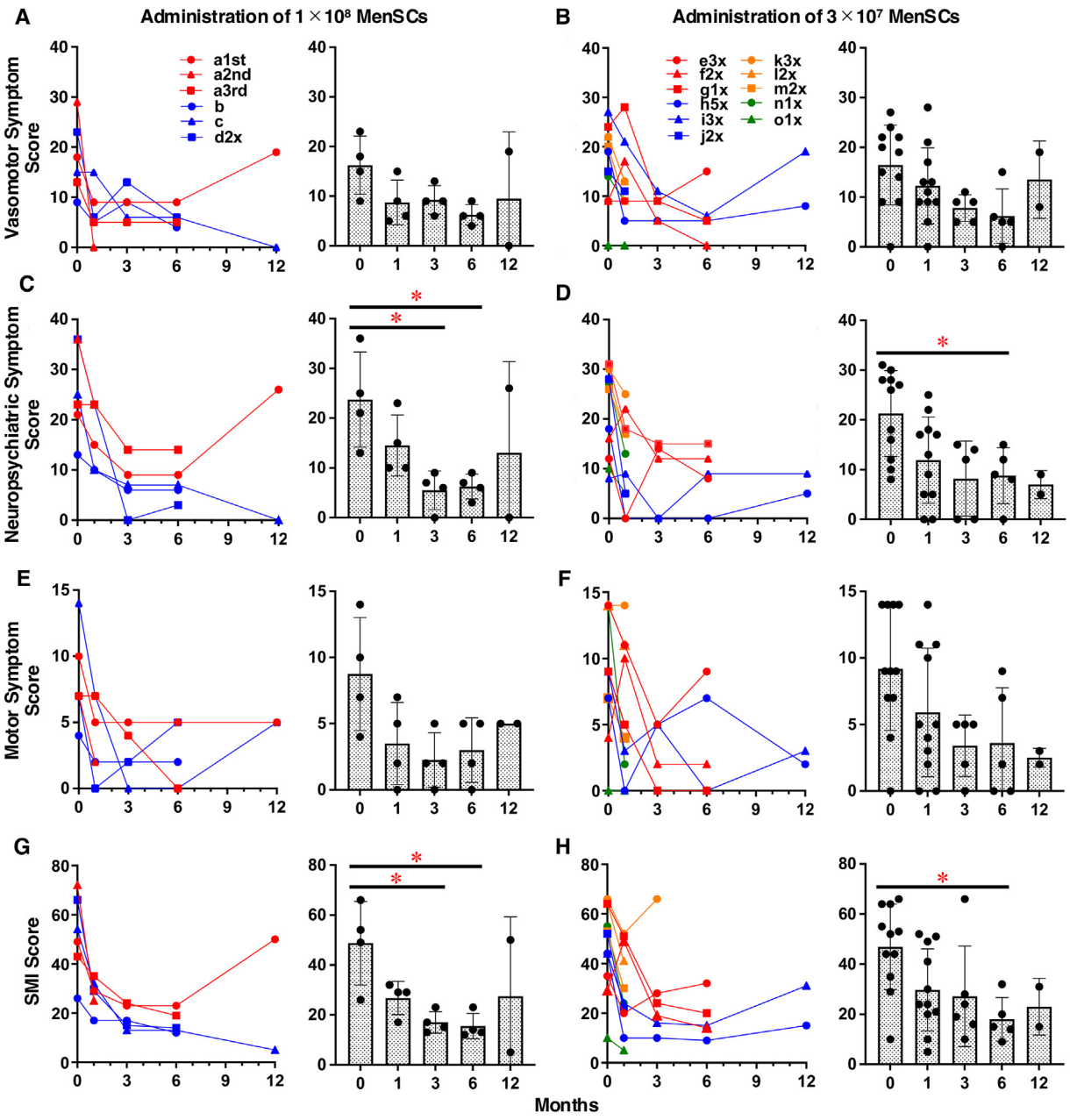

Fig. 3. Intravenous administration of MenSCs improves menopausal symptoms. Menopausal symptoms including vasomotor symptoms (A, B), neuropsychiatric symptoms (C, D), motor symptoms (E, F) and SMI (G, H) were assessed over time following administration of 1×10^8 (A, C, E, G) or 3×10^7 (B, D, F, H) autologous MenSCs. Changes in each score over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline value. Patient a1st, a2nd and a3rd represent the same patient a who received 1×10^8 MenSCs three times at intervals of 1 and 2 years, respectively. Only data from a1st among them were used for statistical analysis. For patients e ~ o, 1 × ~5 × means that each patient received 3×10^7 MenSCs 1 to 5 times at intervals of 1~4 months (Table 1). Data are shown as the mean ± SD [$n = 4$ (A, C, E, G), $n = 11$ (B, D, F, H)], and *P*-values were determined by one-way analysis of variance with the Dunnett's multiple comparisons test. $*P < 0.05$, $**P < 0.01$.

These results suggest that improving hormonal balance in postmenopausal patients can be achieved without relying on external hormone supplementation. This makes it an important option for patients who want to minimize the risk of hormone-related side effects.

3.3. No serious adverse events are observed with treatment of MenSCs

Safety outcomes such as thrombosis, infections, allergic reactions and fever were monitored during treatment. No serious adverse events were observed in any of the cases. In particular, safety was confirmed in the group receiving repeated doses of 3×10^7 cells, supporting the clinical significance of treatment safety and sustainability.

4. Discussion

This report highlights the potential efficacy of intravenous administration of autologous MenSCs in relieving menopausal symptoms and improving ovarian function. These results show that intravenous administration of MenSCs effectively improves menopausal symptoms and ovarian function decline. Intravenous administration of MenSCs tended to increase E2 levels and decrease

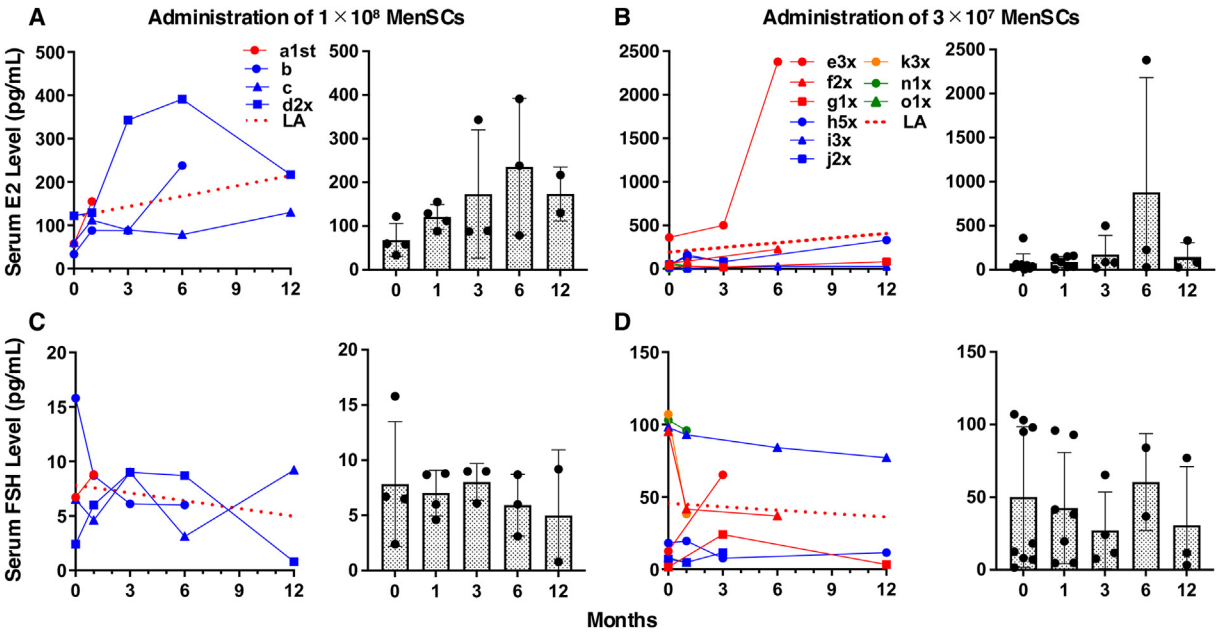

Fig. 4. Intravenous administration of MenSCs improves female hormonal balance. Serum E2 levels (A, B) and FSH levels (C, D) were determined over time after administration of 1×10^8 (A, C) or 3×10^7 (B, D) autologous MenSCs. Changes in each serum level over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline values before treatment. Data are shown as the mean ± SD [$n = 4$ (A, C, E, G), $n = 9$ (B, D, F, H)], and *P*-values were determined by one-way analysis of variance with the Dunnett's multiple comparisons test. LA, linear approximation.

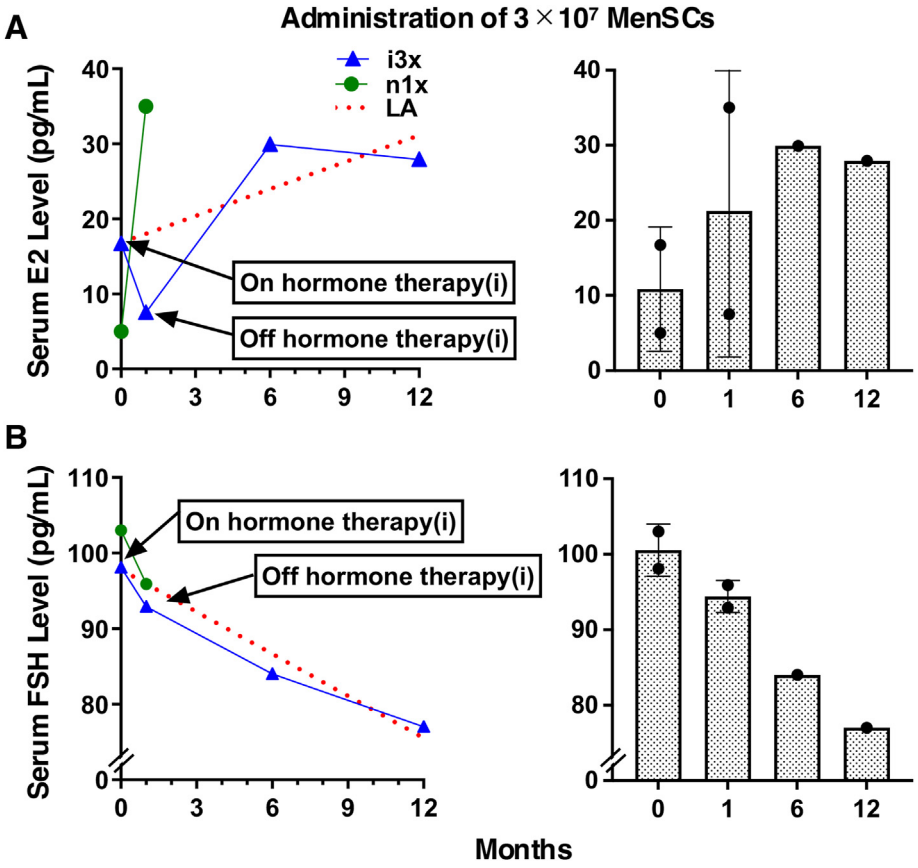

Fig. 5. Intravenous administration of MenSCs improves female hormonal balance in postmenopausal patients (i and n). Serum E2 levels (A) and FSH levels (B) were determined over time after administration of 3×10^7 autologous MenSCs. Changes in each serum level over time for individual patients are shown on the left and the statistical results are shown on the right. The time point at 0 month indicates the pre-treatment baseline values before treatment. Data are shown as the mean ± SD ($n = 2$). LA, linear approximation.

FSH, suggesting the potential for partial restoration of endogenous hormone regulation and ovarian function. While an increase in ovarian mass following intravenous administration has been reported in mouse models of ovarian failure [18], to our knowledge, this is the first clinical report demonstrating such effects in humans. These findings suggest a novel therapeutic approach that differs from traditional HRT in that it promotes the patient's own hormone secretion. Taken together, these results represent a significant advance in regenerative medicine research by expanding the use of non-invasive and sustainable stem cell sources for therapeutic purposes.

In this study, the administration strategy was defined with two dose levels: a high dose of 1×10^8 cells and a low dose of 3×10^7 cells. This classification is based on the assumption that the effects of intravenous cell transplantation may be temporary. Therefore, multiple administrations may help to achieve sustained therapeutic benefit. Regarding the optimal cell dose and frequency of administration, the previous paper on 16 clinical trials of intravenous MSC administration suggested an effective dose range of 1×10^8 to 1.5×10^8 cells per infusion [16]. Based on this, we administered 1×10^8 cells per treatment or 3×10^7 cells per treatment 3 times. One patient h who received five relatively evenly spaced doses of 3×10^7 MenSCs maintained marked improvements in vasomotor, psychological and motor symptoms for up to 12 months after starting treatment (Fig. 3B–D, F, H). This suggests that multiple lower doses of 3×10^7 MenSCs given at regular intervals could reduce SMI scores and improve symptoms. However, the number of treatments is influenced by the patients' schedule and lifestyle. Compared to the single administration of 1×10^8 MenSCs, the multiple administration of 3×10^7 MenSCs may make it difficult to establish a consistent treatment schedule due to variable patient availability (Fig. 3). Therefore, although multiple administrations of 3×10^7 MenSCs would help reduce risks such as embolism while maximizing therapeutic effects and duration of symptom relief, overall, a single administration of higher dose of 1×10^8 MenSCs would be effective and benefit patients if sufficient numbers of MenSCs can be prepared. Further research into more flexible and personalized treatment protocols is needed.

Intravenous administration exerts systemic therapeutic effects rather than being confined to specific organs, making it particularly effective for conditions such as menopausal symptoms, which involve multiple problems at the same time. Menopausal symptoms manifest in several areas, including the vasomotor (e.g., sweating, hot flushes), psychological (e.g., anxiety, depression), and motor systems (e.g., joint pain, muscle weakness) [17], requiring a comprehensive systemic approach to treatment. In addition to intravenous delivery, localized delivery options may increase the flexibility of MenSC-based therapies. These methods directly target specific organs, potentially improving therapeutic outcomes [19,20]. By appropriately combining local and intravenous administration, it is hoped that a treatment strategy can be developed to improve both systemic and localized symptoms simultaneously. Thus, the application of MenSC therapy is not limited to menopausal symptoms but also has the potential to be extended to various conditions such as infertility treatment, gynecological disorders, multi-organ failure and chronic inflammatory diseases. Combining flexibility and versatility, this therapy has the potential to become a new foundation for personalized medicine, offering hope to many patients.

The benefits of MenSC therapy go beyond relieving menopausal symptoms and may help maintain bone density and reduce the risk of cardiovascular disease. In particular, improved estrogen secretion may promote bone formation and help prevent osteoporosis and related conditions [21]. Improving mental health is another important effect of estrogen. Estrogen regulates neurotransmitters such as serotonin and dopamine, and its decline is associated with increased anxiety and depression [22]. The present study revealed the elevated E2 levels following MenSC administration, suggesting potential improvements in neurotransmitter metabolism and mental stability. The psychological benefits observed with intravenous administration of MenSC may alleviate the psychological distress associated with menopausal symptoms and significantly improve patients' quality of life. This effect may be particularly beneficial in postmenopausal women. Estrogen improves the contractility of vascular smooth muscle cells, enhances elastin fiber formation and increases the expression of cell adhesion proteins [23]. Exosomes released from MenSCs have also been shown to promote angiogenesis and activate critical signaling pathways [24]. These effects suggest the potential to reduce the risk of cardiovascular diseases such as atherosclerosis and hypertension, while improving vascular health and longevity.

MSCs are adult stem cells with the capacity for self-renewal and multipotent differentiation, and their many clinical trials have been conducted worldwide [12–14,25,26]. The first report on MenSCs was published in 2007 by Meng et al. [27]. MenSCs are also known by various other names, including menstrual-derived stem cells, menstrual blood stem cells, endometrial stem cells, menstrual blood-derived endometrial stem cells and menstrual blood-derived mesenchymal stem cells [28]. MenSCs provide an alternative source of adult stem cells for research and applications in regenerative medicine [28]. Unlike bone marrow- and adipose-derived stem cells, MenSCs are naturally shed from the body, making them non-invasive from a collection standpoint. This property has led to high expectations for their use in various regenerative medicine therapies [29]. In addition, MenSCs can differentiate into a variety of cell types, including cardiac, neural, bone, cartilage and adipose cells. No cases of teratoma formation, ectopic tissue development or immune reactions have been reported after transplantation in animal models. Thus, their cellular plasticity and safety have been demonstrated in several studies [28]. In addition to the direct effects of cell transplantation, MenSCs exert extensive therapeutic effects beyond mere cell transplantation through the secretion of growth factors, cytokines, and extracellular vesicles (e.g., exosomes) [30]. These secreted substances include several bioactive molecules, such as vascular endothelial growth factor, which promotes angiogenesis, and interleukin-10, which suppresses inflammation [31,32]. Exosomes are small extracellular vesicles, ranging from 30 to 150 nm in diameter, secreted by various cell types, including MSCs [31]. These vesicles play an important role in intercellular communication by transferring bioactive molecules such as proteins, lipids, mRNAs and microRNAs to recipient cells, therby influencing their function. Exosomes can suppress immune responses, reducing the risk of post-transplant rejection and being a key factor in realizing systemic therapeutic effects [33]. Therefore, through the action of these bioactive molecules secreted by MenSCs [34], MenSC therapy has the potential to restore hormonal balance and provide a systemic anti-aging treatment option. The underlying mechanism may involve the possible expression of embryonic stem cell-like markers such as Oct4, SSEA and Nanog in MenSCs, but further research is needed to elucidate this possibility [35].

The importance of using MenSCs as a stem cell source goes beyond conventional cell therapy. The non-invasive collection of menstrual blood, a biological material that is often discarded, using menstrual cups [36,37], opens new avenues for improving the sustainability of medical resources. This approach is very safe because it uses cells collected from the patient and is also excellent from an ethical point of view [38]. The non-invasive collection of MenSCs has attracted attention for its potential to overcome the challenges associated with traditional stem cell therapies. Despite advances in collection techniques, adipose-derived stem cells and bone marrow-

derived stem cells still require invasive surgical procedures. These methods are physically demanding for patients and carry risks such as infection and pain at the collection site [39,40]. In contrast, menstrual blood is regularly expelled by women of reproductive age, and the collection process involves minimal physical and psychological strain. In addition, because menstrual blood can be collected regularly and consistently, it offers high reproducibility and sustainability of treatment, further enhancing its potential in regenerative medicine [29]. This feature allows multiple cell collections from the same patient, enabling the development of long-term treatment plans. Moreover, harvested cells can be cryopreserved [41] and thawed when needed. Advances in cryopreservation technology greatly increase the flexibility of cell-based therapies, making it a critical factor in their practical application. Previous studies have shown that menstrual blood-derived stem cells expanded under standard culture conditions typically do not express HLA-DR, consistent with the broader MSC profile [27,42]. In this study, we did not assess HLA-DR expression, it is important to confirm the immunophenotype in future investigations. The use of MenSCs is thus significant for its medical benefits and its potential to improve the efficient use of medical resources and enhance ethical standards in healthcare. This feature is critical to extending the applicability of regenerative medicine to broader patient population, thereby promoting its wider adoption.

For the practical implementation of MenSC therapy, more research is needed. In particular, it is essential to elucidate the specific mechanisms of action of the cytokines, growth factors and extracellular vesicles secreted by these cells, to establish optimal dosing protocols and to confirm efficacy and safety in large-scale clinical trials. Additionally, reducing the cost of treatment and developing standardized cell culture technologies are essential to make this therapy available to more patients. The results of this study suggest that MenSC therapy could usher in a new era in women's health. This therapy has the potential to the quality of life of postmenopausal women and open up new horizons in the future of regenerative medicine. Further research and clinical application of MenSC therapy is expected to benefit more patients and expand the role of regenerative medicine in healthcare.

5. Conclusions

This is the first report to demonstrate the promising potential of autologous MenSC therapy to improve menopausal symptoms and ovarian function. Intravenous administration of MenSCs has been shown to reduce the severity of menopausal symptoms and improve hormonal balance (increase in E2, decrease in FSH). In addition, no serious adverse events were observed, indicating that this is a very safe treatment. These results suggest that this treatment could be a non-invasive, personalized and sustainable alternative to conventional treatments such as hormone replacement therapy. In the future, larger-scale clinical trials will be needed to determine the optimal dosage and administration protocol for long-term efficacy and safety. This research lays the groundwork for expanding the clinical application of MenSCs in regenerative medicine and women's health.

Declaration of competing interest

The authors have no conflicts of interest to declare. Hiromi Izawa is the director of Jingu-Gaien Woman Life Clinic, where the study was conducted.

Acknowledgments

The authors thank the Specific Certified Regenerative Medicine Committee of the Japanese Society of Skin Regenerative Medicine (Certification Number: NA8190009) for evaluating our clinical protocol.

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