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Menstrual blood transplantation for ischemic stroke: Therapeutic mechanisms and practical issues

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Abstract

Cerebrovascular diseases are a major cause of death and long-term disability in developed countries. Tissue plasmin activator (tPA) is the only approved therapy for ischemic stroke, strongly limited by the short therapeutic window and hemorrhagic complications, therefore excluding most patients from its benefits. The rescue of the penumbra area of the ischemic infarct is decisive for functional recovery after stroke. Inflammation is a key feature in the penumbra area and it plays a dual role, improving injury in early phases but impairing neural survival at later stages. Stem cells can be opportunely used to modulate inflammation, abrogate cell death and, therefore, preserve neural function. We here discuss the possible role of stem cells derived from menstrual blood as restorative treatment for stroke. We highlight the availability, proliferative capacity, pluripotentiality and angiogenic features of these cells and explore their present and future experimental and clinical applications.

Keywords

stroke; penumbra area; cell-based therapy; menstrual blood stem cells; endometrium-derived stem cells; restorative treatment

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Disclosures

CVB and PRS serve as consultants, PRS is a co-founder of Saneron-CCEL Therapeutics, Inc., and CVB, PRS, NK and JGA have a patent application in this area, owned jointly by Cryo-Cell International, Inc. and Saneron-CCEL Therapeutics, Inc. NK is currently employed by Saneron-CCEL Therapeutics, Inc. Cryo-Cell International, Inc. provided the foundational menstrual stem cell technology in the patent applications of M.A. Walton and JGA wholly owned by Cryo-Cell International, Inc.

Introduction

Stroke is characterized by an acute blood supply interruption to the brain, due to either blockage of the blood flow or rupture of an artery, leading to neural cell death. Cerebrovascular diseases are the third leading cause of death [1] and the primary cause of long-term disability in the United States [2]. Permanent disability affects 15–30% of first-time stroke patients and 20% of those still require institutional care 3 months after the event [3]. Despite preventive measures and effective reduction in incidence and mortality, stroke remains a major concern in the clinical setting largely due to the limited treatment available and to the progressive aging of the population.

Tissue plasminogen activator (tPA) is currently the best available therapeutic agent for ischemic stroke. Studies support the early use of tPA, demonstrating a direct correlation between time elapsed to begin treatment and long-term neurological impairment [4–6]. However, the therapeutic window for the administration of the drug is limited to 3 h after onset of symptoms [7, 8]. Estimates from 2001 to 2004 show that only 1.8–2.1% of all patients affected by ischemic strokes in the United States had received the therapy [9], indicating that most patients are not able to reach an emergency room and complete the neurologic triage within such narrow extent of time. Further studies have tried to evaluate the possibility of extending the limit beyond 4.5 h, but there were conflicting results, with an increase in mortality due to hemorrhagic complications [10–12].

Opportunity for Cell Therapy in Stroke

The ischemic lesion of stroke may be divided in the infarct core and the penumbra area, differentiated by their reversibility potentials. The core comprises the tissue promptly affected by the ischemic insult, with irreversible cell death within the first hour of ischemia. The surrounding penumbra area still retains structural integrity but lacks function. It is partially maintained by dilatation of patent vessels and blood supply from neighboring collateral arteries [13] and may evolve to death or to recovery depending on the severity of the ischemia and reestablishment of blood flow [14]. Treatment with tPA, applied early after stroke, contributes to the rescue of the penumbra area [15]. However, as most patients are excluded from such treatment, worse outcomes are inevitable.

While the infarct core is hardly salvageable after the onset of stroke, the penumbra area is potentially restorable. Hess and Borlongan [16] established three consecutive stages after stroke, each one associated with different therapeutic opportunities. Immediately after stroke and within 24 h, restoration of the blood flow would be neuroprotective, restricting neuronal death and decreasing the extent of the final infarct area. Although tPA administration is limited to the first hours after the onset of symptoms, new recanalizing agents, yet to be developed, may still be beneficial within the 24 h that follow the stroke. Thereafter, with the establishment of inflammation in the ischemic tissue, cell-based therapies would have their best indications, since inflammatory signals produced by the injured tissue would attract systemically injected cells [17]. Cell therapy would be most effective during the first week after stroke, with maximum cell migration and still tolerable tissue damage. At the end of 1 month, inflammation decreasing and scars and structural damage persisting, stem cells

would still have a possible therapeutic role, if delivered directly into the nervous tissue through the aid of scaffolds and surgical procedures.

Inflammatory Aspects of Stroke

Immediately following the ischemic insult, neuronal depolarization takes place in the affected area, mainly as consequence of glutamate excitotoxicity [18]. A massive influx of ions then unleashes catabolic processes [19], activates multiple cell death pathways and increases the production of nitric oxide and free radicals, all of which lead to neuronal apoptosis and necrosis. Astrocytes and oligodendroglia, which express *N*-methyl-D-aspartate and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, also die in consequence to glutamate excitotoxicity [20].

In parallel, the microglia have an early participation in inflammation, following the initial hypoxic insult [21, 22]. They stimulate the infiltration of immune cells and release toxic molecules such as free radicals, arachidonic acid and proinflammatory cytokines, therefore contributing to further cell death. On the other hand, microglia phagocytize debris and neurotoxic substances [23] and produce neurotrophic factors important for tissue repair. Astrocytes have supportive functions in the central nervous system (CNS), including scavenging of neurotransmitters released during synaptic activity, water and ion homeostasis, production of neurotrophic factors, integrity of the blood–brain barrier and control of the microvascular tonus in the CNS [24]. Failure of any of the supportive functions jeopardizes neuronal survival. Additionally, reactive astrocytes contribute to the formation of glial scar, therefore limiting the extension of injury. In the long term, however, the scar mechanically restrains the blood supply and cell migration and, therefore, hampers repair of the injured tissue. Astrocytes also secrete inflammatory cytokines, free radicals and proteases, stimulating the inflammatory reaction. Interestingly, Faulkner et al. [25] demonstrated that the inhibition of astrocyte activation following spinal cord injury increased neuron death, possibly because even in the injured environment, astrocytes still maintain some supportive functions, including the secretion of neurotrophic factors (nerve growth factor [NGF] and brain-derived neurotrophic factor [BDGF]), which are important for tissue repair and modulation of synaptic plasticity [26, 27].

Inflammation, therefore, plays a dual role after stroke. While beneficial to the brain during the early stages of neural cell death, it may be deleterious and exacerbate disease progression during the chronic period. Interventions to correct this aberrant immunological response are then warranted, aiming to provide best recovery to the affected patient.

The Therapeutic Potential of Stem Cells in Stroke

To date, numerous studies using stem cells for experimental stroke have been published [28–30] and their beneficial effects are becoming well established. Bone marrow-derived cells are the most frequently studied, because of the extensive previous knowledge from bone marrow transplantation for hematologic diseases. The hematopoietic and non-hematopoietic fractions of cells available within the bone marrow have both been applied in experimental studies of stroke. Bone marrow-derived cells enriched with hematopoietic precursors injected intravenously enhanced survival of mice with lethal stroke induced by

middle cerebral artery ligation [29]. Similarly, bone marrow cells decreased the size of the ischemic injury in animal models of induced-stroke and improved functional recovery [31]. Mesenchymal bone marrow cells prevent neuronal apoptosis and stimulate endogenous repair and angiogenesis, thus improving survival and neurological outcome [32]. The mesenchymal cells have also been cultured and differentiated *in vitro* into neuronal-marker expressing cells and, when injected in animal models of stroke, decreased the size of the ischemic injury and improved neurobehavioral outcome [33].

Neural stem cells are also investigated as a promising source of repair, based on observations that endogenous neural progenitors proliferate after cerebral ischemia. Attempts to stimulate endogenous neurogenesis in ischemic brains include growth factors, anti-inflammatory drugs, galectin-1, substance-P and nitric oxide, among others [34]. Exogenous transplantation of immortalized neural stem cells has improved the outcome of rodents with induced stroke. Borlongan et al. [35] reported functional and histopathological improvement of ischemic stroke in rats, after the transplantation of the NT2N lineage of immortalized human neural cells. Similarly, ischemic rodents transplanted with neural progenitor cells from fetal tissues presented a significant reduction of the infarct volume, which correlated with behavioral improvement [36]. Clinical application of these cells, however, is hampered by the little availability of donor tissues.

Embryonic cells provide the most exciting results, due to the extensive pluripotentiality of these cells. Unfortunately, due to their lack of adequate proliferative control and potential teratogenicity, some investigators have been using *in vitro* differentiated cells into neuronal progenitors, enabling a safer application. When injected into injured brain sites of rodents, the embryonic stem cells promoted transdifferentiation into neural and neuronal cell types, which were functionally active and improved neurological outcome [37].

Taguchi et al. [38] suggested an angiogenic effect of CD34+ cells from umbilical cord blood on the ischemic area of stroke. They observed that the cells injected systemically into a mouse model of stroke secreted growth factors (vascular endothelial growth factor [VEGF], fibroblast growth factor 2 [FGF2] and insulin-like growth factor [IGF]-1) induced formation of vascular channels and, secondarily, promoted the migration of neuronal precursors into the injured areas, which differentiated and improved nervous function. The addition of anti-angiogenic agents abolished the beneficial effect of the cells, demonstrating the importance of vessels in nervous repair. The issue was later discussed by Saghatelian [39], which suggested that vasculature-guided neuronal migration could be observed not only following stroke, but also as part of the normal brain development. More recently, endothelial progenitor cells injected into the systemic circulation of mice migrated to the stroke area, promoted repair and improved behavior, reinforcing the importance of angiogenesis [40]. Finally, Nakagomi et al. [41] demonstrated that the addition of endothelial precursors to neural stem cells, engrafted in mouse models of brain ischemia, enhanced cell survival, proliferation and differentiation, when compared to injections of neural stem cells alone.

Until recently, it was believed that cell effectiveness would be conditional on their migration to the site of injury. In fact, several authors observed a direct relationship between cell migration to the site of injury and behavioral improvement [36, 42]. However, Borlongan et

al. [28] observed, in rat models of stroke, that umbilical cord blood cells were able to promote repair even when not detected in the tissue, probably through the production of growth factors, cytokines and other therapeutic molecules that were able to reach the target. Adding importance to that idea, neurotrophic agents have been extensively researched in stroke, as it happened in basal ganglia disorders. Neurotrophic agents influence cell survival, proliferation, differentiation, function and plasticity [43, 44]. They also have a role in physiological endogenous repair and increased levels can be detected in injured neuronal sites [45]. They protect neurons from the cytotoxic insults generated during inflammation, with anti-excitotoxic and anti-oxidant functions, besides improving mitochondrial function.

Inflammation as a Target for Stem Cells

In the last few decades, research has targeted inflammatory components of stroke, aiming to attenuate the secondary cell death associated with ischemic stroke and decrease neurological impairments and disabilities. Experimental studies have shown that suppression of the inflammatory response after stroke leads to reduction of the infarct size [46, 47]. However, the translation of such approaches into the clinics has not, so far, been as successful [48–55].

Recently, stem cell therapy has been evaluated as a restorative approach. Stem cells are attracted and opportunely interact with the inflammatory dynamics of stroke, modulating its harmful effects and maximizing its regenerative potential. As an advantage upon tPa, cell therapy is available during longer periods after stroke and may be especially oriented to those patients who missed or who did not fully benefit from the thrombolytic treatment.

A key feature of stem cells is the ability to modulate the immune response, suppressing deleterious mechanisms without affecting beneficial functions. These unique properties are based on the fact that the suppressive capacity of the stem cells is also regulated by the inflammatory environment. Stem cells are able to control the further generation of pro-inflammatory events and therefore limit the progression of the inflammatory response. Neural stem cells, for example, decrease the expression of TNF- α and, in consequence, reduce neutrophil infiltration into the CNS of rat models of hemorrhagic stroke [56]. Later in the course of inflammation, stem cells also suppress reactive lymphocytes while enhancing the activity and proliferation of their beneficial, regulatory subsets [57]. Moreover, trophic factors secreted by the stem cells stimulate angiogenesis and repair [58]. Stem cells are, therefore, a very powerful therapeutic tool that still requires further studies to be properly applied with healing purposes. Their potential effects on either acute or chronic inflammatory settings make them useful as treatment not only for stroke but also for other neurodegenerative conditions in which inflammation is present.

Although the knowledge about cell-based therapy for stroke and other neurological diseases has increased over the years, there is no consensus about how the cells should be administered [59]. In the past years, several studies have addressed the issue, some with contributions that may favor the systemic route of cell administration. First, stem cells are attracted to the site of inflammation by agents such as monocyte chemoattractant protein-1 (MCP-1), stromal cell-derived factor (SDF) and macrophage inflammatory protein (MIP-1 α) [60]. Second, undifferentiated cells survive longer and migrate farther in the host

tissue than previously differentiated cells [61]. Finally, some tissues secrete differentiation stimulating factors, which could be effective *in vivo*, avoiding the necessity of previous differentiation of the cells [62]. Third, transplanted cells have limited survival in the host, whether injected locally or systemically [63, 64]. This observation, although at first seemingly discouraging, may be important in the clinical practice, since it indicates that the host immune system is able to control the presence of an allogeneic cell, avoiding undesired proliferation and possible malignancy. On the other hand, the presence of the cell during a minimum period is necessary, enabling therapeutic effects.

Another point of investigation discusses which is the best type of cells for regenerative purposes [59]. Although a significant number of recent studies evaluate the therapeutic effects of mature or differentiated cells, more immature cell lines also present their advantages. Less differentiated cells maintain stem cell markers, including the stem cell factor receptor, which aid in migration to the sites of injury [65]. These cells usually have higher differentiating potential, compared to the already committed predifferentiated cells [66]. This property may allow the differentiation of the transplanted cells into more than one cell type, in response to the cytokine and chemokine profile determined by the injured tissue and, therefore, provide better repair. Finally, more immature cell types are usually able to secrete a wider range of growth factors, which are also imperative for tissue regeneration [60]. In fact, although predifferentiated cells may seem functional *in vitro*, some studies *in vivo* fail to detect integration with the local cells, even when they maintain expression of the differentiation markers, suggesting that their restorative results are mostly mediated by paracrine effects on endogenous precursors [67, 68].

Embryonic stem cells, on the most immature end of the stem cell spectrum, combine high differentiation potential, ability to migrate to inflammatory sites, secretion of trophic factors and reduced immunogenicity [69]. However, major difficulties, associated to uncontrolled cell proliferation and the risk of malignancy, have hindered research using those cells. Because of ethical and safety reasons associated with embryonic stem cells, the last decade has witnessed a shift of cell-based therapies toward the use of adult stem cells.

Sources of Cells for Transplantation

For decades, the bone marrow has been used as the stem cell reservoir for diverse types of therapy. In recent years, however, new sources of stem cells have been investigated, as an attempt to avoid the hurdles associated to hematopoietic stem cell harvesting. Moreover, bone marrow-derived cells may have their proliferative potential impaired by aging, smoking and chronic illnesses, such as diabetes mellitus and hypertension, conditions very frequently associated to neurovascular disorders [70–73]. Opportunely, some disposable tissues, such as the umbilical cord blood, placenta, amniotic fluid and, more recently, the menstrual blood, have provided less mature cells than the bone marrow, some of which express embryonic-like markers [74, 75].

Ideally, autologous sources would be preferred, avoiding rejection and allowing longer permanence of engrafted cells in the targeted tissue. Concerning stroke, however, the short time window after the event frequently limits the use of autologous cells, which in most

cases need to be collected and expanded before delivery. Fortunately, mesenchymal or stromal cells are suitable candidates for allogeneic application, due to their low immunogenicity [76]. Although ultimately rejected by the host immune system, these cells remain long enough within the tissue to promote tissue repair, not requiring immunosuppression [77].

Despite the multiple ongoing studies involving stem cells in CNS disorders, a long-standing challenge in cell therapy is to find the perfect cell graft, which should be immature enough to hold multipotential differentiation properties and yet safe to not induce malignancy. It should also modulate the immune system, decreasing destructive aggression but preserving its ability to fight pathogens. Finally, it should be able to induce changes in the targeted tissue, either restoring its function or promoting repair. Several cell types match the above criteria and have been applied in experimental and clinical research; however, in most cases, ethical and practical issues are a concern. Stem cells from bone marrow, for instance, work well on most studies, but cell harvesting through bone marrow aspiration or leukapheresis is needed and the number of cells obtained may be not enough, besides the need of HLA matching in some cases. Other sources, like the liver, skin, heart, or even induced pluripotent stem cells (iPS), are also available, but the isolation and culture of those cells is currently costly and technically complicated [78–80]. There is interest, therefore, in acquiring stem cells from disposable and easily accessible tissues, such as the amnion and amniotic fluid, placenta, adipose tissue and, more recently, menstrual blood.

Stem Cells Derived from the Endometrium: Characterization and Applications

More than 30 years ago, Prianishnikov [81] described the presence of stem cells in the endometrium, from the observation that the upper layers of this tissue shed and were renovated each month. Part of the endometrium is composed of epithelial cells, which are found in the superficial layers of the tissue, extending through the tubular glands to the interface with the myometrium. The rest of the endometrium consists of stromal cells, smooth muscle cells, endothelial cells and leukocytes [82]. Functionally, the endometrium can be divided in two main layers. The upper layer, named functionalis, contains mostly glands loosely held together by stromal tissue while the lower layer, basalis, contains dense stroma and branching glands. The functionalis is eliminated monthly, as menstruation and the basalis persists and gives rise to the new endometrium, under hormonal influence.

Only in the last few years, Chan et al. [83] better characterized the endometrial stem cells, reporting epithelial and stromal cells that were isolated from the endometrium and cultured *in vitro*. Both were clonogenic and proliferated in laboratory, but the epithelial cells lost part of their phenotypic markers and needed a feeder layer as the cultures progressed. Meng et al. [84] published a study with stem cells obtained from the menstrual blood, which showed similar properties. The cells were differentiated into tissues from the three germ layers, indicating their multipotentiality *in vitro*, and therefore were named endometrial regenerative cells (ERC). Shortly after, in 2008, Patel et al. [75] published a more complete study, in which stromal stem cells, again isolated from menstrual blood (MenSCs), were expanded *in vitro*, and showed clonogenic properties and ability to differentiate into

mesoderm and ectoderm-derived tissues. Of note, they also demonstrated that MenSCs expressed markers of pluripotency, such as Oct-4, stage-specific embryonic antigen (SSEA)-4 and c-kit, which are frequently found in more immature cell types, including the embryonic stem cells.

Cervelló et al. [85] isolated, through flow cytometry of Hoechst-stained endometrium cells, epithelial and stromal-cell-enriched side populations. The cells were characterized *in vitro* and showed a high clonogenic and proliferative potential, especially when exposed to hypoxic conditions, which mimic the endometrial environment. However, when the cells were studied *in vivo*, injected subcutaneously in immunodeficient mice, they showed limited proliferative and differentiation potentials. Masuda et al. [86] studied the same side population of cells and conducted similar studies. The cells were implanted under the kidney capsule of female mice, and, after estrogen stimulus, human tissue development was observed in few animals. The authors demonstrated the differentiation of the side-population cells into glandular epithelial, stromal and, for the first time, endothelial cells, since small and medium sized vessels co-expressing CD31 and human vimentin were observed. Although detectable, their differentiation capacity *in vivo* was considered poor and better proliferative results were obtained when the cells were combined with the remaining population (main population) of endometrial cells. These findings, taken together with existing data from literature, suggest that multiple factors derived from the endometrium, instead of a single cell type, cooperate for the therapeutic properties of this tissue.

The categorization of stem cells derived from menstrual blood, based on their phenotypic and proliferative properties, has been an issue of discussion. As an example, Murphy et al. [87] believe that the endometrial regenerative cells (ERC) isolated by them are not the same as the endometrial stromal cells described by Taylor [88], but may share overlapping properties and may even be equivalent cells as those reported by other studies [75, 84, 89]. Endometrial regenerative cells, for instance, express low concentrations of the Stro-1 marker and exhibit higher proliferative capacity than other endometrial-derived cells. According to Taylor [88], stromal cells found in the endometrium originate from the bone marrow, as observed in recipients of allogeneic bone marrow transplantation. The findings were later reproduced in female rats transplanted with GFP bone marrow cells, which presented GFP cells in the endometrium long after transplantation [90]. In practical matters, however, they seem to have similar effects and comparable therapeutic abilities to promote repair when applied *in vivo*.

The angiogenic potential of the endometrium-derived cells is relevant for the experimental investigations of vascular growth and remodeling and perhaps, even for designing clinical therapeutic studies, as these cells might be applied to cardiovascular diseases. Hida et al. [91] published their experience with menstrual-blood-derived stromal cells in damaged heart tissue, in which they were able to *in vitro* differentiate the cells into spontaneously beating cardiomyocyte-like cells. When menstrual blood cells were injected in the ischemic tissue of myocardial infarct rat models, functional improvement was noted, differently than what was observed when bone marrow stromal cells were used. Finally, the authors also reported evidences of cell engraftment and transdifferentiation into cardiac tissue (Table I). Some authors propose to take advantage of the angiogenic potential of these cells, applying them

to the treatment of chronic limb ischemia [87] and, more recently, severe skin burns, using the cells associated to intelligent artificial films [92].

Regarding central nervous system disorders, Borlongan et al. [93] recently published the results of menstrual blood cell transplantation in experimental stroke. Stromal-like menstrual blood stem cells were isolated, expanded and, at last, selected for CD117, a marker associated with high proliferation, migration and survival [94]. *In vitro* studies showed that the expanded cells maintained expression of embryonic-like stem cell phenotypic markers, such as Oct-4, SSEA-4 and Nanog, even when cultured up to nine passages, as an evidence of the safety and reliability of these cells and some were induced to express neural markers (microtubule-associated protein 2 and Nestin). Moreover, when added to cultured rat neurons exposed to a hypoxic insult, the menstrual blood cells provided neuroprotection and when applied to rat stroke models, less neurologic deficit was observed on functional tests, irrespective of the injection site, i.e. systemic or local administration into the striatum. However, analysis of the tissue, after animal sacrifice, revealed that although human cells were detected in the rat brain, some migrating to areas other than the injected, they did not show signs of differentiation, expressing their original markers. Once more, there is evidence that cell differentiation is not the main pathway of neuroprotection or neuroregeneration. Figure 1 illustrates the possible therapeutic pathways for menstrual blood cells in stroke.

Wolff et al. [89] reported the use of endometrial derived neural cells in a Parkinson's disease mouse model. Endometrial-derived stromal cells were differentiated *in vitro* into dopamine-producing cells and then engrafted into the brain of the animals. Migration, differentiation and production of dopamine were detected *in vivo*, demonstrating the therapeutic potential of these cells to functionally restore the damaged tissue, either through cell replacement or endogenous repair.

The only clinical study yet published evaluated the safety aspects of endometrial-derived stromal cells administration [95]. Four patients with multiple sclerosis were treated with intrathecal injections of 16–30 million cells and one of the patients also received an additional intravenous injection. No adverse events were registered, as expected and the authors reported functional stabilization. However, the longest follow-up reached 12 months and any conclusions about effectiveness of the treatment seem premature in this long-term and slowly progressive illness.

Taken together, the available evidences regarding menstrual-blood-derived cells favor their future application in clinical studies. In comparison to stem cells from other sources, especially those from the bone marrow, menstrual-blood-derived stem cells have the advantage of presenting a more immature phenotype, through the expression of embryonic-like surface markers. Their immature behavior is confirmed by *in vitro* differentiation studies, in which menstrual-blood-derived cells originate diverse tissue types from all three germ layers [75, 84]. Moreover, they seem to have a higher proliferative capacity, above 30 population doublings, when compared to stromal cells from other sources, such as the bone marrow and dental pulp, which are limited to approximately 20 population doublings [96]. Additionally, cultured menstrual blood cells maintain longer telomerase activity than bone

marrow-derived cells [75, 97], indicating delayed senescence. These observations may reflect higher regenerative and differentiation potentials *in vivo*, yet to be confirmed by comparative studies between stromal cells from different sources.

Adipose tissue mesenchymal stem cells (MSCs) are strong competitors to menstrual blood cells and have been lately investigated as alternatives to bone marrow MSC. These cells present high proliferative capacity and angiogenic potential possibly through expression of VEGF and hepatocyte growth factor (HGF). Experimental models of chronic myocardial infarction [98], limb ischemia [99, 100], stroke [30, 101], spinal cord injury [102, 103] and retinal lesions [104], among others, have shown optimistic results about the regenerative potential of adipose tissue cells. The few human studies available establish safety of these cells and reproduce the animal outcomes [105, 106]. *In vitro* studies, however, have revealed high proliferative rates, but with conflicting results regarding senescence [96, 107]. Comparative evaluations among cells from different sources, especially concerning disposable tissues, are necessary to effectively determine which is the best cell type. In the future, better understanding the properties and mechanistic pathways may allow the cell types to be chosen according to their application. Meanwhile, abundance, frequency and expansion potential of the cells seem to be important criteria in establishing the best cell source.

Practical Issues

Menstrual cells are a novel therapeutic option in this field and have great potential, as already demonstrated through experimental studies. In the clinic, the application of autologous stem cells derived from menstrual blood would be ideal to avoid graft rejection issues. However, the low yield and difficulty in expansion of ample supply of stem cells from this source is a barrier to be transposed. Although presenting high proliferation rates, the cells require time to multiply and achieve sufficient quantities for clinical applications, therefore limiting autologous use. Moreover, this approach would be restricted to the female population. Males and post-menopausal women, which are the main targets of stroke and neurodegenerative diseases, would be excluded from the therapy. A feasible solution would be to educate the female pre-menopausal population about the potential of the menstrual cells and, therefore, stimulate the anticipated harvesting and cryopreservation of the cells, for future autologous use. For the male population, however, there remain the options of using allogeneic menstrual blood cells and of searching for an alternative source of cells or a male counterpart cell.

An ideal situation would be a woman, recently affected by a stroke, with autologous menstrual-blood-derived cells previously collected, expanded up to third passage and cryopreserved. In a few days these cells would be thawed, further expanded if necessary and made available for intravenous delivery in adequate time for their best effectiveness in the rescue of the penumbra area. In a more realistic scenario, the patient would be a man or a woman without stored cells and lacking enough time for harvesting and expansion. These would still benefit from the use of allogeneic cells which, being stromal cells, present low immunogenicity and, therefore, tolerable rejection rates.

Investing in cell banking as a safety measure against possible future events may be a wise and even profitable step. While cell-banking is already widely accessible for umbilical cord blood, only recently has it also become available for menstrual blood cells and, yet, limited to autologous or, at most, familiar use. It is possible, however, to expand the availability of menstrual blood cells to a wider population of allogeneic recipients. Women in childbearing age may donate samples of menstrual blood, enabling storage for future use. As a further possibility, the cells could be expanded and differentiated into specific tissues and be ready for eventual necessities [108, 109]. An efficient banking system for menstrual blood cells would require an organized and updated registration system, enabling prompt localization and rapid retrieval of the cryopreserved cells, just in time for therapeutic use.

Conclusions

Research on cell therapy for stroke has evolved lately, progressively decreasing the distance toward the clinics. It seems clear that the rescue of the penumbra area after stroke is decisive for functional outcome and a great opportunity for cell application. Stem cells promote neuroprotection especially through modulation of the activated immune system and secretion of neurotrophic factors. Tissue repair is also described and, although cell differentiation is observed in the experimental setting, its contribution to the outcome of the treatment is still unclear.

Menstrual cells combine characteristics that are convenient for clinical application and, in parallel with cells derived from other disposable tissues, may have a role in future investigations. Despite the potential challenges still to be solved, menstrual blood cells represent an important therapeutic tool that may improve the outcome of stroke and decrease the disability of future patients.

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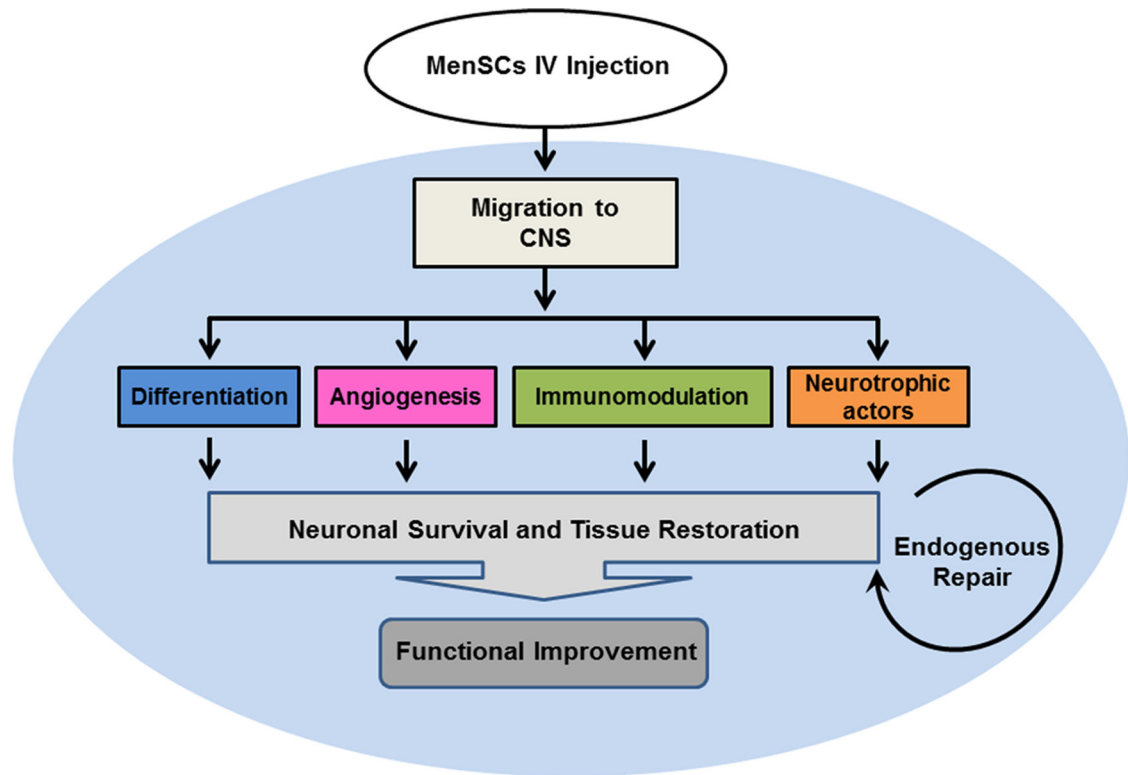


Fig. 1. Mechanistic pathways for menstrual blood stem cells. Intravenously injected menstrual blood cells migrate to the site of ischemic injury in the central nervous system, interacting with the inflammatory tissue and promoting repair. Immunomodulation and secretion of neurotrophic factors are the main mechanisms, improving neural survival and stimulating endogenous repair pathways, with the secondary support from angiogenesis. The contribution of cell differentiation to repair is still unclear and may depend on the tissue and type of injury. The endpoint would be functional improvement, therefore decreasing disability after stroke

Table IInvestigations on endometrial/menstrual blood stem cells^a

Year	Event	Reference
1978	First description of stem cells in the endometrial tissue based on the observations of the monthly tissue shedding	[81]
1991	Supports the idea of stem cells in the endometrial tissue based on the potential of the endometrial cells to migrate and generate new tissue in endometriosis	[82]
2004	Isolation and culture of epithelial and stromal cells from the endometrium. Observed clonogenicity and <i>in vitro</i> proliferation	[83]
2007	Isolation and culture of cells from menstrual blood, similar to cells isolated from the endometrium. Differentiation into the three germ layers	[84]
2007	Intramuscular application of human endometrial and menstrual blood-derived cells in a murine model of Duchenne muscular dystrophy. <i>In vitro</i> and <i>in vivo</i> demonstration of fusion of injected cells to myoblasts and production of human dystrophin by the treated muscle	[109]
2008	Isolation, culture and differentiation of menstrual blood stromal cells into mesodermal and ectodermal tissues. Expression of markers of immature cell types	[75]
2008	Differentiation of menstrual blood stromal cells into spontaneously beating cardiomyocytes <i>in vitro</i> . Functional improvement of myocardial infarct rat models and <i>in vivo</i> evidence of cell engraftment and differentiation	[91]
2009	Safety study of intravenous/intrathecal administration of endometrium-derived stromal cells in refractory multiple sclerosis patients. No adverse effects observed. Clinical stabilization in short follow-up period	[95]
2010	Menstrual blood stromal cells expressed neural markers <i>in vitro</i> . Neuroprotection of neural tissue cultures when menstrual blood cells were added. Functional improvement of rat model of stroke, with migration of cells to the site of injury, but without evidence of cell differentiation	[93]
2010	Endometrial-derived stem cells injected in a rat model of Parkinson's disease. Evidence of migration, engraftment and differentiation, along with increased concentrations of striatal dopamine	[89]

^aList of the publications on endometrium-derived stem cells, from the first report of the existence of stem cells in such tissue, to the clinical applications of these cells