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Original article

Safety and clinical efficacy on intranasal administration of mesenchymal stem cell-derived secretome in patients with Alzheimer's disease and its future prospect

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Abstract

***Objectives*:** Research on neurocognitive disorder, which is a significant barrier against a healthy life span, has been intensively conducted in this aging society. Problems, however, are not yet solved for treatments. The purpose of the present study is to evaluate safety and clinical efficacy on intranasal administration of stem cell secretome (stem cell culture supernatant/exosome) in patients with Alzheimer's disease, the primary disease which causes cognitive disorders. Furthermore, this study explores future prospects of stem cell secretome therapy.

***Methods*:** Research participants were patients with Alzheimer's disease who scored approximately 10 – 20 points of 30 (the full score) on the Hasegawa's dementia scale-Revised (HDS-R). Among 18 initial participants, 5 participants dropped out because of aggravation in pre-existing diseases and other factors. The number of final analysis participants was 13. Adipose-derived mesenchymal stem cell secretome was prescribed with intranasal administration at 3 vials/week for 8 weeks. (one vial included exosome: approximately 1×10^8, HGF, which is one of major hepatocyte growth factors: 1.2×10^5 pg, and multiple high-concentrated bioactive substances). Check list items were HDS-R, blood pressure, pulse, blood biochemistry tests, measurements of hormones, and MCI screening test (measurement tests of 9 types of proteins, which are related to Alzheimer's disease). Evaluations were conducted before and after treatments to confirm safety of nasal drip therapy and to examine alternations in HDS-R scores, blood tests, and alternations of each protein in MCI screening test.

***Results*:** Scores of HDS-R were significantly improved: mean values $15.6 \pm 1.0 \rightarrow 17.5 \pm 1.4$ ($p = 0.030$). More obvious significant differences were recognized in a subclass analysis of mild and moderate cases for patients who had around 15 – 20 scores in HDS-R: $17.2 \pm 0.6 \rightarrow 20.0 \pm 0.8$ ($p = 0.006$). Among blood tests, significant differences with a significant level of 1% or less than 1% were shown in platelet (decrease), albumin/globulin ratio (increase) and LDL cholesterol/HDL cholesterol ratio (decrease). MIC screening test provided, among 9 types of proteins, a significant increase in apolipoprotein A-1, which is related to amyloid β excretion: $131.6 \pm 6.1 \rightarrow 150.3 \pm 7.0$ ($p = 0.010$). No adverse events considered to be related to treatments were observed in 13 research participants during the treatments.

***Conclusions*:** Intranasal administration of adipose-derived mesenchymal stem cell secretome for 8 weeks observed no safety problems and provided significant increases in scores of the Hasegawa's dementia scale-Revised (HDS-R). Therapy of intranasal administration of stem cell secretome (stem cell culture supernatant/exosome) is a safe and simple method, which can be performed at home. Judging from improvement effectiveness on cognitive functions, its possibility as a new treatment was suggested for Alzheimer's cognitive disorder. Further studies are required to solve multiple problems and tasks, establish credible scientific evidence and organize environments for safety. It is our hope that stem cell secretome treatments will be implemented and widely introduced to clinical settings in an appropriate manner.

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KEY WORDS: secretome, stem cell culture supernatant, exosome, Alzheimer's disease, intranasal administration, clinical trial

Introduction

Medications for neurological disorders that have been used could just retard the symptom progression to some extent. Therefore, we hope a robust, new method can be developed. Through these situations, perfect human lgG1 monoclonal antibody (gantenerumab), as a new medicine which targets amyloid-beta (Aβ) for early Alzheimer's disease was examined by a clinical trial, where a large phaseIII double-blind placebo-controlled clinical study (GRADUATE I and II) was conducted and its examination results were published in November of 2023. Clinical effect of progression suppression was not observed for cognitive impairment, although a reduction of the quantity of amyloid aggregation on PET in the active treatment group, where treatments were provided to 1965 research participants aged from 50 to 90 were enrolled from 288 sites in 30 countries for 116 weeks [1]. Lecanemab, which is a monoclonal antibody directed against aggregated soluble Aβ aggregation with intravenous infusion therapy, was approved in Japan in December of 2023, after the U.S.A. However, improved effectiveness was not clearly recognized over conventional medicine. Furthermore, a phase 1 feasibility trial of senolytic therapy, which uses dasatinib/qercetin to lead to the apoptosis of senescent cells, and to selectively clear senescent cells, was conducted. However, significant improvements were not provided in measurement values of phosphorylated tau protein and Aβ protein in cerebral spinal fluid, cognitive function tests, and MRI findings, while decreasing trends were observed in cytokines and chemokines, which are related to aging [2].

Diverse clinical fields require research on regenerative medicine to overcome the current situation. Furthermore, treatments with mesenchymal stem cell-derived secretome (stem cell culture supernatant/exosome) have been drawing attention as a cell-free therapy, which employs no stem cells [3,4].

This study verifies safety and clinical efficacy on intranasal administration of treatment employing adipose derived mesenchymal stem cell secretome for the target of patients with Alzheimer's disease, and explores future prospects for treatment.

Methods

Research participants

The target was 13 patients who received a diagnosis of Alzheimer' disease with scores of approximately 10-20 of 30 points of the full score on Hasegawa's Dementia Scale-Revised (HDS-R), which is a widely employed questionnaire test such as Mini-Mental State Examination (MMSE) in Japan. Subjects were aged from 66 to 97 years old (mean age: 84.8 years old). The number of subjects in the cases was 13 (3 males and 10 females). Exclusion criteria were patients who had cognitive impairments except for Alzheimer' disease, patients who had internal diseases and/or nervous system diseases which could induce cognitive disorder in prior to the onset of Alzheimer's disease, patients who had serious neurological disorders and/or severe mental disorders, and individuals who were considered inappropriate for this study by the investigators. Research participants or legal representatives of participants who were not competent to consent, were fully informed through understanding of the trial and this trial was conducted with written consent.

Among the 18 initially enrolled subjects, 5 subjects dropped out because some had aggravation in pre-existing diseases and some were hospitalized due to an infectious disease or other reasons. Final analysis subjects were 13 participants (***Table 1***).

Table 1. Subject profile.

ID#	Age	Sex	HDS-R (before treatment)
1	79	F	14
2	87	F	16
3	92	F	20
4	97	F	11
5	81	M	17
6	94	F	18
7	80	F	9
8	88	M	17
9	87	F	15
10	66	F	18
11	90	F	11
12	80	M	21
13	82	F	16

Cases: n = 13, Male (M)/Female (F) = 3/10, mean age 84.8 years old. HDS-R, Hasegawa's Dementia Scale-Revised. The perfect score of HDS-R is 30 points.

Stem cell-derived secretome (stem cell culture supernatant/exosome)

The test product was freeze-dried adipose-derived mesenchymal stem cells secretome (Nature Bionix Co., Ltd. Tokyo, Japan). Contents of 1 vial were exosome: 1×10^8, HGF, hepatocyte growth factor, which is one of the major growth factors: 1.2×10^5 pg, and multiple highly-concentrated substances such as diverse growth factors, neurotrophic factors and cytokine. The product was manufactured for any typical constituents to satisfy each indicated quantity. Therefore, active elements were almost quantitatively equal and there was no difference among test product lots.

Donors of stem cells were healthy Japanese females in

their 20–30s and serum-free culture medium was employed not using animal or human serum to avoid risks of viral infection. Unnecessary constituents, which were produced in large quantities such as ammonia, were eliminated to the limit of detection and active constituents such as multiple growth factors and exosome were condensed.

Clinical trial protocol

Stem cell secretome in vial was dissolved with saline solution and was poured into nasal containers. The nasal spray treatments were conducted continuously every day at 3 vials/week for 8 weeks. Evaluations were performed before the commencement of nasal drops (0 week) and 1–2 weeks after the completion of nasal drops. Research participants did not change their lifestyle except when necessary, and new health food ingestions were prohibited during the test. When participants underwent a change for disease complication treatments, the principal investigator judged for continuation or drop-out.

Assessments at 0 week conducted on HDS-R, blood pressure, pulse, and examinations of the blood of the following items: white blood cell (WBC), differential count of leukocytes, red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), total protein (TP), albumin (Alb), albumin/globulin (A/G) ratio, total-bilirubin (T-Bil), direct bilirubin (D-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactase dehydrogenase (LDH), creatinine phosphokinase (CPK), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), amylase (AMY), blood urea nitrogen (BUN), creatinine (Crea), uric acid (UA), estimated glomerular filtration rate (eGFR), Na, K, Cl, Ca, P, fasting plasma glucose (FPG), C-reactive protein (CRP), somatomedin C (insulin-like growth factor-I: IGF-I), dehydroepiandrosterone-sulfate (DHEA-s), cortisol, thyroid stimulating hormone (TSH), free-triiodothyronine (F-T3), free-thyroxine (F-T4), insulin (immunoreactive insulin: IRI) and adiponectin. Furthermore, MCI screening test was conducted. This test was a blood test, which measured and evaluated Alzheimer's disease-related proteins such as transthyretin (TTR), Alb, apolipoproteinA1 (ApoA1), apolipoprotein C1 (ApoC1), complement c3 (C3), Alpha-1B-glycoprotein (A1BG), hemopexin (HPX), alpha-2-antiplasmin (A2AP), and alpha-2-macroglobulin (A2M)[5].

Statistical analysis

Statistical analysis employed a paired t-test. Significance level, statistical difference, was 5 % (p value $\leqq 0.05$).

Ethical Examination

The present study was conducted in compliance with ethical principles based on the Declaration of Helsinki referring to "Ministerial Ordinance on Good Clinical Practice for Drugs" and "Ethical Guidelines for Epidemiology Research." Approval of ethics and validity for the trial was obtained from the committee regarding "medical research involving human subjects" of general incorporated association: Society for Glycative Stress Research (GSE #2024-001). Pre-registration for a clinical trial was conducted (UMIN #000053551).

Results

Hasegawa's Dementia Scale-Revised: HDS-R

Scores of HDS-R were significantly increased at the 8-week assessment, before and after intranasal administration: mean value $15.6 \pm 1.0 \rightarrow 17.5 \pm 1.4$ ($p = 0.030$). Cognitive function improvements were recognized with a significant increase. Further, more obvious significance was recognized in HDS-R in a subclass analysis of ten patients with mild and moderate symptoms with approximate scores of 15-20: $17.2 \pm 0.6 \rightarrow 20.0 \pm 0.8$ ($p = 0.006$, ***Fig. 1-a, b***).

Blood pressure and pulse rate

No changes were observed in particular for blood pressure and pulse.

Blood tests and hematological examinations

Among blood tests, three items of significant differences with significant level $p \leqq 0.01$ were shown, in platelet decrease ($24.7 \pm 2.0 \rightarrow 20.8 \pm 1.3 \times 10^4/\mu L$, $p = 0.010$,) albumin/globulin ratio increase ($1.22 \pm 0.07 \rightarrow 1.38 \pm 0.10$, $p = 0.010$), and LDL-C/HDL-C ratio decrease ($2.50 \pm 0.25 \rightarrow 2.30 \pm 0.22$, $p = 0.002$). No significant difference was observed in hormone examinations (***Fig. 2***) .

MCI screening test

MCI screening test provided, among 9 types protein, a significant increase in apolipoprotein A-1 ($131.6 \pm 6.1 \rightarrow 150.3 \pm 7.0$, $p = 0.010$, ***Fig. 3***).

Adverse event and mental and physical activities

There was no change in physical symptoms and/or examination values caused by intranasal administration of stem cell secretome. However, revitalization due to nasal treatments in mental physical activities was recognized in some research participants.

Discussion

Diverse clinical trials on the use of stem cell secretome for various diseases such as exosome are currently in progress worldwide. To name some published works, there are three studies, a trial of phase II/III RCT on Chronic Kidney Disease by Cairo University, Egypt[6], a cohort study on patients with COVID-19 having acute respiratory distress syndromes from moderate to severe, which was conducted by New York University[7], and a phase I/II clinical trial on collunarium for patients with Alzheimer's disease, which was

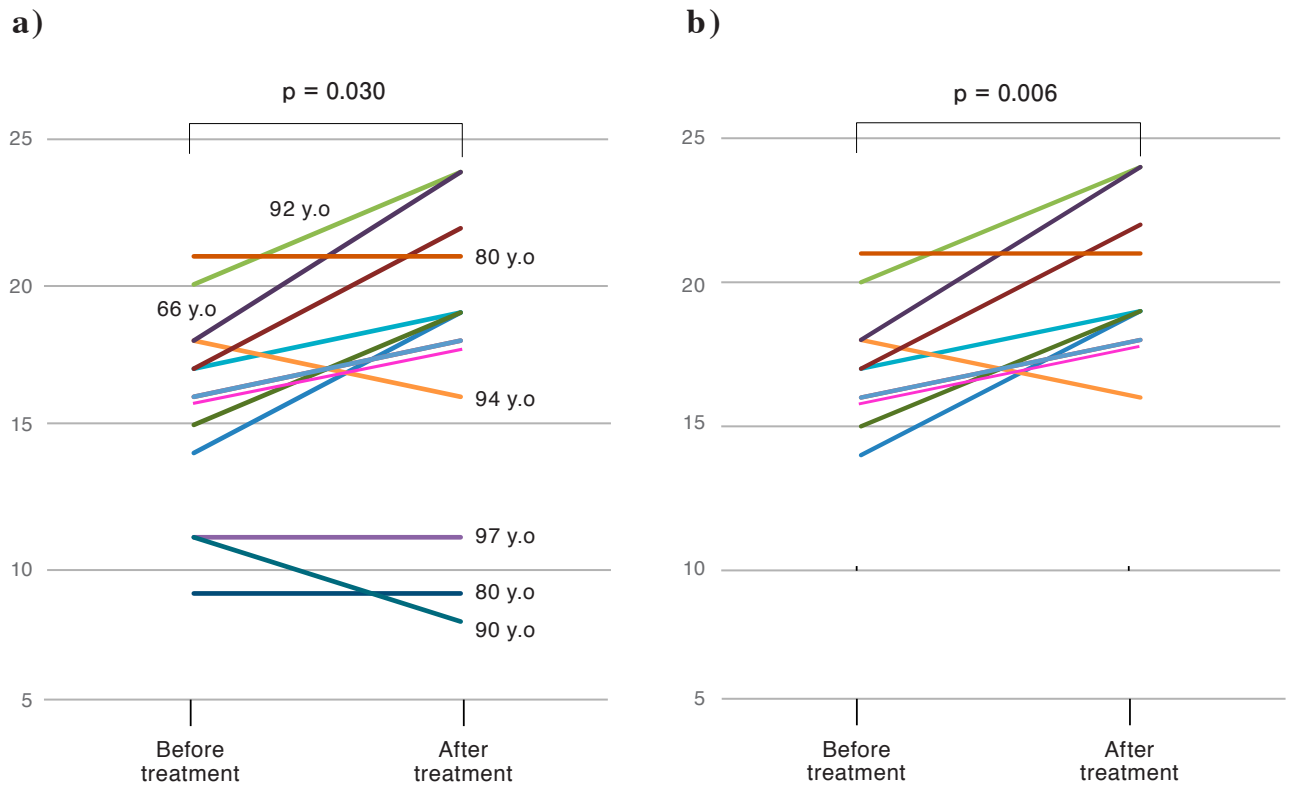

Fig. 1. **Score change of HDS-R**

a) Whole cases (n = 13), Before: 15.6 ± 1.0, After: 17.5 ± 1.4, **b)** mild to moderate cases (n = 10). Before: 17.2 ± 0.6, After: 20.0 ± 0.8. Results are expressed as mean ± SEM, n =13, paired t test. SEM, standard error mean; HDS-R. Hasegawa's Dementia Scale-Revised.

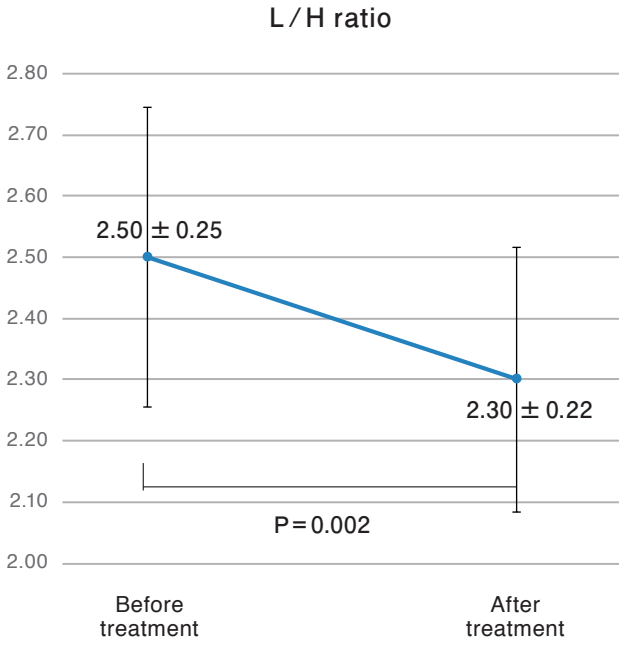

Fig. 2. **Change in serum L/H ratio.**

Results are expressed as mean ± SEM, n = 13, paired t test, SEM. standard error mean; L/H ratio, Low-density lipoprotein-cholesterol/ High-density lipoprotein-cholesterol ratio.

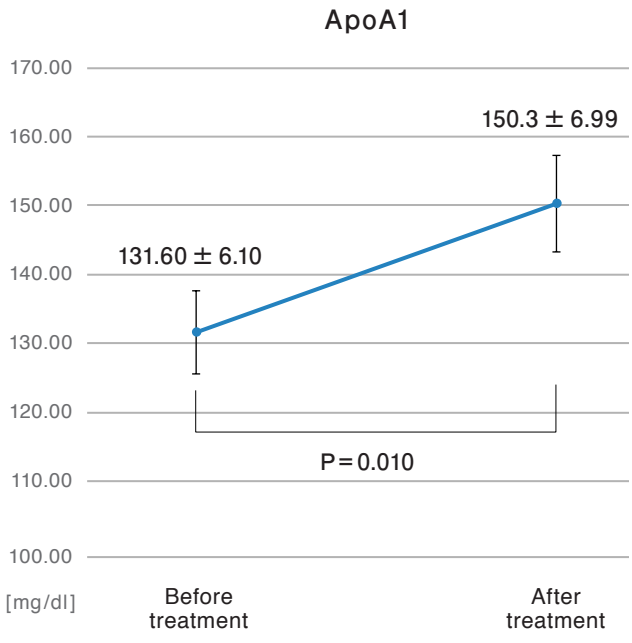

Fig. 3. **Change in serum serum ApoA1.**

Results are expressed as mean ± SEM, n = 13, paired t test, SEM, standard error mean; ApoA1. apolipoproteinA1.

conducted by a Shanghai Chinese team [8]. Other than these, a meager number of studies have been conducted [9,10]. These three studies have no safety problems and show improvements in conditions of diseases. The study by Cairo University, in particular, has confirmed safety and efficacy of one year. The present study was conducted with a purpose of contributing to the establishment of clinical evidence, to evaluate the safety and clinical efficacy on intranasal administration of mesenchymal stem cell-derived secretome, based on findings hitherto regarding Alzheimer's disease.

There was no aggravation of physical conditions and no abnormality in blood test results, which were related to the stem cell secretome treatment through 8 weeks during intranasal administration and several weeks after the completion of the treatment. Intranasal administration of mesenchymal stem cells has been confirmed as safe in animal experiments [11]. Clinical trials on intranasal administration of adipose-derived stem cell exosome for patients with Alzheimer's disease, which had similarity to the present study, did not show any adverse events through the 48-week follow-up [8]. Other clinical studies on inhalation (spray to the respiratory system) did not have any problems [12,13]. Judging from these findings, it is suggested that spray to the upper respiratory tract (intranasal administration) would have no problems regarding clinical safety.

Assessments of HDS-R provided a significant increase in score after 8-week treatment (mean score: from 15.6 ± 0.94 to 17.5 ± 1.4, $p = 0.006$). Severe cases, however, with approximately ten scores of HDS-R among the 13 subjects showed no effects. Then, excluding the 3 subjects from the original 13 who had severe symptoms, a subclass analysis was performed for the remaining ten subjects with mild to moderate symptoms. Consequently, a significant difference was clearly recognized (from 17.2 ± 0.6 to 20.0 ± 0.8, $p = 0.006$). That is, it was effective on most patients at 80s or younger with mild to moderate Alzheimer's disease, and these findings suggested the effectiveness of intranasal administration of stem cell secretome on Alzheimer's disease. Taking age and the level of severity in diseases could be constructive to judge whether or not the treatment would be applicable to the disease.

Three access routes for drug delivery from the nasal cavity to the cranial nervous systems are known a pathway to convey along with axon of olfactory nerve and trigeminal nerve, a pathway to move through perivascular space, and a pathway to enter the cerebrospinal fluid circulation via perineural space and subarachnoid space [14-16]. In comparison with exosome and cytokine, by far larger stem cells, at intranasal administration, has been confirmed to move into intranasal blood vessels and be conveyed to distant organs [17]. Thus, a vascular route could function. Furthermore, the route to drop down to the oral cavity and be absorbed via vessels of the hypogrottis has a possibility of more rapid delivery to the brain. While intranasal administration is recommended for nervous system disorders of the central nervous system and the craniocervical region, so there is a possibility that intranasal administration could be an option for treatment of other organs/tissues and systemic illnesses.

Major pathological findings of Alzheimer's disease were the aggregation and accumulation of Aβ (senile plaques), the aggregation and accumulation of phosphorylated tau protein (neurofibrillary tangle), and synapse elimination. Induction of biological reactions is required for qualitative and functional improvements with inhibitions of these alterations. Systematic review (13 papers) on administration of stem cell secretome (stem cell supernatant/exosome) for patients with Alzheimer's disease, which was published from April of 2020–May of 2022, suggested improvement effects of cognitive functions and memory functions, showing the following diverse direct and indirect phenomena: inhibition of neuroinflammation, inhibition of inflammatory cytokine production, inhibition of oxidative stress, apoptosis suppression, reduction of cytotoxicity, improvement in mitochondrial functions, control of the number and functions of microglia, induction of synapse formation, promotion of neurotrophic factor expression, inhibition of Aβ formation, and increase of neurotransmitters [18].

Hepatocyte growth factor (HGF), a growth factor containing a large amount of stem cell secretome employed in this clinical trial, was identified as a growth factor exerting protective effects on hepatocytes. However, HGF is widely distributed in all organs including the brain other than the liver. It is known that HGF has diverse roles such as anti-inflammatory, anti-apoptosis and angiogenesis, and as a nerve growth factor, brain-derived neurotrophic factor (BDNF,) is intensively related to the synaptic plasticity [19]. The decrease of LDL-C/HDL-C ratio in blood tests as well as the increase of ApoA1 [20], which has depressant activities of Aβ aggregation and toxicity, would positively affect lipid metabolism. Cognitive functions could improve via diversified mechanisms with these multiple factors.

A brain in a healthy condition plays diverse roles, where Aβ is normally degraded and excreted via glial cell and Aβ clearance is maintained. In a brain containing rich lipid, due to the exposure of free radical and aldehyde (carbohydrate-derived aldehyde, alcohol-derived aldehyde, and cigarette smoking-derived aldehyde,) fatty acid-derived aldehyde (methylglyoxal, acrolein, and malondialdehyde) are formed [21]. The structure of Aβ is carbonylation-modified and abnormal Aβ is formed. Consequently, insoluble fiber is formed and is deposited as Aβ polymer [22]. The authors conducted experiments on Aβ phagocytosis of primary cultured microglia cells. As a result, it is confirmed that microglia phagocytize intact Aβ but has difficulties regarding phagocytosis of glycated Aβ with aldehyde modification. Furthermore, it was clarified that Aβ phagocytosis of microglia was promoted by melatonin [23]. The authors are examining effects of enterobacteria-derived exosomes employing the present model. Findings have suggested that phagocytosis activities of microglia are divergent depending on types of bacteria. It is highly possible that exosomes, which are secreted from human mesenchymal stem cells, would positively affect phagocytosis activities of microglia. We are awaiting further examination.

It is necessary that therapies using stem cell secretome establish a position as a field of regenerative medicine. In order to organize its evaluations, its advantages, problems, and tasks, they are explained in comparison with stem cell therapy (***Table 2, 3***) [24]. Stem cell therapy, which uses induced pluripotent stem cells (iPS cell) and embryonic stem cells (ES

Table 2. Advantages of stem cell therapy and stem cell secretome therapy.

Stem cell
1) Conducted under the control of the Law for Regenerative Medicine
2) There is a lot of credible evidence
3) Differentiation into specific lineages to treat various diseases
4) Homing effect to concentrate on the target area
5) Long-lasting effects due to living cells

Stem cell secretome
1) Manipulation of the manufacturing process allows for some customization of the therapeutic ingredients
2) Quick and continuous response to needs
3) Can be provided at a significantly lower cost
4) Many routes of administration such as intravenous, subcutaneous, nasal, ophthalmic, local injection, inhalation, and application
5) Easy to store, manage, and transport

Table 3. Problems/issues on stem cell therapy and stem cell secretome therapy.

Stem cell
1) Risk of mutation, carcinogenesis, or metastasis induction
2) Risk of vascular embolization
3) Local conditions can affect the effectiveness of treatment.
4) Requires strict preservation and management to maintain cell viability
5) Inability to respond quickly
6) Expensive (cost-effective)

Stem cell secretome
1) Because it is not under the control of the Law for ensuring the safety of regenerative medicine, it is a medical treatment that is left to the ethical considerations of physicians and manufacturers, and is spreading unregulated
2) Lack of evidence
3) It is an extremely heterogeneous product that is difficult to standardize
4) Donor selection and quality issues
5) Issues of disparity among organs of origin and lots
6) Possibility of contamination by infectious agents (because, like the placenta, it is derived from living organisms)
7) Requires frequent administration due to a short half-life in the body

cell), has a significant advantage and an achievability where cellular differentiation could respond to diversified diseases. Stem cell therapy, in a background with a large quantity of credible evidence, is an accepted clinical treatment under the legal regulation and oversight in Japan. However, as for therapies using adipose-derived mesenchymal stem cells which are available at clinical sites, the number of stem cells is augmented to several thousand–one or two hundred million without cellular differentiations. These cells are appropriately provided for diseases in agreement with clinical conditions under provision plan guidelines of medical institutions, which is reported to regional bureaus of health and welfare. In other words, it is under the control of the law for Regenerative Medicine. However, some institutions deceivingly describe their therapies as if they were regenerative medicine authorized by bureaus. That is, the current situation, which could cause misunderstanding, must be corrected. As for therapy using stem cell secretome, the following are varied advantages;

1) Manipulation of the manufacturing process enables some customization of the therapeutic ingredients
2) Quick and continuous responses to needs are available
3) It can be provided at a significantly lower cost in comparison with stem cell therapy
4) It is easy to store, manage, and transport
5) There are many routes of administration such as intravenous drip, subcutaneous injection, local injection, as well as collunarium, ocular instillation, inhalation, and application, which can be administered at home

Therapy with stem cells have problems and issues to be solved. Diverse disadvantages of stem cell therapy are as follows;

1) Risks of mutation, carcinogenesis, or metastasis induction[25)]
2) Risks of vascular embolization
3) Local conditions can affect the effectiveness of treatment
4) Strict preservation and management are required to maintain cell viability, which could be a hindrance from facility and technical aspects
5) Inability to respond quickly
6) Expensive (cost-effectiveness)

Therapy using stem cell secretome has the following disadvantages;

1) Donor selection and quality issues
2) Issues of disparity among organs of origin and lots
3) Possibility of contamination by infectious agents
4) Requirement of frequent administration due to a short half-life in the body
5) It is an extremely heterogeneous product, so it is difficult to standardize for refining specific active component
6) Insufficient evidence regarding understanding of mechanism and long-term safety
7) Because it is not under the control of the Law for ensuring the safety of regenerative medicine, medical treatments are left to ethical considerations of physicians and manufacturers, which is the most significant problematic issue [26, 27]

One of the problems regarding research on Alzheimer's disease, including the present clinical trial, is accuracy of clinical diagnosis of Alzheimer's disease. It is considered that among patients who receive diagnosis of Alzheimer's disease, some patients have pathologically different diseases or mixed symptoms, which causes impairments of cognitive functions [28]. There was a possibility in the present study that all the research participants had not only Alzheimer's disease but also mixed symptoms with diverse apparent and/or latent diseases, which might have affected the results of the study. This type of bias would be difficult to deal with in clinical trials where not healthy persons but patients with diseases are research participants.

Medical institutions of stem cell therapy are limited in number because strict regulations and judgements are required by examination councils to provide stem cell therapies, and high medical expenses are not covered by health insurance. On the other side, clinical and social risks of stem cell secretome therapy are not appropriately managed. There are no regulated or defined standards for its designation and the therapies have spread and are widely supplied by not only clinical sites but also businesses in the beauty field. We would have to say that clinical and social risk management has not been prepared yet.

Although both stem cell culture supernatant and exosome are used in most cases, it can be said that accurate distinctions are not conducted and definition of terms is imprecise. They are both substances which are obtained as follows: culture solution, which is used in the process of stem cell culture, is purified and biologically active substance is condensed. The former, stem cell culture supernatant, contains exosome as well as other extracellular vesicles, diversified cytokine, and protein components such as growth factors and nutritional factors. Exosome is a small vesicle, with cell surface markers such as CD9, CD63, CD81. Positive small vesicles with the size of approximately 100nm, among extracellular vesicles with various sizes, are highly contained. However, it is difficult to separate and extract exclusively exosomes via ultracentrifugation or ultrafiltration. Other extracellular vesicles, cytokine, and other substances are inevitably mixed in. Exosomes enclose messenger RNA, which promotes protein synthesis within cells. MicroRNA inhibits the expression of genes which are transcribed from DNA, and inhibits protein synthesis, targeting messenger RNA in the cells. Cytokine, growth factors, and nutritional factors, which are contained in the culture supernatant, exert effects respectively. Therefore, from the viewpoint of therapeutic effects, it is more effective to use all physiological active substances in the culture supernatant, as for usage purposes they do not need to examine individual components for research. Regarding unification of terminology, "stem cellsecretome" is recommended, as it stands for the whole of secreted materials from stem cells.

The way for therapy with stem cell secretome has not been paved yet at present, as it has the above said problems and issues. However, it has been exhibited that exosomes have enhancing abilities of sirtuin gene expression, which is inextricably related to longevity and anti-aging [29, 30]. Exosomal therapy is a new frontier in regenerative medicine, contributing to disease treatments and health and longevity. This unprecedented method is emerging worldwide with vigorous competition for development. It is challenging to standardize this heterogeneous product. To begin with, multiple designations, which have been used with unclear definitions, are needed to be unified. Typical component contents should serve as a standard guideline such as exosome, HGF, VEGF (vascular endothelial growth factor,) and BDNF. Thus, an adequate standardization for products is able to be implemented.

Conclusion

The present study suggested safety and clinical efficacy on intranasal administration of mesenchymal stem cell secretome in patients with Alzheimer's disease. It is our hope that diverse problems and tasks will be solved and credible scientific evidence will be established, and an environment for safety will be organized. Therefore, stem cell secretome therapy will be implemented and widely introduced to clinical settings in an appropriate manner.

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Conflict of Interest Statement:

Authors have no conflict of interest in this study.

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