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Sex Differences in Neuroinflammation and Neuroprotection After Ischemic Stroke

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Abstract

Stroke is not only a leading cause of mortality and morbidity worldwide it also disproportionately affects women. There are currently over 500,000 more women stroke survivors in the US than men, and elderly women bear the brunt of stroke-related disability. Stroke has dropped to the fifth leading cause of death in men, but remains the third in women. This review discusses sex differences in common stroke risk factors, the efficacy of stroke prevention therapies, acute treatment responses, and post-stroke recovery in clinical populations. Women have an increased lifetime risk of stroke compared to men, largely due to a steep increase in stroke incidence in older postmenopausal women, yet most basic science studies continue to only evaluate young male animals. Women also have an increased lifetime prevalence of many common stroke risk factors, including hypertension and atrial fibrillation, as well as abdominal obesity and metabolic syndrome. None of these age-related risk factors have been well modeled in the laboratory. Evidence from the bench has implicated genetic and epigenetic factors, differential activation of cell-death programs, cell-cell signaling pathways, and systemic immune responses as contributors to sex differences in ischemic stroke. The most recent basic scientific findings have been summarized in this review, with an emphasis on factors that differ between males and females that are pertinent to stroke outcomes. Identification and understanding of the underlying biological factors that contribute to sex differences will be critical to the development of translational targets to improve the treatment of women after stroke.

Keywords

Cell death; hormones; X-chromosome dosing; epigenetics

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Conflict of Interest

The authors declare no conflict of interest.

Role of Authors

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Introduction

Stroke is a major cause of death and disability in the United States. Stroke is the 3rd leading cause of death in women in the United States, but has fallen to the 5th leading cause of death in men (Bushnell et al. 2014; Writing Group et al. 2016). Female stroke survivors are more likely to be disabled and living alone after stroke, contributing to the 3.5 higher rates of nursing home placement compared to male stroke survivors (Gargano et al. 2007; Lai et al. 2005; Sohrabji et al. 2013; Writing Group et al. 2016). The National Institute of Health has recognized that understanding the biological differences between sexes is imperative to development of effective therapies (Clayton and Collins 2014; Manwani et al. 2013). There are more than 3.8 million women and 3 million men living in the United States today as stroke survivors, and caring for survivors with significant disability has placed a large burden on the health care system (Liu and McCullough 2012; Writing Group et al. 2016). Importantly, these numbers will only continue to increase in both men and women as our society continues to age, highlighting the urgent need for more therapeutic options for stroke patients (Oertelt-Prigione 2012).

Currently, tissue plasminogen activator (tPA) is the only FDA approved pharmacological therapy for the treatment of acute ischemic stroke. Due to the narrow post-stroke therapeutic time window and numerous contraindications however, less than 10% of patients with acute ischemic stroke qualify for tPA administration (Boehme et al. 2014; Writing Group et al. 2016). In addition, the use of thrombolytic agents increases the risk for other complications, in particular intracranial hemorrhage. Other ischemic stroke patients may be eligible for endovascular intervention, angioplasty or stents. Experts agree however, despite the progress in understanding the etiology of stroke, clinical treatment options remain limited for most patients. Identifying and developing novel, efficacious, and safe neuroprotective agents is critical if we hope to reduce stroke-related morbidity and mortality. Due to the differences in prevalence of stroke in male and female populations and the variation in ischemic sensitivity across the lifespan, research efforts with a focus on sex differences in neuronal cell death signaling, immune responses and inflammation have recently received more attention (Klein et al. 2015; Liu et al. 2009; Manwani et al. 2013; Mirza et al. 2015). These investigations have aimed to understand sex-specific neuroprotection in the context of stroke. New bench studies and clinical trials are emerging with the conclusion that sex does matter in the response to ischemic stroke.

In general, ischemia causes two types of tissue damage: the initial damage from loss of blood flow resulting in neuro-glial apoptosis and necrosis, and a secondary, more prolonged phase of tissue damage due to the immune response induced by injury. Both types of damage vary by sex (Ahnstedt et al. 2016; Boehme et al. 2014; Crislip and Sullivan 2016). Initially, it seems intuitive that an identical hypoxic event should lead to similar neuro-glial responses in males and females, until one considers the dramatic differences in hormonal exposure, physiology, and biology between sexes. Existence of significant sexual dimorphism in a variety of cell types and their function has now been supported by evidence from fields of genetics, epigenetics, immunology, cellular physiology and neuroscience.

This review summarizes sex differences in selected cellular mechanisms that may contribute to clinical differences between male and female stroke patients. These mechanisms include apoptotic signaling cascades in neurons and glia, resident microglial activation, neuro-glial response to ionic imbalance, autophagy, and mitochondrial toxicity (Ahnstedt et al. 2016; Demarest and McCarthy 2015). Further progress in understanding and manipulating these mechanisms can lead to the identification of endogenous and therapeutic neuroprotective agents with successful clinical applications.

We will also discuss recent studies that show that sex-specific immune response that may contribute to outcomes after ischemic stroke. Sex differences in immunity have been documented for decades, although the consequences of these differences have only recently been investigated. These differences are important from an evolutionary standpoint, as women must be able to prevent “refection” of fetal tissue, but may contribute to increased rates of autoimmune diseases due to exposure of women to foreign antigens from paternally derived tissue/antigens (Hendrickson and Delaney 2016). The development of the immune system occurs in the setting of the XX versus XY chromosomal background, with an additional contribution from gonadal hormones (Arnold et al. 2016; Fischer et al. 2015; Trigunaite et al. 2015). Such differences contribute to sex differences in the response to vaccination. For example, vaccine-triggered antibody responses to diverse list of vaccines including the polio vaccine, the influenza vaccine, and the yellow fever virus vaccine are different in men and women (Ainbender et al. 1968; Furman et al. 2014; Klein et al. 2015). Sex differences have also been well documented in autoimmune diseases such as lupus, where incidence rates are drastically higher in women (Der et al. 2014; Trigunaite et al. 2015). The etiology underlying differences in the immune response in males and females is not known but likely multifaceted. Factors that differentially modulate the immune system will be briefly discussed: the different endocrine states of the system (i.e. hormone levels), sex-specific genetic coding (XY vs. XX), and epigenetic control of gene expression to name a few. There are other possible contributors to sex differences in the response to ischemic stroke, such as sex-specific changes in the microbiome, metabolism, and coagulation that will not be discussed here but have been recently reviewed (Maney 2016; Roy-O'Reilly and McCullough 2014).

Sexually Dimorphic Cellular Mechanisms Relevant in Ischemic Stroke

An essential step in the development of effective neuroprotective therapies is to better understand cell mechanisms that lead to cell death during ischemia and how sex differences impact on these mechanisms. Fundamental cell signaling pathways such as mitochondrial metabolism and apoptotic cascades (Demarest and McCarthy 2015) have been shown to be different in males and females and more specifically, it is increasingly recognized that the response to cerebral injury *in-vivo* and *in-vitro* is at least partially a function of the sex of the *cell*. These differences in activation of apoptotic pathways also vary with age. Work in our lab and by other research groups has demonstrated sex differences in ischemia-induced cell death pathways in young, adult, and aged animals (Hagberg et al. 2004; Liu et al. 2009; Renolleau et al. 2007a). The response to stroke is sexually dimorphic even in neonatal models, suggesting that changes are patterned early in development and are in part, independent of the activational effects of gonadal hormones (Hagberg et al. 2004; Rosen et

al. 1999). The growing list of signaling molecules with sexually dimorphic role in cerebral ischemic cell death include Nicotinamide adenine dinucleotide (NAD⁺), apoptosis-inducing factor (Zhu et al. 2006), caspase 3 (Liu et al. 2009), poly ADP ribose polymerase (PARP) (Siffrin et al. 2010), nitric oxide synthase (NOS) (McCullough et al. 2005; Park et al. 2006), glutathione (Siffrin et al. 2010), Akt (Kitano et al. 2007), astrocytic aromatase (Liu et al. 2007), glial fibrillary acidic protein (GFAP) (Cordeau et al. 2008), angiotensin II type 2 receptor (Sakata et al. 2009), and the soluble epoxide hydrolase (sEH) (Zhang et al. 2009). As an example, NAD⁺ levels are different at baseline and after experimental stroke between males and female animals. Treatment with nicotinamide preferentially protects males while it shows minimal benefits in the wild-type female mice (Siegel and McCullough 2013), however clinical trials rarely evaluate men and women independently.

Sex Specific Analysis is Lacking in Many Clinical Trials

Despite urging from the NIH and basic scientists, clinical trials continue to “lump” men and women together. One recent example is the SPRINT trial, reported in the New England Journal of Medicine (Sprint Research Group et al. 2015). This trial will change the way we treat patients with hypertension. Blood pressure goals have already been adjusted based on the results that demonstrated that a systolic blood pressure of 120 or below reduced the risk of the primary outcome (a composite of myocardial infarction, acute coronary syndrome, stroke, heart failure, or death from cardiovascular causes. However, the benefit of lower blood pressures was only significant in men (HR = 72; CI = 0.51-0.86 in men; HR = 84; CI = 0.62-1.14 in women) (Sprint Research Group et al. 2015). The trial was stopped early, but the benefit of targeted blood pressure reduction to 120 was never proven in women (as the CI crossed 1.0). In the future trials, it will be critical to allow for adaptive trial design based on emerging results, which would allow for continued enrollment of women until significance (or lack of an effect) is seen.

Sex Differences in Cell Death

The activation of cell death signaling pathways after a vessel occlusion event can induce sex-specific combinations of cell death programs. Next, we will briefly discuss sex differences between caspase-independent, caspase-mediated, and hypoxia-induced cell death programs.

Caspase-Independent Cell Death and its Modulating Factors

Neuronal death in the hippocampal CA1 region increases in females or in estradiol-treated surgically ovariectomized rats after global cerebral ischemia. One hypothesis is that estradiol might have differential actions in CA1 hippocampus (Zuo et al. 2013). Both receptor subtypes for estrogen, ER- α and ER- β , have been implicated in estrogen mediated neuroprotection. Our group and others have reported the effects of estrogen replacement therapy on stroke incidence and severity in animals (Oertelt-Prigione 2012). Our studies have shown a sexual dimorphic effect of estrogen replacement that is dependent on the age of the animal examined. Males benefit from estrogen, regardless of the timing or age of initiation, but females have a paradoxical response with aging. Estrogen given at the time of ovariectomy in young mice, or supplemented in middle age reduced injury from an induced stroke, but exacerbated injury when given after a prolonged period of gonadal senescence. We found that a pro-inflammatory milieu develops with age in females, and estrogen

interacts, in a negative way, with the aging in the female brain (Liu et al. 2012). This suggests the timing of initiation of estrogen is critical, and demonstrates the importance of studying sex differences with the appropriate animal model.

Stroke, Aging, and Hormonal Replacement

Since stroke is much more common in aging populations, the dramatic changes in physiology resulting from gonadal senescence need to be recapitulated in the laboratory. For instance, the negative results of estrogen therapy in the Women's Health Initiative (Hendrix et al. 2006) showed an increase in stroke risk in women if estrogen treatment was initiated many years after menopause. Despite these results, it was recently reported that oral estradiol therapy was associated with less progression of subclinical atherosclerosis, measured as carotid-artery intima-media thickness (CIMT) as compared to placebo when therapy was initiated within 6 years after menopause but not when it was initiated 10 or more years after menopause (Hodis et al. 2016). Thus, it is safe to assume that the effect of estrogen is highly dependent on the age of the animal or patient examined. Clinical trials must take pre-clinical findings into account and pre-clinical studies must be designed to mirror co-morbidities seen in patients. In addition to effects of estrogen, other factors have been reported that demonstrate sexually dimorphic cell death pathways are activated following stroke, which have largely been ignored in pre-clinical studies. PARP-mediated neuronal death is one example.

Sex Differences in Cell Signaling Pathways

Poly ADP-ribose polymerase (PARP), a DNA repair enzyme, is a mediator of caspase-independent apoptotic signaling. PARP induces a well-recognized programmed cell death involving neuronal nitric oxide synthase (nNOS), PARP-1, and apoptotic inducing factor (AIF). PARP-induced cell death is sexually dimorphic and plays a key role in the male brain (Yuan et al. 2009). nNOS inhibition has been shown to be neuroprotective in adult male mice but exacerbates the stroke-induced damage in adult female mice. Interestingly, this effect of nNOS inhibition seems to be hormone-independent (McCullough et al. 2005). Other studies have shown protective effects of PARP-1 deletion in male pups in neonatal hypoxic ischemic (HI) injury models with no effects on female pups (Hagberg et al. 2004). The same group has shown female P9 mice tend to have significantly more caspase-3 activity after a hypoxic ischemic injury. Intriguingly, these PARP-1-induced effects show not only sex-specific patterns but are also different between pups and adult models. Deletion of PARP exacerbates injury rather than simply being ineffective in adults, suggesting that aging might modify the sex differences. Our work suggests that in young adult females, loss of PARP allows for the enhancement of caspase-mediated cell death, which is more detrimental in females (Liu et al. 2009). How this "shunting" occurs is unknown.

Minocycline, a PARP-1 inhibitor has been investigated as a potential treatment for stroke patients (Lampl et al. 2007; Liu et al. 2009). However, no pre-clinical trials have been published in females. Work in our lab has shown that minocycline does not protect ovariectomized female mice despite having a robust effect in reducing the infarct size in male mice (Li and McCullough 2009). Further, sex differences were found when inhibition effects of PARP-1 on the SRY gene, the sex-determining region of Y chromosome, were

experimentally eliminated. The results show that the reduction in PAR polymer formation is not different in males and females given minocycline, suggesting a non-linear relationship between PAR polymer and infarct size, at least in females (Li et al. 2006). These sex differences have now been shown to be important factors in the response to neuroprotective agents in clinical populations as illustrated by the recent results of an open-label evaluator-blinded trial of Minocycline. The National Institute of Health Stroke Scale (NIHSS) score in ischemic stroke patients was significantly lower in Minocycline-treated patients compared with control-treated individuals on day 90. However, when analyzed based on the sex of the patient treated, women had no significant clinical improvement with treatment. The beneficial effects of Minocycline were solely driven by the beneficial response observed in men (Amiri-Nikpour et al. 2015). Including women in clinical trials in sufficient numbers to allow for sex specific analysis is critical, as therapies effective in one sex may be ineffective, or may even worsen, patient outcomes in the other sex.

Caspase-Mediated Cell Death and its Modulating Factors

A primary process causing neuronal damage following ischemia is excitotoxicity. Loss of oxygen and glucose supply to the neurons prevents their ATP production. Lower ATP levels slow down Na^+/K^+ ATPase pumping action leading to accumulation of Na^+ inside the cell leading to accumulation of Ca^{2+} in the cytosol and swelling of the cell. Higher Ca^{2+} levels in the neuron leads to hyper-excitability and activation of proteases and caspases, which mediate organelle damage and eventually programmed cell death (Manev et al. 1989). Caspase-dependent neuronal death triggered by the influx of Ca^{2+} into the cell, induces the formation of the mitochondrial pore opening, permeabilization of the mitochondrial membrane, and the release of cytochrome c into the cytosol, which activates a cell death program. Critical to note is that caspase-mediated signaling pathways predominate in females, supporting sex differences in the interplay between caspase-dependent and caspase-independent pathways (Cheng and Hurn 2010; Koellhoffer and McCullough 2013; Liu et al. 2009; Whitacre et al. 1999). Interestingly, it has also been shown that female pups have more caspase activation than males, implicating once again that age modifies these sex differences (Le et al. 2002).

Studies using selective pan-caspase inhibitor, QVD-OPH, have shown higher sensitivity to caspase-dependent cell death in females (Liu et al. 2009; Renolleau et al. 2007b). More importantly, this effect of QVD-OPH was shown to be estrogen-independent, providing more plausibility that organizational or epigenetic differences exist in the caspase-dependent cell death between males and females (Liu et al. 2009). This may be related to expression of XIAP, the primary endogenous inhibitor of caspases, which is differentially regulated in males and females (Siegel et al. 2011).

Hypoxia-induced cell death

In another study using hypoxic-ischemic encephalopathy mouse model, Mirza et al. found that female neonates exhibited significantly smaller infarct size and fewer seizures compared to males 3 days after the insult. Females had less brain tissue atrophy and behavioral deficits compared to males. Male animals had increased microglial activation and higher inflammatory response compared to females after insult. Although primary brain injuries in

male and female neonates at the acute stages were equivalent, sexually dimorphic outcomes at later time points were demonstrated that were due to enhancement of inflammation (Mirza et al. 2015), suggesting sex differences in hypoxia-mediated cell death pathways. Defining specific mechanisms underlying sex differences in cell death mechanisms is necessary and urgent for development of sex-specific neuroprotective therapies, which may improve our ability to treat stroke patients.

Further investigation of sex differences in cell death pathways is necessary but insufficient since it yields a partial understanding of the overall sex differences in the outcome of ischemic stroke. The other equally significant component is the sex differences in the immune response to stroke, which could be both dependent and independent of differences in stroke-induced cell death pathways.

Sex Differences in Immune Response after Ischemic Stroke

Endocrine Influence and the Immune Response

Sex hormones not only influence neuronal death pathways but also exert their effects indirectly by modulating the immune system. Estrogen receptors expressed in T cells, B cells, NK cells (Ibanez et al. 2004), macrophages, and neutrophils indicate their responsiveness to estrogen. The effects of these sex hormones are dose-dependent, further supporting age-related differences in stroke outcome. For example, exposure of helper T cells to low dose of estrogen promotes a Th1 response whereas higher dose of estrogen favors a Th2 polarization (Ginhoux et al. 2010; Klein et al. 2015). As another example, progesterone reduces levels of pro-inflammatory cytokines (Robertson et al. 2015). In conjunction to modulating neurotransmission and suppressing microglial activation, progesterone also aids in myelin repair, suggesting its potentially positive effects on stroke outcome (Ghoumari et al. 2005; Ibanez et al. 2004).

Androgen levels drop immensely after both clinical and experimental ischemic strokes, raising the question whether ischemia-induced androgen loss may be as important as the steady levels of androgen prior to the ischemia. In bench studies that controlled for androgen levels, the data seem conflicting and indicate that androgens can either protect or exacerbate ischemic damage (Zuo et al. 2013). Interestingly, the androgen receptor gene is on the X chromosome, as are several other important apoptotic signaling molecules (like AIF, XIAP, etc.). The effect of X inactivation skewing on the response to androgens in females has not been investigated. In addition, as many androgens are aromatized to estrogen, the observed effects could be due to estrogen-mediated effects (Hurn 2014; Li et al. 2011; Persky et al. 2013).

Sex differences in inflammation, cytokines, and immune cells

In general, inflammatory mechanisms aim to clear pathogenic cells. The major pathways activated during inflammation are mediated by reactive oxygen species (ROS), apoptosis-inducing receptors, perforin and granzyme internalization and complement system activation. Thus far, some sex differences have been identified in the inflammatory response. For instance, cardiomyocytes from female mice exhibit greater survival in response to H₂O₂,

an ROS precursor, compared to male mice cardiomyocytes (Crislip and Sullivan 2016). This suggests innate cellular differences allowing cells from females to tolerate cellular stress and to resist inflammation-induced cell death longer than their male counterparts. This could also explain, at least in part, the differences in the T cell response between males and females. Detailed reviews of sex differences in T cell response have been recently published (Crislip and Sullivan 2016). Further, it has been shown that testosterone affects pathways of Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), activated following ischemia (Crislip and Sullivan 2016). Sex differences in the activation of complement system and other components of inflammation require future studies. Sex differences in macrophages (Mantovani et al. 2005), mast cells (Lindsberg et al. 2010; Strbian et al. 2006), dendritic cells, T cells (Felger et al. 2010; Wan 2010), and NK cells (Biron et al. 1999) have been recently identified (Bonneville et al. 2010; Yilmaz and Granger 2010). Table 1 summarizes reported sex differences for each subtype of immune cell.

One example of recently identified sex difference in inflammatory signaling after stroke is the cytokine IL-10, which is produced by T regulatory cells and Th2 CD4⁺ helper T cells. In ischemic stroke, an excessive IL-10 response contributes to post-stroke immunosuppression, which is known to worsen outcomes in terms of recovery (Conway et al. 2015). Similar but sex-specific results have been observed in murine models where female mice show an increase in a subset of IL-10 secreting CD8⁺ T cells after stroke, which is not seen in male mice (Banerjee et al. 2013). This supports the hypothesis that different amount of IL-10 secretion following stroke may be a contributing factor to the different clinical outcomes between males and females, but future studies are required. In addition to the endocrine influence and differences in inflammatory signaling, sex differences also result from differential genetic and epigenetic coding.

Sex-specific Genetic and Epigenetic Influences and Immune Response in Stroke

X chromosome dosing and the Four Core Genotype Mouse Model

Relative contributions of X and Y chromosomes to stroke outcomes have only recently been explored. The generation of the novel four core genotype (FCG) mouse model has allowed us to investigate the relative contributions of biological sex (XX vs XY) to stroke outcomes. FCG mouse are created by mating a WT female (XXF) and a transgenic male (XYM) in which the testis determining gene, *Sry* has been deleted from the Y chromosome and is inserted onto an autosome (Arnold et al. 2016; Li et al. 2014). The resulting four mouse lines are XXF (WT), XXM (with the *Sry* inherited on an autosome so the resulting animals develop testis and are phenotypically male despite the XX chromosome complement), XYF (that have the Y chromosome but no *Sry* and thus develop ovaries and secrete estrogen), and XYM (with *Sry* inherited on an autosome) (McCullough et al. 2016). Early works suggest that the contribution of chromosomal complement differs in the aged brain compared to that seen in young. Using the FCG mouse model, Liu et al. showed that in terms of infarct size, neurologic deficit score and immune cells infiltration and activation, the aged animals with XX chromosomes had worse stroke outcomes when compared to animals with XY chromosome (McCullough et al. 2016). In the same model, when we studied young

gonadally intact and gonadectomized mice, we found that in the young brain, the infarct size is predominantly driven by gonadal sex (ovaries versus testis) rather than chromosomal sex (Manwani et al. 2015). Therefore the difference in stroke outcomes is driven by different factors throughout the lifespan, i.e. gonadal sex in the young and chromosomal sex in the aged models. This also suggests an unappreciated effect of chromosomes on stroke sensitivity.

Histone Methylation

Epigenetic modifications are known to dictate patterns of gene expression in a variety of physiologic responses. Epigenetic regulation may play a role in the coordinated immune response in ischemic stroke. Specifically, when blood transcriptional profiles of the Toll-like receptor (TLR), T-cell receptor (TCR), and B-cell receptor (BCR) signaling pathways are examined, and there is a high correlation with DNA (cytosine-5)-methyltransferase 1 (DNMT1). Thus, epigenetic regulations may contribute to the coordinated response of innate and adaptive immunity in ischemic stroke and some other atherosclerotic diseases (Barr et al. 2015). Experimental studies show that activation of TLRs can, through epigenetic modification, induce diverse inflammatory responses (Foster et al. 2007). Histone methylation signatures have been correlated with regulation of transcriptional programs in effector and memory T cells (He et al. 2013) and the overall response following ischemic stroke (Chisholm et al. 2015; Zhao et al. 2016). Furthermore, recent evidence hints toward sexual dimorphism of these epigenetic codes. Histone-3 lysine-4 trimethylation (H3K4me3), a histone marker near the transcription start site of active genes is an example of a marker with sex differences. Shen et al. (2015) micro-dissected the bed nucleus of the stria terminalis and preoptic area in adult male and female mice and used ChIP-Seq to compare the genome-wide distribution of H3K4me3 (Shen et al. 2015). More than 200 genes and loci with a significant sex difference in H3K4me3 were found. Of these, the majority shows larger H3K4me3 peaks in females. At the baseline conditions however, only a minority of genes with a sex difference in H3K4me3 showed detectable sex differences in expression. Their data suggest that although baseline expression levels may not be significantly different, there may be sex biases in the use of epigenetic marks in the period following an ischemic insult on the brain tissue (Shen et al. 2015).

Together, these reports indicate substantial age- and sex-related differences in cell death pathways and immunity that are important in ischemic stroke outcome. These results and their relevance to clinical populations must be defined further but should, in the meantime, serve as a cautionary note for those investigators designing pharmaceutical trials.

Conclusion

Whether the ischemia-induced tissue damage is due to direct loss of blood flow or mediated by the subsequent immune response, there is no doubt that similar ischemic insults lead to different outcomes in males and females. Caspase-independent and caspase-mediated cell death programs initiated after ischemia are sexually dimorphic. The molecular mediators of inflammatory response to cerebral ischemic injury play sex specific roles. Similar primary brain injuries in male and female neonates lead to sexually dimorphic outcomes, suggesting sex specific inflammatory signaling, both independent of gonadal hormone activation and

modified with age. Although the effects of sex hormones have been under investigation for several years, results have been difficult to interpret, primarily due to high degrees of variability of these hormones with age and sex. The unappreciated effects of genetic coding and epigenetic control on stroke outcome throughout the lifespan have only recently been explored. In conclusion, sex differences in cell death signaling, immune response and post-stroke recovery require sex specific studies in patients and at the bench with hope of identifying sex specific translational targets and more efficacious stroke management.

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Reference for schematic image-pieces

- Neuron: <http://cinpla.org/2015/02/10/creating-a-3d-neuron-scene-in-blender-3>
- Astrocyte: <http://www2.eonexperience.com/eon-models/details.aspx?cid=2196>
- Microglia: <http://www.keyword-suggestions.com/bWljcm9nbGllh/>

Significance

Stroke disproportionally affects women. Women have an increased lifetime risk of stroke compared to men, due to a steep increase in stroke incidence in older postmenopausal women. However the vast majority of studies performed in the laboratory use young male animals or utilize cell culture systems where the sex of the cell is not known or reported. Growing recognition that the cell death pathways and inflammatory signaling cascades triggered by an ischemic stroke differ in males and females now mandates sex specific stroke management and drug development. Fundamental differences in how male and female cells handle ischemic stress has far reaching consequences that are relevant to multiple areas of medicine and biology, not just to stroke or to diseases that affect the brain. This review highlights identified sex differences in ischemic stroke in clinical populations and basic science/animal studies. There is a critical need for robust clinical trial design where sex differences can be properly investigated throughout the drug development process.

Figure 1.
Sex differences and the general immune response

Figure 2. Local and Immune Response to Ischemic/Reperfusion Injury; TGF (transforming growth factor); TNF (tumor necrosis factor); ROS (reactive oxygen species); NO (nitric oxide); IL (interleukin);

Table 1**Sex Differences in Specific Immune Cells**

Cell Type	Identified Sex Differences	Reference
Microglia	<ul style="list-style-type: none"> Female mouse microglia had higher expression of IL-4 and IL-10 receptors and increased production of IL-4, after IL-10 treatment; Males show higher Iba1, a microglia/macrophage-specific calcium-binding protein, in the proximity of the wound; Local microglia express arginase-1 and Neuroglobin, in a greater proportion in males than in females; 	(Ginhoux et al. 2010)
Macrophages	<ul style="list-style-type: none"> Sex differences in gene expression in macrophages grown from the bone marrow of male and female chickens after LPS stimulation; 	(Mantovani et al. 2005)
Mast Cells	<ul style="list-style-type: none"> xenoestrogens can induce mast cell degranulation, and its effects are additive with other xenoestrogens or estrogens; Presence of sex-related differences in the cardiotoxic effects elicited by doxorubicin and identified variations in the level of cardiac mast cells activity; 	(Lindsberg et al. 2010), (Strbian et al. 2006)
Monocytes	<ul style="list-style-type: none"> Female mice show reduced circulating neutrophilia and monocytosis vs. males, despite similar mobilization from BM stores following zymosan-induced peritonitis; 	(Felger et al. 2010)
Dendritic Cells	<ul style="list-style-type: none"> Males show higher % dendritic cells in intestinal Peyer's patches and spleen; In female HIV patients, dendritic cells produced larger interferon when TLR7 activated; DCs from hypertensive male mice have increased surface expression of the B7 ligands CD80 and CD86; 	(Felger et al. 2010), (Vinh et al. 2010)
Neutrophils	<ul style="list-style-type: none"> Little conclusive data on sex-differences was found; 	(Yilmaz and Granger 2010)
B Cells	<ul style="list-style-type: none"> B cell antibody responses to vaccines were consistently higher or equivalent in girls compared with boys when vaccinated for diphtheria toxoid, capsular group A, W, and Y meningococcal, and pneumococcal vaccines; 	
Helper T Cells	<ul style="list-style-type: none"> Males show lower % T cells in intestinal Peyer's patches and spleen; 	(Wan 2010), ***
Cytotoxic T Cells	<ul style="list-style-type: none"> T-cell population's origin of sex determines effect in hypertension; 	***
Regulatory T Cells	<ul style="list-style-type: none"> Females may selectively up-regulate. 	***
NKT Cells	<ul style="list-style-type: none"> Relatively little is known about sex differences. 	***
NK Cells	<ul style="list-style-type: none"> Males have less NK cells in spleen but more in intestinal Peyer's patches than females; Elderly women have more robust natural killer lymphocytes than do elderly men; 	(Biron et al. 1999), ***
$\gamma\delta$ T cells	<ul style="list-style-type: none"> Relatively little is known about sex differences. 	(Bonneville et al. 2010)

(Crislip and Sullivan 2016); abbreviations: IL (interleukin); NK (natural killer); NKT (natural killer T); Iba1 (ionized calcium binding adaptor molecule 1); HIV (human immunodeficiency virus); TLR (toll-like receptor);