mast cells, while papain- and kallikrein-induced itching was not.74,75 The involvement of proteinases in pruritus went unnoticed for many years thereafter. In the 1990s, molecular cloning identified four PAR family members, PAR1-4; PAR1, PAR3, and PAR4 are thrombin receptors, while PAR2 is activated by mast-cell tryptase.76 We wondered whether tryptase played an active role in mast cell-mediated itching. Intradermal injections of tryptase, but not chymase, elicited scratching in ICR mice, and the action of tryptase was inhibited by the serine protease inhibitor nafamostat, anti-PAR2 antibody, and the PAR2 antagonist FSLLRY.77 Scratching induced by intradermal compound 48/80 was also inhibited by nafamostat, anti-PAR2 antibody, and FSLLRY, and coadministration of nafamostat and terfenadine exerted an additive inhibitory effect on compound 48/80-induced scratching.77 Those results suggested that tryptase participates in mast cell-mediated itching in cooperation with histamine. PAR2 activation by mast-cell tryptase may play an important role in scratching in NC mice with chronic allergic dermatitis.78,79

4. ANIMAL MODELS OF PATHOLOGICAL ITCH

Animal models of pathological itch are necessary for anti-pruritic drug development and elucidation of the pathophysiological mechanisms of itching in pruritic diseases. Therefore, we sought to develop mouse models of pathological itch.

4.1. Dry Skin Itching is a common symptom in various dermatoses characterized by dry skin, such as senile xerosis, seasonal xerosis, and atopic dermatitis. Dry skin is also a common cutaneous manifestation in pruritic internal diseases, such as chronic renal failure and chronic cholestasis. However, it was unclear whether dry skin is an underlying cause of itching in patients with these conditions.80,81 Therefore, we tried to establish an animal model of pruritic dry skin. Disruption of the cutaneous barrier function by the removal of lipid components from the stratum corneum,82 gradually increased spontaneous hind-paw scratching.82 Skin dryness was observed from the day after the start of the treatment, and an increase in spontaneous scratching became obvious after 3 d.82 An increase in spontaneous scratching was not obvious in a high-humidity environment (unpublished observation), a feature similar to seasonal xerosis in winter. The numbers of total mast cells and degranulated mast cells did not increase in this animal model of pruritic dry skin and an increase in spontaneous scratching was also observed in mast cell-deficient mice.82 The daily removal of lipid and aqueous components was also shown to increase spontaneous hind-paw scratching in rats.84

4.2. Mosquito Allergy Although the bites of many insects generally elicit pain as well as itch, the bite of female mosquitoes mainly causes itching in humans.85,86 Mosquito bites induce both immediate and delayed cutaneous reactions.86 When bitten by a mosquito for the first time in their life, humans develop a delayed cutaneous reaction but no immediate reaction, including itching.85 However, repeated mosquito bites within a short period of time change the delayed cutaneous reactions to immediate reactions.85 ICR mice bitten by female mosquitoes (Aedes albopictus) for the first time in their life did not develop a hind-paw scratching response within a 1-h period, but repetition (twice a week) was found to increase hind-paw scratching of the affected skin gradually.24

Repeated pretreatment with cetirizine, a nonselective H1 histamine receptor antagonist that inhibits the chemotaxis of T cells and monocytes,87 was reported to inhibit immediate itching following mosquito bites in humans.86 Although plasma extravasation was minute following mosquito bites in naïve ICR mice, it was markedly increased in sensitized mice and suppressed by pretreatment with terfenadine.24 Hind-paw scratching following mosquito bites was not inhibited by terfenadine at a dose that suppressed plasma extravasation and histamine-induced scratching.24 As mentioned above, azelastine inhibited mosquito bite-induced scratching, which may be due in part to its direct inhibitory action on primary sensory neurons.24 An extract from the salivary glands of female mosquitoes also increased hind-paw scratching as the intradermal injection was repeated. This was not affected by a deficiency in mast cells.24 Mosquito allergy scratching may involve lipoxin A, a 5-lipoxygenase metabolite, and the serine protease granzyme A, both of which are T-cell mediators.88,89 After repeated mosquito bites, the serum concentration of total immunoglobulin G1 (IgG1) markedly increased, and that of total IgE also tended to increase.24 The high-affinity IgG receptor FcεRI was found to be present in small-sized dorsal root ganglion neurons.91 Challenge with antigen activated dorsal root ganglion neurons that were previously exposed to antigen-specific IgG.91 Therefore, direct activation of primary afferents with mosquito allergen may also play a role in mosquito allergy itching.

4.3. Pollen Allergy Although mosquito bite allergy induced immediate itch responses, type I allergy and mast cells may not play important roles. Pollinosis including allergic conjunctivitis is a typical type I allergy, and its symptoms are due to the release of histamine and other active substances by mast cells.92 To confirm the roles of mast cells and histamine in pollinosis-associated pruritus, we sought to develop a mouse model of pruritic pollen allergy. One study showed that...
applying a solution of ovalbumin onto the ocular surface elicited hind-paw scratching in sensitized BALB/c mice, whereas application of a ragweed pollen suspension did not.\(^93\) We found that a subconjunctival injection of an extract of ragweed pollen elicited scratching in ICR mice that had been immunized with ragweed pollen in combination with an aluminum hydroxide adjuvant, but not in mice immunized with ragweed pollen alone.\(^94\) In contrast to mosquito bite allergy, pollen allergy-induced scratching was almost completely abolished by mast-cell deficiency.\(^94\) Terfenadine showed a statistically significant but partial inhibition of scratching at a dose that almost completely suppressed plasma extravasation.\(^94\) Leukotriene B\(_4\) has been shown to be produced in RBL-2H3 cells (a cell line with mucosal mast-cell characteristics) following immunoglobulin E receptor activation\(^95\) and to be increased in the tears of patients with giant papillary conjunctivitis.\(^96\) We found that leukotriene B\(_4\) was also involved in pollen allergy-induced scratching in mice.\(^94\)

4.4. Cleanser Eczema The use of toiletries and cosmetics can cause adverse effects such as skin irritation and itching.\(^97\) Many cleansers contain surfactants, which irritate the skin and change cutaneous functions. These effects of surfactants are thought to be due to their interaction with stratum corneum proteins and lipids, and the negative charge on their hydrophilic head plays an important role in the interaction.\(^98\) To elucidate the mechanisms of surfactant-associated itching, we attempted to develop an animal model of topical surfactant-induced pruritus. Single topical application of the surfactants 10% sodium laurate and 10% N-lauroylsarcosine sodium salt, pH 10.1 and 7.7, respectively, to the shaved skin of ICR mice immediately increased hind-paw scratching, which gradually decreased within 1.5 h after application. Sodium laurate increased scratching again 2 to 3 h after application.\(^99\) Two-hours topical application of 10% sodium laurate significantly increased the pH of the skin surface from 5.1 to 6.0, but the pH nearly normal (pH 5.0–5.2) 2 h after the application of 10% N-lauroylsarcosine sodium salt.\(^99\) Delayed scratching after sodium laurate application was markedly inhibited by terfenadine but not affected by mast-cell deficiency.\(^99\) Sodium laurate application increased the histamine content of the epidermis, but not that of the dermis, in normal and mast cell-deficient mice.\(^99\) The increase in epidermal histamine was at least partly due to increased processing of the 74-kDa precursor l-histidine decarboxylase to the 53-kDa active form in epidermal keratinocytes.\(^99,100\)

Sodium dodecyl sulfate (SDS) is a neutral anionic surfactant, and a single topical application of SDS did not induce delayed scratching.\(^100\) However, daily topical application of 10% SDS gradually increased hind-paw scratching and skin surface pH (from 5.0 to 6.0).\(^101\) Daily topical application of 10% N-lauroylsarcosine sodium salt did not increase scratching and skin surface pH.\(^101\) Chronic scratching induced by SDS was also markedly inhibited by terfenadine but not affected by mast-cell deficiency.\(^101\) The gene expression and posttranslational processing of l-histidine decarboxylase and histamine content in the epidermis were increased after daily treatment with SDS.\(^101\)

4.5. Herpes Zoster Pain and itch are two frequent complaints associated with herpes zoster.\(^102\) We established a mouse model of herpes zoster pain; transdermal inoculation of human herpesvirus 1 to the hind paw causes herpes zoster-like lesions in the inoculated dermatome and mechanical allodynia.\(^103,104\) We observed that herpetic mice bit and licked the lesional skin, raising the possibility that they felt spontaneous itch and pain. Therefore, to observe scratching as an itch-related behavior, we inoculated the herpesvirus on the midflank, a region that mice can scratch. A rash erupted on day 4 after inoculation and extended within the dermatome on days 5 to 6. After onset on day 4, scratching and licking of the lesional skin peaked on days 5 and 6, respectively.\(^105\) Spinal neurons expressing BB2 bombesin receptors (receptors for gastrin-releasing peptide) were shown to play a key role in itch signaling.\(^106\) Ablation of BB2 receptor-expressing spinal neurons decreased the scratching but not the licking.\(^105\) Oxidative stress in the affected skin and TRPA1 channels may be involved in herpes-associated itch and pain.\(^107\)

![Epidermal Keratinocytes Release Several Itch Mediators, Many of Which Act in an Autocrine or Paracrine Manner to Enhance Itching](image)

5-LOX, 5-lipoxygenase; BLT1-R, BLT1 leukotriene B\(_4\) receptor; GC, guanylyl cyclase; LTB\(_4\), leukotriene B\(_4\); NO, nitric oxide; NOS1/2, nitric oxide synthase 1 or 2; ORL1-R; ORL1 nociceptin receptor; TP-R, TP prostanoid receptor; TXA\(_2\), thromboxane A\(_2\).
5. ROLES OF EPIDERMAL KERATINOCYTES IN PRURITUS

As mentioned above, substance P-induced scratching was not primarily mediated by mast cells and their mediator histamine and was inhibited by the NK_{1} tachykinin receptor antagonist in mice.\textsuperscript{23} Therefore, we further investigated the mechanisms underlying substance P-induced scratching. It is possible that primary afferents are a site of action of intradermal substance P because low levels of NK_{1} receptor mRNA are expressed in the dorsal root ganglion.\textsuperscript{108} However, since we found that leukotriene B\textsubscript{4} had potent pruritogenic activity after intradermal injection in mice,\textsuperscript{109} we examined the involvement of leukotriene B\textsubscript{4} in substance P-induced scratching. Although substance P increased the levels of leukotriene B\textsubscript{4} and prostaglandin E\textsubscript{2} in the skin, the former, but not the latter, was found to be involved in substance P-induced scratching.\textsuperscript{110} Blockade of leukotriene B\textsubscript{4} may contribute to the antipruritic effects of some H\textsubscript{1} histamine receptor antagonists such as azelastine, emedastine, and bepotastine.\textsuperscript{26,111,112} Epidermal keratinocytes may play an important role in the production of pruritogenic leukotriene B\textsubscript{4} (Fig. 4). Under normal conditions, mRNA encoding the BLT1, but not BLT2, leukotriene B\textsubscript{4} receptor was found to be expressed in the dorsal root ganglion and skin.\textsuperscript{113} Leukotriene B\textsubscript{4} acts on dorsal root ganglion neurons,\textsuperscript{114} and leukotriene B\textsubscript{4} produced by epidermal keratinocytes may be a final itch mediator. Leukotriene B\textsubscript{4} is involved in the scratch-inducing actions of other keratinocyte-producing substances such as sphingosylphosphorylcholine and nociceptin\textsuperscript{27,114} (Fig. 4). Leukotriene B\textsubscript{4} was also shown to be involved in pathological scratching in mouse models of chronic allergic dermatitis and dermatophyte-associated itching.\textsuperscript{115,116}

An intradermal injection of U-46619, a stable analogue of thromboxane A\textsubscript{2}, elicited scratching mediated by TP prostanoid receptors in mice.\textsuperscript{117} Thromboxane synthsase was expressed in keratinocytes, and the TP receptor was mainly expressed in nerve fibers in the skin and epidermal keratinocytes, suggesting that thromboxane A\textsubscript{2} synthesized by keratinocytes acts on primary afferents to induce itching and acts on keratinocytes themselves to enhance itching\textsuperscript{117} (Fig. 4). Substance P acted on keratinocytes to produce nitric oxide via the activation of NK\textsubscript{1} receptors and nitric oxide synthase 1, which enhanced substance P-induced scratching\textsuperscript{118,119} (Fig. 4). Nitric oxide synthase 2 was induced in the epidermal keratinocytes of mice with pruritic dry skin, and nitric oxide was involved in spontaneous scratching.\textsuperscript{120} Collectively, those results suggested that epidermal keratinocytes release several itch mediators, many of which act in an autocrine or paracrine manner to enhance itching. Considering that the cutaneous receptor level for itching is located very superficially\textsuperscript{5} and removal of the epidermis and the subepidermal nerve network abolishes itching,\textsuperscript{121} itch mediators of epidermal keratinocytes may play important roles in pathological itch.

6. CONCLUSION

Itch can be evaluated behaviorally in animals; hind-paw scratching and biting of the rostral and caudal parts of the body, respectively, can be used as an index of itching. The role of histamine in pathological itch may be underestimated in mice because of their low histamine sensitivity. However, animal experiments on itch have revealed many itch mediators, the roles of which vary depending on the etiology of pruritus. Therefore, appropriate animal models of pathological itch are needed for the pharmacological evaluation of the antipruritic efficacy of chemical agents.

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