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ORIGINAL ARTICLE

EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Investigator-initiated clinical study of a functional peptide, SR-0379, for limb ulcers of patients with Werner syndrome as a pilot study

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Aim: An investigator-initiated clinical study was carried out to evaluate the therapeutic potency of SR-0379 for the treatment of leg ulcers in patients with Werner syndrome.

Methods: A multicenter, open-label study was carried out from September 2017 to February 2018. The inclusion criteria for leg ulcers were: (i) leg ulcers in patients with Werner syndrome, diabetes or critical limb ischemia/venous stasis; and (ii) a wound size of >1 cm and <6 cm in diameter. Four individuals with Werner syndrome and diabetic ulcers, respectively, were enrolled. SR-0379 (0.1%) was sprayed on skin ulcers once per day for 4 weeks. Efficacy was evaluated by determining the rate of wound size reduction as a primary end-point at 4 weeks after the first treatment compared with the pretreatment wound size. As secondary end-points, the DESIGN-R score index, the 50% wound size reduction ratio, time to wound closure and quantification of wound bacteria were also evaluated. The safety of SR-0379 was evaluated during the study period.

Results: The reduction rate of ulcer size treated with 0.1% SR-0379 was 22.90% (mean) in the Werner syndrome ulcers group (n = 4) and 35.70% (mean) in the diabetic ulcers group (n = 4), respectively. The DESIGN-R score decreased by 4.0 points in the Werner syndrome ulcers group and 4.3 points in the diabetic ulcers group. Two mild adverse events were reported in two patients, and causal relationships were denied in any events.

Conclusion: Treatment with SR-0379 was safe, well-tolerated, and effective for leg ulcers of both Werner syndrome and diabetes patients. **Geriatr Gerontol Int 2019; 19: 1118–1123**.

Keywords: dermatology, diabetic mellitus, leg ulcers, pharmacology, Werner syndrome.

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Introduction

Werner syndrome is a very rare autosomal recessive disorder[1] caused by the *WRN* gene,[2] which encodes a RecQ DNA helicase. Patients with Werner syndrome have several major complications, including juvenile bilateral cataracts, diabetes, dyslipidemia, atherosclerosis, malignancy and refractory leg ulcers.[3–6] Among them, the incidence of severe leg ulcers markedly reduces patient activity and quality of life. Severe leg ulcers are usually associated with diabetes or ischemia; however, Werner syndrome is also characterized by delayed wound healing, which involves thin connective tissues, physiological overpressure by bone transformation and delayed skin fibroblast cell growth.[3,7] Thus, it is imperative to prevent infections in leg ulcers associated with Werner syndrome during the long treatment period.

We previously carried out an investigator-initiated clinical trial to test a novel functional peptide, named SR-0379,[8] which exerts both antimicrobial action and wound healing functions.[9–11] In a multicenter, double-blinded, randomized phase I/IIa clinical study to evaluate the safety, efficacy and pharmacokinetics of SR-0379 for the treatment of leg ulcers, the skin ulcer reduction rates (%) were 44.73 ± 41.26 (mean $\pm$ SD) for the 0.02% SR-0379 group ($n = 3$), 68.25 ± 28.98 for the 0.1% group ($n = 3$) and 71.61 ± 49.17 for the 0.5% group ($n = 3$), compared with 9.95 ± 65.49 for the placebo group ($n = 3$), respectively. Although the number of participants was limited, treatment with SR-0379 for chronic leg ulcers was safe, well-tolerated and effective. Of importance, the SR-0379 treatment group included patients with Werner syndrome from a previous study. One patient with Werner syndrome was in the 0.1% SR-0379 treatment group and showed a 34.92% size reduction, and another patient was in the 0.02% SR-0379 treatment group and showed a 61.45% size reduction. Because ulcers associated with Werner syndrome are reported to be drug-resistant, in the current study the potential effects of SR-0379 on leg ulcers of patients with Werner syndrome were evaluated in comparison with the effects of SR-0379 on diabetic ulcers.[7,12]

Methods

Preparation of a GMP-grade SR-0379 solution

GMP-grade SR-0379 peptide was synthesized by Bachem (Torrance, CA, USA). The peptide was dissolved in a physiological salt solution, and spray-type containers were filled with 10 mL of the SR-0379 solution under GMP conditions by Nagase Medical (Itami, Hyogo, Japan). A total of 0.05 mL of SR-0379 solution was administered per spray using this container.[13] According to the protocol, each patient received 0.25 mL of SR-0379 solution by five sprays once per day for 28 days.

Study design and patients

The clinical trial was carried out as an investigator-initiated, phase IIa, open-label study (unblinded) at three hospitals (Osaka University Hospital, Osaka; Chiba University Hospital, Chiba; and Kasukabe Chuo General Hospital, Saitama) in Japan. Four participants each with Werner syndrome leg ulcers and diabetic ulcers were enrolled and treated with 0.1% SR-0379. The inclusion criteria for this clinical trial were: (i) adults (age ≥20 years); (ii) a diagnosis of Werner syndrome, diabetes or critical limb ischemia/venous stasis; and (iii) wound size with >1 cm and <6 cm in diameter. Individuals with severe infection, deep skin ulcers (reaching bone tissue), malignancy, organ failure (heart, liver, kidney or blood), malnutrition (serum albumin ≤2 g/dL), poor management of diabetes mellitus (glycated hemoglobin ≥9.0%), pregnancy or those who were judged to be inappropriate for the study by physicians were ineligible for inclusion. Individuals who switched from an oral drug to an intravenous drug to regulate blood flow (i.e. prostaglandin E1, prostacyclin I2), received surgery for wound care or used topical FGF2 (trafermin) after obtaining consent were also excluded. The study protocol was approved by the institutional review board of each hospital. Participants gave written informed consent before enrollment.

The clinical trial was registered at UMIN. The title of the registry is "Treatment with a novel peptide, SR-0379, for the Werner syndrome patients with limb ulcers". The trial number is UMIN000028750, and the registry URL is https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000032842.

Procedures

SR-0379 (0.1%) was topically administered after cleaning with soap once per day for 4 weeks. After administration, the wound was covered with gauze. Participants who received drugs to regulate blood flow (i.e. prostaglandin E1, prostacyclin I2) did not alter their regimen 2 weeks before the start of SR-0379 administration through the final treatment. No topical wound healing drugs were used in combination with SR-0379 or placebo for the same skin ulcer, and the topical use of FGF2 (trafermin) was prohibited after obtaining informed consent through the final treatment.

Upon study entry, clinical assessments, skin ulcer evaluations, blood and urine tests, and electrocardiograms were carried out 11–21 days before treatment. Before the first treatment, clinical assessments and skin ulcer evaluations were carried out. After treatment, clinical assessments, skin ulcer evaluation, blood and urine tests, and electrocardiograms were carried out. During the follow-up period (1 week after treatment), patients received appropriate treatment, including FGF2 (trafermin).

Clinical study outcomes

The primary end-point was the rate of wound size reduction with 4 weeks treatment compared with the pretreatment wound size. Additionally, the following secondary end-points were measured at 4 weeks after the first treatment and compared with pretreatment status: time to wound closure; 50% wound size reduction ratio; quantification of *Pseudomonas aeruginosa*, *Staphylococcus*

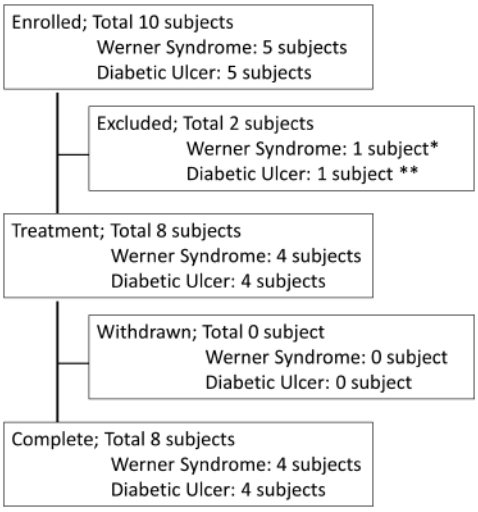

Figure 1 Profile of the patients included in the trial. A total of 10 patients fulfilled the eligibility criteria. Because two patients were excluded due to contracting acute cellulitis and acute limb ischemia before treatment with SR-0379, eight patients were treated with 0.1% SR-0379 for 28 days. Thus, data were collected from eight patients for the full analysis set for the efficiency and safety evaluations. *Excluded before treatment due to cellulitis. **Excluded before treatment due to acute limb ischemia.

aureus and methicillin-resistant *S. aureus*. Skin ulcer photographs were taken after a defined procedure, and ulcer size was quantified and calculated as the percentage change using ImageJ software (NIH, Bethesda, MD, USA). We also used the DESIGN-R score index, which is calculated based on six components (exudate, size, infection/inflammation, granulation tissue, necrotic tissue and pocket size), as a tool to score pressure ulcer severity.[14] Ulcer depth was excluded from the calculation. The total score was calculated by weighing the score of each item; and a high score indicates greater severity. The safety of SR-0379 was evaluated every 7 days during the treatment period (28 days) and post-treatment (7 days), and the adverse events were recorded according to the Common Terminology Criteria for Adverse Events (version 4.0).

Table 1 Baseline characteristics

Characteristics	Werner syndrome	Diabetic ulcer
	(*n* = 4)	(*n* = 4)
Male (%)	3 (75%)	4 (100%)
Female (%)	1 (25%)	0 (0%)
Age (years)	51.5 ± 8.3	62.8 ± 9.9
Height (cm)	159.1 ± 10.8	168.3 ± 2.4
Weight (kg)	52.5 ± 14.5	72.63 ± 4.2
BMI (kg/m^2)	20.6 ± 4.1	25.7 ± 2.0
Target ulcer max diameter (cm)	1.5 ± 0.7	2.3 ± 0.8
Target ulcer position: malleolus	2 (50%)	0 (0%)
Finger	1 (25%)	2 (50%)
Sole	0 (0%)	2 (50%)
Metatarsals 1 (25%)		0 (0%)
Other ulcer existence	3 (75%)	1 (25%)
Non-existence	1 (25%)	3 (75%)
Other ulcer numbers[†]	3.3 ± 3.2	2.0

[†]Calculation only in patients with other ulcer existence. BMI, body mass index.

Results

Characteristics of the clinical study patients

A total of 10 participants (five patients with Werner syndrome and five patients with diabetic ulcer) were recruited between September 2017 and February 2018 at three hospitals in Japan. Figure 1 shows the disposition of the participants. All participants fulfilled the eligibility criteria. However, because two participants were excluded due to developing acute cellulitis and acute limb ischemia before treatment with SR-0379, eight participants were fully treated with 0.1% SR-0379 for 28 days. Thus, data were available for eight participants for the full analysis set for the efficiency and safety evaluations. Table 1 summarizes the basic characteristics of the participants in both groups. The height, weight and body mass index of Werner syndrome patients were low, which is consistent with published reports.[1] In addition, Werner syndrome patients frequently had multiple skin ulcers; however, the SR-0379 treatment was limited to one target ulcer in the present study.

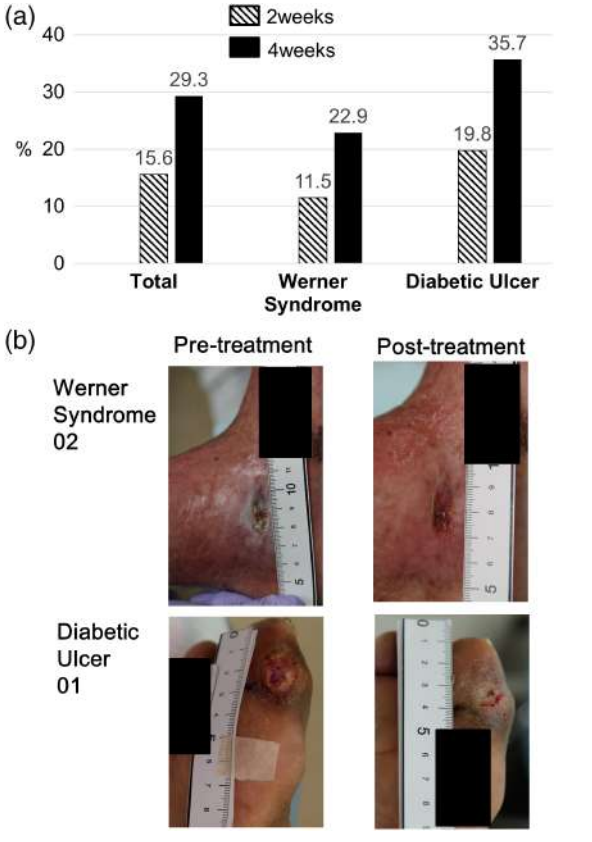

Figure 2 Size reduction (percentage decrease) of skin ulcers with typical pictures. (a) The mean values of the percentage decrease in size by the 0.1% SR-0379 treatment are shown at 2 or 4 weeks after treatment in the Werner syndrome and diabetic ulcer groups. (b) Pictures of the skin ulcers at pretreatment and post-treatment are shown in the patients with Werner syndrome ulcers or diabetic ulcers. One ulcer in a Werner syndrome patient (02) showed a 55.7% reduction in size, and one diabetic ulcer (01) showed total closure.

Table 2 Each patient data

Patient number	Sex	Age (years)	Ulcer area (cm^2) (pre /2 weeks /post)	Decrease, % (2 weeks/post)	Ulcer position/other ulcer numbers
Werner syndrome					
01	Male	50	0.4/0.08/0	80/100	Malleolus/0
02	Female	63	0.97/0.76/0.43	21.6/55.7	Finger/7
03	Male	43	1.51/1.19/1.19	21.2/21.2	Malleolus/2
04	Male	50	0.61/1.08/1.13	−77.0/−85.2	Metatarsals (lateral)/1
Diabetic ulcer					
01	Male	62	1.88/0.48/0	74.5/100	Finger/0
02	Male	77	0.46/0.53/0.56	−15.2/−21.7	Finger (lateral)/2
03	Male	57	0.79/0.48/0.67	39.2/15.2	Sole/0
04	Male	55	3.16/3.77/1.6	−19.3/49.4	Sole/0

Efficacy evaluation

The primary end-point was the skin ulcer rate of reduction (%) induced by 0.1% SR-0379 at the final evaluation, which was 22.90% (mean) for Werner syndrome ulcers (n = 4) and 35.70% (mean) for diabetic ulcers (n = 4; Fig. 2a). All data corresponding to the reduction rate at 2 weeks and 4 weeks after treatment and the average wound size at all time points are shown in Table 2 and Table S1. One participant in each group showed complete ulcer closure at 4 weeks, and two participants in each group showed moderate recovery at 4 weeks (Table 2; Fig. 2b). Conversely, one participant in each group showed increased ulcer size at 4 weeks (Table 2; Fig. S1).

At the secondary end-point (Table 3), the wound closure rate was 25% (1/4 participants) in both groups. The time to wound disclosure was 31 days in the Werner syndrome group and 29 days in the diabetic ulcer group. A significant size reduction (>50%) was observed in 50% (2/4 participants) of Werner syndrome ulcers and in 25% (1/4 participants) of diabetic ulcers. Two participants (one from the Werner syndrome ulcer group and one from the diabetic ulcer group) showed increased local bacterial cultures, whereas the other participants showed no changes or decreased local bacterial cultures. DESIGN-R scores were calculated based on six components (exudate, size, infection/inflammation, tissue granulation, necrotic tissue and pocket size; Tables S2,S3). The DESIGN-R score was decreased by 4.0 points (from 8.8 to 4.8 points) for Werner syndrome ulcers, and by 4.3 points (from 8.0 to 3.8 points) for diabetic ulcers (Table 3). Although two participants (participant number 04 in the Werner syndrome group and participant number 02 in the diabetic ulcer group) did not show a wound size reduction in 4 weeks after treatment compared with pretreatment (Table 2), ulcer depth and granulation were improved (Table S3). Overall, there was no remarkable difference in each efficacy end-point between the Werner syndrome ulcers and the diabetic ulcers.

Table 3 Summary of secondary end-points

	Total (n = 8)	Werner syndrome (n = 4)	Diabetic ulcer (n = 4)
Wound closure	2 (25%)	1 (25%)	1 (25%)
Time to wound closure[†]	31 days	29 days	
50% reduction in wound size	3 (37.5%)	2 (50%)	1 (25%)
Wound bacteria: Decrease	2 (25%)	0 (0%)	2 (50%)
No change	4 (50%)	3 (75%)	1 (25%)
Increase	2 (25%)	1 (25%)	1 (25%)
DESIGN-R score: Pre	8.4 ± 3.1	8.8 ± 2.6	8.0 ± 3.8
Post	4.3 ± 3.0	4.8 ± 3.8	3.8 ± 2.5
Change	−4.1 ± 3.8	−4.0 ± 4.8	−4.3 ± 3.3

[†]Days to wound closure in one patient with wound closure.

Safety evaluation

In the safety evaluation, two adverse events were reported, as shown in Table S4. None of the adverse events were serious, and all were unrelated to SR-0379 treatment. The adverse events comprised of skin abrasions or ulcers in a different position from the SR-0379-treated ulcer; showing that these patients were at high risk for skin ulcers. Thus, there were no apparent safety concerns regarding local or systemic adverse events.

Discussion

We have developed a number of novel peptide drugs to treat skin ulcers over the past 10 years, beginning with a discovery in basic science experiments and extending to clinical trials to evaluate the safety and efficacy for the treatment of severe limb ulcers.[8,13,15–17] In the present study, we evaluated the effect of SR-0379 on limb ulcers in Werner syndrome patients.

The development and approval process for wound-healing drugs has yielded a high number of drug failures.[18] Only one drug, recombinant human PDGF-BB (becaplermin [Regranex]; Healthpoint, Ft. Worth, TX, USA), has obtained Food and Drug Administration approval for a chronic wound treatment indication in the past 20 years.[19] However, topical PDGF-BB results in limited healing of leg ulcers in Werner syndrome patients.[12] In a case report, Bosentan, an endothelin receptor agonist, was reported to be effective for the treatment of severe skin ulcers in Werner syndrome patients.[20] Because it is imperative to prevent infections in leg ulcers associated with Werner syndrome during the long treatment period, we hypothesized that both wound healing and antimicrobial actions might be effective for the treatment of severe leg ulcers.

The percentage decrease in wound size after 4 weeks of treatment was defined as a primary end-point in the efficiency assessment, because previous reports suggested that early changes in wound area reduction (%) are predictive of complete healing.[21,22] We found that the average wound size of both types of Werner syndrome and diabetic leg ulcers decreased by treatment with SR-0379. In particular, one participant from each group showed complete healing within 4 weeks. When comparing Werner syndrome ulcers and diabetic ulcers, there was no remarkable difference in ulcer size reduction (Tables 2, 3). However, two participants

showed increased wound size, and these participants appeared to be non-responders. Importantly, both of these ulcers were located on the lateral side of the metatarsals or finger, which suggests that shoe sores due to poorly fitting shoes might influence the size of the wound by physical damage (Table 2).

In the present study, several secondary exploratory end-points were assessed as the evaluation of wound healing. Although the Japanese Society of Pressure Ulcers originally developed the DESIGN-R score as a tool to score the severity of pressure ulcers and to monitor their healing, the system is also useful in evaluating wound healing.[14] In our previous study, improvements in depth, size and granulation according to the DESIGN-R score were more reliable for evaluating SR-0379. Indeed, the DESIGN-R score decreased by 4.0 points (from 8.8 to 4.8 points) for Werner syndrome ulcers, and by 4.3 points (from 8.0 to 3.8 points) for diabetic ulcers (Table 3). Importantly, two participants (participant number 04 in the Werner syndrome group and participant number 02 in the diabetic ulcer group) showed increased ulcer size (Table 2); however, ulcer depth and granulation improved from D3 to d2 and from g3 to g0 (a score decrease of 3 points) for the Werner syndrome ulcers, and from D3 to d2 and from G5 to g1 (a score decrease of 4 points) for the diabetic ulcers (Table S3). The discrepancy between wound size reduction and DESIGN-R score suggests the limitation of this study in evaluating wound healing drugs in the initial phase. If the depth of the wound is severe, more time is required for granulation by the activation of fibroblasts and angiogenesis. During this process, the wound size does not change, although the depth and granulation of the wound are improving. After the remodeling process is terminated in the wound, wound healing is completed by epithelization, which is fully reflected by the wound size. Thus, we speculate that the two participants who showed increased wound size might still be responsive to SR-0379, because improved wound depth and granulation were observed. In addition, the ulcer position in both participants were lateral metatarsals and lateral finger, respectively (Table 2), which are often affected by physical damage, such as foot blister or pressure. During the foot ulcer treatment process, physiotherapy and equipment method of treatment, as well as drug treatment, are required as total foot care.

In terms of antibacterial action, two participants (one from the Werner syndrome ulcer group and one from the diabetic ulcer group) showed increased local bacterial cultures. However, in the evaluation of the DESIGN-R score, all participants with inflammation and infection improved to i0 for the Werner syndrome ulcers and the diabetic ulcers (Tables S2,S3). Thus, we speculate that local bacteria did not affect the local inflammation and infection, which might lead to delayed wound closure in all patients.

In the safety assessment, just two adverse effects were observed during SR-0379 treatment; however, a causal relationship with SR-0379 treatment was denied. Treatment with SR-0379 did not induce any local adverse effects (i.e. pain, rubor or calor), suggesting that SR-0379 is a safe drug for patients. The limitations of this trial included the small number of patients and the short treatment period. However, the results showed the potency of SR-0379 for the treatment of leg ulcers in Werner syndrome patients. We expect that this academia-derived peptide drug will meet the requirements of a safe and effective pharmacological treatment to improve the treatment outcomes of severe leg ulcers in the future.

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Disclosure statement

Funpep possesses the patent for SR-0379 for clinical study. There are no conflicts of interest among any authors and Funpep, as judged by the committee of Conflict of Interests at Osaka University. The sponsor was not involved in the "design and conduct of the study", "collection, management, analysis and interpretation of data", "preparation, review, or approval of the manuscript" or "decision to submit the manuscript for publication".

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1 Pictures of skin ulcers at pretreatment and post-treatment are shown for a patient with Werner syndrome and a patient with a diabetic ulcer. One ulcer in a Werner syndrome patient (04) resulted in an 85.2% increase in size, and one diabetic ulcer (02) resulted in a 21.7% increase in size.

Table S1 Average wound size in each point.

Table S2 DESIGN-R score. Six of the DESIGN components (depth was excluded) were weighted according to their relationship to healing rate, and their scores were summed to create a total DESIGN-R score ranging from 0 (healed) to 66 (greatest severity).

Table S3 DESIGN-R data of each participant in seven DESIGN components.

Table S4 List of all adverse events.

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