

Appendix S1.

MATERIALS AND METHODS

Study participants

A total of 33 patients diagnosed with HZ between October 2013 and February 2014 at the Department of Dermatology, Incheon St Mary's Hospital, The Catholic University of Korea were included in the study. Exclusion criteria were: (i) patients who came in with PHN; (ii) those with psychotic illness or other communication problems; (iii) age below 20 years; and (iv) patient refusal. All participants underwent physician survey and skin biopsy at first visit.

Ethics

This study was approved by the institutional review board of Incheon St Mary's Hospital, The Catholic University of Korea. Eligible patients were informed about the study protocol in clear, simple language before their informed consent was obtained.

Baseline evaluation

One's age, sex, presence of an underlying disease, medication history (i.e. antivirals and painkillers), the degree (estimated using the 10-point visual analogue scale (VAS) from 0 (no pain) to 10 (worst pain imaginable)), duration, and the neuropathic symptoms and signs of pain (using the Self-completed Leeds Assessment of Neuropathic Symptoms and Signs pain scale (S-LANSS), range 0–24) and the distribution and duration of the skin lesions were assessed by physician survey. S-LANSS is a pain scale for distinguishing neuropathic dominant pain from nociceptive dominant pain (12). It consists of 7 items, termed dysaesthesia, autonomic, evoked, paroxysmal, thermal, allodynia, and tender/numb, where each item is assigned a score from 1 to 5, thus the total score ranges from 0 to 24. The higher scores suggest that the pain is predominantly neuropathic, not nociceptive. Clinical photographs were taken and assessed later on by dermatologists to score the severity of the HZ skin lesions (based on the percentage of erythema in the lesional dermatome; number of vesicles, pustules, erosions and crusts; fusion of vesicles; ulcer formation; number of lesional dermatomes; generalized eruption) (range 0–10) (13). Four-mm punch biopsies were taken from the skin lesions and the contralateral unaffected skin.

Follow-up

Study participants were asked to visit the dermatology outpatient clinic one week later and after 3 weeks. An individual phone call was made by a physician at 3 months to check the presence of PHN. For those with PHN, monthly phone calls were made for up to 6 months.

Immunofluorescence (IF) staining

The skin biopsy specimens were embedded in OCT compound (Sakura Finetek, Torrance, CA, USA), sectioned 4- μ m thick, and mounted onto silane-coated slides (Dako, Glostrup, Denmark). The slides were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and blocked with a pre-blocking solution (GBI Labs, Mukilteo, WA, USA) for 30 min. The slides were then incubated with a rabbit polyclonal antibody against TRPV1 (Santa Cruz Biotechnology, Paso Robles, CA, USA) in 4°C for 48 h, followed by incubation with an Alexa Flour 488-conjugated anti-rabbit antibody (Life Technologies, Carlsbad, CA, USA) for 1 h at room temperature. Nuclei were counterstained with DAPI (Vector Laboratories,

Burlingame, CA, USA). After staining, specimens were examined using a fluorescence microscope (Leica, Solms, Germany).

Haematoxylin and eosin staining

The biopsy samples were fixed with 10% formalin for 24 h and processed into paraffin wax. Paraffin-embedded samples were cut into 4- μ m thick sections and mounted onto silane-coated slides. Haematoxylin and eosin (H&E) staining was performed.

Skin sample processing

For quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot analysis, the epidermis was separated from the dermis by the heat separation method (14). In brief, the skin biopsy samples were incubated in 55°C phosphate-buffered saline (PBS) for 2 min, and the epidermis was subsequently separated from the dermis using forceps.

Quantitative real-time PCR

Total RNA was isolated from the epidermal skin samples using Trizol (Life Technologies, Carlsbad, CA, USA), and 1 μ g total RNA was converted to cDNA using First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania). To estimate the mRNA expression quantitatively, PCR was performed on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) with the following primer pairs: TRPV1 (forward, 5'-CCCCGATAGCTCCTACAACA-3'; reverse, 5'-GCAGCAGGATGATGAAGACA-3'), tumour necrosis factor (TNF)- α (forward, 5'-TCCTTCAGACACCCCTCAACC-3'; reverse, 5'-AGGCCCCAGTTTGAATTCTT-3'), interleukin (IL)-1 β (forward, 5'-CTGTCCTGCG TGTGAAAGA-3'; reverse, 5'-TTCTGCTTGAGAGGTGCTGA-3'), cyclooxygenase (COX)-1 (forward, 5'-GAGCGTCAGTATCAACTGCG-3'; reverse, 5'-ATTGGAAGTGGACACCGAAC-3'), IL-8 (forward, 5'-CAGGAATTGAATGGGTTTGC-3'; reverse, 5'-AAACCAAGGCACAGTGGAAAC-3'), COX-2 (forward, 5'-TTCAAATGAGATTGTGGGAAATTGCT-3'; reverse, 5'-AGATCATCTCTGCCTGAGTATCTT-3') and 36B4 (forward, 5'-TGGGCTCCAAGCAGATGC-3'; reverse, 5'-GGCTTCGCTGGCTCCAC-3'). The PCR conditions were 50°C for 2 min, 95°C for 2 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. The data were analysed using the comparative Ct method, presented as mean $\pm$ standard error of relative mRNA expressions against corresponding controls, normalized to 36B4.

Western blot analysis

Epidermal skin samples were homogenized and protein extracted using RIPA buffer (Merck Millipore, Billerica, MA, USA) with the protease inhibitor mixture (Roche Applied Science, Rockford, IL, USA) and phosphatase inhibitor mixture (Sigma Aldrich, St Louis, MO, USA). Extracts were centrifuged at 13,000 rpm, 4°C for 15 min, and the supernatants were collected. Protein concentration of samples was determined by the Bradford protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts (30 μ g) of proteins were loaded onto 8% sodium dodecyl sulphate (SDS) polyacrylamide gels, and transferred to polyvinylidene difluoride membranes (Roche Applied Science, Penzberg, Germany). Membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% skimmed milk and incubated with a rabbit polyclonal antibody against TRPV1 (Novus Biologicals, Littleton, CO, USA), and a goat polyclonal antibody against actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Horseradish peroxidase-conjugated anti-rabbit or anti-goat IgG (Santa Cruz Biotechnology) were

used as secondary antibodies. Blots were visualized by enhanced chemiluminescence detection system (Thermo Scientific, Waltham, MA, USA). Signal intensity was quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

The results obtained from the HZ skin lesions and the unaffected contralateral skin were expressed in relative ratios and

the significance of the values in the HZ lesions were identified using the *t*-test. Spearman's rank test was used to detect correlation between the protein TRPV1 ratio (HZ lesion/ control), pain, zoster skin severity score and S-LANSS. Independent samples *t*-test and analysis of variance (ANOVA) followed by a *post-hoc* Dunnett T3 were used to estimate the relationship of protein TRPV1 ratio, pain and S-LANSS with age, sex, location, and medication history. *p*-values <0.05 were considered statistically significant.