Increase in sensory sensitivity around the hyperkeratotic papule may be involved in pruritus in lichen amyloidosis

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Aim of this study

Lichen amyloidosis (LA) is an itchy skin disorder characterized by amyloid deposits in the superficial dermis. Pruritus is the presenting symptom in 90% of LA patients. However, **the underlying mechanisms and mediators of pruritus in LA remain unclear.**

One LA patient complained of severe pruritus in the hyperkeratotic papules, especially around the papule. In pruritic skin diseases, skin sensitivity is increased against tactile stimulation. Thus, **in this study, we evaluated in detail the sensitivity against tactile stimulation in the skin, especially the hyperkeratotic papule and its edge, of LA patients.**

Materials & Methods

Subjects

Six patients with LA (six men; mean age, 47 years; range, 27-77 years) enrolled at Toyama University Hospital and five healthy subjects (Two women and three men; mean age, 50 years; range 34-65 years) employed at the University of Toyama were underwent touch test. Skin biopsy was performed in six patients with LA (two women and four men; mean age, 51 years; range 23-77 years) enrolled at Toyama University Hospital. This study was approved by the Medical Ethics Committee of the University of Toyama, Toyama, Japan.

Touch test

Non-noxious mechanical stimulation was given to patients with LA using a fine von Frey filament (North Coast Medical Inc). **A von Frey filament with a bending force of 0.69 mN** was applied perpendicularly against the central part of each point of the hyperkeratotic papule or

its periphery: **R1, center of the hyperkeratotic papule; R2, edge of the hyperkeratotic papule; R3, approximately 1–0.5 mm from the edge of the hyperkeratotic papule; R0, healthy skin** in patients with cutaneous amyloidosis. Responses to the stimuli were scored as follows: **0, patient did not feel the stimulus; 1, patient felt the stimulus.** The test was performed in six hyperkeratotic papules or healthy skin per patient with cutaneous amyloidosis.

Immunohistochemical analysis

Skin samples were fixed in 10% neutral buffered and then embedded in paraffin. The paraffin blocks were cut with a microtome and the sections were placed on standard slides. After deparaffinization, these sections were stained Dylon or toluidine blue. For immunohistochemical stain, the sections were reacted with several antibodies (e.g. anti-protein gene product 9.5 (Ultraclone), anti-nerve growth factor (Merck), or anti-5-lipoxygenase antibody (Santa Cruz Biotechnology)). And then these sections were reacted with horseradish peroxidase-conjugated avidin (DAKO) following by reacting with biotin-conjugated anti-IgG antibody (DAKO). The sections were subsequently treated with 3,3'-diaminobenzidine tetrahydrochloride (DAKO) and counterstained with hematoxylin. The preparations were mounted with Canada balsam and observed using a light microscope (Olympus) with a CCD camera.

Typical examples of histochemical staining in skin with

lichen amyloidosis

- These skin sections were obtained from one patient with LA.
- DN, Dylon stain; TB, toluidine blue stain (arrow head: mast cells). Immunohistochemical staining for protein gene product 9.5 (PGP9.5) (arrow head: primary sensory neuron), nerve growth factor (NGF), and 5-lipoxygenase (5-LOX).
- The center and right panels are magnification of the areas of the left panel enclosed by red and blue squares, respectively. The area enclosed by black square in the upper left portion of the center panels shows the magnification of the areas enclosed by green square. Scale bar: black bar, 200 μ m; white bar, 20 μ m

- **[DN stain]** Amyloid deposition was observed in the papillary dermis under the hyperkeratotic papules, but not around them.
- **[TB stain]** A small number of mast cells were distributed widely in the dermis.
- **[PGP9.5 immunoreactivity]** The immunoreactivity of PGP9.5, which is a marker of neuron, was strongly observed on the epidermis of the edge of the hyperkeratotic papules, but not under the hyperkeratotic papules.
- **[NGF immunoreactivity]** The NGF-immunoreactivity at the edge, especially the epidermal ridges, of the hyperkeratotic papules was observed. However, the immunoreactivity was not detected under the hyperkeratotic papules.
- **[5-LOX immunoreactivity]** High 5-LOX immunoreactivity was detected around the hyperkeratotic papules, especially at their edges, in LA patients. However, under the hyperkeratotic papules, there was little immunoreactivity.

PGP 9.5-, NGF- and 5-LOX-immunoreactivity in each patients

with lichen amyloidosis

Patients	PGP9.5			NGF			5-LOX		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
No1	–	+	n.d.	–	+	–	–	++	n.d.
No2	–	++	–	–	+	–	+	++	+
No3	+	++	++	–	+	–	+	++	++
No4	+	++	++	–	+	–	+	+	++
No5	–	+	–	–	+	+	+	++	n.d.
No6	+	+	+	–	+	+	+	+	+

- n.d., no area in the section, Immunoreactivity: –, no or little stained; +, stained; ++, highly stained.
- PGP9.5, protein gene product 9.5; NGF, nerve growth factor; 5-LOX, 5-lipoxygenase

Results

Cutaneous sensory sensitivity hyperkeratotic papule of lichen amyloidosis

In the center of the hyperkeratotic papules, the patients' response against von Frey filament was low. However, when the edge of the hyperkeratotic papule was stimulated with von Frey filament, the LA patients responded highly. In addition, the LA patients also responded against the stimulation of the periphery of the hyperkeratotic papule with von Frey filament ($P=0.0784$).

- Percentage of the response to the stimulus from the von Frey filament. Dotted line shows the data obtained from healthy subjects. Dashed line shows the data obtained from each LA patient. Solid line shows the mean value of the response in each evaluated point. Values represent the mean and SEM. * $P < 0.05$, as compared with R0.

A schematic conclusion

In summary, we demonstrated that the sensory sensitivity at the edge, but not at the center, of hyperkeratotic papules in LA patients was increased through the epidermal elongation of peripheral sensory neurons. Moreover, we also found an increase in 5-LOX expression, which was related to the production of a potent itch mediator leukotriene B₄ (LTB₄). Taken together, it is suggested that, at the edge of hyperkeratotic papules in LA patients, the epidermal elongation of peripheral sensory neurons and the production of itch mediators (e.g. LTB₄) are involved in itch in LA patients.