

Gender-Linked Stem Cell Alterations in Stroke and Postpartum Depression

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Keywords

Cerebral ischemia; Gender; Psychiatric disorder; Stem cell biology; Stem cell therapy.

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Received 13 June 2014; revision 19 September 2014; accepted 23 September 2014

SUMMARY

Stroke is a significant unmet clinical need. The current stroke treatment of tissue plasminogen activator is limited to the very acute 4.5 h after disease onset which benefits only less than 3% of ischemic stroke patients. Our overarching hypothesis advances the notion that gender, which has been established as a comorbidity factor of stroke, plays a key role in regenerative medicine, in particular stem cell therapy. We hypothesize that gender is a key factor in culture-induced stemness of adult stem cells. Our goal is to provide new evidence supporting gender effects on stroke and stem cells for the purpose of enhancing our understanding of the pathophysiology of the disease and developing novel stem cell-based therapeutics targeting gender-relevant stress hormones as manifested in a stroke-postpartum depression paradigm.

doi: 10.1111/cns.12339

Introduction

Stroke is a major health problem that affects both males and females, yet many preclinical studies have only tested experimental therapeutics in male stroke animals. Current U.S. statistics reveal that stroke is more prevalent among women than men, and even more devastating, the death rate of women suffering from stroke is twice as high as breast cancer. Furthermore, women are at a greater risk of suffering a major disability from stroke and are more likely to die from stroke than men. One significant disparity between genders is that women have shown increase disability when performing everyday task such as eating, walking, or dressing than men after stroke [1]. Data from different studies demonstrate that stroke severity, comorbidities, and mental health status of women were higher than men [2]. Eventually more women depend on assisted living facilities than men after stroke [1,2]. Of note, there is a higher incidence for stroke during pregnancy and the postpartum period in cases of preeclampsia and postpartum angiopathy. Clinical data have shown that there are 8.1 strokes per 100,000 pregnancies. The occurrence of stroke increases with

age and the frequency is also higher because women live longer than men [3]. Moreover, the incidence of stroke in postpartum women is 8.7 times higher than in pregnant or nonpregnant young females [4]. The incidence of stroke in clinically depressed middle-aged women is 1.9 times higher, and women with depression are more likely to have a stroke by a factor of 2.4, compared to women without depression [5]. That postpartum depression influences stroke outcome led us to hypothesize that postpartum depression directly alters stem cell fate, which should be manifested in an animal model of postpartum depression. Our overarching hypothesis builds upon accumulating evidence that gender (i.e., female) is a comorbidity factor in stroke. Because the underlying mechanisms of gender-mediated stroke deficits are not well understood and with our long-standing interest in stem cells, in particular neurogenesis, we embarked on this research theme of gender and neurogenesis in stroke with emphasis on gender-associated hormonal shifts inherent in postpartum depression. Our hypothesis if proven true will lead to a better understanding of gender as a comorbidity factor in stroke. The overall impact is that this hypothesis will answer translational relevant questions,

in particular whether gender, especially the female-inherent postpartum depression, affects the viability, proliferation, migration, and differentiation of stem cells and their functional benefits in repairing the brain after stroke. The envisioned results will also directly impact on advancing our knowledge on stem cell-based therapeutics for stroke and postpartum depression.

Gender, Stroke, and Stem Cells

Our overarching hypothesis is that gender influences stroke symptoms by altering the stem cell regenerative capacity. Gender is a major comorbidity factor in stroke. Stroke affects women at a later age than men and is more prevalent during the menopausal period, which may be attributed to decreasing hormone levels [6,7]. As females get older the incidence of stroke doubles [8], likely due to the postmenopausal reduction in female hormones that normally protect against stroke [6]. In ovariectomized rats, there is more severe stroke deficits than those with intact ovaries due to ovariectomy-induced reduction in progesterone and estrogen which, respectively, exert antiedema and perivascular stabilizing effects against ischemic brain injury [9,10]. Our preliminary data show that *in vitro* endothelial progenitor cell (EPCs) derived from postmenopausal females displayed less viability, decreased proliferative, migratory, and differentiation capacity, and reduced therapeutic benefit compared to aged-matched male-derived EPCs. These pilot observations prompted us to examine the mechanisms underlying these gender-mediated alterations in stroke. In particular, we draw upon exciting clinical findings noting that postpartum depressed women show massive shifts in stress hormonal levels. To this end, our overarching hypothesis of testing the effects of gender on endogenous stem cells from both genders after stroke embraces a dual-pronged approach, addressing both basic science and translational research gaps of stem cell biology and its therapeutic applications for stroke.

Gender-Linked Alterations are Magnified After Postpartum Depression

Our corollary hypothesis stipulates that gender-associated postpartum depression that could exacerbate stroke symptoms and can be manifested in reduced neurogenesis capacity. The translational relevance of this hypothesis directly applies to stem cell therapy for stroke, in that the gender of stem cell donors and transplant recipients may affect the therapeutic outcome in stroke, with female-derived EPCs when transplanted into postpartum depressed stroke female rats will produce significantly less behavioral and histological improvements compared to male-derived EPCs transplanted into stroke male rats. If proven true, another corollary hypothesis is that increasing the cell dose, accelerating the timing of cell delivery, and transplanting male-derived EPCs may be required to enhance the stem cell therapeutic outcome in postpartum depressed female stroke rats. Alternatively, treating postpartum depression as an adjunct to stem cell therapy may prove efficacious. Clearly translational experiments addressing these speculative scenarios are warranted to further advance the concept of gender-associated postpartum depression in stroke as key contributing factor on the therapeutic outcome of stem cell transplantation. Interestingly, a classic example of hormonal shifts

in women is well-documented during postpartum depression [11]. Women diagnosed with postpartum depression display a decrease in hippocampal neurogenesis [12–14]. Accordingly, gender-dependent stem cell alterations are likely to be recognized during postpartum depression, which represents an enhanced state of hormonal shifts in females [9,10]. Although it is challenging to detect neurogenesis in human adult brain, several studies have demonstrated that neurogenesis, indeed, is altered in human stroke patients. Accumulating evidence shows active cell proliferation after ischemic stroke in the ipsilateral side of the SVZ in human postmortem tissue [15,16]. Interestingly, whether enhancement of this endogenous response would improve recovery remains unresolved. The hypothesis we advance here is that this endogenous neurogenesis after stroke is affected by gender.

The recognition that gender-dependent stroke outcomes are magnified during postpartum depression provides a novel platform to reveal gender as a key comorbidity factor to the disease pathology and treatment of stroke. This hypothesis recognizes the importance of gender in cell therapy for stroke, specifically the characterization of the gender effects on the stem cell donor and the transplant stroke recipient. To date, there is no study investigating the transplantation of stem cells harvested from female bone marrow into aged female stroke rats. Our hypothesis places “gender” as a critical translational gating item in selecting the appropriate stem cell donor, as well as in testing the safety and efficacy of the stem cell transplants in a stroke setting. Indeed, the bulk of the literature on experimental stroke therapeutics, not only on stem cell transplants but novel treatments in general, reveals an overwhelming choice of “male subjects” and an almost complete neglect of the females. This gender imbalance in sampling of transplant recipients is further skewed by the lack of systematic study on the stem cell donor gender. Our innovative approach of studying gender effects bridges both basic science and translational research gaps that will help us better understand stem cell biology and its therapeutic applications in stroke. Our corollary hypothesis recognizes postpartum depression as a model of gender-specific hormonal shifts in stroke. While the aged or ovariectomized female rat has been used widely as a model of gender-specific hormonal shifts [9,17], postpartum depression is equally associated with hormonal shifts, yet an underexplored research subject.

The overall impact of our hypothesis is the demonstration of differences in pathological outcomes between genders after stroke. Stroke incidence in females doubles and may be more damaging as they get older [8], this may be due to menopause-associated changes in hormonal levels which can worsen disease manifestations. That hormonal alterations are equally rampant in postpartum depression should allow investigations into the contribution of gender (i.e., female) to stroke pathology. This impact is two-pronged, in that our hypothesis will advance both basic science knowledge and clinical treatment of stroke patients. From the basic science standpoint, we will gain a better understanding of the cellular mechanisms (i.e., neurogenesis) underlying gender effects on stroke outcome. From the clinical perspective, we will be able to provide insights on the need to further consider gender as a major comorbidity factor not just in the diagnosis of the disease, but also its treatment especially as it relates to ongoing clinical trials of stem-based therapy (transplantation of stem cells or

administration of drugs, like G-CSF, designed to boost neurogenesis). As we too often see in research testing drugs, dosing has been routinely carried out on males rather than females; thus, there is a need to expand from this selectivity and tailor pharmacological screening to females as well. Our preliminary results reveal that there are gender differences when cells were exposed to OGD conditions as compared to control. With these results and our further observations on the pathological interactions across hormones, stress, and neurogenesis in a gender-specific manner, it is logical to speculate the importance of gender in the study of stroke and postpartum depression.

Gender-Linked Stem Cell Alterations in Stroke

Estrogen enhances stem cell proliferation and migration, specifically facilitating EPC migration to the site of injury and subsequently enhancing neovascularization [18,19]. EPCs differentiate into endothelial cells which may help in remodeling of the damaged vasculature and heal injured vessels [20]. An advantage of EPCs compared to other stem cells is their potential to repair neurovasculature and reconstitute the blood–brain barrier (BBB) via vasculogenic and angiogenic properties [21]. When EPCs are peripherally transplanted following stroke, they localize to the site of injury by utilizing the CXCR4/SDF-1 pathway that both bone-marrow-derived EPCs and endogenous stem cells also use, suggesting a specificity to the impaired neurovasculature for repair [22]. However, throughout the chronic stage of injury, the quantity of EPCs that assemble and home to the ischemic site decreases. Levels of circulating EPCs are greater in premenopausal women compared to postmenopausal women [23]. EPC concentrations also correlate with the level of circulating estrogen 2 (E2) during the menstrual cycle and are elevated in fertile women compared to young men or postmenopausal women [24]. Estrogen functions as accelerator of functional endothelial recovery after vascular injury via signaling of estrogen receptor (ER) α and β , both found in EPCs and other stem cells, to increase re-endothelialization and vasculoprotection. Thus far, there has not been difference in the levels of EPCs detected between postmenopausal women and age-matched men [25]. There is limited data regarding androgen receptor expression on EPCs. However, one study demonstrated that the low levels of circulating EPCs seen in hypogonadal males were reversed when testosterone was used as a treatment. Its effect was further extended to estrogen as its levels also increased [26].

It seems that to be efficient in obtaining a greater quantity of EPCs the donors should be premenopausal women or women who are going through their menstrual cycle at the time of EPC harvest. It also seems reasonable to assume that EPCs will exert their maximum effect in stroke when the donor cells are from younger, premenopausal women. Thus, postmenopausal women and age-matched men would display a worse stroke outcome compared to younger women due to the low levels already endogenously present before the transplantation. An alternative to boost and enhance the effects of EPCs in these groups could therefore be steroid treatment after transplantation and during the chronic stage of injury.

In cell culture experiments, we observed that female EPCs exhibited reduced proliferation and migration capacity compared to male EPCs when exposed to experimental stroke model, which resembles the observation that females who suffer from postpartum depression display a decrease in neurogenesis. Because circulating EPCs play an important role in vascular remodeling after a stroke [27], the reduced proliferation of EPCs in postpartum depressed females suggests that impaired stemness property of female endogenous EPCs is key factor that needs to be considered for stroke therapy. EPCs have been shown to commit toward a neural lineage after injury [28]. Accordingly, if the proliferation and migration of these cells are decreased in postpartum depressed women, then the endogenous repair mechanism after stroke is compromised. Moreover, female mice suffering from ischemic stroke and treated with estrogen display increased survival rates compared to male mice subjected to the same treatment condition [29,30]. Relevant clinical studies support such gender-mediated hormonal effects on stem cell fate, in that androgens have been shown to increase the number of circulating EPCs [18,31]. Unfortunately, although histological and functional outcomes of stroke are now recognized as being influenced by the gender and age of the patient, the preclinical research arm lags behind in incorporating these comorbidity factors into experimental design. Stem cell therapy may be able to restore brain function in the subacute and chronic phases. In particular, adult human bone-marrow-derived EPCs may help repair the vasculature that is compromised after stroke. Furthermore, gender-associated postpartum depression can exacerbate stroke symptoms and can be manifested in reduced neurogenesis capacity. Depression is characterized by increased morbidity and mortality in vascular diseases [32,33]. The mechanisms of action underlying depression-induced vascular dysfunction are still not well understood but depressive disorders were found to be associated with dysfunction of the immune system and bone-marrow-derived cells, such as EPCs [34]. EPCs may contribute to ongoing vascular repair by providing circulating cell population that home to blood vessel walls and incorporate into the injured endothelial niche to replace dysfunctional endothelial cells [35]. It is possible that factors, such as VEGF, support neuronal cell proliferation indirectly through the stimulation of EPCs which sequentially induces neural progenitor cell division. Indeed, a study has found that neural progenitor cells tend to proliferate in dense clusters associated with vasculature [36]. These studies provide support to our thesis that the healthy conditions of EPCs, with key interaction with VEGF, is essential to proper neuronal function and that impaired EPCs may contribute to neurological and psychiatric disorders (e.g., stroke and depression). This concept also directly links our thesis of gender-associated postpartum depression to stroke, manifested as altered neurogenesis (i.e., impaired EPCs). After the initial stroke insult, endogenous neurogenesis and angiogenesis [37–39] – become activated as compensatory neuroprotective strategies against the injury, but such host defense mechanisms are not sufficient to combat stroke necessitating the need for exogenous stem cells (i.e., EPCs). Microchimerism, defined as the presence of cells originating from another individual therefore genetically distinct

from the host cells, is a phenomenon seen during pregnancy whereby fetal stem cells pass bidirectionally from fetus to mother and these microchimeric stem cells persist for many years, influencing the immune status of females in relation to transplant therapy [40]. Thus, determining if the gender of the stem cell donor or transplant recipient affects the therapeutic outcome will provide broader knowledge on the use of stem cells and will help identify appropriate target patient populations (Figure 1). Figure 1 predicts what would happen if males EPCs were transplanted into female rats after injury and vice versa. The figure also foresees the beneficial effect on the animal if the transplanted EPCs were from the male donor (i.e., male EPCs to male transplant recipients or male EPCs to female transplant recipients) to address whether the effect is due to the EPCs from one's gender or if the effect is due to the gender of the rat and the interactions between the transplanted EPCs' microenvironment. Conceivably male EPCs in male rats will produce a more significant improvement because it better matched for the male anatomy. Our preliminary data support such gender-linked stem cell alterations (Figure 2).

Understanding the genotypic and phenotypic differences of dimorphisms [18,41–43], likely to be reflected in the gender of stem cell donors and recipients, will further optimize the safety and efficacy of stem cell therapy. Based on our preliminary *in vitro* data (Figure 2), we hypothesize that female-derived EPCs transplanted to an aged stroke female will produce therapeutic effects, but may be suboptimal compared to male-derived EPCs if it were transplanted to an aged stroke male rat. For this reason, studies should assess the therapeutic benefits of endogenous stem cells after stroke in both male and females to better reveal the role of the gender on brain remodeling following a brain insult.

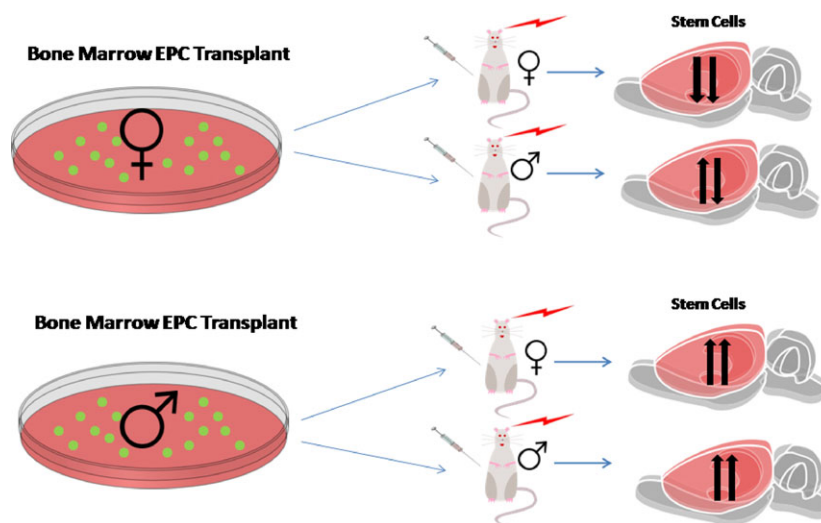
The initial logical step in revealing gender effects on stroke and stem cells is by studying the effects of stroke on endogenous stem cells in both genders. Our preliminary *in vitro* data suggest that under OGD conditions, female-derived stem cells (EPCs) showed resistance to the OGD conditions but male-derived EPCs exhibited better migration and proliferation. We expect to see *in vivo* that the neurogenic niche will be prompted by the experimental stroke

to mount a neuroprotective response in both genders, but that male-derived EPCs will be able to migrate and proliferate better and likely result in superior functional recovery in transplanted stroke animals, than those stroke animals that will receive female-derived EPCs.

Gender-Related Periods of Neuroendocrine Stress (i.e., Postpartum Depression) as a Model of Gender-Specific Hormonal Shift in Stroke

Next, we hypothesize that the reduced robustness of female stem cells is likely due to stress hormonal fluctuations. Such interaction of stress hormones and stem cell function has been implicated in stroke pathology [44–46], but yet to be directly examined in a postpartum depression and stroke paradigm. As with many disorders, there is an important interplay between genetics and the environment that can have an effect on the incidence of the disease. Risk factors for stroke differ in that they can be regulating (i.e., hypertension) and nonregulating (i.e., genetic predisposition [5HTT gene or noradrenaline mutations]) [47,48]. There is evidence that some people are genetically reactive to the environment, resulting in a crossover of risks for postpartum depression for the most reactive groups. Moreover, close relatives of postpartum depressed patients are two to six times more probable to develop postpartum depression than individuals without a family history of depression [47]. This risk is augmented as people are exposed to a negative environment (i.e., stress, low education and economic status, abuse, and failure to breast feed). Women are also more susceptible due to environmental stressors, hormones, being physically unhealthy, and other biological factors. Comasco et al. proposed a correlation between the brain-derived neurotrophic factor (BDNF) gene, as the BDNF Met66 carrier condition progressed postpartum depression symptoms after delivery, and seasonal delivery, as there was a greater increase in incidence of symptoms during autumn and winter [49].

Figure 1 Our hypothesis provides scenarios of treatment iterations to reveal the effects of “gender” in cell therapy for stroke (lightning symbol). The gender of stem cell donor and stroke transplant recipients will be manipulated. The area in the brain enriched with stem cells is the subventricular zone (SVZ). The vertical upward arrows depict a possible increase in endogenous neurogenesis after transplant of endothelial progenitor cell (EPC), and upward/downward vertical arrows represent a balance level of neurogenesis after male or female EPCs transplantation.



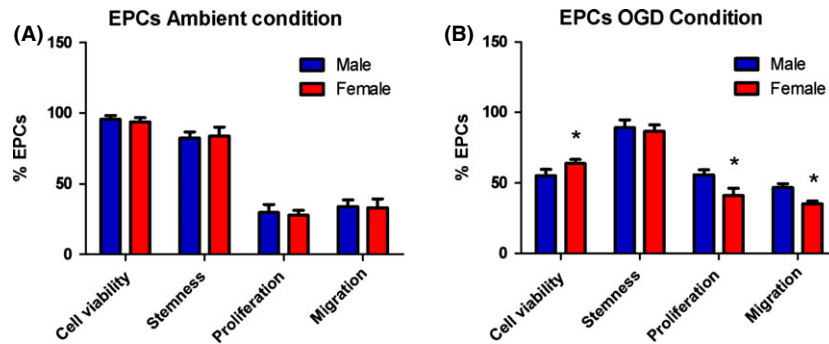


Figure 2 Under ambient conditions (A) both male- and female-derived endothelial progenitor cells (EPCs) did not differ in viability (Trypan Blue), stemness (Oct4), proliferation (BrdU), and migration (Boyden chamber); the latter measuring the amount of cells that migrated from top of the chamber to the bottom chamber. Interestingly, when EPCs were exposed to oxygen glucose deprivation (OGD), an experimental *in vitro* stroke model, the cultured female-derived cells showed an increased resistance to OGD, but male-derived cells displayed better proliferation and migration levels (B). Asterisk denotes significance ($P < 0.05$). These results suggest gender-mediated stem cell effects under *in vitro* experimental stroke, necessitating the need for investigation of male- and female-derived stem cells, as well as transplant recipients, in testing the *in vivo* safety and efficacy of EPCs in stroke therapy.

Influence of Hormonal Shifts

Neuroendocrine changes become more pronounced, and thereby more influential in directing the fate of stem cells during and following pregnancy [13,50]. In particular, the postpartum period is characterized by moderate to severe depression affecting 10–15% of new mothers within the first year after giving birth [13,50]. Aging and postmenopausal hormonal shifts have been implicated to contribute to this female gender as stroke comorbidity factor, and accumulating evidence suggests that pregnancy predisposes women to altered stem cell regenerative activity after stroke, which are not observed in men [41]. Postpartum depression is associated with significant hormonal changes, which remain as an underexplored subject in relation to stroke and neurogenesis. Interestingly, there is also a higher incidence for stroke during pregnancy and the postpartum period in cases of preeclampsia and postpartum angiopathy [51]. Clinical data have shown that there are 8.1 strokes per 100,000 pregnancies [4]. There is an increase in incidence for stroke in depressed women [52]. Women who show massive shifts in hormonal levels postpartum likely present with dysfunctional neuroendocrine axis following physiological stress [5]. As noted above, the incidence of stroke in postpartum depressed women is significantly much higher than in pregnant or nonpregnant young females [4], implicating that postpartum depression influences stroke outcome. Ischemic stroke during pregnancy has been shown to be associated with an increase in hypercoagulability factors as the body prepares for child delivery [53].

During postpartum, two-thirds of the stroke cases occur during the first week after delivery, and interestingly often occur following a normal pregnancy [54,55]. In other cases, stroke onset is associated with drugs such as vasoconstrictors used to treat postpartum hemorrhage. Thus, the physiological status of women during pregnancy is likely critical to stem cell outcome, in that stress during pregnancy and postpartum can affect fetomaternal microchimerism. Fetomaternal microchimerism contributes to the increase in stem cells during pregnancy and the postpartum period [56], but fluctuating stress hormones, common in neuropsychiatric conditions such as postpartum depression [13,57–60] may

dampen such fetomaternal microchimerism-mediated stem cell enhancement. For example, pregnant rats exposed to an experimental model of stress via treatment with high levels of glucocorticoids (CORT) give rise to offspring with reduced hippocampal cell proliferation, a putative marker of depression. When CORT is administered postpartum, depressive symptoms in the dam emerge, suggesting deleterious effects of dysregulated maternal glucocorticoids during gestation and the postpartum period [13,61]. Of note, hippocampal neurogenesis has been recognized as sensitive to hormonal levels during pregnancy, with increased cell proliferation in neurogenic brain niches during early gestation and the immediate postpartum period in maternal mice [12]. However, neuroendocrine dysfunction due to changing levels of stress hormones is associated with the onset of postpartum depression [12,58,62] or glucocorticoid exposure during pregnancy [13,14], which will likely reduce neurogenesis [43]. Our corollary hypothesis if proven true will decipher the effects of postpartum hormonal shifts on endogenous stem cell status, particularly in animals exhibiting depressive-like features (Figure 3).

Postpartum depression may also accompany men. The prevalence of elevated depressive symptoms among expectant fathers was 9.8% prenatally compared to 7.8% postnatally. The fathers who were interviewed during their partners pregnancy up to 6 weeks postpartum [63] had concerns regarding birth, anxiety, partnership satisfaction, personal history of depression, poor relationship with spouse, lack of support from others, and economical status. Surprisingly, partners of patients hospitalized for preeclampsia or preterm premature rupture of membranes had symptoms of PTSD and depression at a similar rate to partners of healthy pregnant women. Moreover, increased paternal age predicted more symptoms and at 6 weeks postpartum, a strong association was found between men and women in symptoms of PTSD and depression [64]. Fathering appears to be particularly affected by the loss of a close adult relationship and be exacerbated by the age of the father, increasing the morbidity of stroke in men. Because postpartum depression is not only limited to women nor to men whose partners have been hospitalized for pregnancy complications, the possibility of both partners displaying symptoms of PTSD and depression at the same time postnatally is of

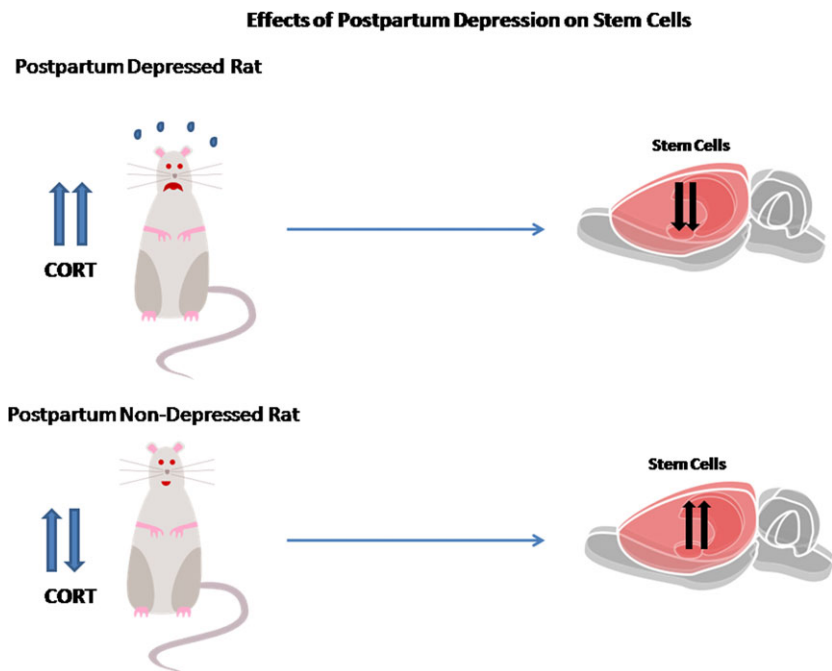


Figure 3 Our corollary hypothesis advances the notion of severe alterations in stem cells in the brain of postpartum depressed rats versus postpartum nondepressed rats. The blue vertical upward arrows depict increased levels of CORT, and the vertical upward/downward arrows depict a steady level of CORT. The black vertical upward arrows depict a possible increase in endogenous neurogenesis after transplant of endothelial progenitor cell (EPC), and upward/downward vertical arrows represent a balance level of neurogenesis after male or female EPCs transplantation.

critical importance to treat. Early intervention would lessen the health risks (i.e., stroke) and the burden of rearing a child during such vulnerable time.

Our desire to assess the contribution of postpartum depression in stroke outcome is within our collaborative effort to better understand the role of gender in stroke and the endogenous repair process of neurogenesis accompanying the disease pathology. We expect to see higher levels of neurogenesis markers in males as compared to postpartum depressed rats. We also anticipate that postpartum depression will exacerbate stroke outcome and worsen the impairment in neurogenesis. In contrast, those animals that are not exhibiting the postpartum depression will show less stroke deficits and better neurogenesis compared to the postpartum depressed animals subjected to stroke. The caveat here is identifying depressed versus nondepressed postpartum female rats. The availability of a postpartum depression rodent model has demonstrated the validity and reliability of CORT levels and sensitive behavioral assays in identifying postpartum depressed animals. Maternal postpartum behavior in a rodent model is monitored via video recordings from a closed-circuit television feed in an undisturbed vivarium housing unit. Recordings are taken twice daily for 1 h during light and 1 h during dark phases. Observations take place for 12 consecutive days postpartum. Each dam's behavior must be assessed for 10-s period at 10 time points per hour with 5–6 min between observations (approximately 45 min) by two independent observers. Monitored postpartum behaviors include the following: (1) mother in the nest; (2) mother in contact with at least one pup; (3) mother contacting more than half the litter; (4) mother grooming a pup; (5) mother transporting a pup; (6) mother manipulating nonnest bedding; (7) mother manipulating nest bedding; (8) mother eating; (9) mother drinking; (10) mother self-grooming; (11) mother rearing or exploring cage; (12) mother resting away from the litter; (13)

mother passively nursing pups; (14) mother arched-back nursing pups; (15) mother blanket nursing pups. Pup-oriented behaviors then are summated from variables 4–5, 7, and 13–15. Self-oriented behaviors are then summated from 6 and 8–12 [65].

Another caveat is the possibility that a few male rats may also display depression which may skew the results. Accordingly, employing the same CORT and behavioral depression criteria should identify any male rat that may be showing signs of depression. Pending demonstration of worsened stroke outcomes in postpartum depressed rats, the next logical step of evaluating efficacy of EPC transplants in this group of animals will be pursued in a subsequent study.

In summary, our hypothesis captures translational relevant questions (Figure 4), in particular whether gender, especially the female-inherent postpartum depression, affects the viability, proliferation, migration, and differentiation of stem cells and their functional benefits in repairing the brain after stroke. Animal models of female stroke are needed. We have recently developed a behavioral paradigm by which to study postpartum depression and stroke in rat dams. When EPCs were exposed to OGD, an experimental model of stroke, cultured male-derived EPCs displayed better proliferation and migration than female-derived EPCs which displayed an increased resistance to physiologic stress. Understanding the genotypic and phenotypic differences imparted by the gender of stem cell donors will further optimize the safety and efficacy of stem cell use for stroke. Using this behavioral paradigm will enable us to delineate mechanisms by which postpartum depression may exacerbate stroke morbidity through alterations in stem cell function. Overall, confirmation of our hypotheses will directly impact on advancing our knowledge on stem cell-based therapeutics for stroke and postpartum depression. *In vitro*, under ambient conditions both male- and female-derived EPCs did not differ in viability, stemness, proliferation, and migration.

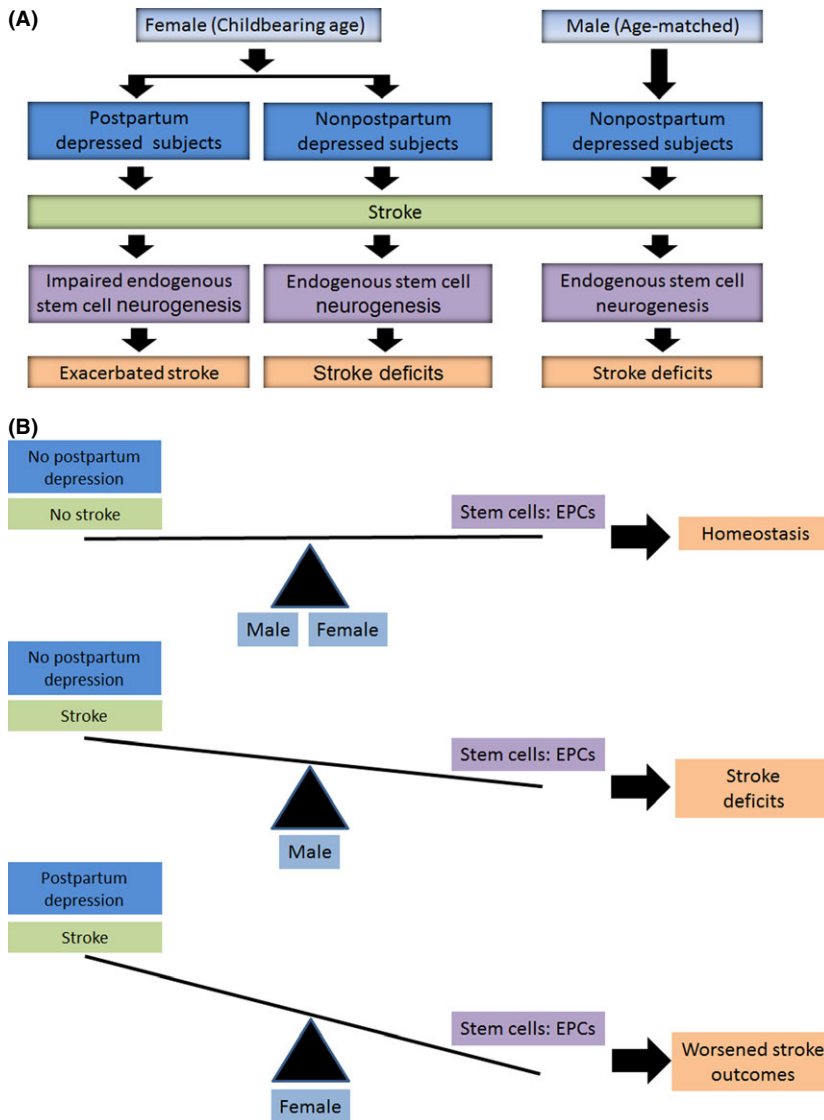


Figure 4 Our overarching hypothesis highlights the pathological link between postpartum depression and stroke via stem cell alterations. Panel (A) shows the sequence of events in female and male subjects capturing the interaction of postpartum depression and the resulting endogenous stem cell neurogenesis and stroke outcomes. Panel (B) provides scenarios of gender-linked alterations in stem cells (e.g., endothelial progenitor cells or endothelial progenitor cells) in normal and stroke conditions, with or without postpartum depression and the envisioned stroke outcomes.

Acknowledgments

Financial support for this study was provided by the University of South Florida Women’s Health Collaborative Grant, the University of South Florida Signature Interdisciplinary Program in Neuroscience funds, the University of South Florida and Veterans Administration Reintegration Funds, and the University of South Florida Neuroscience Collaborative Program.

Conflict of Interest

CVB is funded by the National Institutes of Health 1R01NS071956-01A1, James and Esther King Biomedical

Research Foundation 1KG01-33966, SanBio Inc., KMPHC and NeuralStem Inc. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures

The authors have read the journal’s policy and have the following conflicts: CVB is supported by National Institutes of Health, National Institute of Neurological Disorders and Stroke 1R01NS071956-01, James and Esther King Foundation for Biomedical Research Program, SanBio Inc., KMPHC and Neural Stem Inc.

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