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Effect of an Intravenous Administration of Adipose-Tissue-Derived Autologous Stem Cells on the Skin Temperature of Paralyzed Limbs of Stroke Patients

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1. Abstract

1.1. Background: Cold or warm feelings were reported to be common symptoms of stroke patients. We recently reported that intravenous administration of autologous Adipose-Tissue-Derived Stem Cells (ADSCs) significantly improves neurological functions, including a skin temperature increase, shortly after treatment.

1.2. Aim: To confirm the effect of ADSCs on the skin temperature of stroke patients, we measured skin temperatures immediately before and shortly after ADSC therapy and evaluated the clinical effect on stroke patients in relevance with the skin temperature.

1.3. Patients and Methods: The skin temperature of 16 stroke patients was evaluated. The skin temperature of 9 healthy subjects without ADSC treatment was measured as a control. Skin temperature was measured on healthy and paralyzed limbs of stroke patients, immediately before, during and shortly after treatment with ASDCs at 8 skin location. Before ADSCs treatment, patients and their families are fully explained of safety, efficacy and possible side effects of cell therapy, and patients and their families are explained about possible presentation and publication of their clinical data under the privacy protection of subjects.

1.4. Results: The mean skin temperature of healthy big toes was lowest among the 8 measured locations. The mean skin temperature of paralyzed big toes was lower compared to the healthy side, and increased significantly shortly after ADSC infusion. Skin temperature of patients with large cranial tissue damage did not increase in 4 of 5 cases. Patients with NIH stroke scale recovery and skin temperature increase of paralyzed limbs suggested common recovery mechanism.

1.5. Discussion: Our study is the first report in the world indicating

that the skin temperature of the toe of stroke patients significantly elevated shortly after ADSC therapy. Further, our study also indicated that skin temperature regulation by ADSCs may correlate with the recovery of other skin functions, such as tactile. In conclusion, we speculate that ADSCs may exert beneficial therapeutic effects at least partly via an increased blood circulation immediately after ADSC treatment, although the detailed mechanism of the skin temperature increase and its effect on other neuronal functions remains to be clarified.

2. Introduction

Autologous mesenchymal stem cells have been reported to be effective in the recovery on sensory and motor functional outcomes mostly at subacute stages and rarely in chronic stages of stroke patients [1-3]. Further, human adult mesenchymal stem cells are known to be highly resistant to spontaneous transformation, strongly indicating that mesenchymal stem cell transplantation may be a promising and safe therapeutic modality for strokes [4,5]. To our knowledge, most clinical studies on mesenchymal stem cell therapy of strokes evaluated the efficacy one week to a year after stem cell therapy. So, there is little information available about the early effects of mesenchymal stem cells during or immediately after intravenous transplantation.

We recently reported the characteristics of the recovery of motor, sensory and cognitive functions of 21 stroke patients who were observed during and shortly after Adipose-Tissue-Derived Stem Cells (ADSCs) transplantation therapy. Further, we suggested a possible role for biological small molecules secreted from stem cells suspended in 200 ml saline during the 90-minute-drip-infusion.6 In addition, we experienced several cases who exhibited visible reddish changes of paralyzed limb skin during or immediately after ADSC infusion, suggesting an increase of peripheral blood flow and skin temperature of the paralyzed extremities.

Recently, extracellular vesicles derived from mesenchymal stem cells were suggested to mediate the cell therapeutic effects on stroke by facilitating intercellular communications in a paracrine fashion, and to regulate intrinsic cell functions, so exosomes have been extensively studied and reported to be major mediators of stem cell therapy in stroke and other disorders including skin diseases [7-12]. We speculate that in addition to miRNAs, other small molecules, such as cytokines, growth factors and other factors released from ADSCs, may play a pivotal role in ADSC-induced early functional recovery of stroke patients, since small trophic molecules can travel throughout the body within a minute and affect the biology of both proximal and distant responder cells.

To better understand the efficacy of therapy of stroke patients with ADSCs, we studied the relationship between early clinical recovery of motor/sensory functions and blood circulation of paralyzed limbs of stroke patients after intravenous ADSCs administration, by measuring the skin temperature of healthy and of paralyzed limbs before, during and 30 min after ADSC therapy.

The mean temperature of the paralyzed big toes of 16 stroke patients significantly increased immediately after ADSC therapy compared to before treatment, whereas the temperature of the contralateral healthy big toes showed a tendency to increase, but not at a statistically significant level.

Our study for the first time in the world demonstrated that the skin temperature of the big toe of paralyzed leg significantly elevated during and shortly after a single intravenous drip infused of ADSC. In addition, the study also indicated that the homeostatic recovery of skin temperature by ADSC therapy may correlate with the recovery of other skin functions, such as tactile and temperature sensations of paralyzed limbs. Further, our study suggest that treatment with ADSCs may exert early therapeutic beneficial effects at least partly via increased blood circulation, possibly due to small bioactive molecules secreted from ADSCs during and after ADSC administration. In addition, we speculate that small molecules may be key effectors that exert their biological functions quickly, within several hours and even one month after ADSC therapy, although the detailed molecular mechanisms of the effect of ADSCs on skin temperature and other functional recovery remains to be elucidated.

3. Patients and Methods

3.1. Patients and Skin Temperature Measurement

We report an unblinded clinical study of 16 stroke patients who wanted to be treated with ADSCs, expecting some motor and sensory functional recovery of stroke symptoms which remained after common therapy for strokes. The mean age of the subjects was 54.6 (range: 37~80 years) at the time of their stroke and 57.5 (37~84 years) at the time of ADSC treatment. Demographic and clinical characteristics of the patients are shown in Table 1.

Autologous ADSCs were administered to treat chronic stroke patients who had a stroke onset more than 7 months and less than 9 years earlier. In the present clinical study, 16 cases of 3 ischemic and 13 hemorrhagic stroke patients were included.

We analyzed the effects of ADSC therapy on skin temperature and functional recovery of 16 chronic stroke patients who had severe to moderate neurological abnormalities of hemi-paralysis that remained after common therapy including rehabilitation for acute, subacute and chronic stages, by comparing the effects shortly before and immediately after ADSC therapy.

For skin temperature measurements, we used a combined instrument of LT-8 series and LT-ST08-12 (Gram Corporation, Saitama, Japan), which is designed to measure skin temperature at an accuracy of ±0.01℃. We measured the skin temperature of each patient lying on their back on a bed at a room temperature of 24±0.5℃.

The severity and functional evaluation of each patient was detected by the NIH Stroke Scale (NIHSS), and skin temperature was measured at the middle finger and big toe of healthy and paralyzed limbs of each patient. We evaluated the NIHSS and measured skin

temperature shortly before and immediately after ADSC therapy, since we experienced and reported a case who had a quite rapid recovery of more than 3 NIHSS score within two hours after ADSC therapy.6 The study was conducted according to the Declaration of Helsinki Principles using a protocol ethically reviewed and approved by the Arts-Ginza Clinic Ethics Committee. Informed consent to participate in this study was obtained from each subject or family member before commencement of the study.

Table 1: Skin temperature of 9 healthy subjects.

Subject	Age	Sex	Skin temperature [℃]							
			Upper arm	Forearm	Hand	Mid finger	Upper leg	Lower leg	Foot	Big toe
1	47	M	31.5	32.1	32.1	32.9	31.8	32.1	31.4	30.0
2	42	F	31.5	31.3	30.9	30.5	31.6	31.9	31.3	29.7
3	52	F	31.8	32.4	33.1	33.5	32.1	32.2	32.1	32.3
4	32	F	30.5	30.9	29.8	30.6	31.7	32.1	31.6	29.3
5	32	F	30.6	31.2	31.4	32.4	30.6	31.3	31.2	29.4
6	46	F	30.8	31.5	30.4	29.5	30.5	31.3	31.3	28.8
7	81	M	29.8	31.1	31.4	32.6	29.5	30.1	30.1	30.3
8	61	M	31.5	32.0	32.2	32.8	31.7	31.4	30.8	30.7
9	51	M	30.3	30.8	31.0	31.6	30.2	30.7	30.4	27.8
		mean	30.9	31.5	31.4	31.8	31.1	31.5	31.1	29.8
		SD	0.7	0.6	1.0	1.3	0.9	0.7	0.6	1.3
		n	9	9	9	9	9	9	9	9

Mean values of right and left side of each location are listed.

3.2. Preparation of Autologous ADSCs

Briefly, ADSCs were prepared from the subcutaneous fat tissue of the abdominal skin of each patient. Patients were treated with a local anesthetic patch and injection in the skin approximately 10 cm to the side of the umbilicus, and 2~3 rice-sized pieces of subcutaneous fat tissues were surgically obtained from a 0.7 cm incision. The fat tissues were cut into 15-20 small pieces and were placed on a scaffold of nonwoven fabric painted with hydroxy apatite (BioMiraiKobou, Tokyo, Japan) in culture dishes and were cultured at 5% CO2 and 37℃ in medium supplemented with 4% autologous serum for 11 to 13 days. They were then trypsinized (0.25% trypsin, BioMiraiKobo, Tokyo, Japan) and reseeded in T75 flasks and further cultured for approximately 3 days in medium containing 2% serum, after which they were re-trypsinized and cultured in T300 flasks (BM Equipment, Tokyo, Japan), and then trypsinized again and cultured for 3 days in HyperFlasks (Corning Japan, Tokyo, Japan), before the final cell preparation for the treatment. At the day of transplantation, cells were trypsinized and washed 4 times with saline, then passed through two filters (40 μm and 100 μm) and an average number of 1.0 x 108 (0.6~1.4 x 108) ADSCs were prepared and resuspended in 200 ml saline and administered intravenously to the patients. The cells were further filtered through a 180 μm pore size-mesh during the drip infusion to remove clusters of cells. Stem cell characteristics of collected cells were confirmed with flow cytometry using CD 73, CD90, and CD105 for positive antibodies, and CD45 for negative antibody against stem cells, respectively.

3.3. Statistical Analysis

A probability value of <0.05 is considered statistically significant. All values are presented as means ± SD. Comparisons between groups were made using one-way analysis of variance (ANOVA, nonparametric) with statistical software.

4. Results

4.1. Skin Temperature of the Extremities of Healthy Subjects

Nine healthy subjects were enrolled in the study and the skin temperature of the extensor surface of the elbow, middle forearm, hand joint, middle finger, thigh, lower leg, foot joint and big toe was measured (Table 2). The mean temperature of the big toe skin, including both the right and left big toe of each subject, was the lowest among the skin locations measured, and the mean temperature of the middle fingers of all 9 subjects was higher than all other locations (Figure 1). Based on these results, we selected the middle finger and the big toe of each healthy and paralyzed side of stroke patients to evaluate the effects of ADSC administration on skin temperature.

Table 2: Demographics and clinical characteristics of stroke patients in this study.

Patient No.	Age	Sex	m-finger healthy		m-finger paralyzed		b-toe healthy		b-toe paralyzed		Paralysis side	brain damage characteristics 1	brain damage characteristics 2	Date of stroke onset	Date of ADSC therapy	ADSC cell number	NIHSS		Skin tactile	
			before	after	before	after	before	after	before	after							Before	After	Before	After
No 1	60	Female	31.88	31.12	31.97	▼30.32	32.05	▼30.36	31.3	31.34	left	right putamen bleeding	venticular perforation, large volume	●2019/12/27	10/30/2020	$1.0x10^8$	25	25	—	—
No 2	61	Male	33.48	▼32.46	32.65	32.5	31.02	31.43	31.05	31.32	left	right m edulla oblongata ischemia	vertebral artery heangioma 6mm	●2019/12/4	8/13/2020	$1.0x10^8$	3	3	3	3
No 3	42	Female	31.32	31.44	31.48	△32.5	29.19	29.89	28.62	28.49	right	left putamen bleeding	less than 5cm in diameter	●2019/12/17	7/17/2020	$1.0x10^8$	7	6	1	3
No 4	71	Male	32.82	33.8	32.58	△33.79	31.66	△33.29	31.24	△33.17	right	left putamen bleeding	less than 5cm in diameter	2011/7/18, 2017/5/17	8/19/2020	$1.0x10^8$	3	3	10	10
No 5	66	Female	29.78	29.96	30.09	△32.14	26.58	26.67	25.76	26.34	left	brainstem bleeding	hemangioma 1.8mm	3/1/2016	11/6/2020	$7.4x10^7$	7	7	3	4
No 6	45	Male	31.31	30.64	30.64	△32	27.04	△29.16	26.39	△28.03	left	right AVM rupture bleeding	less than 5cm in diameter	●2019/10/6	5/29/2020	$1.0x10^8$	2	1	16	10
No 7	68	Female	27.96	△29.3	28.59	28.34	26.42	△27.68	25.91	26.66	right	left middle cerebral artery ischemia	large volume	12/9/2018	4/15/2020	$1.0x10^8$	19	18	—	—
No 8	66	Female	31.07	▼29.78	31.26	▼29.56	31.81	31.23	27.61	△31.7	left	right middle cerebral artery ischemia	large volume	12/21/2004	2/27/2020	$1.0x10^8$	3	3	0	0
No 9	50	Female	32.31	▼28.91	33.5	▼30.03	31.92	▼30.71	29.79	29.63	right	left putamen bleeding	large volume	7/21/2017	3/25/2020	$1.0x10^8$	5	4	2	3
No 10	80	Female	31.63	△33.99	32.98	▼29.63	30.29	30.04	29.23	△30.74	right	left thalamic bleeding	less than 5cm in diameter	1/22/2016	10/30/2020	$4.0x10^7$	6	6	1	1
No 11	57	Male	30.55	△33.63	30.89	△33.06	27.61	△29.64	25.98	△29.77	left	right putamen bleeding	less than 5cm in diameter	●2019/6/3	1/20/2020	$1.0x10^8$	4	3	—	—
No 12	37	Male	31.88	▼30.12	31.97	▼30.82	32.05	▼30.36	31.3	30.36	left	right putamen bleeding	large volume	●2020/3/22	11/20/2020	$1.0x10^8$	6	5	0	0
No 13	53	Male	31.26	31.13	31.23	32.09	28.26	△29.56	29.27	28.84	right	left thalamic bleeding	less than 5cm in diameter	1/10/2018	8/23/2019	$1.0x10^8$	2	2	10	10
No 14	51	Female	28.48	△30.35	32.55	△34.39	32.49	32.26	30.55	△32.51	left	right thalamic bleeding	venticular perforation, large volume	11/4/2014	10/28/2020	$1.0x10^8$	1	1	1	5
No 15	56	Male	30.23	▼28.9	30.7	30.77	26.28	△29.03	27.33	△29.05	right	left thalamic bleeding	less than 5cm in diameter	1/9/2019	10/29/2020	$1.0x10^8$	2	2	4	6
No 16	59	Male	34.63	▼32.26	33.31	33.36	34.02	33.46	33.93	33.76	both sides	rapture of right vertebral artery	less than 5cm in diameter	●2019/12/31	11/20/2020	$1.0x10^8$	32	32	—	—

increase (△) and decrease (▼) of temperature after ADSC therapy at healthy skin, increase (△) and decrease (▼) of temperature at paralyzed skin m-finger: middle finger, b-toe: big toe, ●: indicates that time lag betweeen stroke onset and ADSC therapy was less than one year —: Not done, Skin tactile: 10 indicate healthy sensations, 0~9 means comparative sensation.

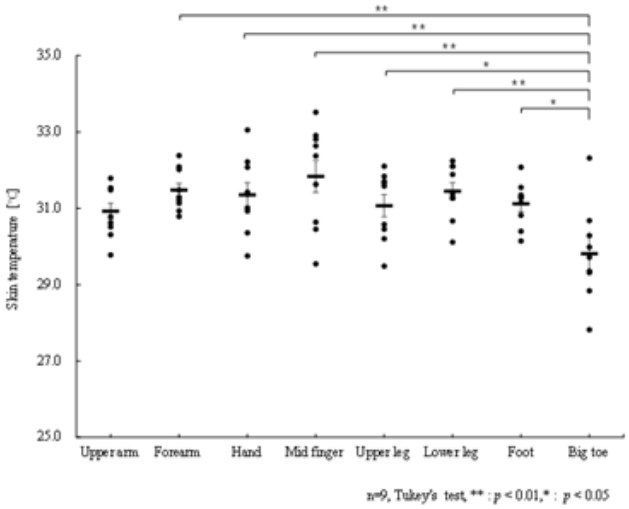

Figure 1: Skin temperature of upper and lower extremities of 9 healthy subjects.

Skin temperature was measured at 8 locations: extensor surface of the upper arm, forearm, hand joint, middle finger, upper leg, lower leg, foot joint and big toe. The middle finger temperature was higher than the other 7 locations, and the big toe temperature was statistically the lowest among the 8 locations (except the upper arm). Statistical analysis was conducted using Tukey's test; ** indicates p<0.01, * indicates p<0.05

4.2. Effect of ADSCs on the Skin Temperature of the Paralyzed Extremities of Stroke Patients

Seven of the 16 stroke patients showed a skin temperature increase at the big toe on the paralyzed side, and the mean skin temperature of the big toe on the paralyzed side of 16 stroke patients was significantly lower than the healthy side before treatment with ADSCs, but it increased to a significantly higher level comparable to the non-treated healthy toe skin temperature after treatment with ADSCs (Figure 2). The mean skin temperature of the paralyzed middle finger, forearm and lower leg of each stroke patient, however, did not increase significantly after ADSC therapy. Interestingly, the skin temperature of the big toe on paralyzed side did not decrease more than 1.0℃ in any patient after ADSC therapy, whereas, the skin temperature of the paralyzed and healthy middle fingers of some stroke patients decreased more than 1.0℃, even to 3.0℃, after ADSC therapy, although the mechanism of the ADSC-induced skin temperature change remains to be clarified.

The mean skin temperature of the middle finger and the big toe on the healthy sides of stroke patients before ADSC therapy was statistically at a level similar to healthy subjects (Figure 3).

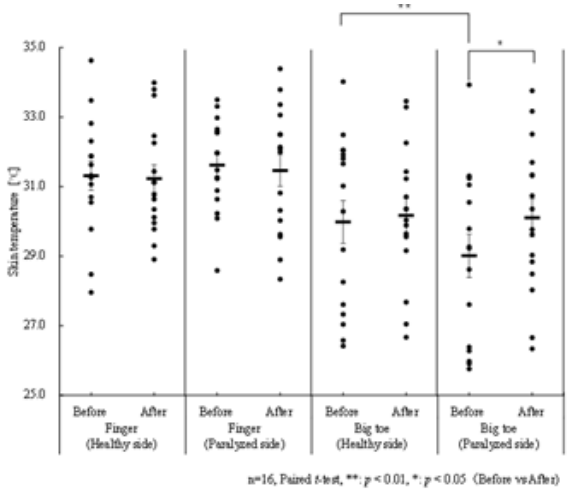

Figure 2: Skin temperature (℃) of the middle finger and big toe of healthy and paralyzed side of stroke patients.

Skin temperature measured at the middle finger and the big toe of healthy (H) and paralyzed (P) limbs of 16 stroke patients, before and after ADSC administration are shown as filled circles and the average of each group is shown by a thick bar (—). Temperature after ADSC administration increased significantly compared to the temperature before ADSC treatment only at the big toe on the paralyzed side. The average skin temperature of pretreatment paralyzed big toes was significantly lower compared to healthy limbs.

** indicates a significant difference between the mean values of healthy and paralyzed big toe skin temperature before ADSC treatment.

* indicates a significant difference between the mean big toe skin temperature between pre- and post- ADSC therapy.

Statistical analysis of skin temperature before and after treatment was performed using t-test, and statistical analysis of skin temperature on the healthy side and on the paralyzed side at the beginning and the end of the treatment was performed using a Paired t-test. A p-value of less than 0.05 is considered statistically significant (**:p< 0.01, *: p<0.05).

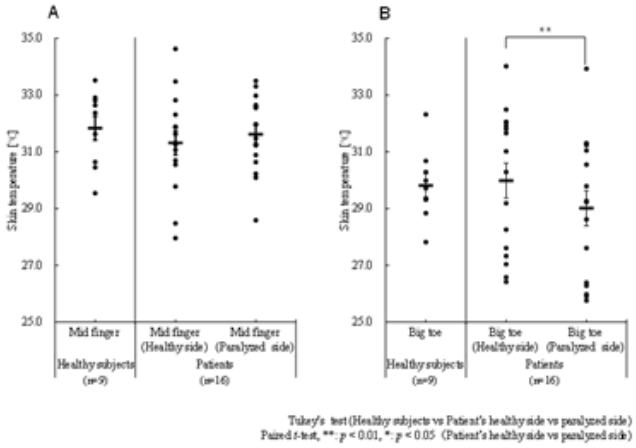

Figure 3: Middle finger and big toe temperatures of healthy subjects and stroke patients on both the healthy and the paralyzed sides.

Mean skin temperature of the middle finger (A) and the big toe (B) on the healthy side of stroke patients was statistically at the level of healthy subjects, whereas the temperature of the big toe on the paralyzed side of stroke patients was statistically lower than healthy subjects and the healthy side of stroke patients. Statistical analysis was conducted according to Tukey's test.

Statistical analysis of skin temperature on the healthy side and paralyzed side was performed using a Paired t-test, and statistical analysis of skin temperature of healthy subjects, healthy side and paralyzed side, was performed using a Paired Tukey's test. A p-value of less than 0.05 is considered to indicate a statistically significant difference (**: $p < 0.01$, *: $p < 0.05$).

4.3. Effect of ADSC Treatment on Skin Temperature and other Functional Recovery of Stroke Patients in Relevance to Brain Lesion Characteristics

Six of the 16 stroke patients showed a functional recovery by NIHSS assay shortly after ADSC therapy, and in 3 of those 6 patients, the skin temperature of the big toe on the paralyzed side was significantly elevated (Table 1). These results suggest that the skin temperature increase of paralyzed toes immediately after ADSC therapy may be correlated with other functional recoveries of stroke patients. 3 of those 7 had putamen bleeding, while the remaining 3 and 1 cases had thalamic bleeding and rupture at the anterior cerebral artery, respectively. The skin temperature of the 3 putamen bleeding cases who showed increased toe skin temperature decreased (Table 1). These results suggest that injured locations of the brain, putamen or thalamic areas may affect via a common route the skin temperature alteration of paralyzed limbs after ADSC therapy.

4.4. Effect of Patient's Clinical Characteristics, Age, Sex and Brain Lesion Volume on Skin Temperature after ADSC Treatment

The effect of clinical characteristics of stroke patients on immediate skin temperature changes after ADSC therapy was studied focusing on age, sex, ischemia or hemorrhage, and brain lesion volume examined by MRI and CT at stroke onset. The age, sex and brain locations of patients with paralysis had no effect on skin temperature change after ADSC therapy. Interestingly, the skin temperature of patients with brain lesion volumes larger than 5 cm in diameter showed no increase of skin temperature at any location after ADSC treatment, but the temperature decreased at 4 of the 5 paralyzed middle fingers. The mechanism of the temperature decrease of the middle finger on the paralyzed side of stroke patients having large brain damage volume remains to be clarified (Table 1).

4.5. Skin Temperature Alteration at Different Locations of Stroke Patient's Extremities after ADSC Treatment

The middle finger of 6 patients and the big toe of 7 patients on the paralyzed side of the 16 patients showed an apparent skin temperature increase during or immediately after ADSC treatment, but 2 middle fingers and 3 big toes on the healthy side of those patients also showed a temperature increase, respectively (Table1).

Two of the 6 patients with paralyzed-middle finger temperature increase and 3 of the 7 patients with paralyzed-big toe temperature increase showed skin temperature increases at both the paralyzed and healthy middle fingers and the paralyzed and healthy big toes, respectively. These results suggested that the skin temperature increase elicited by ADSC therapy takes place simultaneously or independently at the paralyzed and healthy limbs. Further, the temperature increased only in 4 of 16 cases at both the middle finger and the big toe of the paralyzed limbs (Table1). These results suggest that the effect of ADSCs on skin temperature increase may be initiated by biological factors acting not only at the brain lesion penumbra alone, but also at the contra-lateral brain area responsible for skin temperature homeostasis, although the detailed mechanism of the effect of ADSCs on skin temperature remains to be clarified.

4.6. Effect of the Time Lag Between Stroke Onset and ADSCs Therapy

The skin temperature of both groups treated earlier than a 1-year lag time, and later than a 1-year lag time showed similar increase ratios, 3 of 6 and 6 of 10, respectively (Table1). Based on these results, we concluded that the shorter time lag between stroke onset and ADSC therapy may not exert a strong effect on the skin tem-

perature change of the paralyzed limbs. We need to study the effect of lag between stroke onset and ADSC administration on the skin temperature using more stroke cases, since ADSC therapy shorter than a one-year lag time showed better NIHSS recovery compared to a longer than one year lag time in our previous study [6].

5. Discussion

As far as we know, our study shows for the first time in the world that ADSCs administered intravenously increaseatatistically significantly the skin temperature of the big toe on the paralyzed side of stroke patients at the chronic phase shortly after drip infusion. Based on these results, we suggest that there is an increase of blood flow at the paralyzed limbs shortly after the systemic infusion of ADSCs. Further, the results show that the mean skin temperature of the big toe on the healthy side of stroke patients was statistically at the same level of healthy subjects, and the mean skin temperature on the paralyzed side of stroke patients was statistically lower than that on the healthy side (Figures 2,3).

Studies of skin temperatures on hemiplegic limbs reported previously are conflicting [13,14]. Earlier studies claimed an increased temperature of the hemiplegic arm, and one study suggested a two-stage theory of initial warmth of the paralyzed limb at the acute phase and coldness in the chronic phase. Most previous studies were conducted based on the perception of coldness or warmness by stroke patients. Wanklyn et al. showed that stroke patients with coldness had a lowered skin temperature and a markedly lower blood flow in the paralyzed limbs compared to the unaffected limbs [15]. Naver et al. reported that 43% of stroke patients had a sensation of coldness in their analysis of 37 cases and further they reported that no patients with right cortical hemispheric lesions had noticed coldness in the contralesional side [16]. In our study, we observed patients with coldness who had a cortical hemispheric lesion even on the right side.

The detailed mechanism causing the coldness of limbs of stroke patients is still unknown, but it may be caused by disturbed sympathetically mediated vasomotor tone, leading to reduced blood flow, initially triggered by damaged brain lesions. The signaling systems that modulate the structural and functional plasticity of Autonomic Nervous System (ANS) are largely unknown. The ANS is easily influenced by Central Nervous System (CNS) and by neuroendocrine system involved in stress response and, also greatly influenced by neurotrophic factors [17]. Therefore, we speculate that ADSCs-induced skin temperature increase could be caused by some neurotrophic factors, such as BDNF (brain derived neurotrophic factor), NGF (nerve growth factor), IGF-1 (insulin-like growth factor), and CNTF (ciliary neurotrophic factor) which are expected to be secreted from ADSCs into the medium during drip infusion.

The present study also suggests that a skin temperature increase observed at the paralyzed limbs of many stroke patients, particularly of the big toe, after ADSC therapy may contribute to recovering the warm sensation of cold extremities of stroke patients. Finger coldness was reported to be a major sign of stroke patients in a previous study [14], but we observed a more severe skin temperature decrease of the big toe compared to the finger. We propose that the big toe skin may be the best location for temperature measurement of stroke patients, at least those at the chronic stage.

It is not yet clear enough from our study that the skin temperature increase after ADSC therapy may play a role in the recovery of other neuronal functions of stroke patients since we identified only 3 cases who showed a temperature increase of their paralyzed limbs among the 6 cases who had recovery of their NIHSS. In addition, our study indicates that the effect of ADSC therapy on the skin temperature of paralyzed limbs may be affected by the characteristics of the injured brain including lesion volume, which may cause a severe circulation disturbance of brain tissue at the chronic stage of stroke, since the skin temperature of the big toe on the paralyzed side did not increase after treatment with ADSCs in patients having brain lesion volumes larger than 5 cm in diameter. The present study also suggests that the skin temperature increase observed at the paralyzed limbs shortly after ADSCs therapy may be correlated with the functional recovery of skin tactile sensations, but it is not clear how ADSC therapy may correlate with other functional recoveries, since the number of patients we examined was too small to reach a conclusion about that.

Importantly, our results strongly indicate that extracellular small molecules secreted from stem cells suspended in 200 ml saline, begin to circulate during the 90 minutes of drip infusion and may play a pivotal role in the recovery of neuronal and circulatory functions following ADSC therapy, since most of the stem cells administered are considered to be trapped in the lungs at least for the first 24 hours, and only a few percent of cells are expected to recirculate to other organs including the brain, after a 1 to 2 day lag time [18-20]. We speculate that biological molecules secreted from ADSCs composed of miRNAs, growth factors, cytokines and other small molecules, contribute directly and/or indirectly to the functional recovery shortly after ADSC infusion. Further, based on the present study, we speculate that miRNAs may not be a major contributor to the skin temperature increase immediately after drip-infusion, since the biological effects of miRNAs may appear at least a few hours after drip infusion to exert clinical functional recovery.

Our study also contributes to the promotion of the application of ADSC therapy for hemorrhagic strokes, since clinical reports of mesenchymal stem cell therapy of hemorrhagic strokes are still limited [21-27] compared to studies of ischemic strokes. Further, preclinical studies showed the functional effects of systemically administered mesenchymal stem cells on traumatic and hemorrhagic strokes [28-32], although the pathophysiology and mechanisms of recovery are reported to differ between ischemic and hemorrhagic strokes [33]. for example, the lack of salvageable

penumbra with intracerebral hemorrhage in contrast to ischemic stroke [34]. In addition, a review of mesenchymal stem cell therapy for ischemic strokes that analyzed 13 clinical trials between 2014 and 2020 based on searching the PubMed database concluded that more evidence regarding the safety and efficacy of mesenchymal stem cell therapy of stroke is needed [35].

In future studies, we aim to confirm the effect of ADSCs on the skin temperature of stroke patients and its relationship to other neurological functional recoveries by analyzing more clinical cases. To use skin temperature as a non-invasive biomarker evaluating the effects of ADSC therapy of chronic stroke patients, it is required to show how skin temperature correlates with other biomarkers of stroke patients and brain injury [36-40]. Further, we aim to understand the mechanism of the effects of ADSCs on stroke patients using in vitro and in vivo studies, specifically focusing on small molecules that are released from ADSCs during treatment that might play an important role in the temperature increase and in the functional recovery of strokes even during and shortly after the therapy.

6. Conclusion

Our study indicated that a single intravenous drip infusion of ADSCs recovers the skin temperature and other functions of paralyzed limb of stroke patients shortly after administration, and a possible pivotal role of trophic factors and other small molecules secreted from stem cells in ADSCs therapy, although the precise role of ADSCs remains to be clarified by further studies.

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