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Efficacy of prosultiamine treatment in patients with human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis: results from an open-label clinical trial

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Abstract

Background: Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic myelopathy characterized by motor dysfunction of the lower extremities and urinary disturbance. Immunomodulatory treatments are the main strategy for HAM/TSP, but several issues are associated with long-term treatment. We conducted a clinical trial with prosultiamine (which has apoptotic activity against HTLV-I-infected cells) as a novel therapy in HAM/TSP patients.

Methods: We enrolled 24 HAM/TSP patients in this open-label clinical trial. Prosultiamine (300 mg, orally) was administered once daily for 12 weeks. We monitored changes in the motor function of the lower extremities and urinary function as well as copy numbers of the HTLV-I provirus in peripheral blood mononuclear cells (PBMCs).

Results: Improvement in the motor function of the lower extremities based on a reduction in spasticity (for example, decrease in time required for walking and descending a flight of stairs) was observed. In an urodynamic study (UDS), bladder capacity and detrusor pressure and then maximum flow rate increased significantly. Detrusor overactivity and detrusor-sphincter dyssynergia improved in 68.8% and 45.5% of patients observed at pretreatment, respectively. Improvement in UDS corresponded with improvements in the score of nocturia-quality of life questionnaire. HTLV-I proviral copy numbers in PBMCs decreased significantly (approximately 15.4%) compared with pretreatment levels.

Conclusions: These data suggest that prosultiamine can safely improve motor dysfunction of the lower extremities and urinary disturbance as well as reduce HTLV-I provirus levels in peripheral blood. It therefore has potential as a new therapeutic tool for HAM/TSP patients.

Trial registration: University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) number, UMIN000005969.

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Keywords: HAM/TSP, HTLV-I, Prosultiamine, Treatment

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Background

Human T lymphotropic virus type I (HTLV-I) infects approximately 10 to 20 million people worldwide, mainly in large endemic areas such as southern Japan, the Caribbean, Central and South America, the Middle East, Melanesia, and equatorial regions of Africa [1,2]. HTLV-I is a human retrovirus and the causative agent of adult Tcell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [3,4]. HAM/TSP is a chronic progressive myelopathy characterized by bilateral pyramidal tracts involved with sphincteric disturbances [5]. Only a small proportion of HTLV-I-infected individuals develop HAM/TSP. However, the main neurological symptoms (for example, motor dysfunction of the lower extremities accompanied by urinary disturbance) are progressive and lead to a deterioration in the quality of life (QoL) of patients once the myelopathy develops. Therefore, novel and safe therapeutic regimens are needed for HAM/TSP patients to use as treatment, or to prevent disease progression.

The primary neuropathological feature of HAM/TSP is chronic inflammation in the spinal cord caused by high HTLV-I proviral load in peripheral blood. Immunomodulatory therapy such as corticosteroid hormones and interferon (IFN)α has been the main treatment administered to HAM/TSP patients to date [6]. Although these treatments have produced good results in the short term, their overall efficacy is controversial [6,7]. In addition, it is not known if these treatments can be tolerated as a long-term or lifelong treatment against HAM/TSP, or whether they are necessary in the therapeutic strategy against HAM/TSP. When treating HAM/TSP, the optimal treatment is elimination of HTLV-I-infected cells from peripheral blood because HTLV-I-infected $CD4^+$ T cells are the first responders in the immunopathogenesis of HAM/TSP [8].

(*N*-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-*N*-[4-hydoxy-1-methyl-2-(propyldithio)-1-butenyl]-formamide) is known as prosultiamine and as Alinamin®. It is a product of Takeda Pharmaceutical Company Limited (Osaka, Japan). Prosultiamine is a homolog of allithiamine, which was originally synthesized from thiol-type vitamin B1 and allicin [9]. For stability in the blood and efficient access of vitamin B1 to tissues, prosultiamine was developed after allyl disulfide derived from allicin was substituted with propyl disulfide in the structure of allithiamine [10]. Importantly, prosultiamine is pharmacologically stable and is readily available for the treatment of Wernicke's encephalopathy and polyneuropathy induced by deficiency of vitamin B1. Moreover, it has been shown to be safe for use in Japan. Therefore, this drug could be utilized immediately for conducting clinical trials in individuals with HAM/TSP.

Recently, we demonstrated that prosultiamine can induce the caspase-dependent apoptosis of HTLV-I-infected cells through disruption of intracellular redox reactions by a disulfide moiety in its structure [11]. Based on these data, we undertook a clinical trial based on the intravenous administration of prosultiamine in HAM/TSP patients with the purpose of targeting HTLV-I-infected cells [11]. We found that prosultiamine administration for 2 weeks was safe and induced clinical improvement. Examples of such improvement included a decrease in (i) spasticity of the lower extremities and (ii) levels of HTLV-I provirus in peripheral blood mononuclear cells (PBMCs) to 30% to 50% of pretreatment levels.

As mentioned above, we do not know if prosultiamine can be tolerated as a long-term treatment against HAM/TSP or indeed if it is necessary in the therapeutic strategy against HAM/TSP. Therefore, in the present study, we administered prosultiamine *via* the oral route for 12 weeks in subjects with HAM/TSP. We found that such treatment could result in (i) improved motor function in the lower extremities based on a decrease in spasticity, (ii) appreciable amelioration of associated urinary disturbance, and (iii) a decrease in the level of HTLV-I provirus in peripheral blood.

Methods

Ethical approval of the study protocol

This study protocol was approved by the clinical studies review boards of Nagasaki University Hospital (Nagasaki, Japan). The clinical trial was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) UMIN000005969. Written informed consent was obtained from all patients enrolled in the study for both participation in the study, and for inclusion of personal data as shown in Table 1.

Patients

We enrolled 24 HAM/TSP patients (17 women and 7 men; 31 to 80 years (mean ± SD; 60.1 ± 11.2 years)) who fulfilled criteria described previously [12]. The duration of illness was 3 to 51 years (mean ± SD; 20.9 ± 12.1 years). Motor function scores were rated from 0 to 13 according to the motor disability score described by Osame *et al.* [13]. Concomitant therapies such as immunomodulators and drugs for the neurogenic bladder were continued on the condition that the dose was kept constant during the study period. Intermittent self-catheterization with regard to voiding was noted except in cases 7, 8, 10, 17, 21, 22, and 24. Patient profiles are shown in Tables 1 and 2.

Study design

Treatment protocol

Prosultiamine was imported from Ildon Pharmaceutical Co., Ltd (Seoul, South Korea). Capsulated prosultiamine (300 mg, orally) was administered once daily for 12 weeks.

Table 1 Profile of HAM/TSP patients enrolled and improvement of motor function in the lower extremities 12 weeks after treatment

Case no.	Age (years)	Sex	Duration of illness (years)	Concomitant therapy			Intermittent self-catheterization	OMDS[a]		Spasticity of the lower extremities	
				Immunomodulator	Drug for neurogenic bladder			Before treatment	After treatment	Before treatment	Improvement[b]
					Anticholinergic	α1 blocker					
1	80	Female	23	No	Yes		Yes	6	6	Yes	Yes
2	64	Female	16	No	Yes		Yes	6	6	Yes	Yes
3	57	Male	51	PSL/ IFN-α	Yes		Yes	6	6	Yes	Yes
4	51	Female	36	No	Yes		Yes	9	9	Yes	Yes
5	67	Female	3	No			Yes	3	3	No	
6	61	Female	30	No			Yes	5	5	Yes	Yes
7	68	Female	12	No		Yes	No	4	4	Yes	Yes
8	64	Male	11	PSL		Yes	No	5	5	Yes	No
9	66	Male	23	PSL	Yes		Yes	9	9	Yes	Yes
10	76	Male	23	No			No	6	6	Yes	Yes
11	53	Female	7	No			Yes	6	6	Yes	No
12	62	Female	12	PSL		Yes	Yes	4	4	No	
13	44	Female	22	No			Yes	6	6	Yes	No
14	56	Male	10	No	Yes		Yes	5	5	Yes	Yes
15	71	Female	45	No	Yes		Yes	9	9	No	
16	78	Female	18	No	Yes		Yes	5	5	Yes	Yes
17	50	Female	19	No			No	5	5	Yes	Yes
18	63	Female	29	No	Yes		Yes	8	8	Yes	Yes
19	62	Female	9	PSL	Yes		Yes	8	8	Yes	No
20	60	Female	34	No	Yes		Yes	2	1	No	
21	46	Female	26	PSL		Yes	No	2	1	Yes	Yes
22	31	Female	7	No		Yes	No	4	3	Yes	Yes
23	56	Male	18	No			Yes	10	10	No	
24	56	Male	16	IFN-α			No	2	2	Yes	Yes
Remarks	mean ± SD; 60.1 ± 11.2		mean ± SD; 20.9 ± 12.1								% improvement: 78.9 (P = 0.0003)[c]

[a]Osame's motor function score (OMDS) was rated from 0 to 13 according to the disability grade [13].
[b]Improvement in spasticity of more than 1 grade according to the modifiedAshworth scale [14].
[c]Statistical significance was determined by the McNemar test.
IFNα interferon α, *PSL* prednisolone.

Table 2 Improvement of motor function in the lower extremities and urinary function 12 weeks after treatment

Case no.	Time required to walk 10 m (sec)			Time required to walk downstairs (sec)			Detrusor overactivity		Detrusor-sphincter dyssnergia	
	Before treatment	After treatment	% improvement	Before treatment	After treatment	% Improvement	Before treatment	After treatment	Before treatment	After treatment
1	26.5	21.6	18.5		N.E.		No	No	No	No
2	15.5	9.8	36.8		N.E.		Yes	No	No	No
3	11.5	10.5	8.7	8.6	7.7	10.5	Yes	No	Yes	Yes
4		N.E.			N.E.		Yes	No	Yes	Yes
5	5.3	4.9	7.5	3.8	3.7	2.6	No	No	Yes	No
6	5.9	6.2	−5.1	4.1	4.2	−2.4	No	No	Yes	No
7	8.9	9.5	−6.7	9.2	7.9	14.1	No	No	No	No
8	12.6	13.3	−5.6	9.5	8.6	9.5	Yes	No	Yes	Yes
9		N.E.			N.E.		Yes	No	No	No
10	20	25.1	−25.5		N.E.		No	No	No	No
11	29.5	32.5	−10.2		N.E.		Yes	No	Yes	Yes
12	6.6	6.9	−4.5	4.4	4.3	2.3	Yes	No	Yes	No
13	22.8	21.3	6.6		N.E.		Yes	No	No	No
14	15.4	11.3	26.6	14.3	11.4	20.3	No	No	No	No
15	N.E.	N.E.			N.E.		Yes	No	No	No
16	13.7	20.9	−52.6		N.E.		Yes	Yes	Yes	Yes
17	13.3	11.5	13.5	10.0	7.3	27	Yes	Yes	No	No
18		N.E.			N.E.		Yes	Yes	No	No
19		N.E.			N.E.		Yes	Yes	Yes	No
20	6.9	5.7	17.4	4.5	3.5	22.2	No	No	No	No
21	5.7	4.1	28.1	3.6	3.5	2.8	Yes	No	No	No
22	10.3	6.8	34	9.4	4.4	53.2	Yes	Yes	Yes	Yes
23		N.E.			N.E.		No	No	Yes	No
24	4.5	4.3	4.4	3.2	3.4	−6.3	Yes	No	No	No
Remarks								% improvement: 68.8 (P = 0.0094)[a]		% improvement: 45.5 (P = 0.0736)[a]

[a]Statistical significance was determined by the McNemar test. N.E.; not evaluated because of high OMDS.

Assessment of effect

Neurological assessment

We monitored changes in neurological signs, motor disability scores, time required for walking 10 m, and time required for walking down a flight of stairs at 4-week intervals. Spasticity of the lower extremities was graded using the modified Ashworth scale (MAS) [14].

Urological assessment

Subjective symptoms were evaluated using the scores of the Nocturia Quality of Life (N-QoL) questionnaire [15-17] at 4-week intervals. The N-QoL questionnaire comprised 13 items and dealt with daytime energy, worry, productivity, sleep, and vitality. The total score ranged from 0 (poorest QoL) to 100 (best QoL). The Duet® Logic G2 system (Mediwatch UK Ltd., Rugby, UK) was used for the urodynamic study (UDS). Bladder capacity, detrusor pressure, maximum flow rate, detrusor overactivity (DO) and detrusor-sphincter dyssynergia (DSD) were evaluated by UDS.

Quantification of HTLV-I proviral load

For quantitative analyses of HTLV-I proviral loads, real-time quantitative polymerase chain reaction (RT-qPCR) was carried out in a Light-Cycler® FastStart DNA Master (Roche Diagnostics, Mannheim, Germany) based on fluorescence detection with SYBER® Green, as described previously [11]. Briefly, genomic DNA samples from PBMCs from HAM/TSP patients were prepared using a Genomic DNA Extraction kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and were subjected to RT-PCR in a LightCycler PCR system using *Tax*-specific primers, that is, forward primer (5′- AAACAGCCCTGCAGATACAAAGT-3′) and reverse primer (5′-ACTGTAGAGCTGAGCCGATAACG-3′), as well as β-actin-specific primers, that is, forward primer (5′-GCCCTCATTTCCCTCTCA-3′) and reverse primer (5′-GCTCAGGCAGGAAAGACAC-3′). The PCR condition for *Tax* was 40 cycles of denaturation (95°C, 15 s), annealing (55°C, 5 s), extension (72°C, 10 s). That for β-actin was 32 cycles of denaturation (95°C, 15 s), annealing (62°C, 5 s), and extension (72°C, 15 s). The HTLV-I proviral load per 10,000 cells was calculated according to the following formula: (copy number of *Tax*)/(copy number of β-actin/2) × 10,000

Statistical analyses

The Wilcoxon signed-rank test was used for statistical analyses of the change of HTLV-I proviral copy numbers and on the N-QoL scores or the urodynamic study except for DO and DSD. The McNemar test was used for statistical analyses of improvement of spasticity, DO and DSD. JMP 10 (SAS Institute Inc., Cary, NC, USA) was used as the software for statistical analyses. $P < 0.05$ was considered significant.

Results

Improvement of motor function of the lower extremities

Improvement in Osame's motor function score (OMDS) was observed in three patients during treatment (Table 1). After 12 weeks of treatment, improvement of more than 1 grade of the degree of spasticity (evaluated according to MAS) was observed in 15 of 19 patients in whom spasticity of the lower extremities was observed before treatment (% improvement; 78.9, $P = 0.0003$, McNemar test) (Table 1). In time required for walking 10 m in 18 ambulatory patients, the decrease ranged from 4.4% to 36.8% was observed in 11 patients although the increase ranged from 4.5% to 52.6% was observed in 7 patients (Table 2). In time required for walking down a flight of stairs in 12 patients, the decrease ranged from 2.3% to 53.2% was observed in 10 patients although the increase of 2.4% or 6.3% was observed in 2 patients (Table 2).

Improvement in urinary function

The conserved overall score of the N-QoL questionnaire was significantly improved, with a significant improvement of subscale scores at 12 weeks post treatment (Table 3). We compared urinary function by UDS at pretreatment with that at 12 weeks after treatment initiation. Bladder capacity and detrusor pressure were significantly increased from 341.3 (SD 127.2) ml to 391.0 (SD 139.9) ml ($P = 0.0097$), and 16.8 (SD 15.6) cm/H_2O to 27.5 (SD 15.3) cm/H_2O ($P = 0.0001$), respectively, by this treatment (Figure 1a,b). As analyzed in 18 patients whose own voiding function was partially reserved, the maximum flow rate was increased significantly from 7.5 (SD 6.2) ml/s to 10.2 (SD 5.6) ml/s ($P = 0.0139$) (Figure 1c). Moreover, DO improved in 68.8% (11 of 16 patients observed at pretreatment) by this treatment ($P = 0.0094$, McNemar test) (Table 2). DSD also improved in 45.5% (5 of 11 patients observed at pretreatment) 12 weeks after the start of treatment ($P = 0.0736$, McNemar test) (Table 2).

Decrease in HTLV-I proviral copy numbers in PBMCs

We monitored changes in copy numbers of the HTLV-I provirus in PBMCs at pretreatment as well as 4, 8, and 12 weeks after treatment commencement (Figure 2a). HTLV-I proviral copy numbers in 10^4 PBMCs decreased gradually from 2,127 (SD 1,932) at pretreatment to 1,961 (SD 1,692) ($P = 0.2776$, vs pretreatment), 1,845 (SD 1,693) ($P = 0.0152$, vs pretreatment) and 1,799 (SD 1,676) ($P = 0.0207$, vs pretreatment) at 4, 8, and 12 weeks after treatment, respectively. The level of HTLV-I proviral copy numbers 12 weeks after treatment decreased significantly (approximately 15.4%) from pretreatment levels. Figure 2b shows the changes in HTLV-I proviral copy numbers in each case between pretreatment and 12 weeks after treatment commencement. A decrease

Table 3 Changes in N-QoL scores after 12 weeks treatment with prosultiamine

Question	Before treatment	After treatment	*P*value
Q1 Concentration	0.6 ± 1.0	0.3 ± 1.6	0.1235
Q2 Low in energy	0.9 ± 1.1	0.4 ± 0.6	0.0077
Q3 Sleep during the day	1.5 ± 1.4	1.0 ± 1.1	0.0229
Q4 Productiveness	0.7 ± 0.9	0.3 ± 0.6	0.0830
Q5 Physical activities	1.0 ± 1.2	0.5 ± 0.8	0.0505
Q6 Fluid restriction	0.8 ± 1.2	0.7 ± 0.9	0.3270
Q7 Inadequate sleep at night	1.6 ± 1.5	0.7 ± 1.0	0.0070
Q8 Disturbance of others	0.8 ± 1.9	0.5 ± 1.8	0.0277
Q9 Preoccupation with waking at night	0.6 ± 1.1	0.3 ± 0.6	0.1235
Q10 Worry over condition worsening	1.5 ± 1.5	0.8 ± 1.1	0.0032
Q11 Worried over treatment options	1.5 ± 1.6	1.0 ± 1.3	0.0303
Q12 Overall bother	1.3 ± 1.3	0.8 ± 0.8	0.0238
Q13 Overall impact on everyday life	2.6 ± 2.8	0.9 ± 1.0	0.0023
Converted overall score (Q1 to 12)	73.2 ± 21.0	85.3 ± 19.9	0.0001
Subscale scores:			
Sleep/Energy (Q1 to5, 7)	74.0 ± 20.7	87.0 ± 15.0	0.0001
Bother/Concern (Q6, Q8 to12)	72.4 ± 25.7	83.7 ± 21.2	0.0028

Subjective symptoms were evaluated using the scores of the Nocturia Quality of Life (N-QoL) questionnaire [15-17]. The score ranges of each question except Q13 are from 0 to 4. The score of Q13 is from 0 to 10. Converted overall score (0–100) = 100 × total converted scores (Q1 to 12)/4 × question numbers, converted score = 4-each raw score. Subscale score (0 to 10) = 100 × total converted scores/4 × question numbers. Statistical significance was determined by the Wilcoxon signed-rank test.

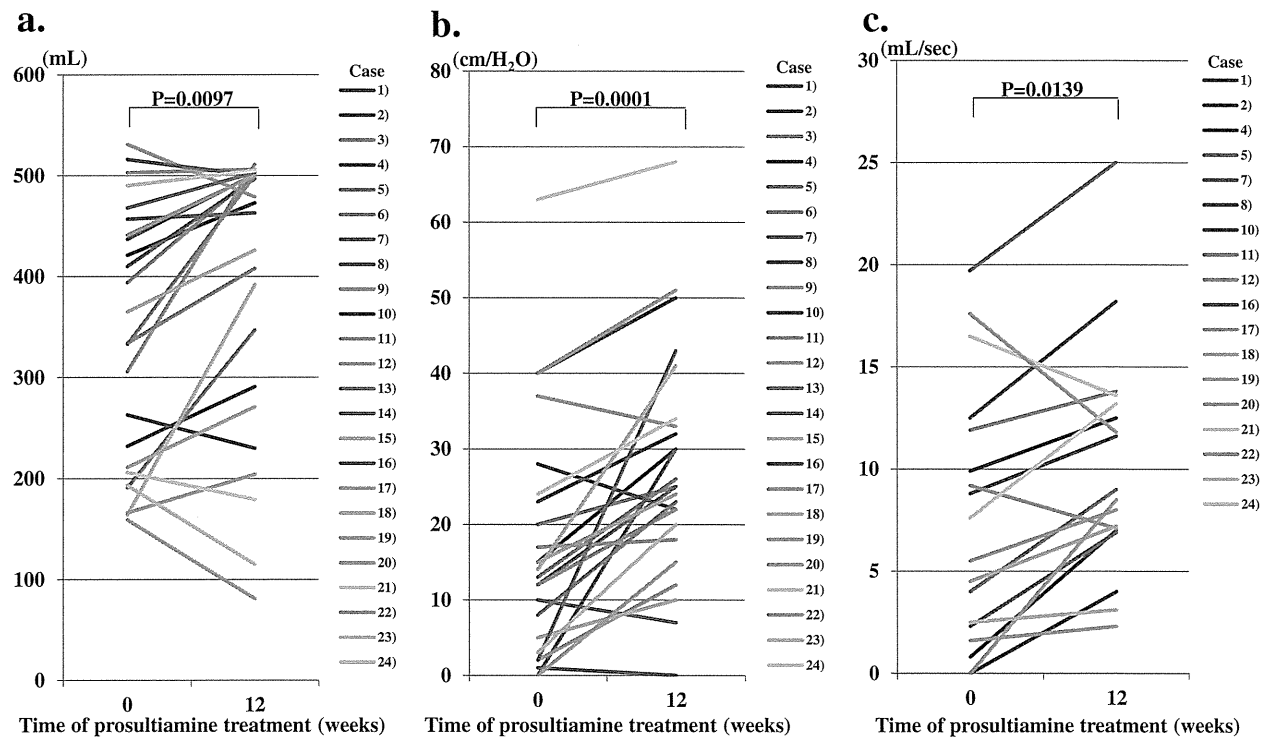

Figure 1 Change in bladder function as evaluated by urodynamic study. Bladder capacity **(a)** and detrusor pressure **(b)** increased significantly 12 weeks after prosultiamine treatment. Maximum flow rate **(c)** increased significantly 12 weeks after prosultiamine treatment as assessed in 18 patients whose voiding function alone was partially reserved. Statistical significance was determined by the Wilcoxon signed-rank test.

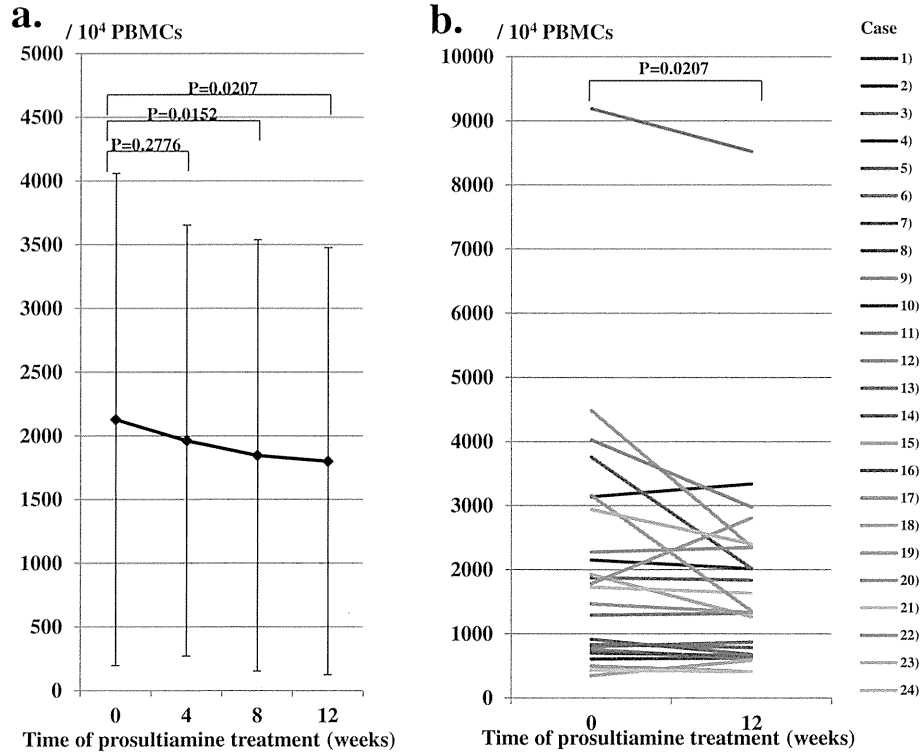

Figure 2 Change in human T lymphotropic virus type I (HTLV-I) proviral copy numbers in peripheral blood mononuclear cells (PBMCs). (a) HTLV-I proviral copy numbers from 10^4 PBMCs decreased gradually until 12 weeks after prosultiamine treatment. The level of HTLV-I proviral copy numbers 12 weeks after prosultiamine treatment decreased by 15.4% compared with the time at pretreatment. **(b)** Changes in HTLV-I proviral copy numbers in each case between pretreatment and 12 weeks after prosultiamine treatment. Statistical significance was determined by the Wilcoxon signed-rank test.

of approximately 30% to 50% in HTLV-I proviral copy numbers was observed in cased 8, 9, 11, 15 and 22.

Adverse effects

There were no serious adverse effects except mild epigastric discomfort rated as '2' evaluated according to the Global Overall Symptom scale [18] in three HAM/TSP patients. This symptom immediately resolved after this clinical trial.

Discussion

Effective therapeutic regimens are needed urgently to treat such myelopathic symptoms of HAM/TSP as spasticity of lower extremities and urinary disturbance. To this end, we administered prosultiamine *via* the oral route for 12 weeks in subjects with HAM/TSP. This treatment improved (i) the motor ability of the lower extremities by decreasing spasticity, and (ii) urinary function. The mean duration of illness of the patients enrolled in this study was relatively long (approximately 21 years), so the efficacy of this treatment is promising. Indeed, these data suggest that the pathological processes in the spinal cord of HAM/TSP patients are partially reversible and treatable even if the tissues are damaged over a long period of time.

The most striking effect in this clinical trial was the amelioration of urinary disturbance in HAM/TSP patients. The common urodynamic findings in HAM/TSP patients are DO, DSD and detrusor hypoactivity [19]. However, as evaluated by UDS, prosultiamine treatment resulted in a significant increase in detrusor pressure and bladder capacity followed by an increase in maximum flow rate with improved DO. DSD also improved in 45.5% (5 of 11 patients observed at pretreatment) (P = 0.0736). Although this value did not reach statistical significance, it showed a tendency of improvement. This is the first time that the therapeutic effect for urinary dysfunction in HAM/TSP patients was evaluated in detail by UDS. With respect to the effect of urinary conditions on the QoL of HAM/TSP patients, nocturia, urgency, increased frequency of urination and dysuria have been reported to be the main problems [20]. Therefore, we evaluated the change in QoL of patients using N-QoL questionnaires during treatment. The improved UDS corresponded with improvements in the score of N-QoL questionnaires. Concomitant pharmacological therapies for the neurogenic bladder were continued during the present study. However, the efficacy of prosultiamine treatment, even in patients who were not having concomitant therapies (cases 5, 6,

11, 13, 23, and 24), strongly suggested that urological improvement was dependent solely upon prosultiamine treatment (Table 1). Overall, these data suggest that prosultiamine treatment can reverse bladder dysfunction in HAM/TSP patients.

Recently, two reports have focused on targeting HTLV-I in therapeutic trials against HAM/TSP. One study used reverse transcriptase (RT) inhibitors, whereas the other used a histone deacetylase enzyme inhibitor for treatment [21,22]. In the former, the results of combination therapy (zidovudine + lamivudine) in a randomized, double-blind, placebo-controlled study suggested that RT inhibitors were not effective for targeting HTLV-I for the treatment of HAM/TSP. In the latter study, long-term treatment using valproic acid did not reduce the number of HTLV-I -infected cells in peripheral blood [22]. A decrease in the HTLV-I provirus in PBMCs was one of the primary endpoints in our recent report [11]. Indeed, oral administration of prosultiamine induced a significant decrease in HTLV-I proviral copy numbers in PBMCs, However, the rate of reduction was not as high as we had expected. This finding might suggest a limitation of the protocol used in the present study. Thus, the remarkable improvement of motor dysfunction and urinary function in the present study cannot be attributed solely to a decrease in HTLV-I proviral copy numbers in PBMCs. The exact mechanism is not known. Prosultiamine was originally developed for efficient access of vitamin B1 to nervous tissues [10]. Although this drug is reduced to a part thiamine and propyl disulfide by the intracellular reducing system after penetration to the cells [10], it is suspected that the disruption of intracellular redox system is induced during reduction of disulfide bond leading to the apoptosis of HTLV-I-infected cells [11]. Therefore, it might be conceivable that, as one of the mechanisms, this drug functions to induce the apoptosis of HTLV-I-infected cells in the spinal cord even if the extent of reduction of the number of HTLV-I-infected cells in PBMCs is relatively small. Further investigations including analysis of cerebrospinal fluid are needed to elucidate the exact mechanism of action of prosultiamine.

Conclusions

In the present work we have demonstrated that oral administration of prosultiamine can safely promote improvement of motor function of the lower extremities based on a reduction of spasticity along with appreciable amelioration of urinary disturbance associated with a decrease in the amount of HTLV-I provirus in peripheral blood. Our results suggest that prosultiamine could be a promising therapeutic tool for HAM/TSP patients. Therefore, further studies are warranted, such as the evaluation of prosultiamine treatment against HAM/TSP in a large-scale, randomized, controlled study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TN designed the study, assessed the neurological findings, analyzed data, and wrote the paper. TMatsuo designed the study, analyzed data, and contributed to the urological studies. HSakai contributed to the urological studies. TF and TN-M assessed the neurological findings. TN, TF and SY managed the blood supply and laboratory studies. KY and HSasaki handled the prosultiamine and enclosed it in capsules. IK, TMatsuzaki, YN, KN, HN, KS, and AK were involved in managing the patients. All authors contributed to the manuscript and approved the final version of the report.

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NEW DEVELOPMENTS FROM ASIA

Prosultiamine treatment as a new therapeutic strategy in human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis

Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic myelopathy characterized by motor dysfunction of the lower extremities and urinary disturbance.[1] The primary neuropathological feature of HAM/TSP is chronic inflammation in the spinal cord caused by high HTLV-I proviral load in peripheral blood mononuclear cells (PBMC). Therefore, immunomodulatory therapy, such as corticosteroid hormones and interferon-α, has been the main treatment for HAM/TSP patients.[2] However, there are many issues in long-term treatment with these drugs, such as insufficient effects and various side-effects. Once the myelopathy develops, the main neurological symptoms, such as motor dysfunction of the lower extremities accompanied by urinary disturbance, are progressive and lead to a deterioration in the quality of life of patients. Therefore, novel and safe therapeutic regimens are urgently required for HAM/TSP patients to use as a treatment, or prevent disease progression.

Prosultiamine (Alinamin), a vitamin B_1 derivative, is safely available in Japan for the treatment of Wernicke's encephalopathy and polyneuropathy induced by deficiency of vitamin B_1. Based on the data that prosultiamine can induce the caspase-dependent apoptosis of HTLV-I-infected cells through disruption of intracellular redox reactions by a disulfide moiety in its structure,[3] we carried out a clinical trial with prosultiamine for 24 HAM/TSP patients using an open-labeled design. Here, I will show the remarkable efficacy of prosultiamine treatment against HAM/TSP patients without serious adverse effects.[4,5]

Prosultiamine 300 mg was given orally once daily for 12 weeks. As a result, improvement in the motor function of the lower extremities based on a reduction in spasticity (e.g. decrease in time required for walking and descending a flight of stairs) was observed. Interestingly, this treatment induced the striking amelioration of urinary disturbance. In an urodynamic study (UDS), bladder capacity and detrusor pressure, and then maximum flow rate, increased significantly. Detrusor overactivity and detrusor-sphincter dyssynergia improved in 68.8% and 45.5% of patients, respectively. Improvement in UDS corresponded with improvements in the score of nocturia quality of life questionnaire. Thus, given that the mean duration of illness of the patients enrolled in the present study was relatively long (approximately 21 years), the efficacy of this treatment is promising.

In the present study, HTLV-I proviral copy numbers in PBMC decreased significantly (approximately 15.4%) compared with pretreatment levels. However, the remarkable clinical improvement in the present study cannot be attributed solely to a decrease in HTLV-I proviral copy numbers in PBMC. Although the exact mechanism is not known, it might be conceivable that, as one of the mechanisms, prosultiamine functions to induce the apoptosis of HTLV-I-infected cells by the disruption of intracellular redox system in the spinal cord, even if the extent of reduction of the number of HTLV-I-infected cells in PBMC is relatively small. Further investigations including analysis of cerebrospinal fluid are required to elucidate the exact mechanism of action of prosultiamine.

Overall, the present results suggest that prosultiamine could be a new promising therapeutic tool for HAM/TSP patients. Therefore, further studies are warranted for the evaluation of prosultiamine treatment against HAM/TSP in a large-scale, randomized, controlled study and long-term treatment.

Competing interests

None.

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ORIGINAL ARTICLE

HTLV-I virological and histopathological analysis in two cases of anti-centromere-antibody-seropositive Sjögren's syndrome

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Abstract
Introduction The aim of this study was to show the clinical and pathological characteristics of anti-centromere-antibody (ACA)-seropositive Sjögren's syndrome (SS) in two anti-human T-cell leukemia virus type I (HTLV-I)-seropositive patients.
Methods One patient was an HTLV-I carrier whereas the other was diagnosed with HTLV-I-associated myelopathy (HAM). Background data including serum HTLV-I titers, viral loads, and cytokine profiles were recorded. Azocarmine with aniline blue (Azan)–Mallory staining and immunohistochemistry of the labial salivary glands (LSGs) and a muscle biopsy specimen from the HAM patient were performed.
Results Serum transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), and HTLV-I viral load were high in the HAM-SS patient compared with the HTLV-I carrier. Fibrous change in LSG was prominent in the HAM-SS patient. Although TGF-β expression was similar in the two patients, expression of HTLV-I-related proteins including p12, p28, group-specific antigen (GAG), and nuclear factor kappa-B (NF-κB) in the LSG were dominantly detected in the HAM-SS patient. Frequency of TGF-β staining in HTLV-I-seropositive SS patients without ACA, HTLV-I-seronegative SS patients with ACA, and HTLV-I-seronegative SS patients without ACA was lower than that of the previous two patients.
Conclusion A high HTLV-I viral load in situ is supposed to promote the production of cytokines, especially TGF-β, resulting in the fibrous change of LSG in ACA-seropositive SS patients.

Keywords HTLV-I infection · Anti-centromere antibody · Sjögren's syndrome · Cytokine

Abbreviations

ACA	Anti-centromere antibody
ANA	Anti-nuclear antibody
CSF	Cerebrospinal fluid
HAM	HTLV-I-associated myelopathy
HTLV-I	Human T-cell leukemia virus type I
IFN-γ	Interferon gamma
MNC	Mononuclear cell
LSG	Labial salivary gland
SS	Sjögren's syndrome
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha

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Introduction

Human T-cell leukemia virus type I (HTLV-I) is known to be one of the causative agents of Sjögren's syndrome (SS) [1, 2]. Our previous epidemiologic studies show a close association between HTLV-I and SS [3, 4]. In addition, we found a significantly high prevalence of SS in patients with HTLV-I-associated myelopathy (HAM) [3, 5]. On the other hand, anti-centromere antibody (ACA) is known as a second class of autoantibodies in SS patients [6, 7]. Our previous report revealed that ACA is detected in only 4 % of HTLV-I-seropositive SS cases, demonstrating that HTLV-I might not be involved in the pathogenesis in ACA-seropositive SS patients [8]. However, if HTLV-I infection coincidently occurs in ACA-seropositive SS patients, the influence of ACA on HTLV-I-associated SS might become obvious. In this study, we report two cases of ACA-seropositive SS patients who were also seropositive for anti-HTLV-I antibody. One patient was complicated with HAM, whereas the other was an HTLV-I carrier. The variation in HTLV-I viral road in these patients appears to explain the differences in labial salivary gland (LSG) histopathology and cytokine profile.

Patients and methods

Patients

Case 1

This was a 61-year-old female patient who complained of sicca symptoms. Both ACA and anti-HTLV-I antibody measured by chemiluminescent enzyme immunoassay (CLEIA) were highly positive, as shown in Table 1. As no other symptoms or signs, including in the neuromuscular systems, were found in this patient, she was classified as an HTLV-I carrier.

Case 2

A 57-year-old female patient who complained of sicca symptoms and myalgia was diagnosed with HAM based on the diagnostic guidance for HAM determined by the Ministry of Health, Labour and Welfare. She had slowly progressive and symmetrical pyramidal tract damage with positive anti-HTLV-I antibody in both serum and cerebrospinal fluid (CSF). Antibodies against gp46, p53, p24, and p19 of HTLV-I in CSF were all positive. Serum ACA was also positive at a high titer (Table 1). She also suffered from inflammatory myopathy as evidenced by the elevation of muscle enzymes and by magnetic resonance imaging and muscle biopsy findings.

Both patients were diagnosed with SS according to the revised criteria [9], as proposed by the American–European Consensus Group. In both cases, HTLV-I viral roads in sera and serum cytokines including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and transforming growth factor beta (TGF-β) were measured. For comparison, we studied the three groups of patients: (1) HTLV-I-seropositive SS patients without ACA, (2) HTLV-I-seronegative SS patients with ACA, and (3) HTLV-I-seronegative SS patients without ACA with respect to TGF-β immunostaining of LSG (four patients each in three groups).

LSG biopsy

LSG biopsy from the lower lip was performed under local anesthesia in SS patients. Informed consent to use biopsy samples was obtained from all participating patients at the commencement of the study. The study was conducted with the approval of the human ethical committee of our institution. The classifications of Chisholm and Mason [10] were used to determine the severity of mononuclear cell (MNC) infiltration.

Azan–Mallory staining and immunohistochemistry of labial salivary glands

Formalin-fixed, paraffin-embedded sections (3-μm thick) from the LSGs of these ACA-seropositive SS patients were used for azocarmine with aniline blue (Azan)–Mallory staining and immunohistochemistry. The sections were then stained using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan) with mouse anti-human CD4, CD8, CD20, and CD68 antibodies (DakoCytomation, Glostrup, Denmark), mouse anti-HTLV-I [p19, p28, and group-specific antigen (GAG)] antibody (Chemicon International Inc., Temecula, CA, USA), mouse anti-nuclear factor kappa B (NF-κB) p65 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and mouse anti-TGF-β antibody (LifeSpan BioSciences, Inc., Seattle, WA, USA). Briefly, endogenous peroxidase was inactivated in a 3 % hydrogen peroxide (H_2O_2) solution after microwave epitope retrieval. These sections were then blocked with 5 % normal horse serum, followed by incubation with monoclonal and polyclonal antibodies in a humid chamber for 60 min at room temperature. After incubation, all sections, including the negative control sections, were treated with peroxidase-conjugated secondary antibodies for 30 min. The color was developed by soaking the sections in 3.3′-diaminobenzidine (DAB) and H_2O_2 for 10 min, followed by counterstaining by soaking the sections in hematoxylin solution. Negative

Table 1 Background information and serum data of the human T-cell leukemia virus type I (HTLV-I)-associated anti-centromere antibody (ACA)-seropositive patients

	Case 1 HTLV-I carrier with ACA-seropositive SS	Case 2 HAM with ACA-seropositive SS
Age and gender	61 years old, female	57 years old, female
Xerostomia	Positive	Positive
Xerophthalmia	Positive	Negative
Schirmer test (right/left mm; <5 mm: positive)	5/4	11/11
Saxon test (g/2 min; <2 g: positive)	1.47	2.7
ANA: pattern	160×, centromere	640×, centromere
Anti-SS-A antibody: normal 10–30 U/ml	0.7	0.9
Anti-SS-B antibody: normal 15–25 U/ml	0.9	0.5
ACA: normal <16 index	172.8	165.0
IgG: normal 870–1,700 mg/dl	1,712	1,623
Rheumatoid factor: normal <15 IU/ml	11.4	17.0
Sialography[a] (Rubin and Holt)	Stage 1	Stage 2
Lip biopsy grade[b] (Chisholm and Mason)	3	3
LST (cpm)	105,936/617	184,859/19,319
PHA(+)/no stimulation		
LST (cpm)	160,934/617	102,299/19,319
ConA(+)/no stimulation		
Serum anti-HTLV-I antibody: normal <1.0 COI	>45	>45
Serum viral load (copies/10^4 cells)	<53	373
Serum TNF-α: normal 0.6–2.8 pg/ml	1.0	2.9
Serum IFN-γ: normal <0.1 IU/ml	<0.1	<0.1
Serum TGF-β: normal 1.56–3.24 ng/ml	2.76	12.6

Anti-SS-A Ab and anti-SS-B Ab (Mesacup SS-A/Ro test and SS-B/La test; Medical and Biological Laboratories, Nagoya, Japan) and ACA (Mesacup-2 test CENP-B; Medical and Biological Laboratories, Nagoya, Japan) were measured using an enzyme-linked immunosorbent assay (ELISA) kit. Serum anti-HTLV-I antibody was measured by chemiluminescent enzyme immunoassay, and HTLV-I viral load was measured by the FastStart DNA Master Hybridization probe method. Serum TNF-α and TGF-β were measured by ELISA. Serum IFN-γ was measured by enzyme immunoassay. Data shown represent the period before treatments with agents such as glucocorticoids or immunosuppressive agents

SS Sjögren's syndrome, *ANA* anti-nuclear antibody, *COI* cutoff index, *ConA* concanavalin A, *cpm* count per minute, *HAM* HTLV-I-associated myelopathy, *Ig-G* immunoglobulin G, *LST* lymphocyte stimulation test, *PHA* phytohemagglutinin, *TNF* tumor necrosis factor, *IFN* interferon *TGF* transforming growth factor

[a] Sialography grading was determined by Rubin and Holt. Stages 1 and 2 represent punctate and globular patterns, respectively

[b] Grading defined by Chisholm and Mason: the presence of at least one focus of mononuclear cells per 4 mm^2 section = grade 3

control sections were treated with mouse immunoglobulin (Ig)G1.

Results

Clinical and serological data with cytokine profile

As shown in Table 1, a high ACA titer was detected in both patients. Serum IgG was almost normal, which is characteristic in ACA-seropositive SS patients [6]. As patient 2 was diagnosed with HAM, spontaneous proliferation of MNCs was significantly higher than in patient 1. Serum HTLV-I viral load was 373 copies/10^4 cells in patient 2, which is obviously higher than in patient 1 (<53 copies/10^4 cells). Serum TNF-α and TGF-β levels in patient 2 were increased compared with those in patient 1, although serum IFN-γ in both patients was within normal limits.

Azan–Mallory staining and immunohistochemical analysis

MNC infiltration was similar in both patients; however, Azan–Mallory staining showed a stronger fibrosis in patient 2 than in patient 1 (Fig. 1). In patient 2, TGF-β was highly stained in infiltrating MNCs and vessels, except in ductal and acinar cells. TGF-β staining, although weaker than MSG, was also performed in the muscle in patient 2. Accordingly, infiltration of CD4+ lymphocytes, which were dominant compared with CD20 and CD68, was shown in the LSGs of both patients (Fig. 2). Although CD8+ lymphocytes were also scattered in LSGs, CD4+

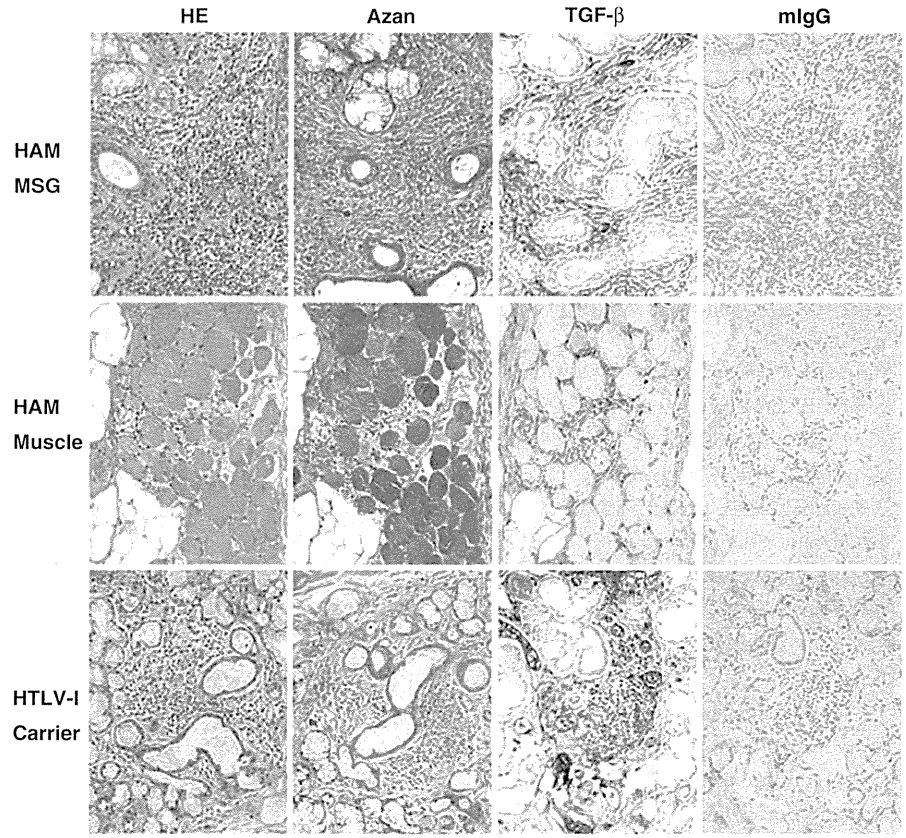

Fig. 1 Azocarmine with aniline blue (Azan)–Mallory staining and transforming growth factor beta (TGF-β) immunostaining in the labial salivary gland (LSG). Azan–Mallory staining and immunohistochemistry after epitope retrieval were performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the LSG using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). The primary antibodies used for immunohistochemistry were TGF-β and mouse immunoglobulin (Ig)G1 (×200). Hematoxylin was used as a counterstain

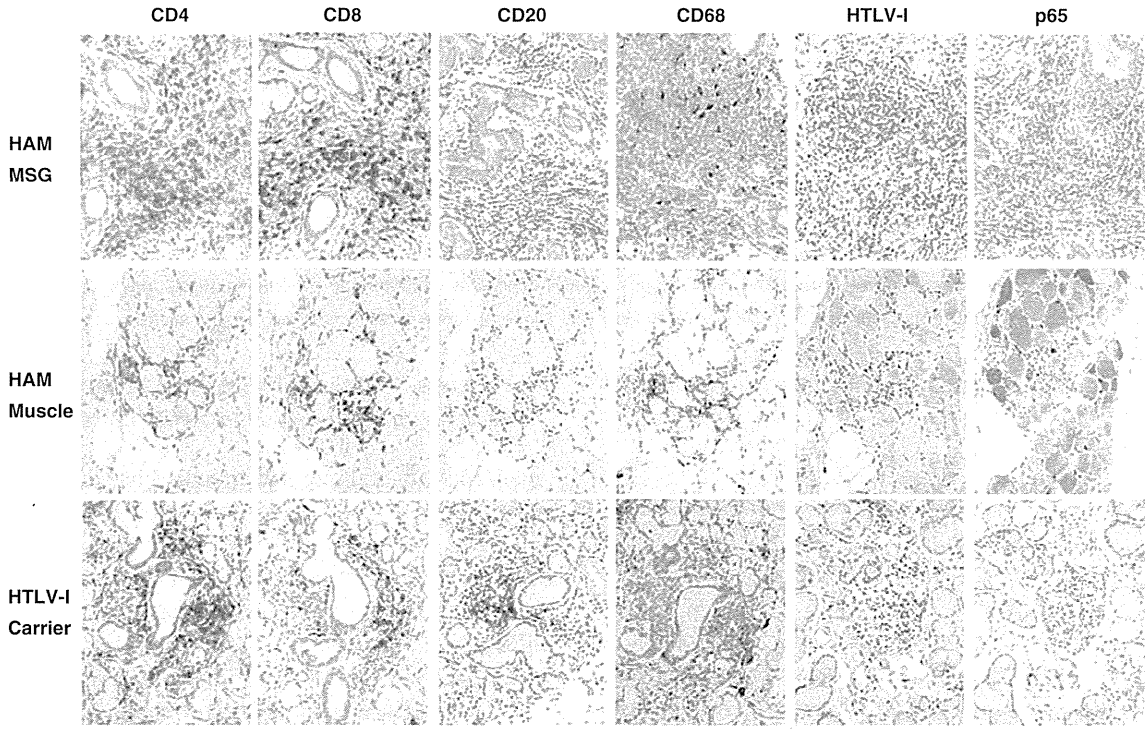

Fig. 2 Immunohistochemistry in the labial salivary gland (LSG). Immunohistochemistry after epitope retrieval was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the LSG using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). The primary antibodies used for immunohistochemistry were CD4, CD8, CD20, nuclear factor kappa B (NF-κB) (p65), and human T-cell leukemia virus type I (HTLV-I) [p19, p28, group-specific antigen (GAG)]. Lymph node from a patient with adult T-cell leukemia was used as a positive control for staining HTLV-I-related proteins (data not shown) (×200). Hematoxylin was used as a counterstain

and CD8+ lymphocytes were found in a muscle specimen from patient 2. It is interesting to note that HTLV-I-related proteins including p19, p28, and GAG were detected in the nuclei of a large percentage of infiltrating MNCs in LSGs and in the muscle specimen in patient 2, which was in accordance with the distribution of NF-κB p65.

TGF-β immunostaining in SS in the presence or absence of anti-HTLV-I antibody or ACA

We finally showed TGF-β immunostaining according to the presence of anti-HTLV-I antibody or ACA (Fig. 3). We performed these experiments in four patients each in three groups and show representative results (Fig. 3). In the HTLV-I-seropositive SS patients without ACA, TGF-β was dominantly found in vascular endothelial cells or fibrous tissues in LSG; however, the frequency of TGF-β+ cells (patients A–D in Fig. 3) appeared to be lower than the patients in cases 1 and 2 in Fig. 1. In the HTLV-I-seronegative SS patients with ACA, TGF-β was seen in infiltrating MNCs, vascular endothelial cells, and fibrous tissues in LSG. Then, in the HTLV-I-seropositive SS patients without ACA, TGF-β expression was similar to HTLV-I-seronegative SS patients with ACA (patients E–H in Fig. 3). In contrast, TGF-β expression was less in HTLV-I-seronegative patients without ACA (patients I, K, L) compared with other groups. In a HTLV-I-seronegative SS patient without ACA (as in patient J), TGF-β was not found in fibrous cells but in MNCs.

Discussion

Both HTLV-I and ACA are known to contribute to SS [1–8]; however, this coincidence of HTLV-I and ACA is supposed to occur with low frequency [8]. Our two cases presented here are rare but may illustrate the in vivo role of HTLV-I in patients with ACA-seropositive SS. Although both patients showed grade 3 MNC infiltration in LSGs, results from exocrine function tests, including Schirmer test and Saxon test in patient 1, were worse than those in patient 2. Except for the degree of MNC infiltration in LSGs, other factors such as aquaporin-5 distribution or type 3 muscarinic receptors [11, 12] might affect lacrimal and salivary secretion. With respect to MNC infiltration into the LSG, both cases showed similar findings. However, there were significant differences in fibrosis determined by Azan–Mallory staining and cytokine profiles.

As patient 2 was diagnosed with HAM, the HTLV-I viral load was high in comparison with patient 1, a finding that is consistent with previous reports [13]. Striking differences were observed in the Azan–Mallory staining findings; however, both patients showed high TGF-β expression in LSGs. TGF-β is a key cytokine for promoting the fibrotic process; thus, the prominent fibrosis of LSG is believed to be driven by TGF-β. Fibrosis was found in the LSG of both patients, which might be explained to some extent by the presence of ACA, as we previously reported [6]. However, a recent report found that HTLV-I basic-leucine zipper (bZIP) factor enhances TGF-β signaling through the p300 coactivator [14]. As strong expression of HTLV-I-related proteins was found in the LSG of patient 2, the TGF-β signaling pathways were suggested to be promoted in situ by HTLV-I, resulting in marked fibrosis. A similar phenomenon might occur in the muscle of patient 2, resulting in inflammatory myopathy. We previously reported that myopathy or uveitis was one characteristic of HTLV-I-seropositive SS patients [15]. With respect to a low level of IFN-γ, Santos et al. [16] demonstrated that administration of exogenous TGF-β induced a decrease of IFN-γ in cells from HTLV-I carriers, suggesting the possibility of the modulation of IFN-γ by TGF-β in HTLV-I-seropositive individuals. The high TNF-α level in patient 2 may also be driven by HTLV-I, as indicated for TGF-β.

To show the involvement of HTLV-I and ACA toward TGF-β expression, we examined TGF-β immunostaining for HTLV-I-seropositive patients without ACA, HTLV-I-seronegative patients with ACA, and HTLV-I-seronegative without ACA (Fig. 3). Although the precise quantitative analysis was not performed in this study, it may demonstrate that TGF-β expression in vascular endothelial cells and fibrous tissues of LSGs is more prominent in SS patients positive for both anti-HTLV-I antibody and ACA (two cases in Fig. 1) compared with SS patients positive for either one alone [two groups (patients A–H in Fig. 3)]. Accordingly, TGF-β expression in the above-mentioned sites was less in SS patients who were not positive for either anti-HTLV-I antibody or ACA (patients I–L in Fig. 3) in comparison with other groups. Therefore, we speculate that the synergistic effect of HTLV-I infection with ACA-carrying status induces the expression of TGF-β in LSGs, especially in vascular endothelial cells and fibrous tissue of SS patients (Fig. 4). However, we also found intense expression of TGF-β in MNCs even in HTLV-I-seronegative patients without ACA. As fibrous change determined by Azan–Mallory staining was not so significant in these patients, TGF-β in MNCs of LSGs may not be directly associated with the fibrotic process. In fact, TGF-β is known to be produced by CD4+ T lymphocytes [17] and influenced by other cytokines, such as IFN-γ [18]. Therefore, the two phenomena—Azan–Mallory-stain-proven fibrosis and TGF-β expression—should be carefully determined in patients with SS. Further studies with a larger number of participants and more definitive qualification approaches are necessary to prove our hypothesis.

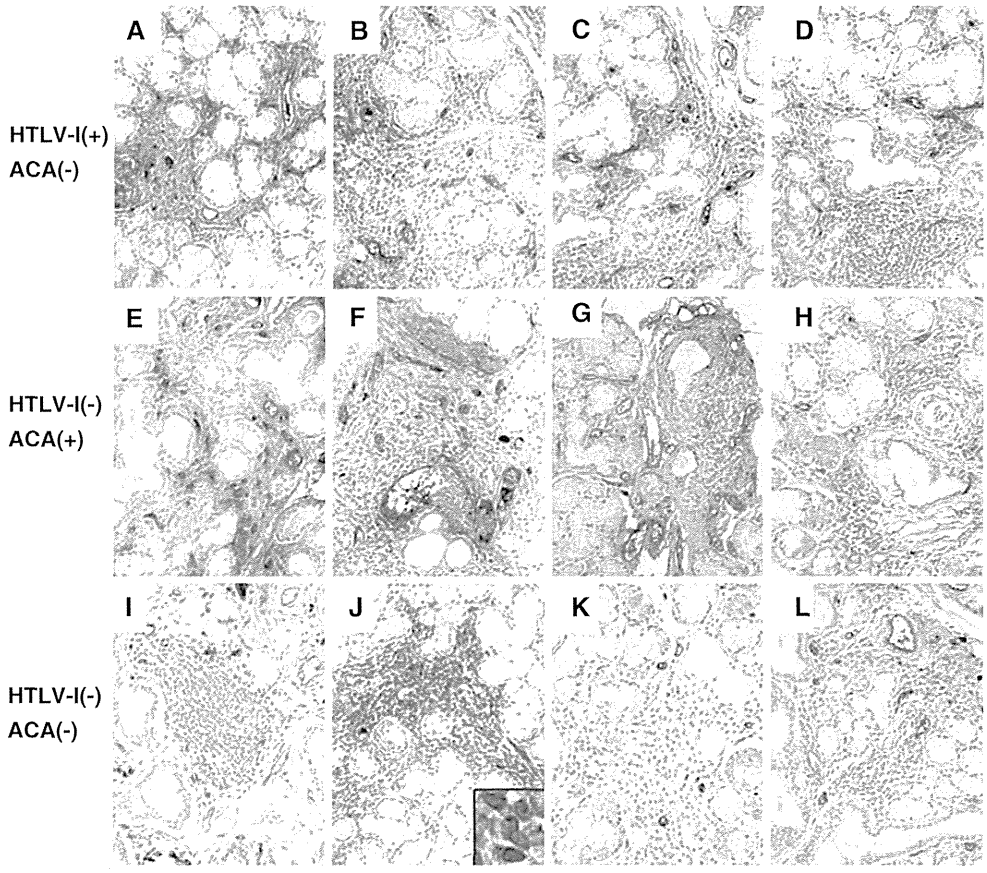

Fig. 3 Expression of transforming growth factor beta (TGF-β) in human T-cell leukemia virus type I (HTLV-I)-seropositive Sjögren's syndrome (SS) patients without anti-centromere-antibody (ACA), HTLV-I-seronegative SS patients with ACA, and HTLV-I-seronegative SS patients without ACA. Immunohistochemistry for TGF-β after epitope retrieval was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the labial salivary gland (LSGs) using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). Staining was performed for four HTLV-I-seropositive SS patients without ACA (patients A–D), four HTLV-I-seronegative SS patients with ACA (patients E–H), and four HTLV-I-seronegative SS patients without ACA (patients I–J). For patient J, TGF-β-positive MNCs are shown in the *inset* (×200). Hematoxylin was used as a counterstain

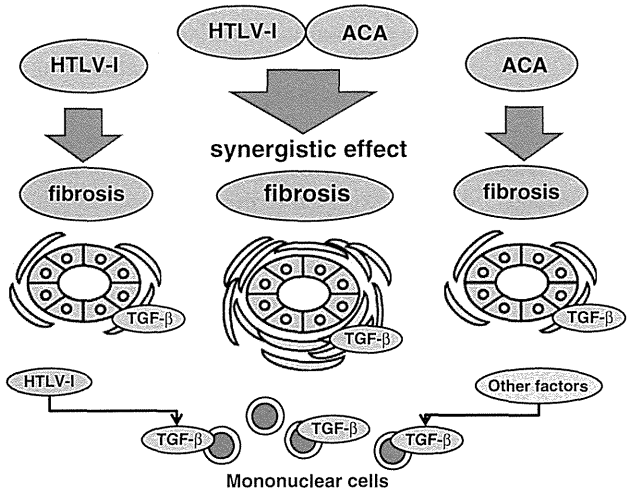

Fig. 4 Hypothesis for fibrotic alternation of salivary glands in Sjögren's syndrome (SS) patients through human T-cell leukemia virus type I (HTLV-I) infection and anti-centromere-antibody (ACA)-carrying status. From the results of the this study, HTLV-I- and ACA-carrying status induce fibrosis in labial salivary glands (LSGs). Furthermore, synergistic effects of HTLV-I infection with ACA-carrying status are assumed from the results of azocarmine with aniline blue (Azan)–Mallory staining. However, transforming growth factor beta (TGF-β), especially in mononuclear cells (MNCs), is also induced in HTLV-I infection and ACA-carrying status

In summary, we report two cases of ACA-seropositive SS found in HTLV-I-seropositive individuals and compared these patients with HTLV-I-seropositive SS patients without ACA, HTLV-I-seronegative SS patients with ACA, and HTLV-I-seronegative SS patients without ACA. The predominant characteristics were found in a patient with HAM, which was believed to have been caused by elevated HTLV-I viral load and subsequent cytokine production. Elements other than TGF-β are also suggestive of influencing fibrotic alternation of LSGs in patients with SS.

Conflict of interest None.

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ORIGINAL ARTICLE

Feasibility of Rehabilitation Training With a Newly Developed Wearable Robot for Patients With Limited Mobility

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Abstract

Objective: To investigate the feasibility of rehabilitation training with a new wearable robot.

Design: Before-after clinical intervention.

Setting: University hospital and private rehabilitation facilities.

Participants: A convenience sample of patients (N = 38) with limited mobility. The underlying diseases were stroke (n = 12), spinal cord injuries (n = 8), musculoskeletal diseases (n = 4), and other diseases (n = 14).

Interventions: The patients received 90-minute training with a wearable robot twice per week for 8 weeks (16 sessions).

Main Outcome Measures: Functional ambulation was assessed with the 10-m walk test (10MWT) and the Timed Up & Go (TUG) test, and balance ability was assessed with the Berg Balance Scale (BBS). Both assessments were performed at baseline and after rehabilitation.

Results: Thirty-two patients completed 16 sessions of training with the wearable robot. The results of the 10MWT included significant improvements in gait speed, number of steps, and cadence. Although improvements were observed, as measured with the TUG test and BBS, the results were not statistically significant. No serious adverse events were observed during the training.

Conclusions: Eight weeks of rehabilitative training with the wearable robot (16 sessions of 90min) could be performed safely and effectively, even many years after the subjects received their diagnosis.

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Rehabilitation robotics emerged in the 1980s with the aim of using robotic technology to assist people with movement dysfunction.[1] Robotic devices have recently been developed for use in clinical settings. Tefertiller et al[2] reviewed 30 articles (14 randomized controlled trials, 16 nonrandomized controlled trials) that examined the effects of locomotor training with robotic assistance in patients after stroke, spinal cord injury (SCI), multiple sclerosis, traumatic brain injury, and Parkinson's disease. The review supports the conclusion that locomotor training with robotic assistance is beneficial for improving walking function in individuals after stroke and SCI.[2] The development of main gait training machines followed. These machines either involve an exoskeleton robotic device (eg, Lokomat, LOPES exoskeleton robot)[3,4] or a robotic device with foot-driven plates (eg, Gait Trainer GT I, Haptic Walker).[5,6] The exoskeleton robotic device is equipped with programmable drives or passive elements that flex the knees and hips during the swing phase, whereas with the other type of robotic device, the feet are placed on footplates, whose trajectories simulate the stance and swing phases. Other than robotic gait training and conventional therapy, another treatment

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