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Original Article

Amelioration of female menopausal syndrome by intravenous administration of autologous menstrual blood-derived stem cells

Hiromi Izawa [a, b, *], Charlie Xiang [c, d, e], Seiji Ogawa [a, f], Ichiro Hisanaga [a, g, h], Takayuki Yoshimoto [b]

[a] Jingu-Gaien Woman Life Clinic, Shibuya-ku, Tokyo, Japan
[b] Department of Immunoregulation, Institute of Medical Science, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan
[c] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
[d] Research Units of Infectious Disease and Microecology, Chinese Academy of Medical Sciences, Hangzhou, Beijing, China
[e] Jinan Microecological Biomedicine Shandong Laboratory, Jinan, China
[f] Fujita Medical Innovation Center Tokyo, Reproduction Center, Ota-ku, Tokyo, Japan
[g] Dai Nippon Printing Co., Ltd., Human Engineering Laboratory, Shinjuku-ku, Tokyo, Japan
[h] Ritsumeikan University, Art Research Center, Kyoto-shi, Kyoto, Japan

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ABSTRACT

Introduction: Menopausal syndrome is characterized by a wide range of physical and psychological symptoms in women aged 40s–50s as a result of hormonal fluctuations and age-related decline. Various treatments have been used to manage the symptoms, including hormone replacement therapy, but no effective causal therapies have yet been identified. Regenerative medicine has gained considerable attention as a promising approach to age-related problems, and mesenchymal stem cell therapies have been extensively studied. Recently, menstrual blood has emerged as a novel cell source of stem cells, called menstrual blood-derived stem cells (MenSCs), due to its non-invasive, regular and consistent collection from women. In this study, we have investigated the therapeutic potential of intravenous administration of autologous MenSCs on female menopausal syndromes.
Methods: Menstrual blood was collected from 15 patients aged 30s–60s with ovarian dysfunction using a menstrual cup, and MenSCs were isolated, cultured and expanded. Patients received either 3×10^7 cells or 1×10^8 cells intravenously 1 to 5 times at intervals of more than 1 month. Patient-reported symptoms were assessed using the Simplified Menopausal Index at pre-treatment and after 1, 3, 6, and 12 months, and safety assessments were performed. Serum estradiol and follicle-stimulating hormone levels were also measured by immunoassay.
Results: Almost all patients who received MenSCs experienced a sharp reduction in menopausal symptoms, including vasomotor, neuropsychiatric, and motor symptoms, one month after the first administration, and these symptoms remained low for 6 months. The Simplified Menopausal Index score was significantly reduced after treatment. The reducing potency of 1×10^8 MenSCs was greater than that of 3×10^7 MenSCs. Patients who received a higher number of MenSCs showed an increasing trend in estradiol levels and a decreasing trend in follicle-stimulating hormone levels. When MenSCs were administered to postmenopausal patients, this trend was more pronounced. Overall, no apparent serious adverse events were observed during these treatments.
Conclusions: The present results suggest that the administration of MenSCs improved menopausal symptoms and regulated hormonal balance without any serious adverse events. This is the first report on

Abbreviations: E2, estradiol; FSH, follicle-stimulating hormone; HR, hormone replacement therapy; MenSC, menstrual blood-derived stem cell; MSC, mesenchymal stem cell; SMI, Simplified Menopausal Index.

* Corresponding author. Jingu-Gaien Woman Life Clinic, 3-39-5 Jingumae, Shibuya-ku, Tokyo 150-0001, Japan.
E-mail address: w.l.c.izawa@gmail.com (H. Izawa).

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the promising therapeutic potential of cell-based therapy using autologous MenSCs for female menopausal syndrome.

1. Introduction

Menopausal syndrome is a complex clinical condition experienced by women in their 40s–50s as they approach the menopause, characterized by a wide range of physical and psychological symptoms resulting from hormonal fluctuations and age-related. Common symptoms include vasomotor disturbances such as hot flushed, sudden sweating and palpitations [1–3]. In addition, women often experience neuropsychological symptoms such as mood swings, difficulty concentrating, memory problems, anxiety, depression and headaches [4]. Sleep problems, including insomnia and poor sleep quality, are also common [2,3]. Moreover, urogenital symptoms such as vaginal dryness, dyspareunia, urinary frequency and urinary incontinence are commonly reported [5]. Musculoskeletal symptoms such as joint and muscle pain, fatigue and bone density loss leading to osteoporosis may occur [6]. Other notable effects include weight gain, reduced skin elasticity and hair thinning, which can have a significant impact on daily life [7].

A variety of treatments have been used to manage such a wide range of symptoms, including hormone replacement therapy (HRT), traditional herbal medicine (Kampo), placental injections, psychosomatic approaches, relaxation techniques and home-based exercise therapy. However, HRT has often been associated with an increased risk of women's cancers, such as breast, uterine and ovarian cancers, leading some patients to use HRT cautiously or avoid it altogether [8,9]. There is a growing need for novel therapeutic approaches that balance efficacy with patient safety. In recent years, new drugs such as elinzanetant and fezolinetant, which act as neurokinin-1 and neurokinin-3 receptor antagonists, have been developed. These agents suppress the hyperactivation of hypothalamic KNDy neurons, offering potential relief from vasomotor symptoms such as hot flushes and night sweats, as well as related problems such as nocturnal awakenings and sleep disturbances [10]. However, these treatments target specific symptoms and do not comprehensively address the wider range of menopausal symptoms, including the decline in ovarian function.

The ovaries are the earliest aging organs in the female reproductive system [11], highlighting the need for treatments that fundamentally restore hormonal balance. Regenerative medicine has gained considerable attention as a promising approach to addressing age-related problems. By promoting tissue repair and regeneration at the cellular level, regenerative medicine aims to improve the function of tissues compromised by aging or disease. Among various cell-based therapies, mesenchymal stem cell (MSC) treatments have been extensively studied in regenerative medicine [12–14]. In particular, menstrual blood has recently emerged as a novel cell source for stem cells called menstrual blood-derived stem cells (MenSCs), due to their non-invasive, regular and consistent collection from women, proliferative capacity, multipotency, anti-inflammatory properties and immunomodulatory functions [11]. Treatment with MenSCs has the potential to be a novel therapeutic option for menopausal symptoms, including ovarian dysfunction. Growth factors and cytokines secreted by these cells are thought to promote tissue regeneration and suppress inflammation, potentially helping to restore ovarian function and hormonal balance and alleviate complex menopausal symptoms. Furthermore, after administration, MSCs migrate to specific tissues or target sites, exert localized effects, undergo apoptosis, and are naturally metabolized and cleared [15]. This characteristic positions them as a potentially safe treatment option [16].

In this study, we have investigated the therapeutic potential of intravenous administration of autologous MenSCs into patients with female menopausal syndrome. The administration of MenSCs improved menopausal symptoms and regulated the hormonal balance without any serious adverse events. To the best of our knowledge, this is the first report on the promising therapeutic effects of the cell-based therapy using autologous MenSCs against female menopausal syndrome.

2. Methods

2.1. Ethical approval

This study protocol has been approved by the Specific Certified Regenerative Medicine Committee of the Japanese Society of Skin Regenerative Medicine (certification number: PB3200082) and submitted to the Ministry of Health, Labour and Welfare. Written informed consent was obtained from each patient, and the treatment was performed in accordance with the Act on Securing Safety of Regenerative Medicine.

2.2. Study period and patient selection

Autologous MenSC therapy was conducted from the date of the treatment plan on August 24, 2020 until October 31, 2024. This study included 15 patients (30 treatments in total) aged 30s–60s with ovarian dysfunction in the female menopausal syndrome (Table 1). Inclusion and exclusion criteria are shown in Table 2.

2.3. Menstrual blood collection

A medical-grade menstrual cup made of silicone rubber, which is flexible and funnel-shaped, was inserted into the vagina during menstruation (Fig. 1A–C). Approximately 10~20 mL of menstrual blood was collected transferred to a container pre-filled with antibiotics (penicillin, streptomycin, and amphotericin B, Wako) and anticoagulant (heparin, Mochida Pharmaceutical), stored in a cold pack, and delivered to the clinic within 72 h (Fig. 1D and E).

2.4. Preparation of MenSCs

MenSCs were prepared from menstrual blood. Briefly, menstrual blood samples were washed with phosphate buffered saline containing antibiotics and anticoagulants. Mononuclear cells were isolated from the menstrual blood samples by a density gradient centrifugation using Lymphoprep (Serumwerk Bernburg AG), and further purified by reaction with magnetic microbead-conjugated antibodies against CD34, CD45, and CD105 (Miltenyi Biotec), followed by passing through an LS column (Miltenyi Biotec) using MidiMACS Separator (Miltenyi Biotec). The resulting $CD34^{-}CD45^{-}CD105^{+}$ cells were cultured in Dulbecco's modified Eagle's medium:Ham's F-12 nutrient mixture (1:1, Wako)

Table 1
Patient information and clinical data. Patients a~d were received 1×10^8 MenSCs, Patients e ~ o were received 3×10^7 MenSCs.

Patient	Age (years)	Number of treatments	Dosing interval	Menopausal status	Collection method	HRT treatment
a	42	3	1 year 2 years	Pre	Menstrual blood	No
b	41	1		Pre	Menstrual blood	No
c	46	1		Pre	Menstrual blood	No
d	39	2	7 months	Pre	Menstrual blood	No
e	44	3	1 month 3 months	Pre	Menstrual blood	Yes
f	48	2	4 months	Pre	Menstrual blood	Yes
g	44	1		Pre	Menstrual blood	No
h	46	5	1 month 4 months 2 months 2 months	Pre	Menstrual blood	No
i	68	3	Monthly	Post	Hormone-induced bleeding	No HRT: None post-intravenous
j	41	2	1 month	Pre	Menstrual blood	No
k	49	3	Monthly	Pre	Hormone-induced bleeding	Yes
l	31	2	2 months	Pre	Menstrual blood	No
m	35	2	1 month	Pre	Menstrual blood	No
n	54	1		Post	Hormone-induced bleeding	No HRT: None post-intravenous
o	37	1		Pre	Menstrual blood	No

supplemented with 5 % autologous serum isolated from own peripheral blood and antibiotics, expanded, and stored as MenSCs at −80 °C or in liquid nitrogen until use. Quality testing was performed prior to treatment, including visual inspection, sterility testing, cell count and viability testing, mycoplasma testing and endotoxin testing to ensure safety. Phase contrast microscopy images of cell morphology and growth are shown in Fig. 2A. Cells were stained using the BD Stemflow Human MSC Analysis Kit [APC mouse anti-human CD73 (clone AD2), FITC mouse anti-human CD90 (clone 5E10) and PerCP-Cy5.5 mouse anti-human CD105 (clone 266), BD Biosciences] and analyzed using a Coulter EPICS XL-MCL (Beckman) and the Kaluza software (Beckman). The purity of cells positive for CD73, CD90, and CD105 was more than 98 % (Fig. 2B).

Table 2
Key inclusion and exclusion criteria for receiving MenSC treatment.

Selection Criteria	
1	Patients for whom intravenous administration of autologous MenSCs is considered an effective means of treating their main complaint or fulfilling their hopes and expectations.
2	Patients with diseases or syndromes primarily characterized by ovarian dysfunction, such as menopausal disorders (menopausal syndrome), menstrual irregularities, infertility or ovarian insufficiency, for whom other treatments are not considered to be sufficiently effective or who may be undergoing treatment for these conditions.
3	Patients in good general health.
4	In the case of minors, those who have the consent of a legal representative.
5	Patients who have given written consent (or consent from a legal representative in the case of minors).
Exclusion Criteria	
1	Absence of Menstruation at the Time of Cell Collection: Cases where menstruation stops for reasons such as menopause or amenorrhea. Note: Withdrawal bleeding or hormone-induced bleeding is acceptable.
2	First Menstrual-like Bleeding Postpartum or After Miscarriage: The first menstrual-like bleeding after childbirth or miscarriage. Note: Approximately one month after childbirth or miscarriage, patients may report bleeding that is clinically difficult to distinguish from normal menstruation. This ensures that contamination by residual fetal-derived tissue is excluded.
3	Sexual Activity Prior to Menstrual Blood Collection: Sexual intercourse occurring between the last menstrual period and the current menstrual blood collection. Note: Ensures no contamination by sperm from a third party.
4	Inability to Attend Follow-up Visits: Patients who cannot regularly visit the clinic during or after the treatment.
5	Lack of Informed Consent: Patients who do not provide written consent for the treatment.
6	Positive Test Results for Certain Infections: Includes syphilis, HBV, HCV, HIV, or genital infections. Note: Patients with HBV infection may be treated if consent is obtained.
7	Pregnancy: Pregnant patients are excluded.
8	History of Allergic Reactions to Treatment: Patients who have previously experienced allergic reactions related to this treatment.
9	High Risk of Blood Disorders or Infections: Patients identified on pre-treatment assessment as having or suspected of having sepsis, bleeding tendency, or high-risk hematological disorders.
10	Other Conditions Deemed Unsuitable by the Physician: Any other cases where the treating physician does not consider the treatment to be appropriate.

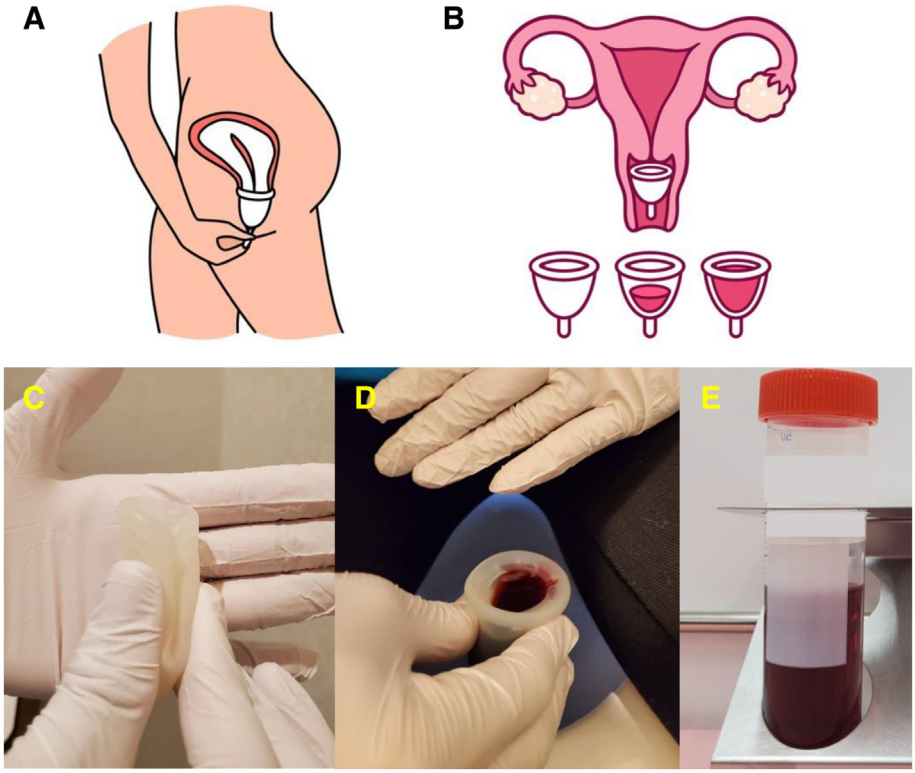

Fig. 1. Non-invasive menstrual blood collection technique using a menstrual cup. (A, B) A medical grade menstrual cup made of silicone rubber and flexible funnel-shaped (C) was inserted into the vagina during menstruation. Menstrual blood (10–20 mL) was collected for the preparation of MenSCs (D, E).

2.5. Intravenous administration of MenSCs

Patients were intravenously administered with either different cell numbers of MenSCs, 3×10^7 cells or 1×10^8 cells, in 100 mL saline containing 1 % autologous serum over 30–60 min, taking into account the general condition of the patient. The number of administrations was adjusted according to the patient's condition, with an interval of at least 1 month between treatments to ensure

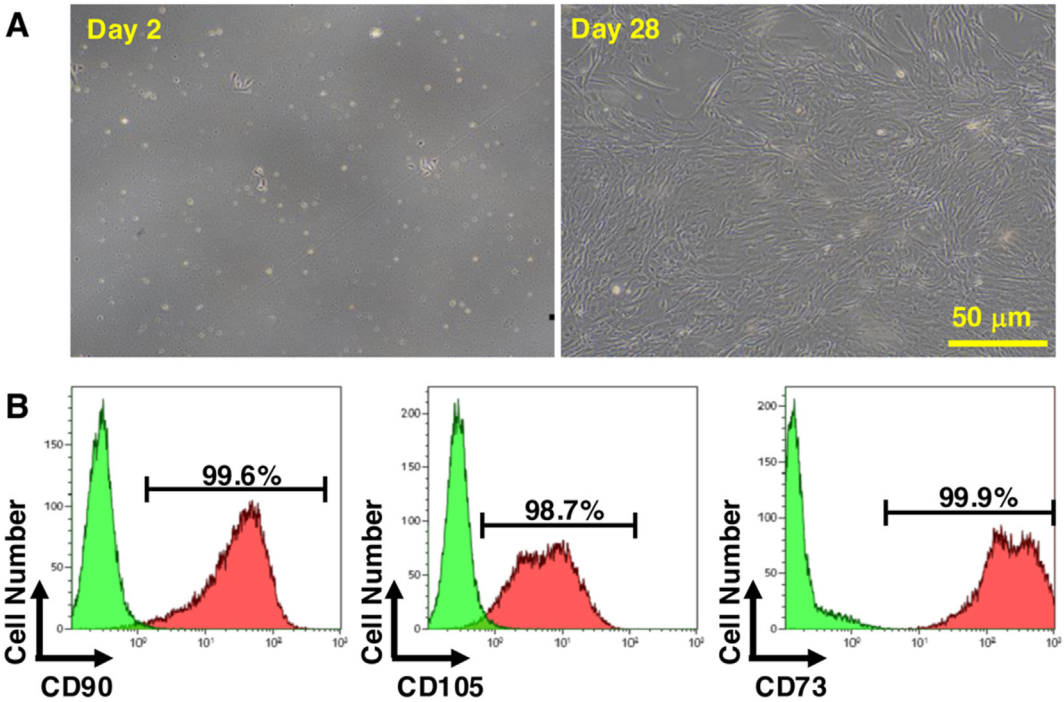

Fig. 2. Cell morphology and purity of MenSCs. Phase-contrast microscopy images of MenSCs on day 2 and day 28 after the cell culture initiation are shown (A). Flow cytometry analysis of MenSCs was performed to determine the purity of cells positive for CD73, CD90, and CD105 and representative data are shown (B).

safety. For 1×10^8 MenSCs, dosing intervals ranged from 7 months to 2 years, and for 3×10^7 MenSCs, dosing intervals ranged from 1 to 4 months (Table 1). MenSCs (1×10^8 cells) were administered to four patients a~d in a total of 6 treatments (1 treatment for two patients, 2 treatments for one patient and 3 treatments for one patient). MenSCs (3×10^7 cells) were administered to eleven patients e ~ o in a total of 24 treatments (1 treatment for three patients, 2 treatments for four patients, 3 treatments for three patients and 5 treatments for one patient). Of the eleven patients, two patients experienced withdrawal bleeding due to hormone replacement therapy (HRT), which was stopped after the start of the cell collection treatment.

2.6. Efficacy and safety evaluation

To assess treatment efficacy, patient-reported symptoms were assessed using the Simplified Menopausal Index (SMI, Table 3) [17] at pre-treatment and 1, 3, 6, and 12 months post-treatment. Follow-up assessments were also carried out to monitor illnesses, infections, allergic reactions, embolisms and fevers for safety assessments. The scientific validity of the treatment was comprehensively evaluated by measuring the patients' serum estradiol (E2) and follicle-stimulating hormone (FSH) levels using electrochemiluminescence immunoassay and chemiluminescent immunoassay, respectively, by SRL, Inc. (Japan).

2.7. Statistical analysis

Data are described as the mean ± standard deviation (SD) for each group. Statistical analysis was performed with the GraphPad Prism software (version 7.05; GraphPad Software) using one-way analysis of variance with Dunnett's multiple comparison test for comparisons involving three groups. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Intravenous administration of MenSCs improves menopausal symptoms

During the treatment, individual symptoms, including vasomotor, neuropsychiatric, and motor symptoms, were assessed over time in each patient (Fig. 3). Patients a~d who received 1×10^8 MenSCs showed an immediate decreasing trend in vasomotor symptom scores, including hot flushes and sweating, 1 month after the first administration compared to pre-treatment levels (Fig. 3A). These symptoms remained low for 6 months, but one patient showed an increase at 12 months. Patients e ~ o who received 3×10^7 MenSCs also showed a decreasing trend in score over 6 months after administration, except for two patients who showed a transient increase in score at 1 month, followed by a decrease over the next 6 months (Fig. 3B). However, two patients showed an increase in score at 12 months. In terms of neuropsychiatric symptom scores, including anxiety and depression which are more common in women, patients a~d who received 1×10^8 MenSCs showed a significant decrease in scores over 6 months after the first administration compared to pre-treatment levels (Fig. 3C). However, one patient showed an increase in score at 12 months. Patients e ~ o who received 3×10^7 MenSCs also showed a significant decrease in score over 12 months (Fig. 3D). In addition, in terms of motor symptom scores, including muscle weakness and joint pain, patients a~d who received 1×10^8 MenSCs showed an immediate trend towards a reduction in scores up to 12 months (Fig. 3E). Patients e ~ o who received 3×10^7 MenSCs showed a similar decreasing trend in score for up to 12 months, with the exception of one patient who showed an increase at 1 month, followed by a decrease over 6 months (Fig. 3F).

Overall, the menopausal symptom score of a total of 15 patients who received 1×10^8 or 3×10^7 MenSCs tended to show an immediate reduction in vasomotor, neuropsychiatric and motor symptoms 1 month after the first administration. These symptoms then remained low for 6 months. The SMI score, which aggregates these individual symptom scores, was significantly reduced after treatment with both 1×10^8 and 3×10^7 MenSCs (Fig. 3G and H), but the reduction was more pronounced when the higher number of cells was administered. Thus, MenSCs therapy has a comprehensive effect in alleviating female-specific symptoms.

3.2. Intravenous administration of MenSCs improves female hormone balance

Next, hormone levels were measured before and after treatment (Fig. 4). In four patients who received 1×10^8 MenSCs, an increasing trend in E2 levels was observed as early as 1 month after treatment (Fig. 4A). Similarly, all but one of the nine patients who received 3×10^7 MenSCs showed an upward trend in E2 levels at the final follow-up point (Fig. 4B). In contrast, a decreasing trend in FSH levels was observed in two patients who received 1×10^8 MenSCs (Fig. 4C) and six patients who received 3×10^7 MenSCs (Fig. 4D) at the final follow-up point.

Since we cannot exclude the possibility that these values are due to random variations caused by menstrual cycle fluctuations in premenopausal women, we next focused on the data from two postmenopausal patients i and n, even without the use of HRT. Administration of MenSCs to these patients more clearly showed an increasing trend in E2 levels (Fig. 5A) and a decreasing trend in FSH levels (Fig. 5B).

Table 3
SMI score for evaluation of the menopausal symptoms. Questions 1–4 are related to vasomotor symptoms, questions 5–8 are related to neuropsychiatric symptoms, and questions 9,10 are related motor symptoms. The score for each symptom was calculated by summing the scores for the individual questions. The SMI was calculated by summing the three symptom scores.

Question	Symptom	Symptom Severity (Score)			
		Severe	Moderate	Mild	None
1	Hot flush	10	6	3	0
2	Increased Sweating	10	6	3	0
3	Cold sensitivity in the back and extremities	14	9	5	0
4	Shortness of breath and palpitations	12	8	4	0
5	Shortness of breath and palpitations	14	9	5	0
6	Increased irritability and a tendency to anger	12	8	4	0
7	Frequent worrying and depressive moods	7	5	3	0
8	Frequent headaches, dizziness, and nausea	7	5	3	0
9	Increased fatigue	7	4	2	0
10	Shoulder tension, back pain, and limb pain	7	5	3	0