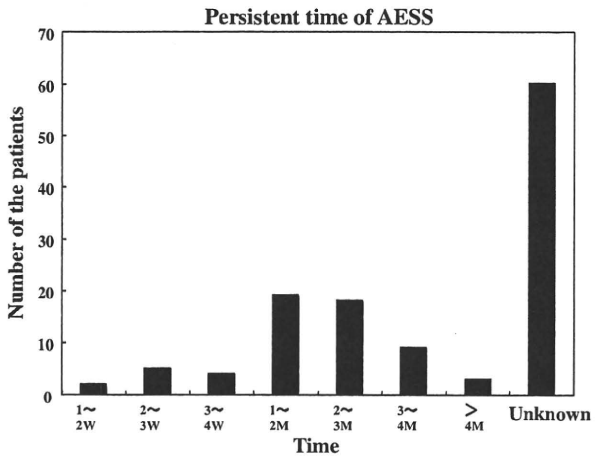

Fig. 3 Clinical course of AESS before the patient visited a dermatology clinic : <1 month, 11 patients; 1–2 months, 19; 2–3 months, 18; 3–4 months, 9; >4 months, 3; unknown, 60. *W* Week, *M* month

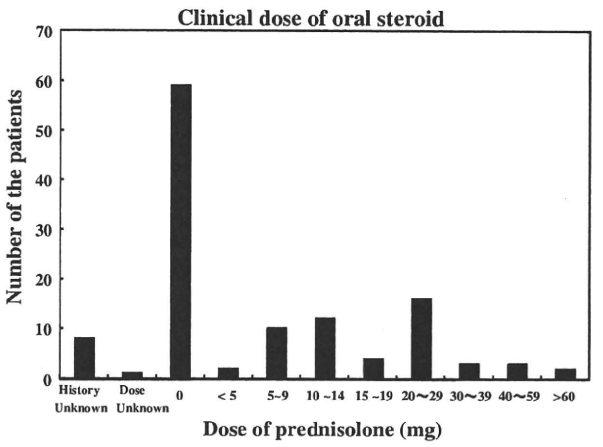

Fig. 5 Clinical dose of oral glucocorticoid. The clinical dose of oral glucocorticoid showed two major peaks around 5–15 and 20–30 mg of predonisolone

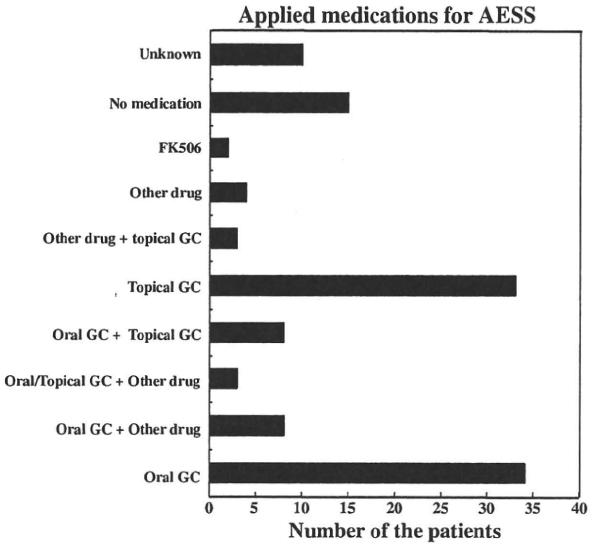

Fig. 4 Management of AESS. Several approaches to managing AESS have been reported in the literature. The administration of either oral or topical glucocorticoid (*GC*) remains the first line of therapy for AESS. About 12.5% of patients received no medication. A recent report noted a beneficial effect of tacrolimus. Chloroquine or thalidomide are preferentially used in Korea or Europe. Other drugs: anti-histamine, aspirin.

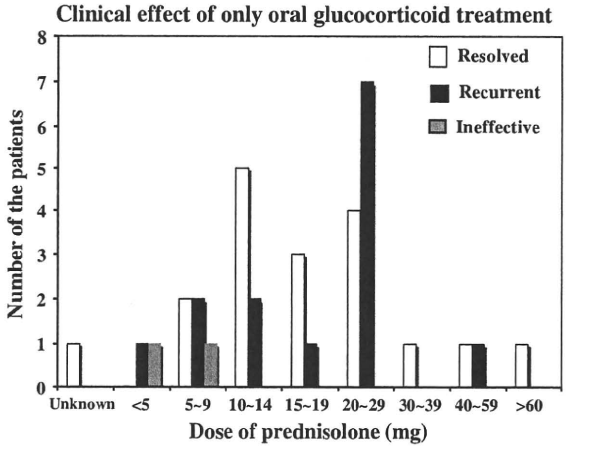

Fig. 6 The most effective dose of oral glucocorticoid to control AESS. The most effective dose of oral glucocorticoid to control AESS was 5–15 mg oral prednisolone. AESS recurred approximately twice in cases treated with more than 20 mg prednisolone, thus suggesting the possibility of several subsets of AESS

patients with SS with the aim of differentiating AESS from SCLE [4]. Annular erythema is characterized by deep perivascular and/or periappendageal infiltration of the lymphocytes with an admixture of neutrophils or plasma cells and, less frequently, epidermal changes suggestive of cutaneous LE. Immunoglobulin or complement deposition along the dermoepidermal junction of lesional skin was observed in eight of 18 cases [4], with most of the dermal infiltrates consisting of CD4(+) and CD45 RO(+) cells [78]. The appearance of anti-SS-A (Ro) (100%) and anti-SS-B (La) (77%) was significantly higher in patients with annular erythema than in those without these skin manifestations. These results suggest that patients with SS may have a distinct annular erythematous lesion that is both clinically and histologically different from SCLE, although close immunologic abnormalities exist in these two diseases [82].

Japanese reports have demonstrated transition cases from type 1 AESS to SCLE with epidermal changes and

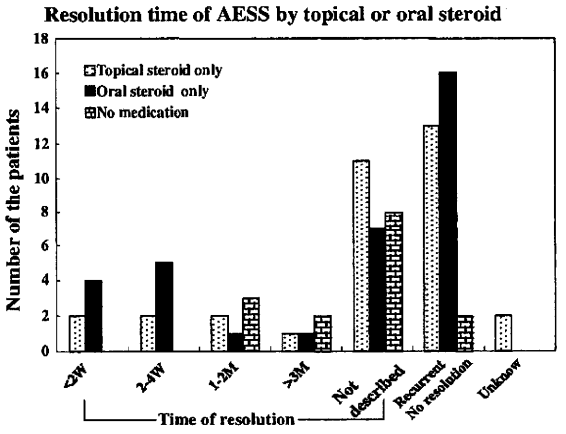

Fig. 7 The clinical effect of oral or topical glucocorticoid therapy on AESS. AESS showed a more rapid clinical response to oral glucocortoid than to topical glucocorticoid (9 cases vs. 4 cases within 1 month), which is also faster than the natural course of AESS

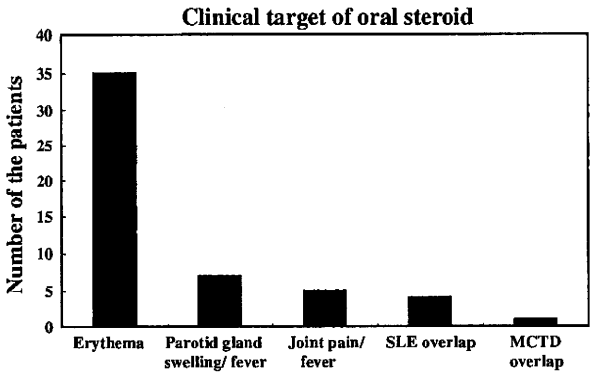

Fig. 8 Clinical target of oral glucocorticoid. In daily clinical practice, oral glucocorticoid should be given to control systemic manifestations of SS, such as parotid gland swelling, joint pain, or high fever unresponsive to non-steroidal anti-inflammatory drugs. However, oral glucocorticoid was applied to the AESS patients without any systemic manifestations in this study (35/52). *MCTD* Mixed connective tissue disease, *SLE* systemic lupus erythematosus

histological LE, thus suggesting that common etiopathological factors may be involved in the pathogenesis of SCLE and AESS, as suggested by McCauliffe et al. [82] and Provost et al. [83].

Oral NSAIDs represent the first-line treatment systemic extra-glandular manifestations, such as inflammatory swelling of the salivary gland or lymph node, or muscle and joint pain. Oral steroids are used to treat more severe manifestations, such as the lung, kidney, or nerve involvement. Oral gold compound (Auranofin) and cyclophosphamide were reported to be effective for recurrent purpura with improved serum immunoglobulin (Ig)G levels [84].

To date, there is no consensus or evidence-based management of AESS because of its rare occurrence in Occidental populations and the fact that most of the Oriental cases are isolated reports. Here, we have summarized the cases of AESS reported up to 2007. As expected, most of the reported patients are Oriental, especially from Japan (113 cases in 120). The age distribution and positive anti-SSA/SSB antibodies are consistent with those reported previously.

The most effective dose of oral glucocorticoid to control AESS was found to be around 10 mg oral prednisolone, while AESS recurred around twice in the cases treated with more than 20 mg of prednisolone. This result implies that several subsets might exist in AESS (Fig. 6). AESS subsided in about 50% of the cases without oral glucocorticoid, which may suggest that oral prednisolone only shortens the persistency of AESS but does not fully control the inflammatory changes underlying AESS. Any evaluation of the clinical effect of topical glucocorticoid to AESS is difficult in this study because the time for resolution of AESS is poorly described in most reports, and the persistence time before resolution is similar to that of the natural course (Figs. 3, 7). In daily clinical practice, oral glucocorticoid should be given to control systemic manifestations of SS, such as parotid gland swelling, joint pain, or high fever unresponsive to NSAIDs. However, oral glucocorticoid was administered to the AESS patients without any systemic manifestations in this study (35/52; Fig. 8). Yokota et al. recently reported that topical tacrolimus improved AESS [14] more effectively in Japanese patients. This new approach should be further evaluated in future studies that included pimecrolimus or other topical agents.

Conflict of interest statement None.

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Effects of nonsedative antihistamines on productivity of patients with pruritic skin diseases

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Keywords: antihistamine; itch; quality of life; skin diseases; WPAI-AS.

The symptoms associated with allergic diseases are recognized to exert a negative social and economic impact on patients as a result of impairments in work productivity (1, 2). Similar negative effects are experienced by patients with skin diseases such as chronic idiopathic urticaria, psoriasis, and chronic hand dermatitis (3–5). However, the consequences of pruritic skin diseases on productivity at work, in the classroom, and in daily activities are not fully understood. We assessed the impact of pruritic skin disease (e.g., dermatitis/eczema, urticaria, atopic dermatitis (AD), pruritus cutaneous) – as well as the effect of antihistamine therapy – on work, classroom, and daily productivity. In addition, we evaluated the effects of antihistamines on the intensity of itch and patient's quality of life (QOL).

Impaired work productivity in patients with pruritic skin diseases is improved significantly by nonsedative antihistamines.

The study design was approved by the Institutional Review Board, and patients with pruritic skin diseases (n = 206; male : female = 93 : 113; mean age ± SD: 52 ± 20 years) gave informed consent to participate in this study. Participants received no medical attention during the week before study initiation. The selection of therapy for each patient – i.e., oral antihistamines versus external medicine (e.g., steroid ointments, tacrolimus ointments, and certain moisturizers) – was left to the physician's discretion (open-label trial). The antihistamines fexofenadine (n = 72) and loratadine (n = 2), for which the package insert contained no cautionary statement regarding sedative actions, were categorized as 'nonsedative'. All other antihistamines were classified as 'sedative'. The effects of pruritic skin diseases on QOL were measured using the Skindex-16 instrument, and the magnitude of the itch sensation was assessed using a visual analog scale (VAS) (0–100). Work, classroom, and daily productivity were assessed by means of the Work Productivity-Activity Impairment-Allergy Specific (WPAI-AS) instrument (6). These instruments were self-administered by patients before (baseline) and 1 month after treatment initiation.

Based on the average baseline WPAI-AS scores for different diseases,

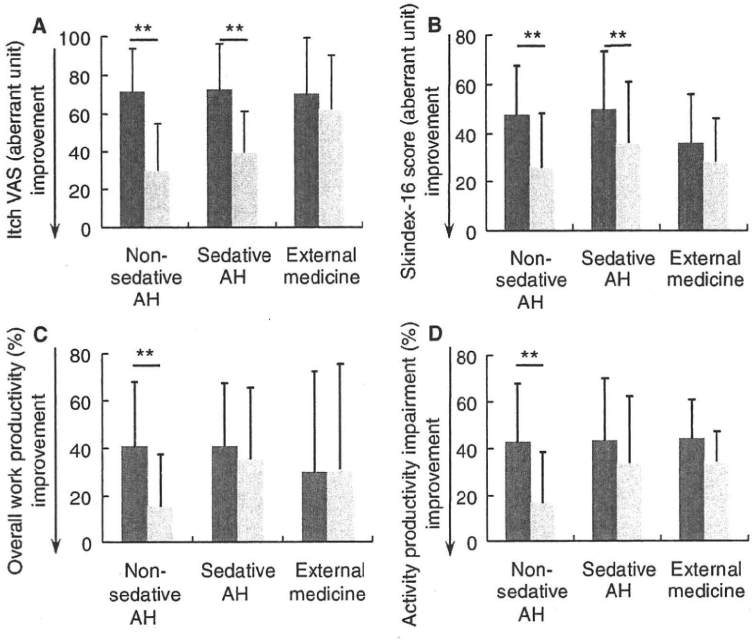

Figure 1 The impact of antihistamines on (A) itch visual analog scale, (B) skindex-16 score, (C) overall work productivity impairment, and (D) daily activity productivity impairment. The data for baseline (dark gray bars) and post-treatment assessment (light gray bars) are shown as mean ± SD. **Statistically significant improvement compared to baseline assessment (P < 0.001, one-sample t-test). AH, antihistamines.

pruritic skin diseases produced impairments in overall productivity in the workplace, classroom, and daily life activity of 39.3 ± 26.5%, 45.0 ± 28.9%, and 42.3 ± 25.1%, respectively. No significant differences between disease groups were identified at baseline. Patients in this study were treated for 1 month with oral antihistamines, with the exception of 11 patients who received only with topical medications. Nonsedative antihistamines were given to 74 patients, and the remaining 121 patients were treated with sedative antihistamines. The effects of these treatments on the intensity of itch, the Skindex-16 QOL score, overall work productivity, and daily activity productivity are shown in Fig. 1. As expected, itch intensity was reduced significantly by antihistamine therapy, while external medicines were ineffective (Fig. 1A). The effects of nonsedative and sedative antihistamines on itch intensity were similar (Fig. 1A). The effects of all treatments on the Skindex-16 QOL measure were similar to those for the itch VAS, with significant improvement from all antihistamines, but not for topical medications (Fig. 1B). As anticipated, impairments in overall work productivity and daily activity productivity were reduced significantly by nonsedative antihistamines, whereas sedative antihistamines failed to improve either measure (Fig. 1C,D).

Our results indicated that pruritic skin diseases negatively impact WPAI-AS scores at baseline. Sedative antihistamines fail to reduce work productivity impairment, despite decreasing itch VAS and Skindex-16 measures. Thus, clinicians should be aware of the potential to overestimate the benefits of sedative antihistamines on work productivity if they rely solely on patient intensity of itch and QOL values. In conclusion, this report highlights benefits in patient productivity as a new goal in the treatment of pruritic skin diseases and provides a rationale for shifting the choice of treatment to nonsedative antihistamines.

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Maize: a new occupational allergen in the pharmaceutical industry

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Keywords: excipient; maize; maize starch; occupational allergy.

Maize is part of contemporary nutrition and can be found in breakfast cereal, snacks, pastries, tortilla chips, polenta and many other foods. Moreover, maize flour and starch are often added to processed food, and starch is a widespread excipient of tablets. Allergic reactions to maize have previously been reported in southern Europe and Mexico where maize is commonly ingested. The major food allergen of maize, Zea m 14 is a heat-resistant lipid transfer protein and has a molecular weight of 9 kDa (1). Three other potential allergens have been detected: a 16-kDa α-amylase trypsin inhibitor, a thioredoxin named Zea m 25 (2) and a not yet characterized 50-kDa protein that belongs to the so-called reduced soluble protein fraction of the corn endosperm (3).

Maize starch should be considered as a potential occupational allergen in tablet manufacturing.

A 19-year-old girl, apprentice of a pharmaceutical company, noticed an erythematous itching rash on both hands and the face associated with dyspnea everyday 2 hours after starting work in tablet manufacturing. She was not known for any kind of seasonal or perennial allergic rhinoconjunctivitis, food or drug allergy, or atopic dermatitis.

Her company produces tablets of acetylsalicylic acid (ASA), nifedipin, acarbose, ciprofloxacin and moxifloxacin. Main excipients of these tablets are

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Original Article

Tumor necrosis factor-α processing inhibitor-1 inhibits skin fibrosis in a bleomycin-induced murine model of scleroderma

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Abstract: Elevated serum concentration of soluble tumor necrosis factor receptor p55 (sTNFRp55) is known to correlate with the severity of systemic sclerosis (SSc). However, it has not been verified whether this increase contributes to the pathogenesis of SSc. In this study, we found that sTNFRp55 also is increased in the bleomycin (BLM)-induced murine model of SSc. Therefore, we examined the effect of tumor necrosis factor-α processing inhibitor-1 (TAPI-1), the inhibitor of TNFRp55 sheddase, in this model. TAPI-1 was administered weekly to mice with skin fibrosis induced by daily BLM injections. TAPI-1 significantly suppressed BLM-induced skin thickness and the number of myofibroblasts. It also inhibited the increase of serum sTNFRp55 after 3 weeks of BLM injections. The mRNA expression of collagen type I α1, transforming growth factor-β1 and alpha smooth muscle actin were decreased by TAPI-1 administration. Taken together, these findings indicate that targeting the TNFα converting enzyme might be a new type of therapy for patients with SSc.

Key words: scleroderma – TAPI-1 – tumor necrosis factor-α – tumor necrosis factor-α converting enzyme – tumor necrosis factor receptor p55

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Introduction

Systemic sclerosis (SSc) is a disease characterized by progressive fibrosis of multiple systems including the skin. Although a small number of studies have observed statistically significant benefits from immunomodulatory treatment, none of the major clinical trials using skin fibrosis as an endpoint have been clinically superior to placebo (1,2).

Skin fibrosis is caused by massive production of fibrous connective tissue in the dermis, which exceeds the rate of degradation (3). Transforming growth factor-β (TGFβ), tumor necrosis factor-α (TNFα), various interferons and interleukins are known to induce or inhibit the expression of extracellular matrix genes or enzymes (4). One of the major cytokines involved in skin fibrosis is TGFβ. It is the most potent inducer of connective tissue growth factor (CTGF), which promotes matrix deposition and fibroblast proliferation (4,5). Disruption of TGFβ prevented the occurrence of fibrosis in 'tight skin' mice (6).

On the contrary, TNFα is known to have an antagonistic effect on TGFβ by suppressing the induction of CTGF (7–9). We previously reported that wild-type mice with a disrupted TNF receptor p55 (TNFRp55) gene exhibited severe skin fibrosis following bleomycin (BLM) treatment (10). The TNFRp55−/− mice exhibited skin fibrosis starting on day 3 vs day 14 in wild-type mice. This result indicates that the TNFRp55 signalling pathway plays an important role in the mechanism of skin fibrosis induced by BLM.

Several previous studies have demonstrated the association of TNFα and TNFRp55 with the clinical symptoms of SSc patients. Expression of TNFα is detectable in the serum of patients at very early stages of SSc (11). The serum level of TNFα increases with the clinical severity and biological activity of the disease (12) and the serum level of soluble TNFRp55 (sTNFRp55) correlates with the severity of disease (13–15). sTNFRp55 is known to neutralize TNFα and inhibit its effects (13). Therefore, we assumed that an increase in sTNFRp55 (which results in TNFα neutralization and reduction of the TNFRp55 signalling) plays a key role in the pathomechanism of SSc.

In this study, we focussed on TNFα converting enzyme (TACE). TACE is a member of the disintegrin and metalloproteinase family that is responsible for the processing of pro-TNFα and TNF receptors (16,17). We hypothesized that TACE activity might be increased in SSc patients, which might result in an increase of serum TNFα and

sTNFRp55. We examined the effect of a TACE inhibitor (TNF-α processing inhibitor-1, TAPI-1) to see whether it has the ability to inhibit BLM-induced skin sclerosis in C57BL/6 mice. TAPI-1 significantly suppressed skin sclerosis induced by BLM and reduced fibrogenic cytokines. Therefore, it has the potential to be a new type of therapy for skin sclerosis in SSc patients.

Materials and methods

Cell culture

Isolation and culture of mouse keratinocytes and mouse fibroblasts were carried out as previously described (18,19). Full-thickness skin harvested from day 2 to day 4 newborn mice was treated with 4 mg/ml of dispase (Gibco; Invitrogen, Paisley, UK) for 1 h at 37°C. Next, the epidermis was peeled from the dermis. The epidermis was trypsinized to prepare single cells. It was then incubated in Human Keratinocyte Serum Free Medium (DS Pharma Biomedical, Osaka, Japan) for 6 h at 37°C under an atmosphere with 5% CO_2. This atmosphere allowed the cells to adhere in the culture dishes precoated with type-1 collagen (Asahi Techno Glass, Funabashi, Japan). Non-adherent cells were washed away with phosphate-buffered saline (PBS) twice, then cultured for 2–3 days in human keratinocyte serum-free medium before use in experiments.

The dermis was placed in PBS + 0.05% type-1 collagenase (Sigma-Aldrich, St Louis, MO, USA) and incubated at 37°C for 30 min with vigorous agitation to prepare single cells. After filtration, cells were centrifuged at 200 *g* for 10 min, resuspended in Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS) and incubated at 37°C and 5% CO_2. Passage one or two fibroblasts were starved for 2 h and then used for experiments. For isolation of splenocytes, C57BL/6 mouse spleens were removed aseptically and passed through a sterile nylon 70 μm cell strainer (BD bioscience, Bedford, MA, USA). The red blood cells were lysed by adding lysis buffer (0.15 M ammonium chloride) followed by centrifugation. The cell pellet was washed with PBS and cultured in RPMI-1640 medium containing 10% FBS. A mouse C3H muscle myoblast cell line (C2C12) was obtained from ECACC (Salisbury, UK), cultured in DMEM + 10% FBS and incubated at 37°C and 5% CO_2. Cells were starved overnight before the experiment.

BLM and TAPI-1 treatment

Six-week-old female C57BL/6 mice were obtained from Clea Japan (Osaka, Japan), Inc. Animal care was in accordance with the institutional guidelines of Osaka University. BLM (Nippon Kayaku, Tokyo, Japan) was dissolved in PBS at a concentration of 1 mg/ml. Daily injections of 100 μl of BLM or PBS were administered subcutaneously to the shaved dorsal area for 3 weeks.

One micro mol of TAPI-1 (Biomol, Plymouth Meeting, PA, USA) was diluted in 25 μl dimethyl sulfoxide (DMSO) and further diluted with 275 μl of PBS and was given by gavage to mice on day 1, 8 and 15. As a vehicle control, 25 μl DMSO diluted with 275 μl PBS was given on the same day.

Histopathological analysis

The dorsal skin was removed 1 day after the final injection. The skin pieces were fixed with 10% formaldehyde for 24 h followed by embedding in paraffin and sectioning using a microtome. Slides were stained with haematoxylin and eosin (H&E). For immunohistochemical analysis, sections were hydrated by passage through xylene and graded ethanols. Next, slides were blocked with 2% bovine serum albumin for 10 min, stained with primary antibody for 60 min (anti-smooth muscle actin 1:50 dilution, DAKO-Cytomation, Carpinteria, CA, USA and mouse monoclonal anti-TACE antibody 1:200 dilution, R&D Systems, Minneapolis, MN, USA). After washing with Tris-Buffered Saline (TBS) containing 0.05% Triton-X100 (TBST), slides were developed using the DAKO ChemMate Envision Kit/HRP (Dako-Cytomation, Carpinteria, CA, USA) followed by counterstaining with haematoxylin. Rabbit IgG was used as the isotype control.

Determination of sTNFRp55, sTNFRp75 and TNFα

Serum samples were obtained from mice before the first injection (day 0) and 1 day after the last injection (day 22). sTNFRp55, sTNFRp75 and TNFα were measured using an enzyme-linked immunosorbent assay (ELISA; R&D Systems). For the *in vitro* assay, mouse primary keratinocytes, mouse primary fibroblasts, mouse splenocytes and C2C12 cells were deprived of serum for 12 h. Variable doses of BLM (100 nM, 1 μM, 10 μM) were added and the cell culture supernatants were collected 24 h later for sTNFRp55 analysis.

In situ hybridization

Tissue sections were de-waxed with xylene and rehydrated through an ethanol series and PBS. The sections were fixed with 4% paraformaldehyde in PBS for 15 min and then washed with PBS. For antigen retrieval, the sections were treated with 10 μg/ml Proteinase K in PBS for 30 min at 37°C. Next, slides were washed with PBS, refixed with 4% paraformaldehyde in PBS, again washed with PBS and placed in 0.2 M HCl for 10 min. After washing with PBS, the sections were acetylated by incubation in 0.1 M triethanolamine–HCl (pH 8.0) with 0.25% acetic anhydride for 10 min. After washing with PBS, the sections were dehydrated through an ethanol series. Hybridization was performed with probes at concentrations of 100 ng/ml in Probe Diluent (Genostaff, Tokyo, Japan) at 60°C for 16 h. After hybridization, the sections were washed in 5x HybriWash (Genostaff) (equal to 5xSSC) at 60°C for 20 min. Subsequently, slides were washed

in 50% formamide (2x HybriWash) at 60°C for 20 min followed by RNase treatment (50 μg/ml RNaseA, 10 mM Tris–HCl, 1 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) for 30 min at 37°C. The sections were washed twice with 2x HybriWash at 60°C for 20 min, twice with 0.2x HybriWash at 60°C for 20 min and once with TBST (0.1% Tween20 in TBS). After treatment with 0.5% blocking reagent (Roche, Indianapolis, IN, USA) in TBST for 30 min, the sections were incubated with anti-DIG AP conjugate (Roche) diluted to 1:1000 with TBST for 2 h. The sections were washed twice with TBST and then incubated in 100 mM NaCl, 50 mM $MgCl_2$, 0.1% Tween 20 and 100 mM Tris–HCl pH 9.5. Colouring reactions were performed with BM purple AP substrate (Roche) overnight and followed by washing with PBS. Sections were counterstained with Kernechtrot stain solution (Mutoh, Tokyo, Japan), dehydrated and mounted with Malinol (Mutoh).

RNA isolation and real-time polymerase chain reaction

Sections of skin lesions removed 1 day after the final injection, and cells incubated with variable doses of BLM (100 nM, 1 μM, 10 μM) for 6 and 24 h were collected. Total RNA was isolated using the SV Total RNA Isolation System (Promega, Madison, WI, USA). The product was reverse-transcribed into first-strand complementary DNA (cDNA). Thereafter, the expression of collagen type I α1 (Col1a1) and TGF-β1 were measured using the Power SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA. Sequence-specific primers were designed as follows: Col1a1, sense 5′-gagccctcgcttccgtactc-3′, antisense 5′-tgttccctactcagccgtctgt-3′; TGF-β1, sense 5′-cgaatgtctgacgtattgaagaaca-3′, antisense 5′-ggagcccgaagcgg-acta-3′; GAPDH, sense 5′-tgtcatcatacttggcaggtttct-3′, antisense 5′-catggccttccgtgttccta-3′. Real-time PCR (40 cycles of denaturing at 92°C for 15 s and annealing at 60°C for 60 s) was run on an ABI 7000 Prism (Applied Biosystems).

Western blot analysis

Skin samples were frozen in liquid nitrogen, then solubilized at 4°C in lysis buffer (0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% sodium dodecyl sulphate, 100 μg/ml phenylmethylsulphonyl fluoride, 1 mM sodium orthovanadate and protease inhibitor cocktail). Ten micrograms of protein were fractionated on SDS-polyacrylamide gels and transferred onto PVDF membranes (Bio-Rad, Hercules, CA, USA). Non-specific protein binding was blocked by incubating the membranes in 5% w/v non-fat milk powder in TBST (50 mM Tris–HCl, pH 7.6, 150 mM NaCl and 0.1% v/v Tween-20). The membranes were incubated with mouse monoclonal anti-TACE antibody (R&D Systems) at a dilution of 1:1000 overnight at 4°C or with mouse monoclonal anti-β-actin (Sigma-Aldrich, St Louis, MO, USA) at a dilution of 1:5000 for 30 min at room temperature. After three 5-min washes in TBST, membranes were incubated with Horse radish peroxidase (HRP)-conjugated anti-mouse antibody at a dilution of 1:10 000 for 60 min at room temperature. Protein bands were detected using the ECL Plus kit (GE Healthcare, Buckinghamshire, UK).

Collagen analysis in the sclerotic skin

Six-millimeter skin punch biopsies were homogenized in acetic acid at 4°C to extract collagen. One milligram of pepsin was added to each homogenized sample, which was incubated at 4°C for 24 h with shaking. The pepsin-solubilized material was collected after removal of the insoluble residue by centrifugation at 35 000 *g* for 60 min at 4°C. The extracted collagen was analysed using 5% polyacrylamide gel electrophoresis, and the gels were stained with Coomassie brilliant blue to identify the pepsin-resistant collagen band.

Statistical analysis

The data are expressed as mean values ± standard deviation (SD). The unpaired Student's *t*-test was used to determine the level of significance between the sample means.

Results

Serum sTNFRp55 is increased in BLM-treated wild-type mice

Initially, we investigated serum concentration of sTNFRp55 in a murine model of skin fibrosis induced by subcutaneous BLM injection. The BLM-treated group started exhibiting skin sclerosis after 2 weeks of BLM injections, meanwhile the PBS-treated group did not. On day 22, a significant elevation in serum sTNFRp55 was observed in the BLM-treated group. Serum sTNFRp75 also was moderately increased in BLM-treated group (Fig. 1a,b). As TNFRp55 and TNFRp75 are processed by TACE to become soluble, we postulated that BLM might have increased the expression or the activity of TACE. We compared the protein expression of TACE and found higher levels in BLM-treated skin compared with skin from PBS-treated mice (Fig. 1c). Therefore, we next investigated whether the TACE inhibitor, TAPI-1, is able to reduce skin fibrosis induced by BLM injection.

Low dose of TAPI-1 inhibits BLM-induced shedding of TNFRp55 but not TNFRp75

We first examined the effect of TAPI-1 alone. As the inhibitory effect of TAPI-1 depends on its dosage (TNFRp55: IC50 = 5–10 μM, TNFRp75: IC50 = 25–50 μM, TNFα: IC50 = 50–100 μM), we started with a very low-dose TAPI-1 that is supposed to inhibit proteolytic release of sTNFRp55 specifically. Administration of 1 μmol TAPI-1 significantly

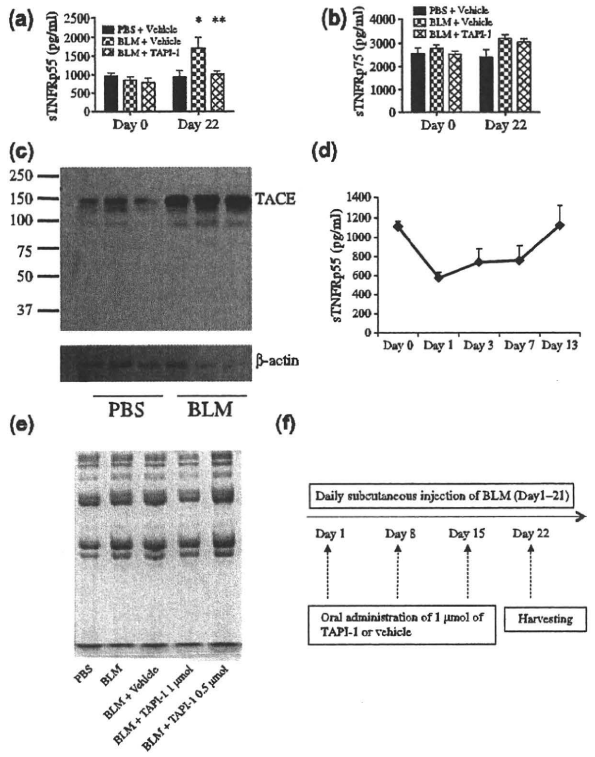

Figure 1. Serum levels of sTNFRp55 and sTNFRp75 in BLM-treated mice. Serum levels of sTNFRp55 (a) and sTNFRp75 (b) were measured by ELISA from mice subcutaneously injected daily with PBS 100 μl/day, or BLM 1 mg/ml, 100 μl/day with or without TAPI-1 administration. Three mice in each group were examined. Each histogram shows the mean (±SD) of each group. $*P < 0.05$ versus PBS group, $**P < 0.05$ versus BLM group. (c) Typical western blot of TACE in PBS-treated and BLM-treated dorsal skin at day 22. (d) Changes in the serum level of sTNFRp55 after administration of TAPI-1 on day 0. (e) Collagen analysis of sclerotic skin. Collagen was extracted from skin lesions and analysed using 5% polyacrylamide gel electrophoresis. (f) Treatment schedule.

reduced the serum level of sTNFRp55 and was effective for up to 7 days (Fig. 1d). Thus, we decided to administer TAPI-1 weekly in the BLM-induced skin fibrosis model. When administered weekly, 1 μmol TAPI-1 reduced the amount of collagen in skin lesions although 0.5 μmol did not (Fig. 1e). From these results, we developed our treatment schedule as shown in Fig. 1f. Wild-type mice received daily subcutaneous injections of PBS or BLM, with or without oral administration of 1 μmol of TAPI-1 on days 1, 8 and 15. At this dose, TAPI-1 inhibited the release of TNFRp55 (Fig. 1a) but not TNFRp75 (Fig. 1b). TNFα was not detectable (<4.3 pg/ml) during this experiment. From this result, we determined that this dosage and interval of TAPI-1 were optimal to decrease the serum concentration of sTNFRp55 alone.

TAPI-1 inhibits BLM-induced dermal thickening

We next investigated the effect of TAPI-1 on skin thickening. The dorsal skin of the mice was harvested 1 day after the last BLM injection for histological analysis. As previously reported, BLM-injected skin showed histopathological features such as acanthosis, dermal thickening and adipose tissue atrophy. To our surprise, BLM-injected skins of TAPI-1-administered group were easy to pinch, which indicated improvement of the skin sclerosis. Histological examination also revealed the symptomatic relief from the BLM-induced events. In particular, TAPI-1 significantly inhibited dermal thickening induced by daily BLM injection (Fig. 2a,b). Administration of TAPI-1 alone did not alter skin thickness ($P = 0.18$, 0.27 ± 0.058 mm in Vehicle + PBS group versus 0.36 ± 0.075 mm in TAPI-1 + PBS group).

Myofibroblasts were decreased in number in the TAPI-1 group

Systemic sclerosis patients' skin is characterized by an increased number of myofibroblasts. These cells are active forms of fibroblasts, which express alpha smooth muscle actin (α-SMA), and are known to contribute to the pathogenesis of SSc (20). To investigate the effect of TAPI-1, the number of myofibroblasts in the skin lesions was counted. As a result, the number of α-SMA-positive cells was significantly increased in the BLM-injected regional skin, while it

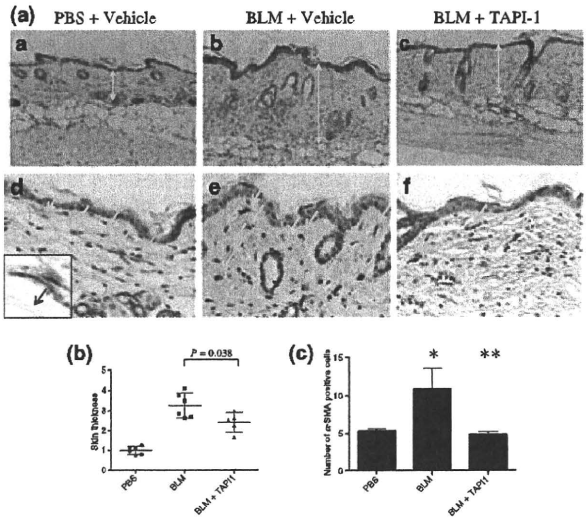

Figure 2. Histological analysis of the skin. (a–c) Haematoxylin and eosin (H&E) staining of PBS + vehicle, BLM + vehicle, BLM + TAPI-1 treated mice skin on day 22 (original magnification ×100), (d–f) immunohistochemistry of α-smooth muscle actin (α-SMA) in myofibroblasts (arrows; original magnification ×100). The small box shows a higher magnification of α-SMA positive cells. (g) Dermal thickness of BLM ($n = 6$) and BLM + TAPI-1 ($n = 5$) treated mice compared with PBS ($n = 6$) treated mice. Two sections from each mouse were evaluated and five locations in each section were measured. The average was calculated and shown as dermal thickness in each mouse. (h) The number of α-SMA-positive cells. $*P < 0.01$ versus PBS group. $**P < 0.01$ versus the BLM group. Horizontal bars represent the mean value and the mean ± SD of each group.

was decreased in the TAPI-1-administered group (Fig. 2a,c). This result indicates that TAPI-1 might be effective during the early fibrosing phase as the number of myofibroblasts is increased in early lesions of skin sclerosis (21).

Expression of collagen-associated genes

Next, to evaluate the effect of TAPI-1 on the synthesis of collagen and induction of fibrogenic cytokines, RNA extracted from the dorsal skin lesions of mice was analysed for the expression of collagen-associated genes. The expression of Col1a1 and the fibrotic cytokine TGFβ1 were lower in the TAPI-1 group than the BLM group (Fig. 3). These results help to corroborate the efficacy of TAPI-1 in this model.

TACE expression is increased in keratinocytes and muscle fibres in skin tissue

To determine the source of sTNFRp55 in our model, we performed *in situ* hybridization (ISH) and immunohistochemical staining (IHC) for TACE in skin tissue. ISH assay revealed the increased mRNA expression in the epidermis and muscle, which was remarkable in the skin lesions of BLM-treated mice (Fig. 4). In support of this observation, increased TACE protein expression in BLM-induced sclerotic skin also was observed by IHC and western blotting (Figs 1c and 5). To investigate whether BLM directly increased sTNFRp55 in these tissues, we compared the concentration of sTNFRp55 in the supernatant and the expression of the TACE mRNA in the cell lysates of primary mouse keratinocytes, primary mouse fibroblasts and mouse skeletal muscle cell lines (C2C12) after adding BLM *in vitro*. We also added BLM to primary mouse splenocytes *in vitro* because it was recently reported that macrophages are activated in the skin of patients with localized scleroderma (22). We did not observe any increase in the level of sTNFRp55 and TACE in any of these culture cells (data not shown). This finding suggests that the increase of sTNFRp55 in BLM-induced skin fibrosis probably requires multiple cell–cell interactions and some humoral factors.

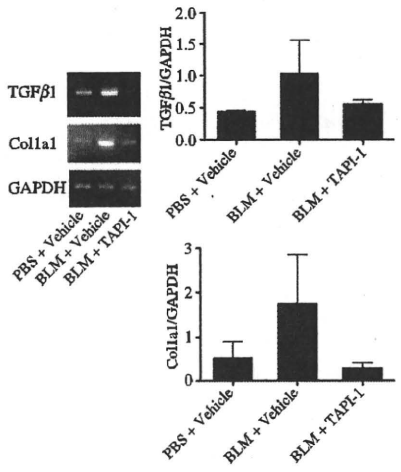

Figure 3. Expression of genes up-regulated in fibrotic tissue. RNA was extracted from the skin lesions. Three mice in each group were investigated. Horizontal bars represent mean values and means ± SD of each group.

Discussion

The skin is severely affected by fibrosis in SSc. Although multiple cytokines and other factors are known to contribute to the development of fibrosis, there are no therapeutic approaches that specifically interfere with any of these factors (2). In this study, we focussed on anti-fibrotic signals and targeted TACE, a sheddase of TNFRp55. We showed that oral administration of TAPI-1 significantly inhibited BLM-induced dermal fibrosis.

It was reported by Bohgaki et al. that there was up-regulated expression of TACE in peripheral monocytes of patients with early SSc (23). The expression of TACE protein in monocytes of early-stage SSc patients (disease duration less than 3 years) was significantly higher than in chronic SSc patients and healthy controls. Its expression in SSc patients who received haematopoietic stem cell transplantation (HSCT) was down-regulated 6 months after HSCT. We investigated the expression of TACE in the skin of mice, which was remarkable in the epidermis and muscle, and was increased by BLM injections (Fig. 4). It is interesting that TACE expression is prominent in muscles,

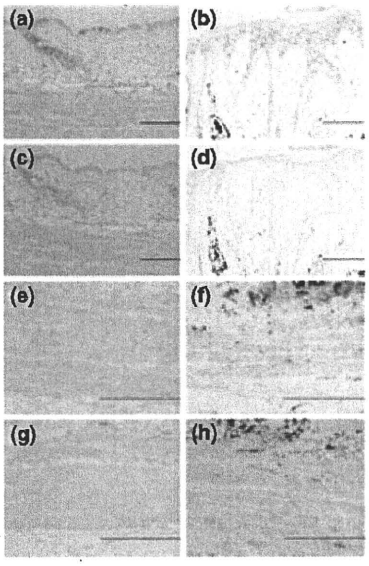

Figure 4. TACE mRNA expression was observed in epidermis and muscle of PBS- and BLM-treated mice by *in situ* hybridization. (Scale bar; 100 μm) Positive reaction appeared as purple to blue colour, and counterstaining reaction appeared as red colour. (a, b) Epidermis of PBS-treated (a) and BLM-treated (b) mice. (e, f) Muscle of PBS-treated (e) BLM-treated (f) mice. (c), (d), (g), (h) are control sense probe of (a), (b), (e), (f).

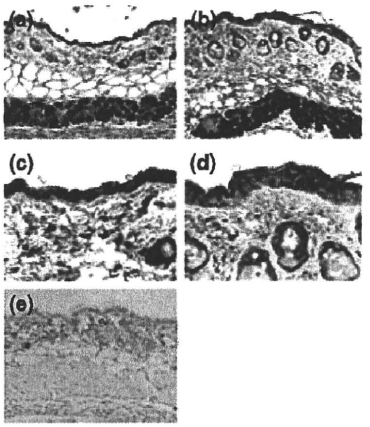

Figure 5. Immunohistochemical staining of the TACE in PBS- and BLM-treated mice. (a) PBS-treated and (b) BLM-treated mice. (original magnification ×100) (c) and (d) are high magnification (×200) of (a) and (b). (e) Isotype control. $n = 6$ in BLM and PBS group and $n = 5$ in BLM + TAPI-1 group. Representative data are shown.

as fibrosis in SSc patients starts in the deep dermis. Further investigation is needed to show the potential involvement of muscle in skin sclerosis.

It is controversial whether blocking TNFα in SSc patients is effective or not. Although not well-designed controlled studies, there are some reports that mention the effectiveness of anti-TNFα therapies in SSc patients (24,25). Recently, it was reported that etanercept was effective in BLM-induced SSc (26). On the contrary, Chizzolini et al. reports that collagen type I production by dermal fibroblasts is inhibited by membrane-associated TNFα, which suggests that TNFα blockade aimed at controlling fibrosis may be unwise (27). Our data and our previous findings (10) support the importance of TNFα/TNFRp55 signalling as an anti-fibrotic signal. The TNFα/TNFR superfamily is known to have a unique and non-redundant function (20). As anti-TNFα therapies block both TNFα/TNFRp55 and TNFα/TNFRp75 signalling, its blockage may have adverse effects. In fact, intraperitoneal injection of TNFα did not reduce skin sclerosis in our BLM-induced skin sclerosis model (data not shown). Therefore, we think that the application of TAPI-1 at a very low dose only to block TNFRp55 shedding or the use of molecules that block only TNRRp55 may be effective in treating skin sclerosis.

In this study, we demonstrated that TAPI-1 significantly inhibits BLM-induced dermal thickening. Although additional experiments are necessary to find the site of action of TAPI-1, therapy targeted at TACE may show promise for SSc patients in the future.

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CED
Clinical and Experimental Dermatology

Epithelioid sarcoma on the foot masquerading as an intractable wound for > 18 years

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Summary

Slow-growing sarcomas may give rise to intractable wounds, which may be attributed to commoner causes. A 57-year-old man with diabetes mellitus presented with a 24-year history of a chronic wound that originated on his left great toe. Because of the long history, the nonspecific histological findings and the complication of ulcerative colitis, we misdiagnosed his ulcer as pyoderma gangrenosum. The wound was eventually diagnosed correctly by histological examination of a skin biopsy and the use of immunohistochemistry to detect cytokeratin, epithelial membrane antigen and vimentin. Specimens obtained 16 years earlier showed the same staining pattern. Radiological examinations revealed no metastasis. The patient received a below-knee amputation without further chemotherapy or radiotherapy. When patients have intractable ulcers, appropriate biopsies and immunohistochemical examinations are sometimes necessary to exclude a malignancy even if the history and symptoms do not suggest a diagnosis of sarcoma.

Intractable wounds can be caused by complicated multiple factors including skin cancers and soft-tissue sarcomas.[1] Although most intractable wounds are relatively easy to diagnose macroscopically, previous inappropriate treatments may mask the true characteristics of the disease. We recently experienced a case of an epithelioid sarcoma (ES) on the foot that masqueraded as an intractable wound over an 18-year period, which is to our knowledge, the longest reported in the literature.

Report

A 57-year-old man had received skin grafts six times over a period of 24 years and eventually had had his great, second and third toes amputated in 1988 at another institution (Fig. 1a,b). He then presented to our clinic in April 2005 with a chronic wound on his left sole with irregular borders and an unhealthy-looking yellowish granulation tissue (Fig. 1c). Diabetes mellitus had been diagnosed 2 years previously. Because of the long history of ulcers, the nonspecific histological findings with aggregates of chronic inflammatory cells (mainly consisting of neutrophils) and the complication of ulcerative colitis, we first misdiagnosed the ulcer as pyoderma gangrenosum.

The patient was admitted to our hospital in December 2006. At that time, the patient did not have the features of aggregated nodules with central necrosis typical of a tumour. Nevertheless, a skin biopsy was taken from the nodular lesion at the wound periphery (Fig. 1d).[2] Histological examination of the lesion at lower magnification after haematoxylin and eosin staining showed no epidermal changes other than pseudoepitheliomatous (pseudocarcinomatous) hyperplasia at the wound edge, a tumour-like nest extending from the wound to the thickest end of the biopsy specimen, and centrally degenerated collagen bundles surrounded by tumour-like cells (Fig. 2a). The tumour-like nest was different

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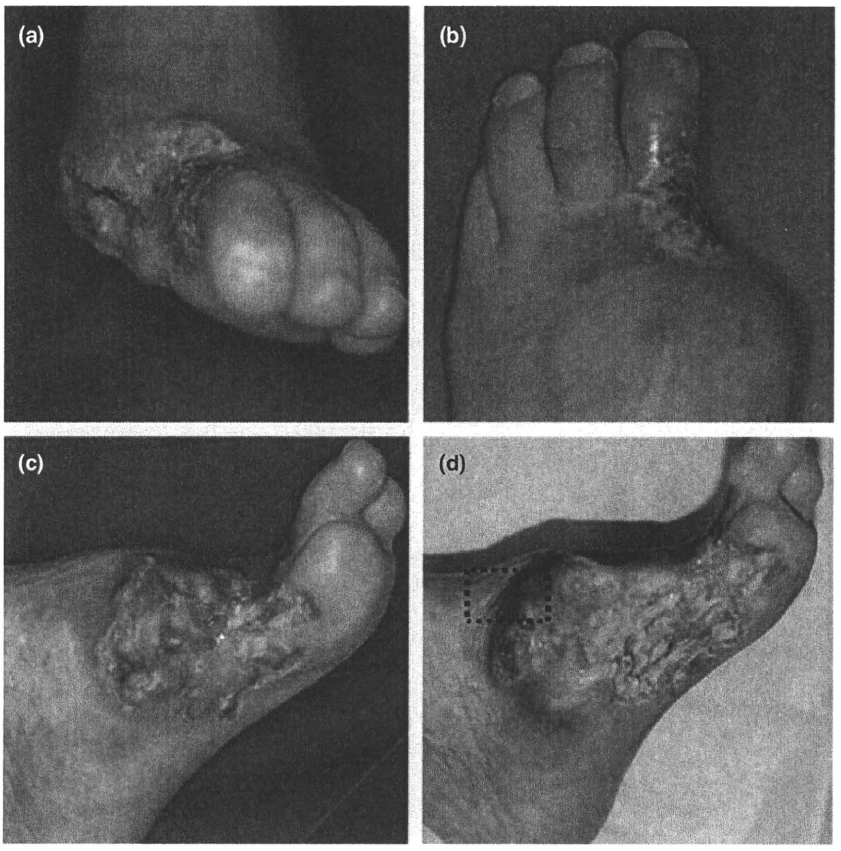

Figure 1 Epithelioid sarcoma on the left foot masquerading as an intractable wound. The ulcers had been intractable for > 3 years when the oldest biopsy available was taken in 1990. Photos taken in 1998 show the recurrence of ulcers from the amputated left great toe: (a) apical view; (b) dorsal view. (c,d) Photographs taken in (c) April 2005 (at another institution) and (d) December 2006 (on admission to our clinic).

from scars and granulation tissues, as it had a high degree of cellularity consisting of fibroblastic cells with few vessels. Fibroblastic tumour cells with high cellularity were also seen in the deep dermis at higher magnification (Fig. 2b), whereas epithelioid cells with several mitotic figures were observed just beneath the wound edge in the epidermis (Fig. 2c), suggesting the transition from spindle-shaped tumour cells to epithelioid tumour cells within the tumour nest. We performed immunohistochemistry and determined that the tumour cells stained positively for pancytokeratin (CK; 1 : 1000; Dako Denmark A/S, Glostrup, Denmark) (Fig. 2d), epithelial membrane antigen (EMA; 1 : 50; Dako) (Fig. 2e) and vimentin (Fig. 2f; 1 : 100; Dako), whereas they were negative for CK7 (1 : 100; Dako), carcinoembryonic antigen (CEA; 1 : 100; Dako), S-100 protein (1 : 200; Dako), HMB-45 (1 : 50; Dako), CD68 (1 : 100; Dako), CD34 (1 : 25; Becton Dickinson Co., Franklin Lakes, NJ, USA), Factor XIIIa (1 : 50; Calbiochem, San Diego, CA, USA) and α-smooth muscle actin (α-SMA; 1 : 100; Dako).

The oldest histological specimen in this patient's records had been taken in 1990 and embedded in paraffin wax. This was taken from scars resulting from intractable ulcers lasting < 2 years and had been stained with haematoxylin and eosin (Fig. 3a). The sample showed the same staining pattern (Fig. 3b) as the most recent biopsy. Taken together, the results allowed us to diagnose the ulcer as a slow-growing distal ES, which had been present for at least 18 years.

Although serum levels of tumour markers, including CEA, CA19-9 and SCC, were within normal ranges, the serum level of CA125 (known as a tumour marker for ES)[3] was high at 119 U/mL (normal range 0–65). Magnetic resonance imaging (MRI) revealed abnormal enhanced lesions around the plantar muscles (abductor hallucis and flexor digitorum brevis) (Fig. 4). Positron emission tomography–computed tomography and bone scintigraphy did not find any metastasis.

Although Whitworth *et al.* [4] reported that wide local excision with free margins is recommended for patients with ES, we performed a below-knee amputation on this patient because of the previous surgical failures and the desire to totally remove any remaining occult tumour cells.

Histological examination of lesions from the amputation showed a typical conglomerate of tumour nodules with central necrosis, as typically observed in a classic

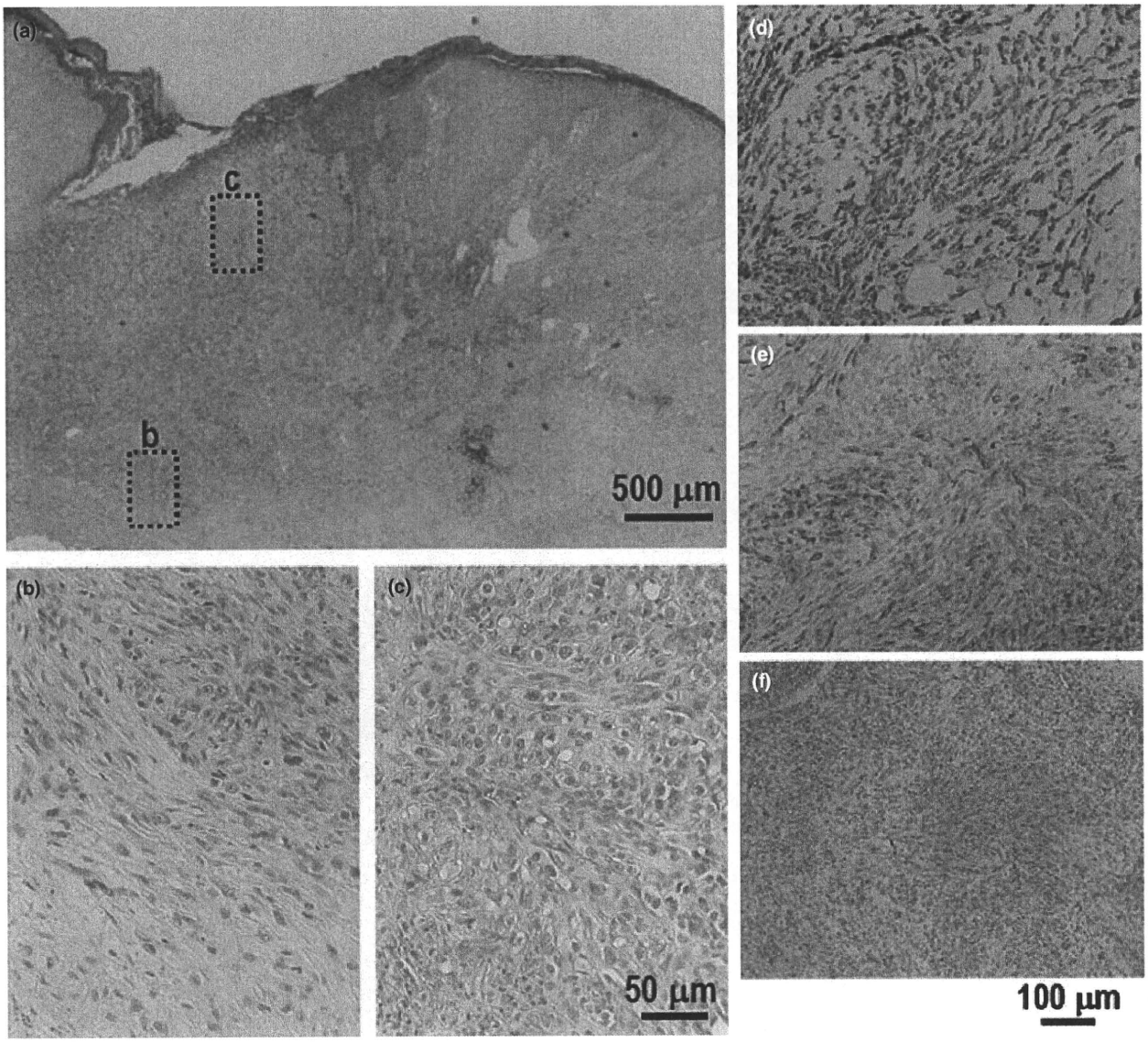

Figure 2 Biopsy taken in 2006 from the area shown in the square in Fig. 1d shows (a) centrally degenerated collagen bundles surrounded by tumour cells, with (b) fibroblastic cells and (c) epithelioid cells. Haematoxylin and eosin original magnification (a) × 20; (b) × 200. (d–f) Tumour cells stained positively for (d) cytokeratin; (e) epithelial membrane antigen and (f) vimentin by immunohistochemistry (original magnification × 100). Bars: (a) 500 μm; (b,c) 50 μm (d–f) 100 μm.

epithelioid sarcoma (Fig. 5a). Immunohistochemistry (Alexa Fluor® secondary antibodies; Molecular Probes Inc., Eugene, OR, USA) revealed that the tumour cells were double-positive for two epithelial markers, CK (Fig. 5b) and EMA (not shown) and for a mesenchymal marker, vimentin (Fig. 5c). A representative merged sample stained with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Youngstown, OH, USA) showed colocalization of both markers (Fig. 5d).

The Ki67 proliferation index is useful to assess the aggressiveness of tumour growth.[5] Double-staining of Ki67 (1 : 50, Dako) (red fluorescence) and CK (green fluorescence) was seen in only 3.8 ± 2.5% (Fig. 5e) of all CK-positive tumour cells, suggesting a less aggressive biological behaviour in this case. The percentage of Ki67-positive epithelioid cells (5.1 ± 2.6%; Fig. 5f) was significantly higher ($P = 0.018$; $n = 11$ nonoverlapping fields) than that of fibroblastic cells (2.6 ± 1.8%; Fig. 5g), indicating a mesenchymal–epithelial transition (MET) rather than vice versa. As a result, we decided not to perform further chemotherapy or radiotherapy. The patient is being reviewed every 3 months by an

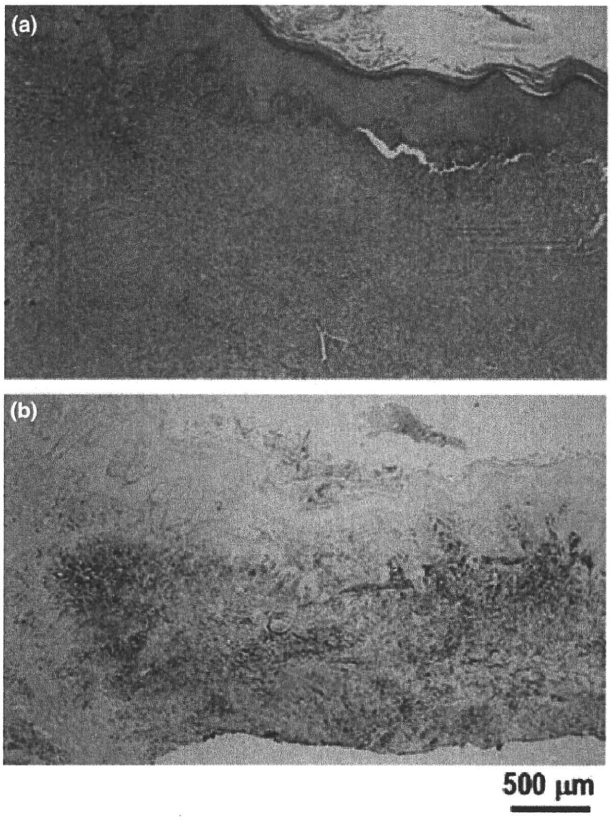

Figure 3 (a,b) Although the sample obtained in 1990 did not show particular signs of epitheloid sarcoma (ES) with (a) haematoxylin and eosin staining (original magnification × 20), it stained positively for (b) epithelial membrane antigen, showing the typical ES staining pattern (original magnification × 100). Bar: 500 μm.

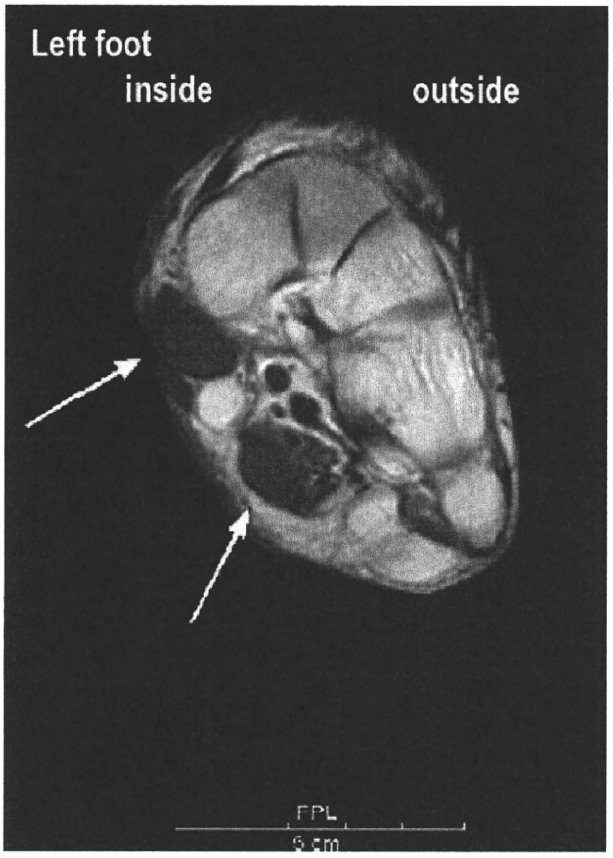

Figure 4 Abnormal enhanced lesions around the plantar muscles visible on magnetic resonance imaging scan. Arrows indicate abductor hallucis for inner muscle and flexor digitorum brevis for middle muscle.

orthopaedic surgeon who specializes in soft tissue tumours. To date no recurrence or metastasis has been noted.

ES are extremely rare sarcomas usually seen in adolescents and young adults, with a 2 : 1 male : female gender ratios. Although the previous biopsies might have been inappropriate samples to reflect the skin lesion, this case was CD34-negative from the beginning, which resulted in the delay in diagnosis; 30–50% of ES are negative for CD34.[6] Enzinger[2] first described ES as a distinct tumour accounting for < 1% of soft-tissue tumours.[7] Chase and Enzinger[8] reported that 9% of those tumours occur in the topographic distribution of the ankle, foot or toes as shown in this case. They reported the largest retrospective study of ES, suggesting that male gender, proximal location, large tumour size (> 50 mm),[9] depth of tumour,[10] high mitotic index, haemorrhage, necrosis, vascular invasion and inadequate initial excision causing local recurrence[9,10] were detrimental prognostic factors. Several of those factors are controversial, especially the location (proximal vs. distal)[9] and the tumour depth (ulcerative vs. nodular). Ulceration occurs when the nodules are located in the dermis as in our patient. Chase and Enzinger[8] and Spillane *et al.*[10] reported increased tumour depth as an adverse prognostic factor.

We chose amputation as the most effective treatment because of the large tumour size and the frequent local recurrences after previous inadequate removal, although conservative surgery has been reported to give better results.[4] We decided not to perform postsurgical treatments, including radiotherapy or chemotherapy, because of (i) the histological low-grade malignancy with few Ki-67-positive tumour cells, (ii) the 24 years of symptom duration before diagnosis and (iii) the distal and superficial ulcerative type of ES. To our knowledge, the duration of > 18 years before diagnosis of a soft-tissue sarcoma

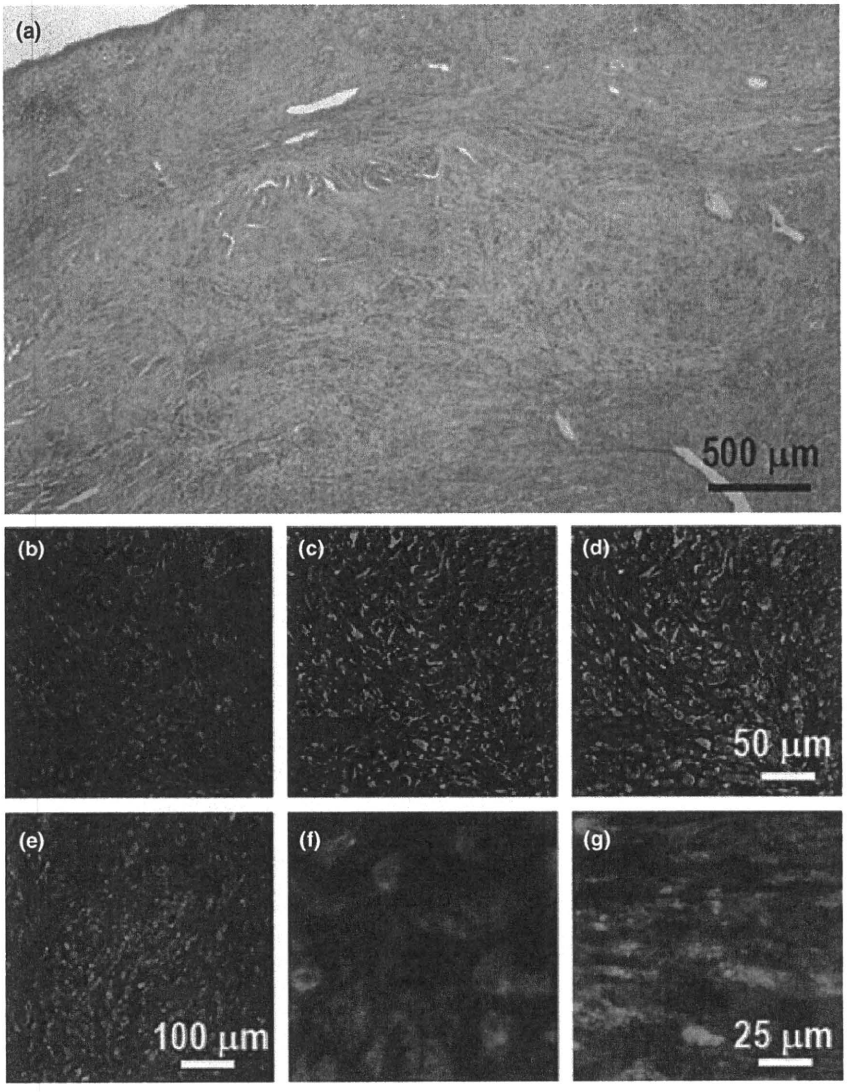

Figure 5 (a–g) Surgically removed tissue. (a) A conglomerate of tumour nodules with central necrosis (haematoxylin and eosin original magnification × 20). (b–d) Immunohistochemical staining with (b) cytokeratin (red) and (c) vimentin (green); (d) 4′,6-diamidino-2-phenylindole (blue nuclear fluorescence) in merged image of (b) and (c). Original magnification (b–d) × 200. (e–g) Immunohistochemical double staining of Ki67 (red) and creatinine kinase (green): (e) a few (3.8 ± 2.5) double-positive tumour cells within the cytokeratin-positive tumour cells; (f) double-positive epithelioid tumour cells (5.1%); (g) double-positive fibroblastic tumour cells (2.6%). Original magnification (e) × 100; (f,g) × 400). Bars: (a) 500 μm; (b–d) 50 μm; (e) 100 μm; (f,g) 25 μm.

masquerading as an intractable wound is the longest reported in the literature. The rates of local recurrence, lymph-node metastases and distant metastases vary from 29% to 85%, 11% to 65% and 21% to 62.5%, respectively.[9] As the most common sites of metastases include the regional lymph nodes (34%) and lungs (51%),[8] careful systematic examinations in addition to regular check-ups for local recurrence will be required for our patient for some time. When patients present with intractable ulcers of unknown causes, appropriate biopsies and immunohistochemical examinations are necessary to exclude neoplasms not only from epithelia (including ulcerative squamous cell carcinoma, basal cell carcinoma and amelanotic melanoma) but also from mesenchyme (including synovial sarcoma and ES), regardless of the patient's history or complications.

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日本皮膚科学会ガイドライン

原発性局所多汗症診療ガイドライン

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1. ガイドライン作成の背景

原発性局所多汗症は，本邦では難治性疾患として認識されておらず，未治療もしくは美容クリニック，エステティックサロンなどで不適切な処置がなされている．しかし，欧米ではすでに原発性局所多汗症に対する適切な診断基準，診療ガイドラインが作成され重症度に応じた段階的な治療がなされている．今回，原発性局所多汗症の診断基準，診療ガイドラインができることにより，本邦における発症頻度が明らかにされ，重症度に応じた治療指針に沿って治療が行われるようになれば，現在のようにボツリヌス毒素局所注射療法，交感神経遮断術などが安易に施行され過剰医療に伴う多くの弊害がもたらされている現状の改善が期待できる．さらには，適切な治療により多汗症に悩む活動期の青年層の精神的苦痛を改善し青年期多汗症患者の勤勉，勤労意欲を高めることが可能である．

2. ガイドラインの位置づけ

本委員会は日本皮膚科学会，日本発汗学会から委嘱された委員らにより構成され，2009年9月から委員会および書面審議を行い，本ガイドラインを作成した．本ガイドラインは現時点に置ける我が国の原発性局所多汗症の基本的，標準的治療の目安を示すものである．

3. 免責条項

本ガイドラインは報告書作成の時点で入手可能なデータをもとに，ガイドライン作成委員の意見を集約的にまとめたものであるが，今後の研究の結果によっては本報告書中の結論または勧告の変更を余儀なくされる可能性がある．また特定の患者および特定の状況によっては本ガイドラインから逸脱することも容認され，むしろ逸脱が望ましいことさえある．従って治療を施した医師は，本ガイドラインを遵守したというだけでは過失責任を免れることはできないし，本ガイドラインからの逸脱を必ずしも過失と見なすこともできない．

4. エビデンスのレベルと推奨度

本ガイドラインのなかで記載されたエビデンスのレベルと推奨度は，皮膚悪性腫瘍グループが作成した「エビデンスのレベルと推奨度の決定基準」（付表1）に基づいて決定した．

5. 概念

エクリン汗腺は発汗により主に体温調節機能を担っているが，その他，皮膚表面の適度な湿度を供給する機能，自然免疫などにより外界の細菌，ウイルスから体を守る作用が注目されている．汗は皮膚が正常な役割を果たすためこのように重要な役割を果たすが，手掌，足底に温熱や精神的な負荷，またそれらによらずに大量の発汗がおこり，日常生活に支障をきたす状態になる状態を多汗症と定義している．

6. 分類

多汗症は，全身の発汗が増加する全身性多汗症と体の一部のみの発汗量が増加する局所性多汗症に分類されている．全身性多汗症には特に原因のない原発性（特発性）全身性多汗症と他の疾患に合併して起きる続発性全身性多汗症がある．続発性には結核などの感染症，甲状腺亢進症，褐色細胞腫などの内分泌代謝異常，神経疾患や薬剤性の全身性多汗症がある．神経疾患では

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7)しのみやクリニック

付表 1　エビデンスのレベルと推奨度の決定基準（皮膚悪性腫瘍グループ）

A. エビデンスのレベル分類	
I	システマティック・レビュー/メタアナリシス
II	1つ以上のランダム化比較試験による
III	非ランダム化比較試験による
IV	分析疫学的研究（コホート研究や症例対照研究による）
V	記述研究（症例報告や症例集積研究による）
VI	専門委員会や専門家個人の意見+

B. 推奨度の分類#	
A	行うよう強く勧められる （少なくとも1つ以上の有効性を示すレベルIもしくは良質のレベルIIのエビデンスがあること）
B	行うよう勧められる （少なくとも1つ以上の有効性を示す質の劣るレベルIIか良質のレベルIIIあるいは非常に良質のIVのエビデンスがあること）
C1	行うことを考慮してもよいが，十分な根拠*がない （質の劣るIII-IV，良質な複数のV，あるいは委員会が認めるVI）
C2	根拠*がないので勧められない（有効のエビデンスがない，あるいは無効であるエビデンスがある）
D	行わないよう勧められる（無効あるいは有害であることを示す良質のエビデンスがある）

+基礎実験によるデータ及びそれから導かれる理論はこのレベルとする．

*根拠とは臨床試験や疫学研究による知見を指す．

#本文中の推奨度が必ずしも上表に一致しないものがある．国際的にも皮膚悪性腫瘍診療に関するエビデンスが不足している状況，また海外のエビデンスがそのまま我が国に適用できない実情を考慮し，さらに実用性を勘案し，（エビデンス・レベルを示した上で）委員会のコンセンサスに基づき推奨度のグレードを決定した箇所があるからである．

表 1　続発性多汗症の原因

全身性：	薬剤性，薬物乱用，循環器疾患，呼吸不全，感染症，悪性腫瘍，内分泌・代謝疾患（甲状腺機能亢進症，低血糖，褐色細胞腫，末端肥大症，カルチノイド腫瘍），神経学的疾患（パーキンソン病）
局所性：	脳梗塞，末梢神経障害，中枢または末梢神経障害による無汗からおこる他部位での代償性発汗（脳梗塞，脊椎損傷，神経障害，Ross syndrome）
	Frey syndrome，gustatory sweating，エクリン母斑，不安障害，片側性局所性多汗（例：神経障害，腫瘍）

大脳皮質の障害により発汗機能亢進や低下が認められる．脳梗塞で麻痺側の発汗量の増加，体温中枢のある視床下部を含む間脳の障害，脊髄損傷による自律神経障害などによって多汗が起きる．一方，局所性多汗症にも原発性（特発性）と続発性がありFrey症候群は続発性局所性多汗症の一つであり耳下腺の手術や外傷の後で食事の時に耳前部が赤くなり多汗がみられる症候群である．損傷を受けた副交感神経が発汗神経に迷入することにより発症すると考えられている（表1）．

7. 病態

原発性局所多汗症では手掌，足底，腋窩に生じることが多く手掌に多汗症がみられるのを手掌多汗症と呼んでいる．掌蹠に分布するエクリン汗腺は700個/cm^2であり，背部の64個/cm^3，前額部の181個/cm^3と比べて圧倒的に多いことがわかる[1)2)]．しかし手掌多汗症の患者と正常人で汗腺の個数，分布，形状については差がみられることはない．原発性局所多汗症で遺伝的要因の関与が示唆されることが多く，手掌と足底に多汗症がみられるのを掌蹠多汗症と呼んでいる．掌蹠や一部腋窩の発汗様式は，コリン作動性交感神経が関与するとともに，情動を反映する精神発汗であることを特徴とし，その責任部位としては前頭葉[3)]，海馬，扁桃核[4)5)]ともいわれているがまだ解明されてはいない．また，近年家族歴がある多汗症の報告があり，患者の一部には何らかの遺伝子関連も背景にあると考えられて