Original Article:

Antipruritic effect of Neurotropin injection on moisturizer and antihistamine-resistant itch in patients with pruritus: A multi-center, open-label, small sample study

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Abstract

Pruritus is a condition in which itch occurs in the absence of apparent skin lesions. It is sometimes unresponsive to treatment with topical moisturizers and often is unresponsive to antihistamines. We evaluated the antipruritic effects of Neurotropin injections in patients with moisturizer- and antihistamine-resistant pruritus. We monitored these patients by itch scores recorded in symptom diaries, as well as reports of quality of life (QOL). This study investigated both the efficacy and safety of Neurotropin injections using an open-label study design. We enrolled 40 patients from six participating study sites. Of the 40 patients that were initially enrolled, 6 patients were ineligible, and ultimately, 33 were included for evaluation after one patient dropped out. Neurotropin was administered by subcutaneous injection to 22 patients and intravenous injection to 11 patients at a frequency of once per week. Compared to data collected during a 1-week observation period prior to treatment, after 7 injections of Neurotropin, there was a significant improvement in the Shiratori symptom severity score and the visual analog scale (VAS) scores for itch symptoms, and the Dermatology Life Quality Index (DLQI) for quality of life. No new adverse events occurred during the period of investigation. A notable benefit to Neurotropin is that it can be used in patients with renal impairment and patients receiving dialysis therapy. Our results demonstrated that Neurotropin is effective in the treatment of moisturizer- and antihistamine-resistant pruritus.

Introduction

Itch has been known to negatively affect the quality of life (QOL) in patients with a variety of dermatoses.[1] According to the International Forum for the Study of Itch, chronic itch is defined as itch lasting for 6 weeks or more.[2] Itch manifests on the skin, palpebral conjunctiva, and nasal mucosa, and is the major symptom experienced in a large number of skin diseases.[3, 4] Pruritus is a condition in which itch occurs in the absence of apparent skin lesions, and can be caused by xerosis, drugs, and many underlying diseases such as diabetes mellitus. Xerosis is a common cause of pruritus in elderly patients.[3] Pruritus is sometimes unresponsive to treatment with topical moisturizers and often is unresponsive to antihistamines.[5]

Neurotropin, a non-protein extract isolated from the skin of rabbits inoculated with the vaccinia virus, is widely used in Japan and China to treat various chronic pain and itch conditions.[6] In Japan, Neurotropin is covered by health insurance for the treatment of pruritus associated with skin diseases, symptomatic neuralgia, lower back pain, neck-shoulder-arm syndrome, allergic rhinitis, and sequelae of subacute myelo-optico-neuropathy (SMON) such as coldness, paresthesias, and pain. A recent report of a dry-skin mouse model has shown that Neurotropin inhibits the extension of nerves from the dermis into the epidermis, thereby decreasing the itch threshold and upregulating epidermal semaphorin 3A, a nerve repulsion factor. [7] The antipruritic effects of

Neurotropin have been evaluated clinically in patients with pruritus,[8] but these studies only evaluated the short-term effects using qualitative scores. In the present study, we aimed to evaluate the effects of Neurotropin on itch in patients with moisturizer- and antihistamine-resistant pruritus using itch scores and QOL score recorded in an itch symptom diary.

Subjects and Methods

1. Study design

A 7-week, multi-center, open-label study was conducted by the Department of Dermatology at Shimane University to evaluate the antipruritic effects of Neurotropin. This study was conducted in accordance with the ethical principles described in the Declaration of Helsinki (UMIN-Clinical Trials Registry: UMIN000021318). The total study duration was 8 weeks comprising a 1-week observation period followed by a 7-week Neurotropin treatment period. This study was approved by the Ethics Committee of Shimane University (approval No. 2459, 2606, 2607, 2608, 2609). The study monitor was Hiroaki Yasumoto of the Department of Urology, Shimane University Faculty of Medicine, and the statistical advisor was Ritsuro Suzuki of the Clinical Research Center, Shimane University Hospital.

2. Inclusion and exclusion criteria

The inclusion criteria of the study were: men and women aged 20 years and over in whom pruritus was clinically diagnosed, those receiving treatment with moisturizers and/or antihistamines for pruritis more than 1 week before the date of study consent, those with at least one of daytime and nighttime average Shiratori severity score (Appendix 1)[9] is 2 or more during the observation period, and those meeting any of the above criteria who provided informed consent.

The exclusion criteria of the study were: those with a history of hypersensitivity to neurotropin (injection and tablet), those with a need of continuous treatment for other diseases which affect neurotropin itself or the evaluation, those with pregnancy, pregnancy wish and breast-feeding, those with difficulty of regular visits, those with complications such as severe heart disease/ liver disease/ kidney disease, and others the doctor judges that it is inappropriate.

3. Drug administration protocol

One ampule of Neurotropin (Nippon Zoki Pharmaceutical Co., Ltd.) was administered once per week by subcutaneous or intravenous injection.

4. Concomitant medications that were permitted or prohibited

Medications that were already being taken by the patients during the observation period for the treatment of itch and its complications were allowed to be used concomitantly. However, the dosage and method of administration of the drugs used to treat itch could not be changed during the study period, and starting new medications (including over-the-counter drugs) to treat itch during the study period was not permitted.

5. Discontinuation criteria

Patient participation was discontinued if a patient requested to withdraw from the study, if an investigator judged that discontinuation was necessary due to adverse events

(including worsening of itch), or if medications being used concomitantly to treat dermal pruritus were stopped, added, or changed during the study period.

6. Observations

6.1 Study period

The evaluations were performed over a total of 8 weeks, including the 1 week prior to the start of treatment (observation period), and the 7 weeks during the study treatment period.

6.2 Evaluation methods

The study patients completed an itch symptom diary based on their own assessment of itch symptoms based on Shiratori severity scores (Appendix 1)[9], visual analogue scale scores (VAS), and the Dermatology Life Quality Index (DLQI).

(1) Shiratori severity score

The Shiratori severity score was determined by the patients based on their itch symptoms between wake-up time and bedtime (daytime symptoms) and between bedtime and wake-up time the next day (nighttime symptoms). The scores were then recorded in the itch symptom diary. The primary endpoint was the greatest change in the Shiratori severity score from the start to the end of the study, evaluated on a 5-grade scale. These 5 grades were marked improvement (4→0, 4→1, 3→0, 2→0), moderate improvement (4→2,

3→1, 1→0), mild improvement (4→3, 3→2, 2→1), unchanged (4→4, 3→3, 2→2, 1→1), or worsened (worsened pruritus).

(2) VAS

The VAS was determined by the patients based on the intensity of itch from breakfast until dinner (daytime symptoms) and from dinner until breakfast (nighttime symptoms). The VAS was represented by a line of 5.6 cm in length with "No itch" shown on the left end and "Intolerable itch" shown on the right end of the line. The VAS score for itch was calculated by measuring the distance from the left-most point on the VAS score line to the point at which a transverse line crossed the VAS. The transverse line represented the most severe itch felt compared to the previous VAS score. Patients placed a transverse line across the VAS line twice each day (after breakfast and after dinner). This VAS score distance was then extrapolated to a 10 cm VAS line.

(3) DLQI

The DLQI questionnaire was answered by the patients at the end of a week (i.e. at the end of observation period and at the end of each treatment week). The DLQI scores were reported as: 3 points for "Very much" or "Yes," 2 points for "A lot," 1 point for "A little," and 0 points for "Not at all" or "Not relevant." Incomplete answers were counted as 0 points. The patients were asked to complete DLQI surveys as described in the previous report.[10]

6.3 Overall evaluation

One investigator evaluated the clinical effects and adverse reactions and determined at the end of the study whether Neurotropin yielded improvement using a 5-level grading scale (1: Marked improvement; 2: Moderate improvement; 3: Mild improvement; 4: Unchanged; 5: Worsened). For those who did not complete the itch symptom diary (i.e. those who did not write in the itch diary at week 7), the last week of data recorded in the diary was used as a substitute for treatment week 7 data.

6.4 Adverse reactions

When adverse reactions were judged to be caused by Neurotropin, the symptoms, onset, timing, severity, treatment, and outcomes were recorded. The principal investigator provided comments regarding any suspected relationship between the adverse effect and Neurotropin. We calculated the percentage of patients with at least one adverse event related to Neurotropin.

7. Subjects

Forty patients aged 20 years or older were enrolled in the six departments (Appendix 2) between March 2016 and June 2017. Patients were included if they had daytime or nighttime itch graded as "mild " (score 2) or more based on the Shiratori severity score (Appendix 1) during the observation week. Of the 40 patients, six patients who did not

meet the inclusion criteria were excluded from the evaluation. One patient dropped out of the study due to the onset of bullous pemphigoid, leaving 33 patients in the study. The 33 patients had a mean age of 76 ± 9.0 (mean ± SD) years (range: 47-88 years). Men accounted for 84.8% of these patients. Complications included diabetes mellitus in 27.3% of patients, hypertension in 39.4%, and other complications in 45.5% (Appendix 3). The mean disease duration was 4.0 ± 4.0 (mean ± SD) years (range: 0.25-17 years). Pre-enrollment treatments included topical steroid therapy in 93.9% and topical moisturizers in 81.8% of the patients. Oral antihistamines were used by 81.8% of the patients whose symptoms were refractory to antihistamines, ultraviolet therapy was used by 15.2%, and the remaining treatments included oral steroid therapy in one patient, oral Remitch® (Nalfurafine Hydrochloride) in one patient, and oral *Toki-inshi* in one patient.

8. Statistical analysis

Changes in the itch severity scores (Shiratori severity score) were tested for significance using the paired Wilcoxon signed-rank test, and the Bonferroni correction was applied to adjust the P-values (i.e. the adjusted P-value was calculated by multiplying the P-value determined in the Wilcoxon signed-rank test by 7). Changes in the VAS score for itch and the DLQI were tested for significance using the paired t-test and paired Dunnett's test. Significance was signified by $P<0.05$. The statistical analysis was conducted using SPSS statistics version 24 (IBM, Armonk, NY, USA) and R version 3.4.3 (2017-12-06) for

Windows ("Frisbee Sailing" Copyright (C) 2017 The R Foundation for Statistical Computing Platform).

Results

All 33 patients were administered one ampule per week of Neurotropin, with 22 patients having received subcutaneous injections and 11 having received intravenous injections. The full evaluation of the itch severity scores is shown in Table 1 and Figure 1. The VAS scores for itch are shown in Table 2 and Figure 2, and the DLQI scores are shown in Table 3 and Figure 3.

The itch severity scores were recorded each week and compared to the score recorded in the observation period. The severity score decreased each week, and a significant decrease was seen from week 3 onward in both daytime and nighttime itch based on the Wilcoxon sign test results (Table 1 and Figure 1). In addition, multiple comparisons using the Bonferroni correction showed that there was a significant reduction in itch severity scores during both daytime and nighttime from week 3 and beyond compared to that in the observation period.

A comparison of the VAS scores for itch recorded each week with the score recorded in the observation period showed that there was a significant reduction in both daytime and nighttime symptoms in week 2 using the paired t-test. However, multiple comparisons

using the paired Dunnett's test showed a significant reduction in the daytime itch severity scores in week 3 and beyond (Table 2 and Figure 2).

A comparison of the DLQI scores recorded each week to those recorded in the observation period showed a significant reduction in weeks 4, 6, and 7 of the treatment period according to the paired t-test. Multiple comparisons based on the paired Dunnett's test showed a significant reduction in itch symptoms in week 4 and beyond (Table 3 and Figure 3).

The investigators' evaluation at the end of the study described improvements (including mild, moderate, and marked improvement) in 84.8% of the patients' symptoms (Figure 4). However, the patients' subjective assessments of symptom improvement (including mild, moderate, and marked improvement) indicated a 15.1% improvement in daytime symptoms and a 21.1% improvement in nighttime symptoms at week 7 (Figure 5).

As for adverse reactions, worsened pruritus was observed in one patient based on the investigator's evaluation (3.0%) and in three patients by their subjective evaluations (9.1%).

Discussion

This study demonstrated that Neurotropin decreases itch and improves QOL with potential placebo effects in patients with moisturizer- and antihistamine- resistant pruritus.

These improvements appeared after 2 or 3 weeks with respect to itch and after 4 weeks based on the DLQI.

Although this study was limited by the absence of a placebo control, the administration of Neurotropin resulted in a significant reduction in daytime VAS scores for itch from 5.0 cm (95%CI: 4.4-5.7) in the observation period to 3.3 cm (95%CI: 2.5-4.0) at the end of the study, with a difference of 1.8 cm (95%CI: 0.6-2.9) as shown in Supplemental Table 2. A significant reduction was also seen in the nighttime VAS scores for itch from the observation period (5.4 cm, 95%CI: 4.8-6.0) to the end of the study (3.5 cm, 95%CI: 2.7-4.3), with a difference of 1.9 cm (95%CI: 0.7-3.1).

In the previous investigation that evaluated intravenous, or subcutaneous Neurotropin administration to 45 patients with intractable pruritic skin disease, moderate or better improvement of daytime symptoms was found in 37.8% of patients and that for nighttime symptoms was found in 40.0% of patients after a 2-week administration.[11] These results show greater improvement than those of the present study where moderate or more improvement of daytime symptoms was achieved in 15.1% of the patients and that for nighttime symptoms was achieved in 21.1% of patients. This difference might be due to a difference in the Neurotropin administration schedule between these two clinical studies. Neurotropin was administered on a daily or every other day schedule in the previous study vs. weekly once administration of Neurotropin in the present study. Since most patients

usually visit hospitals or clinics on a weekly basis, our results showing the effectiveness of a weekly regimen of Neurotropin, is considered valuable. In addition, we demonstrated that weekly administration of Neurotropin significantly improves DLQI in patients with pruritus, indicating its efficacy in actual clinical use.

It is noteworthy that the patients with moisturizer- and antihistamine-resistant pruritus were enrolled in this study. Daily use of antihistamines is considered to be effective and is recommended in the guidelines for generalized skin pruritus.[5] In an open-study investigating the effects of antihistamines on generalized skin pruritus, improvement in itch was found in 52.6% of the patients with pruritic diseases.[12] In the present study we enrolled subjects with pruritus resistant to antihistamines as well as topical steroids and moisturizers. In this study, 81.8% of the subjects received antihistamines and 93.1% received topical application of steroids. The 81.8% used moisturizers in their pre-enrollment treatments. Our study suggests that weekly administration of Neurotropin has additional effects on itch that is refractory after such treatments.

The most frequently presumed cause of itch was senile xerosis in our patients (36.3%). Metabolic syndrome, endocrine disorders, and psychogenic disease were found in 9.0% of the patients (Table 4). The weekly administration of Neurotropin was equally effective for each group as shown in Table 5, indicating that Neurotropin has anti-pruritic effects regardless of the cause of the itch.

In the present study, Neurotropin was administered to one subject who was already taking nalfurafine hydrochloride. In this patient, both the daytime and nighttime Shiratori severity scores fell from 2.6 to 1.1, and the VAS score for itch fell from 7.9 to 1.9 in the daytime observations and from 7.9 to 1.7 in the nighttime observations. This suggests that Neurotropin suppresses itch by a different mechanism than nalfurafine hydrochloride, and also shows its synergistic effect with nalfurafine hydrochloride.

Although worsened pruritus was the adverse reaction observed in 3 patients, it does not imply that there is a causal relationship to Neurotropin use. No other adverse reactions were identified in this study. Neurotropin can be used for patients with renal impairment and is only contraindicated in patients with a hypersensitivity to Neurotropin. Taking these into consideration, Neurotropin is a beneficial drug, even when prescribed to elderly patients taking many other medications.

A limitation of this study was that the analysis was conducted in a study population that only included 33 patients. An adequate subgroup analysis would require a larger number of patients. In addition, the difference in effect between subcutaneous injections of Neurotropin and intravenous injections as reported by Yoshida et al.[14] was not apparent in our study.

In conclusion, administering Neurotropin to patients with refractory pruritus resulted in a significant reduction of itch by all measures (Shiratori severity scale, VAS value for

itch, and DLQI) over the duration of the study period. This reduction was also confirmed after the multiple comparison evaluation. These results demonstrated that Neurotropin is effective in the treatment of moisturizer- and antihistamine-resistant pruritus.

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. Weisshaar E, Gieler U, Kupfer J, et al. Questionnaires to Assess Chronic Itch: A Consensus Paper of the Special Interest Group of the International Forum on the Study of Itch. Acta Derm Venereol 2012;92:493-6.

2. Ständer S, Weisshaar E, Mettang T, et al. Clinical classifications of itch: a position paper of the International Forum on the Study of Itch. Acta Derm Venereol 2007;87:291-4.

3. Yosipovitch G, Greaves MW, Schmelz M. Itch. Lancet 2003;361:690-4.

4. Yosipovitch G, Bemhard JD. Chronic Pruritus. N Engl J Med 2013;368:1625-34.

5. Sato T, Yokozeki H, Katayama I, et al. Guidelines for management of generalized skin pruritus. Jpn J Dermatol 2012;122:267-80. [In Japanese]

6. Yoshii H, Suehiro S, Watanabe K, Yanagihara Y. Immunopharmacological actions of an extract isolate from inflamed skin of rabbits inoculated with vaccinia virus (neurotropin); enhancing effect on delayed type hypersensitivity response through the induction of Lyt-1 + 2- T cells. Int J Immunopharmacol 1987;9:443-51.

7. Kamo A, Tominaga M, Taneda K, et al. Neurotropin inhibits the increase in intraepidermal nerve density in the acetone-treated dry-skin mouse model Clin Exp Dermatol. 2013;38:665-8.

8. Clinical Research Group for Neurotropin: Double blind study on antiprurigous effect of neurotropin on chronic urticaria and eczematous dermatitis Nishinihon J Dermatol 1979;41:552-9. [In Japanese]

9. Shiratori A. Therapeutic effect of pruritic skin diseases by Mequitazine (LM-209) Nishinihon J Dermatol 1983;45:470-3. [In Japanese]

10. Kaneko S, Liu L, Kakamu T, et al. Salivary chromogranin A levels correlate with disease severity but do not reflect anxiety or personality of adult patients with atopic dermatitis. J Dermatol 2017;44:920-6.

11. Yoshida H, Tanaka M, Hirose R et al. On the clinical utility of neurotropin for intractable pruritic skin diseases. Acta Dermatologica (Kyoto) 1990;85:153-9. [In Japanese]

12. Nishiyama S, Okamoto S, Ishibashi Y et al. Clinical study of KW-4679 (Olopatadin Hydrochloride) on Pruritic diseases. J Clin Ther & Med, 1996;12:1615-40

Figure legends

Figure 1. Time course of Shiratori severity scores each week from the start to the end of the study. Both daytime and nighttime itch were found to be significantly improved from week 3 onwards in the multiple comparison evaluation (Bonferroni correction, *: $P<0.05$).

Figure 2. Time course of VAS scores for itch each week from the start to the end of the study. Significant reductions in both daytime and nighttime VAS scores for itch from week 3 onwards were observed in the multiple comparison evaluation (Paired Dunnett's test was used, *: $P<0.05$, error bars: 95% confidence interval).

VAS: visual analog scale

Figure 3. Time course of DLQI each week from the start to the end of the study. Multiple comparisons using the paired Dunnett's test revealed there was a significant improvement from week 4 onwards (*: P<0.05, error bars: 95% confidence interval).

DLQI: Dermatology Life Quality Index

Figure 4. Overall assessment by investigator from the start to the end of the study (N=33). Mild improvement was achieved in 84.8% of patients, and moderate, or better than moderate improvement was achieved in 45.5%.

Figure 5. The graded change in Shiratori severity scores from the start to the end of the study (N=33).

Table 1. Time course of the Shiratori severity score at each week between the start and end of the study.

	Day						Night					
	Number	Percentile			*P-value*		Number	Percentile			*P-value*	
		25	50	75	Wilcoxon	Bonferroni		25	50	75	Wilcoxon	Bonferroni
Observation	33	2.00	2.14	2.86	-	-	33	2.00	2.67	3.00	-	
Week 1	31	2.00	2.29	2.71	0.121	0.844	31	2.00	2.29	3.00	0.154	1.070
Week 2	32	1.93	2.00	2.57	**0.020**	0.139	32	1.69	2.29	3.00	**0.011**	0.080
Week 3	33	1.50	2.00	2.29	**0.001**	**0.010**	33	1.50	2.00	2.71	**<0.001**	**0.003**
Week 4	32	1.00	2.00	2.00	**<0.001**	**0.003**	32	1.00	2.00	2.59	**<0.001**	**0.002**
Week 5	32	1.00	2.00	2.23	**0.001**	**0.004**	32	1.00	2.00	2.57	**<0.001**	**0.001**
Week 6	31	1.00	2.00	2.43	**0.001**	**0.007**	32	1.43	2.00	2.36	**<0.001**	**0.001**
Week 7	29	1.14	2.00	2.00	**<0.001**	**0.002**	29	1.00	2.00	2.29	**<0.001**	**0.001**

Bold: P<0.05, The scores of each measured point in the treatment period against the observation period were compared using paired Wilcoxon sign tests. Bonferroni correction was applied to adjust the multiple comparisons.

Table 2. Time course of VAS values for itch at each week between the start and end of the study.

	Day					Night				
	Number	Average (95%CI)	Decrease from observation (95%CI)	*P-value*		Number	Average (95%CI)	Decrease from observation (95%CI)	*P-value*	
				Paired-t	Paired-Dunnett				Paired-t	Paired-Dunnett
Observation	32	5.0 (4.4-5.7)	-	-	-	32	5.4 (4.8-6.0)	-	-	-
Week 1	30	4.9 (4.3-5.5)	0.2 (-1.0-1.3)	0.825	1.000	29	5.2 (4.6-5.8)	0.2 (-1.0-1.4)	0.473	0.926
Week 2	31	4.3 (3.7-5.0)	0.7 (-0.4-1.8)	**0.037**	0.097	31	4.6 (3.9-5.3)	0.8 (-0.4-1.9)	**0.029**	**0.032**
Week 3	32	3.9 (3.3-4.6)	1.1 (0.0-2.2)	**0.002**	**<0.001**	32	4.2 (3.5-5.0)	1.1 (0.0-2.3)	**0.003**	**<0.001**
Week 4	30	3.8 (3.0-4.5)	1.3 (0.2-2.4)	**0.005**	**<0.001**	30	4.0 (3.2-4.8)	1.4 (0.2-2.6)	**0.003**	**<0.001**
Week 5	30	3.8 (3.1-4.6)	1.2 (0.1-2.3)	**0.01**	**<0.001**	29	3.9 (3.1-4.7)	1.5 (0.3-2.7)	**<0.001**	**<0.001**
Week 6	31	3.7 (3.0-4.5)	1.3 (0.2-2.4)	**0.007**	**<0.001**	31	3.5 (2.8-4.3)	1.9 (0.7-3.0)	**<0.001**	**<0.001**
Week 7	29	3.3 (2.5-4.0)	1.8 (0.6-2.9)	**0.001**	**<0.001**	29	3.5 (2.7-4.3)	1.9 (0.7-3.1)	**<0.001**	**<0.001**

Bold: P<0.05, The VAS value of each measured point in the treatment period against the observation period were compared using paired t-tests. Paired-Dunnett was applied to adjust the multiple comparisons. Decrease from observation was also calculated.

Table 3. Time course of DLQI at each week between the start and end of the study.

	Number	Average (95%CI)	P-value	
			Paired-t	Paired-Dunnett
Observation	30	4.3 (3.3-5.3)	-	-
Week 1	30	4.3 (3.3-5.3)	1.000	1.000
Week 2	29	4.1 (3.0-5.2)	0.419	0.979
Week 3	30	3.6 (2.6-4.6)	0.092	0.223
Week 4	30	2.9 (2.0-3.8)	**0.003**	**<0.001**
Week 5	30	3.3 (2.2-4.4)	0.058	**0.038**
Week 6	29	3.0 (2.0-4.0)	**0.007**	**<0.001**
Week 7	27	2.4 (1.7-3.2)	**0.000**	**<0.001**

Bold: P<0.05, The DLQI of each measured point in the treatment period against the observation period were compared using paired t-tests. Paired-Dunnett was applied to adjust the multiple comparisons.

Table 4 Presumed causes of pruritus in the 33 patients analyzed.

Cause	Number	Women (%)
Senile xerosis	12	1 (8.3)
Idiopathic	9	1 (11.1)
Metabolic syndrome	3	0 (0.0)
Endocrine disorders	3	1 (33.3)
Psychogenic disease	3	1 (33.3)
Localized pruritus	2	1 (50.0)
Polycythemia	1	0 (0.0)

Metabolic syndrome includes one case each of chronic kidney insufficiency, and hepatic cirrhosis and hemodialysis.

Endocrine disorders include one case each of myopathy, and hemodialysis and hepatocirrhosis.

Table 5. Improvement of pruritus grouped by causes

	≥ Moderately improved		≥ Mildly Improved	
	Day	Night	Day	Night
Senile xerosis	2 (16.7)	1 (8.3)	3 (25.0)	9 (75.0)
Idiopathic	1 (11.1)	2 (22.2)	5 (55.6)	7 (77.8)
Metabolic syndrome	1 (33.3)	1 (33.3)	2 (66.7)	2 (66.7)
Endocrine disorders	1 (33.3)	1 (33.3)	3 (100.0)	2 (66.7)
Psychogenic disease	0 (0.0)	1 (33.3)	1 (33.3)	3 (100.0)
Localized pruritus	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)
Polycythemia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Metabolic syndrome includes one case each of chronic kidney insufficiency, and hepatic cirrhosis and hemodialysis.
Endocrine disorders include one case each of myopathy, and hemodialysis and hepatocirrhosis.

Appendix 1 Shiratori severity score（reference 7）

Score (severity)	Daytime symptoms	Nighttime symptoms
4 (severe)	Intolerable itch, not relieved by scratching but instead worsens. Cannot focus on work or study	Can hardly sleep because of itch. Scratching all the time, but itch intensifies with scraching
3 (moderate)	Scratching even in the presence of others. Irritation as a result of itch, continuous scratching	Wake up because of itch. Can fall asleep again after scratching, but continue to scratch unconsciously while sleeping
2 (mild)	Itch sensation is relieved by light, occasional scratching. Not too disturbing	Feel somewhat itchy, but can obtain relief by scratching. Do not wake up because of itch sensations
1 (slight)	Feel itchy sometimes, but tolerable without scratching	Feel slightly itchy when going to sleep, but do not need to scratch. Sleeping well
0 (no symptoms)	Hardly feel itchy or do not feel itchy at all	Hardly feel itchy or do not feel itchy at all

Appendix 2

Participating study site and collaborators

Study site	Principal investigator
Department of Dermatology, Shimane University Faculty of Medicine	Sakae Kaneko
Department of Dermatology, Shimane Prefectural Central Hospital	Yoshio Tsujino
Togi Dermatological Clinic	Kimiko Tohgi
Honda Dermatological Clinic	Sakae Honda
Department of Dermatology, Masuda Red Cross Hospital	Sakae Kaneko
Department of Dermatology, Heisei Memorial Hospital	Yuko Chinuki

Appendix 3

*A list of complicating disease

Hypertension, 13; Diabetes mellitus, 9; Hyperlipidemia, 3; hepatitis C, 2; Sigmoid colon cancer, 1; Descending colon cancer and prostate cancer; 1, Ascending colon cancer, 1; Prostate cancer, 1; reflux esophagitis, 1; Hyperuricemia, 1; Cardiac insufficiency, 1; Atrial fibrillation, 1; Brain infarction, 1; Abdominal aortic aneurysm ruptured, 1; Hemodialysis for chronic kidney insufficiency, 1.