HHS Public Access

Author manuscript

J Neurosci Res. Author manuscript; available in PMC 2017 July 02.

Published in final edited form as:

J Neurosci Res. 2017 January 2; 95(1-2): 681–691. doi:10.1002/jnr.23855.

Sex Differences in Stroke Therapies

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Abstract

Stroke is the 5th leading cause of death and acquired disability in aged populations. Women are disproportionally affected by stroke, having a higher incidence and worse outcomes than men. Numerous preclinical studies have discovered novel therapies for the treatment of stroke, but almost all of these were found to be unsuccessful in clinical trials. Despite known sex differences in occurrence and severity of stroke, few therapeutics, both preclinically and clinically, take into account possible sex differences in treatment. Reanalysis of data from the only currently FDA-approved stroke therapy, tPA, has shown to not only improve stroke outcomes for both sexes, but to also show sexual dimorphism by more robust improvement in stroke outcome in females. Experimental evidence supports the inclusion of sex as a variable in the study of a number of novel stroke drugs and therapies, including preclinical studies of anti-inflammatory drugs (minocycline), stimulators of cell survival (IGF-1), and inhibitors of cell death pathways (pharmacological inhibition of PARP-1, NO production, and caspase activation), as well as in current clinical trials of stem cell therapy and cortical stimulation. Overall, study design and analyses in clinical trials, as well as in preclinical studies, must include both sexes equally, consider possible sex differences in the analyses, and report the differences/similarities in more systemized/structured way to translate promising therapies to both sexes and increase stroke recovery.

Keywords

stroke; sex differences; treatment; preclinical; clinical trials

OVERVIEW

Stroke is a cerebrovascular disease caused by interruption of the blood supply to the brain, resulting in rapid death of neurons and, consequently, a range of neurological problems including loss of sensory or motor function, paralysis, depression, dementia, epilepsy, and even death. Stroke is the 5th leading cause of death and leading cause of disability in the United States (Mozaffarian et al. 2015). Stroke can be classified broadly into 2 types:

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ROLE OF AUTHORS

All authors participated equally in the writing of this review.

ischemic stroke and hemorrhagic stroke. In an ischemic stroke, a portion of the brain is deprived of blood (and therefore glucose and oxygen), due to a clot that obstructs blood flow or due to narrowing of blood vessels. In a hemorrhagic stroke, a blood vessel ruptures, causing blood to flood into the brain, where it eventually clots. Ischemic strokes are more common (87%) as compared to hemorrhagic strokes (13%), however, hemorrhagic strokes are more severe and likely to result in death.

Globally, 15 million people will suffer a stroke every year and approximately 40% of these patients will die and about 30% will be permanently disabled. In the US, the incidence of stroke is higher among older individuals, such that 25% of all strokes occur below the age of 65, while 75% of all strokes occur in populations 65 and older (US Centers for Disease Control and Prevention) (Statistics and Research 2011). However, a principal variable affecting stroke incidence in aging is the biological sex of the patient. Women are more likely to get a stroke (Petrea et al. 2009), to display more non-classical stroke symptoms, and to have worse stroke outcomes. Thus, while stroke is the 4th leading cause of death overall in the US, it is the 5th leading cause of death in men, and the 3rd leading cause of death in women (Statistics and Research 2011). In fact, the rates of stroke-related death have declined over the last 25 years for men but not women (Roger et al. 2011). Women account for 60% of stroke-related deaths (Lloyd-Jones et al. 2010). The 5 year stroke recurrence is also disproportionately higher in females (20%) as compared to males (10%) in the 45–64 age range (Roger et al. 2011). Despite the observation that stroke size tends not to be different in males and females (Silva et al. 2010), a Canadian stroke registry study reported that 10% of women stroke patients were discharged to long-term care as compared to 5% of men (Kapral et al. 2005). Furthermore, since women live longer than men, it is projected that stroke-related disability and institutionalization is likely to affect women more than men (Lai et al. 2005).

Sex differences in the prevalence of stroke may be attributed both biological and sociocultural factors. In part, greater longevity among women ensures that they are overrepresented in the age groups where strokes are common. Other factors such as outliving their spouse and/or living alone may also delay their access to health care facilities when a stroke occurs, resulting in worse outcome and poor functional recovery. This is made worse by the time limitations for current stroke therapy. The most commonly used intervention, intravenous treatment of the thrombolytic tissue plasminogen activator (tPA) has an optimal time window, and is not recommended for use later than 4.5 hours after stroke onset. Therefore, delay in treatment for women with stroke can decrease their chances of receiving tPA treatment and increase the damage from an ischemic stroke.

Irrespective of the reasons underlying the greater prevalence of stroke in women or the more severe outcomes, there is an urgent need for stroke therapies that can improve outcomes and can be delivered on a broader/delayed time frame. Although not systematically studied, existing data on preclinical and transitional therapies suggests that there may be sex-specific effects of stroke neuroprotectants and therapies. This review will focus on current research on stroke therapies with an emphasis on how these therapies affect stroke outcomes in males and females. We have also included a summary of sex differences in stroke therapy in Table 1. Throughout this review, the term ‘(biological) sex’ is used instead of gender. There is an

emerging literature in cardiovascular medicine where gender identity influences disease outcomes, however, in the context of stroke therapies, which is the focus of this review, such distinctions have yet to be studied.

STROKE THERAPEUTICS - CLINICAL

Acute stroke presents a critical challenge for the ER physician and staff. Initially the challenge is to identify whether the patient has had a stroke, excluding mimicking conditions such as Bell's palsy, meningitis, and diabetic confusion, among others. In the case of ischemic stroke, a critical decision is whether the patient is eligible for tPA.

Tissue plasminogen activator (tPA; Alteplase) is the only FDA-approved therapy for stroke, and its mode of action consists of proteolytic degradation of the clot, with the goal of reestablishing circulation, known as recanalization. tPA has also been shown to increase the risk for hemorrhagic transformation, which occurs subsequent to ischemic stroke and cerebral infarction (The NINDS rt-PA Study Group (1995), even in eligible patients (Katzan et al. 2000). Although hemorrhagic transformation (intracerebral hemorrhage) may occur spontaneously after ischemic stroke, thrombolytic therapy occasionally leads to this complication, possibly due to the actions of tPA on matrix metalloproteinases (Tsuruoka et al. 2014). In animal models, tPA increases permeability of the blood brain barrier in aged (18–20 month old) male Wistar rats as compared to young (3–4 month old) males and is associated with disassembly of endothelial tight junction proteins such as claudin and occludin (Kaur et al. 2011).

Sex differences in treatment among patients that receive tPA may also factor into the sex differences in stroke outcomes. In a study spanning over a decade (1997–2006), men were more likely than women to receive intravenous (IV) tPA, angioplasty/stents, carotid endarterectomy, or cardiac reperfusion. However towards the end of the study period, sex differences in the use of IV tPA were eliminated (Towfighi et al. 2013), which suggests that greater overall tPA use and an emphasis on early time-to-treatment may decrease sex differences in acute stroke care. More recently, a comparison of white and black male and female stroke patients found no differences in the outcome of tPA administration in men, but reported that black women were less likely to get tPA than white women (Boehme et al. 2014), and that short-term stroke outcomes among African American women that received tPA were no different from controls (Mandava et al. 2013). In a regional study, women were more likely to be excluded from tPA for hypertension as compared to men, suggesting that under-treatment of stroke risk factors in women may further impact stroke therapies as well (Madsen et al. 2015).

Outside of differences in administration of tPA, clinical studies have indicated that thrombolytics have differing efficacy on stroke outcomes between the sexes. Reanalysis of data from multiple clinical tPA studies (ATLANTIS, ECASS II, and CASES) show that women were more likely than men to have an improved 90 day outcome in response to tPA treatment (Kent et al. 2008; Kent et al. 2005). In other words, placebo-treated women faired significantly worse than men, but there was no difference in outcome between the sexes in the tPA groups, thereby increasing the outcome in women to a greater extent than men. This

was further validated using data from the Safe Implementation of Treatments in Stroke-International Stroke Thrombolysis Register (SITS-ISTR) (Lorenzano et al. 2013). An additional thrombolytic, recombinant prourokinase (r-proUK; Prolyse) showed enhanced effects in women. In a reanalysis of the PROACT-2 clinical trial data, like with the tPA trials, untreated women had significantly worse outcomes than untreated males, but there was no difference between males and females who received r-proUK (Hill et al. 2006). Interestingly, for patients that are less responsive to acute tPA, this sex difference may favor improvement in males instead of females (Elkind et al. 2007), which may be due to improved recanalization rates of women in the acute phase of thrombolytic treatment (Savitz et al. 2005). However, for r-proUK treatment, at 2 hours post-treatment there were no differences in recanalization rates between males and females, suggesting that the difference in recanalization rates does not explain all of the improved outcomes in females in response to thrombolytics (Hill et al. 2006).

Overall, there is strong evidence that thrombolytic therapy has increased therapeutic efficacy in women compared to men. Increased assignment of tPA therapy to women should be encouraged as treatment outcomes do not differ between tPA treated men and women, while in non-tPA administered groups, males were more likely to have a better neurologic score as compared to women (Shobha et al. 2010). This conclusion is also supported by data from endovascular intervention trials, where mechanical removal of the clot is performed to reestablish circulation in large vessel occlusion stroke. Meta-analysis of the 5 recent randomized trials shows that the scores on the modified Rankin Scale (mRS) are significantly improved in both men and women after endovascular thrombectomy and improvement is independent of alteplase treatment (Goyal et al. 2016). Therefore, a push to enhance treatment rates of women, either by mechanical or chemical thrombectomy could greatly improve stroke outcome

FAILED STROKE TRIALS

Although several drugs have been identified in preclinical studies, only a few of these have made it to clinical trials and none have succeeded (Chacon et al. 2008). These include the SAINT I and SAINT II trials that tested the free radical scavenger NXY-059; the RANTTAS trials for tirilazad mesylate, a lipid peroxidation inhibitor; and the INVEST trials for the calcium channel blocker verapamil SR co-administered with the angiotensin-converting enzyme inhibitor trandolapril. While several reasons may explain why the preclinical promise of these drugs was not borne out in clinical trials, in at least one case (tirilazad mesylate), European trials showed the outcomes were much worse in women as compared to men (Tirilizad Steering Committee, 2000). Preclinical studies with these drugs routinely failed to use clinically relevant animal models, such as the aged, and include females and those with comorbid diseases (van der Worp et al. 2005). These and other studies provided the impetus for the STAIR recommendations, which specifically included recommendations for clinically relevant animal models (Fisher et al. 2009)

Since tPA is mainly responsible for recanalization, most preclinical studies have focused on therapies that affect the survival of perilesional tissue. In animal models, cells around the infarct can display enhanced excitability 7 days post incident (Buchkremer-Ratzmann et al.

1996; Centonze et al. 2007), which then decreases but may persist for up to 4 months after stroke (Schiene et al. 1996). The increased calcium influx into neurons from the enhanced excitability can lead to mitochondrial stress and the activation of cell death pathways (Sims and Muyderman 2010; Szydlowska and Tymianski 2010). Therapies to combat the excitotoxicity and restore neuronal count and function have not resulted in consistent translational promise. Interestingly, while these studies have included respondents of both sexes, there has been a lack of subsequent analysis of sex differences in efficacy. Additionally, the efficacy of cortical stimulation and stem cell therapy varies with the sex of the patient and, in the case of stem cells, of the cell host. These will be explored further below (see Table 1 for summary).

Cortical Stimulation

During stroke recovery, there are long-term changes to the excitatory/inhibitory ratio in the cortex. While activity at the perilesional site can be over active and excitotoxic, overall on the ipsilateral hemisphere there is reduced cortical activity, but increased activity at distal brain regions which have circuitry to the affected region, particularly analogous structures in the contralateral cortex (Cramer 2008). These long-term activity changes can be on the scale of months to years, with studies have showing that activity changes in motor cortices in both hemispheres still occur between 2 to 4 months post stroke, and that alterations to activity required for language recovery can persist for a year or longer (Saur et al. 2006; Traversa et al. 1998). Pharmacological intervention to adjust activity after stroke has largely been focused on the acute stroke period but no treatment has made it through clinical trials. But two methods of cortical stimulation, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), have been investigated for their ability to improve stroke recovery.

Cortical activity can be directly stimulated by rTMS, or modulated by rTMS or tDCS (Fregni and Pascual-Leone 2007). In studies using stroke patients, both rTMS, using either high frequency stimulation to stimulate activity in the affected cortex (Khedr et al. 2005) or low frequency stimulation to decrease activity in the contralateral cortex (Conforto et al. 2012) can enhance functional outcome in physical therapy for hemiparesis. In non-stroke subjects, tDCS can control cortical activity, with cathodal stimulation can decrease cortical activity while anodal stimulation increases activity (Nitsche and Paulus 2000). Studies in stroke patients have shown that much like rTMS, tDCS therapies, in concert with physical therapy, have shown that both anodal stimulation of the ipsilateral cortex and cathodal stimulation of the contralateral cortex can have therapeutic effects on motor function (Bastani and Jaberzadeh 2012; Zimerman et al. 2012), and using these stimulation paradigms in concert can result in robust effects, with one study showing effects persisting a week post stimulus (Lindenberg et al. 2010).

These individual studies show the promise of cortical stimulation in stroke recovery. Overall, Cochrane reviews have shown that there is no consistent effect of rTMS (for measurements of daily living, motor function, cognitive function, depression) or tDCS (for aphasia and naming) in comparison to sham-treated patients, but suggest that more randomized control trials are need to fully determine the viability of using cortical stimulation in stroke

rehabilitation (Elsner et al. 2015; Hao et al. 2013; Pollock et al. 2014). A major issue with cortical stimulation and reproducible outcomes is that many variables need to be controlled for including stimulation strength, pattern of stimulation, location of electrodes, and individual variation in recovery (Gomez Palacio Schjetnan et al. 2013). The patients' biological sex has not been included in this list of variables to consider in cortical stimulation therapy, although evidence suggests that stimulus paradigms may have different effects on males and females. In a re-analysis of previous tDCS studies on healthy adult subjects, Kuo et al. (Kuo et al. 2006) showed that cathodal stimulation in the motor cortex cause greater inhibition of motor evoked potentials in females, and that the inhibition had a longer duration in females. Interestingly, there were no sex differences in response to anodal stimulation. Gennaro et al., (Gennaro et al. 2004), using rTMS, found a similar sex differences in cortical stimulation, with enhanced inhibition of motor evoked potentials in females. These sex differences may vary by brain region, such that for tDCS in the visual cortex, anodal, and not cathodal, stimulation showed sex differences, with females having an enhanced cortical excitation in response to visual stimulus, but with no difference in the duration of effect between the sexes (Chaieb et al. 2008). These studies all examined healthy young adults, hence more work needs to be done examining the effects of age and disease on sex differences in cortical stimulation. Since motor function restoration is a key therapeutic target in stroke recovery, leading to enhanced independence and quality of life, the inclusion of sex as a variable as in response to cortical stimulation could be valuable in elucidating appropriate paradigms for reproducible therapeutic effects.

Stem Cell Therapies

To recover cellular loss and repair disruption to neuronal circuitry, there are several clinical trials underway using stem cells to aid in tissue recovery. The sourcing of the cells can be allogenic, from immortalized cells lines and cultured stem cells, or autologous, including bone marrow and adipose tissue derived stem cells. Cells from all of these sources have a sex which could affect the outcome of therapy, and those possible differences have not been well explored.

The usage of immortalized cell lines for cell transplant has had some success in clinical trials. The NT2/D1 human embryonal carcinoma derived cell line (ATCC® CRL-1973™) can be mass differentiated into neurons with retinoic acid exposure (Layton Biosciences). These cells are safe in application for stroke therapy, and transplantation may be able to improve cognitive and motor function even years after stroke incidence (Kondziolka et al. 2005; Kondziolka et al. 2000). The parent cell line for the LBS-neurons was derived from a metastatic testicular tumor in a 22 year-old male, but not considered were sex differences and changes in efficacy of the male cells into male or female patients, especially in the Phase II trial where women were a small proportion of the patients examined (28%). Additionally, these studies present a possible confounding treatment in allogeneic cell application - the usage of anti-rejection drugs. These studies, and others, have used continuous cyclosporine A treatment which may have effects of its own along with differences in metabolism between males and females (Kahan et al. 1986). The inclusion of sex as a variable in studies of *ex vivo* stem cell application both of the cell line and of the patient, could help determine

if cells have better efficacy by genetic sex and if they provide a better outcome in patients in one sex over another.

Autologous stem cells include stem cells that occur in other regions of the body, including bone marrow and adipose tissue, as well as human-induced pluripotent stem cells (hiPSCs) derived from the patient. Studies have shown that stem cells derived from males and females can differ in their pro- and anti-inflammatory markers and may vary in their propensity for particular differentiation fates (Crisostomo et al. 2007; Ogawa et al. 2004). While autologous stem cells transplants have long been preferred because of decreased risk of rejection, recent studies have indicated that autologous transplants can also trigger immune responses, particularly induced pluripotent stem cells (iPSCs), therefore differences inflammatory markers could affect transplant stability even in an autologous transplant (Charron 2013; Zhao et al. 2011). Not only can inflammation arise from iPSC transplant, due to cell to cell variations in epigenetic markers, iPSC expansion can result in alterations to gene expression and an induction of oncogenic potential. For example, hiPSCs from females may lose expression of X-inactive specific transcript (XIST) and have a subsequent increase in oncogene transcription, thereby increasing the risk of tumor formation as a side effect (Anguera et al. 2012). For all stem cell transplants, a rigorous screening of inflammatory markers, alteration to gene transcription, and inclusion of cellular sex as a variable will greatly enhance the chance for success with stem cell therapies.

Of the completed and published clinical trials for stem cell usage, there is a disparity in patient recruitment between males and females, with only 40% (ranging from 16.7% to 56%) of patients receiving treatment being female. Most studies recruiting patients indicate that both sexes will be recruited, but even in the larger Phase II and III trials, only one current study states that they will examine the data to find the most appropriate target population. In the clinical trials of cultured cell lines, with the exception of LBS-neurons, there is no mention of the sex of the originating cells, and for allogenic cells there is no statement of control for the biological sex of the donor cells in comparison to the sex of the recipient. As we move forward with additional clinical trials for stroke treatment, the inclusion, or at least reporting, of sex as a variable will contribute to more efficacious treatment and enhanced recovery

SEX DIFFERENCES IN TREATMENT EFFICACY IN PRECLINICAL STUDIES

Sex differences in stroke outcome are also well recognized in preclinical models. Specifically, young females (rats and mice) have a smaller infarct volume and better cerebral blood flow than age-matched males both in normoglycemic (Alkayed et al. 1998) and diabetic (Toung et al. 2000) animals. Young females sustain a smaller infarct as compared to young males or aged female mice or rats (Selvamani et al. 2014), however, aging reverses this sex differences, such that aged females show poorer stroke outcomes and significantly more mortality compared to older males (Manwani et al. 2011). Sex differences have also been noted at middle age, where adult female rats have smaller infarcts than middle-aged females (Selvamani and Sohrabji 2010a).

The female advantage seen in animal models may be associated with either chromosomal sex or differences in gonadal hormones. In an interesting animal model called the 4-core genotype, chromosomal contribution and gonadal contribution can be evaluated for a specific disease in XX/Sry (genetic female, gonadal male) and XY/Sry$^-$ (genetic male, gonadal female) mice (Arnold and Chen 2009). Using this model, a recent study showed that sex differences in stroke are influenced by sex hormones and not by chromosomes (Manwani et al. 2015). This is consistent with a large body of experimental research that estrogen, the major ovarian hormone, may improve stroke outcomes. In ovariectomized females, replacement with 17-β estradiol, the inactive stereoisomer 17-α estradiol (Simpkins et al. 1997), or the conjugate equine estrogen preparation (McCullough et al. 2001) will reduce infarct volume in female animals. Interestingly, estrogen also reduces infarct volume in males, while the precursor steroid, testosterone, increases infarct volume in this group (Yang et al. 2002).

Thus, while estrogen availability is likely the reason for sex differences in infarct severity in young animals, estrogen treatment is protective for both sexes at this age. In the case of other preclinical stroke neuroprotectants, however, unexpected sex differences have been observed. Preclinical studies have focused on cell death effectors, immune modulators, and neurogenesis-angiogenesis modulators. While the majority of studies have used only males, usually young, several recent studies have included both males and females and those that displayed sex differences are summarized below.

Anti-inflammatory: Minocycline as a Case Study

A tetracycline antibiotic, minocycline, is clinically well tolerated and is shown to have neuroprotective effects on ischemic stroke in experimental models and clinical trials (Lampl et al. 2007; Li and McCullough 2009; Yrjanheikki et al. 1999). It is known to cross the blood–brain barrier, and once in the brain can attenuate neuronal apoptosis, reduce the inflammation response by reducing microglial activation and migration of T-cells, and inhibit matrix metalloproteinase-9, which remodels extracellular matrix (Fagan et al. 2011; Goldstein 2008; Machado et al. 2006; Machado et al. 2009; Matsukawa et al. 2009; Switzer et al. 2011; Switzer et al. 2012; Yang et al. 2015; Yrjanheikki et al. 1999). In experimental models of acute ischemic stroke, minocycline shows neuroprotective effects and improved behavioral outcomes in males (Alano et al. 2006; Li and McCullough 2009; Yrjanheikki et al. 1999).

In 2007, the first clinical trial of minocycline, an open-label blinded end point evaluation trial for acute ischemic stroke, randomly allocated 152 patients (35% female and 65% male) to placebo or oral minocycline 200mg daily for 5 days (Lampl et al. 2007). The study revealed that the patients treated with minocycline (n=74) had significantly improved outcomes as compared to placebo group (n=77) (Lampl et al. 2007). In 2012, a subsequent human trial found similar beneficial outcomes in a randomized, single-blind, open-label trial of oral minocycline 200mg daily for 5 days (n=23, 43% female and 57% male) versus control (n=27, 33% female and 67% male) with acute ischemic stroke (Padma Srivastava et al. 2012). More recently, in 2013, Kohler and colleagues conducted a randomized open-label blinded end point evaluation pilot study of intravenous minocycline 100mg every 12 hours

for a total of 5 doses in acute stroke [n=95, minocycline (n=47, 38% female and 62% male) and routine care control (n=48, 44% female and 56% male)]. The study found that intravenous minocycline was safe, but not efficacious (Kohler et al. 2013).

In this context, a study by Li and McCullough is particularly relevant. In C57BL/6 male and female mice subjected to middle cerebral artery occlusion, minocycline is effective in reducing infarct volume only in male mice. Furthermore, minocycline was also ineffective in ovariectomized female mice even though male and ovariectomized female mice present similar levels of estrogen (Li and McCullough 2009). Following this critical preclinical study, an open-label evaluator-blinded clinical study of minocycline for acute stroke found that oral minocycline, 200mg daily for 5 days, is effective only in male stroke patients, and not in females (Amiri-Nikpour et al. 2015). In this study by Amiri-Nikpour and colleagues, which included virtually similar numbers of females in treatment (53.8%) and control (51.9%). Male patients presented with significantly lower NIH stroke scale (NIHSS) in the minocycline-treated group compared with controls, while no significant clinical improvement was found in female patients between groups (Amiri-Nikpour et al. 2015). The authors also report an important point that the clinical improvement in the minocycline-treated group is significant among all patients (males + females) (Amiri-Nikpour et al. 2015), indicating study design and analyses in clinical trials, as well as in preclinical studies, must include both sexes equally, consider possible sex differences in the analyses, and report the differences/similarities in more systemized/structured way to translate promising therapies to both sexes.

Cell Survival Effector: Insulin-like Growth Factor-1 (IGF-1)

IGF-1 is one of the well-known neuroprotectants for ischemic stroke in young and aging animals for both males and females. Ischemic injury is more severe in older population as compared to the younger, and this difference is associated with reduced availability of IGF-1 (Selvamani and Sohrabji 2010a). Clinical observation supports that the availability of IGF-1, along with its binding protein IGFBP3, is decreased with age (Rosario 2010) and the ratio IGF-1 to IGFBP3 declines faster in males than females (Waters et al. 2003). Studies using male animal models support the hypothesis that IGF-1 plays a pivotal role in maintaining brain functions in acute ischemic stroke through various mechanisms including neuronal survival, anti-inflammation, and/or anti-thrombotic effects (Jin et al. 2013; Li et al. 2010; Patel et al. 2013). Exogenous IGF-1 treatment intranasally, intravenously, or intracerebroventricularly has been shown to decrease ischemic stroke injury (Lioutas et al. 2015; Liu et al. 2004; Rizk et al. 2007; Sohrabji 2015). Female animal models have demonstrated that post-stroke IGF-1 replacement reverses estrogen’s neurotoxic effects in middle-aged ovariecotmized female rats (Selvamani and Sohrabji 2010a; Selvamani and Sohrabji 2010b). IGF-1 adminstered to estrogen deficient (but not ovariectomized) middle aged females is also capable of improving reducing stroke-induced damage and motor impairment in the aging brain, and reduces blood brain barrier disruption and neuroinflammation (Bake et al. 2014).

It is clinically well established that serum IGF-1 levels are positively correlated to stroke outcome. Extensive numbers of clinical studies have shown that lower IGF-1 levels

significantly increase the risk of stroke and higher IGF-1 levels are associated with improved stroke outcomes, suggesting the circulating IGF-1 level may be used as a predictive value for ischemic stroke outcome (De Smedt et al. 2011; Dong et al. 2014; Tang et al. 2014) and post-stroke IGF-1 treatment may be beneficial to both sexes. However, a recent study using miRNA that regulate IGF-1 found sex differences in the efficacy of this treatment. Selvamani and colleagues have shown that intracerebroventricular anti-Let7f injection is effective only in intact females but not in males or ovariectomized females, suggesting that this miRNA action may be influenced by the hormonal milieu (Selvamani et al. 2012).

Cell Death Pathways

Poly (ADP-ribose) polymerase-1 (PARP-1)—Poly (ADP-ribose) polymerase-1 (PARP-1) activation is a major cytotoxic mechanism and plays a key role in the pathogenesis of cardiovascular and inflammatory diseases (Beneke 2008; Peng et al. 2012; Song et al. 2013; Sun et al. 2015). Emerging data suggests that cell death pathways in ischemic stroke are sexually dimorphic (Gibson 2013; Liu et al. 2011; Reeves et al. 2008; Yuan et al. 2009). In the previously cited study (Li and McCullough 2009), Li et al. additionally has found that minocycline does not impact ischemic injuries in PARP-1 null male mice, indicating sexually dimorphic neuroprotective effects may be attributed to PARP-1 inhibition in male mice whereas the pathway is not affected in females (Hagberg et al. 2004; Lang and McCullough 2008; Li and McCullough 2009; Liu et al. 2011; Mabley et al. 2005; McCullough et al. 2005). It has been shown that the downstream pathways of PARP including apoptosis inducing factor (AIF) and poly (ADP-ribose) polymerase (PAR) polymers also mediate cell death after ischemic insult only in males (Yuan et al. 2009).

Several studies suggest that pharmacological inhibitors of PARP-1 differentially affect males and females in response to ischemic stroke (Mabley et al. 2005; McCullough et al. 2005). Studies using male animal models support that classical (3-aminobenzamide) or selective (PJ34) PARP inhibitors reduce infarct volume and enhance long-term stroke recovery by mechanisms including suppression of the post-stroke neuroinflammatory response (Couturier et al. 2003; Hamby et al. 2007; Takahashi et al. 1999). More recently, a novel water-soluble PARP-1 inhibitor, MP-124, confers neuroprotection in focal ischemia by reducing NAD depletion and PAR formation in male Sprague-Dawley rats (Fujio et al. 2009). Interestingly, MP-124, can provide neuroprotection in both sexes by ameliorating the neurological deficits and brain infarct volume in male and female monkeys (Matsuura et al. 2011). Multiple PARP inhibitors are currently in Phase I to Phase III clinical trials for cancer treatment (Dean et al. 2012; Gelmon et al. 2011; Guha 2011; Wang et al. 2012), however, PARP-1 inhibitors for ischemic stroke lag behind in clinical trials (Ford and Lee 2011).

Nitric oxide (NO)—Similar to PARP pathway, nitric oxide (NO) is one of the key components in a mechanism of neuronal cell death in cerebral ischemia. The mechanism includes stimulation of neuronal nitric oxide synthase (nNOS), local accumulation of nitric oxide (NO), peroxynitrite formation and nitrosative DNA damage, and PARP-1 activation (Nemoto 2000; Stagliano et al. 1997; Zhang et al. 2013). Accumulating evidence support sex differences in nitric oxide synthesis levels. Forte and colleagues have measured NO biosynthesis by ^{15}N nitrate excreted in urine after the intravenous administration of L-

$[^{15}N]_2$-guanidino arginine in healthy population (Forte et al. 1998). Total nitric oxide production and release is significantly higher in females (n=11) as compared to males (n=11) (Forte et al. 1998), suggesting sexual dimorphism of NO and its related responses.

Preclinical studies show that reducing nitric oxide is neuroprotective only for males but deleterious for females (McCullough et al. 2005; Yuan et al. 2009). Female nNOS null mice exhibit worse outcome after middle cerebral artery occlusion (MCAO) relative to wild-type females, whereas absence of nNOS in male null littermates have a better outcome compared to wild-type males, suggesting that the neurotoxicity of nitric oxide production in ischemic stroke only occurs male mice, and not in females (McCullough et al. 2005). Estrogen, present in both males and females, promotes protection of endothelial function and vascular reactivity (dilation) by enhancing endothelial nitric oxide synthase functionality and NO production and manipulating endothelium-derived hyperpolarizing factor (EDHF) effectivity. However, testosterone has opposing effects, increasing cerebrovascular inflammation and cerebral artery tone (Haast et al. 2012; Krause et al. 2006).

Caspases—Caspase mediated cell death pathways also show sexual dimorphism. In females, caspase-dependent cell death pathways are initiated in the ischemic brain, whereas males tend to show a preference to PARP- and NO-dependent cell death under ischemic conditions (Gibson and Attwood 2015; Siegel et al. 2010). Evidence utilizing the selective pan-caspase inhibitor, quinoline-Val-Asp(Ome)-CH2-O-phenoxy (Q-VD-OPh), shows neuroprotective effects in both neonatal and adult female mice post ischemic stroke but no effects in males, indicating intrinsic sensitivity to caspase-mediated cell death mechanism in females (Renolleau et al. 2007). Additional evidence by Liu and colleagues shows that there is an early release of cytochrome C and increased caspase activation after stroke in female mice, but not males, and that Q-VD-OPh-treated female C57BL/6 mice have smaller infarcts and better neurological outcome, unlike male mice, where neuroprotection is not observed. (Liu et al. 2009), suggesting caspase inhibition may be beneficial mainly to females. Although caspase pathways are activated in both sexes after stroke, regulating this pathway promotes neuroprotection in females but not males. In contrast to pan-caspase inhibitor, caspase 9-specific inhibitor delivered intranasally spares brain damage and improves neurological outcome post stroke in male mice and rats.

Overall, evidence is accumulating that stroke-induced cell death pathways are sexually dimorphic: PARP- and NO-mediated cell death pathways predominate in males, while the caspase- and mitochondrial cytochrome c-dependent cell death pathways predominate in females (Table 1). Therefore, a push to include both sexes in preclinical research could greatly benefit clinical stroke trials.

CONCLUSIONS

It has been observed for decades that the preclinical promise of many drugs for stroke is not successfully translated to clinical trials. One of the major reasons for failed stroke trials may have stemmed from the exclusion of both sexes in preclinical studies and failure to stratify by sex in clinical trials. Even though aged women have a higher risk for stroke, worse outcomes, and poorer recovery after the insult compared to aged men, preclinical studies

routinely failed to utilize clinically relevant animal models (e.g. aged female model) and ignored sexual dimorphisms in underlying mechanisms. In addition, evidence that estrogen therapy may increase ischemic stroke in primary prevention studies and increase mortality in secondary prevention studies (reviewed in Hurn and Brass, 2003) suggests that hormone usage among women enrolled in clinical stroke trials should also be included. To translate promising preclinical therapies to the bedside, scientists and clinicians should consider sex as a critical biological variable in research design and analyses, should report the outcomes in more structured way and be cognizant of the fact that therapies may be sex specific.

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Significance

Stroke is the fifth leading cause of death in the United States. The incidence, age, presentation, and recovery from stroke differs between males and females. Unsurprisingly, current treatments for stroke have been shown to have a difference in efficacy between males and females, and research indicates that therapies that are in development or discovery should also consider differences in efficacy between the sexes. This review outlines the current literature on therapies for ischemic stroke and specifically illustrates that sex differences in treatment efficacy should be acknowledged and incorporated in study design to improve eventual stroke outcomes.

Table 1

Summary of sex differences in stroke therapies.

Therapy	Males	Females
Clinical		
Thrombolytics		
Tissue Plasminogen Activator	• Improves outcome	• Improves outcome • Females fair worse than men without tPA administration
Recombinant Prourokinase Endovascular Intervention	• Improves outcome • Improves outcome	• Improves outcome • Females fare worse than men without r-proUK administration • Improves outcome • Beneficial to both sexes independent of tPA treatment
Cortical Stimulation		
rTMS	• Can enhance recovery	• Can enhance recovery, • More effective, longer lasting inhibition in normal subjects
rTDS	• Can enhance recovery	• Can enhance recovery • More effective, longer lasting inhibition in normal subjects
Stem Cells		
Effects of Cellular Sex	• Different pro- and anti-inflammatory markers – effects not yet studied	• Different pro- and anti-inflammatory markers than females • May have a higher risk of tumor formation
Transplantation	• May improve recovery – sex differences not yet examined	• May improve recovery – sex differences not yet examined
Preclinical		
Ant i-Inflammation		
Minocycline	• Varying efficacy in human trials without examination of sex differentiation • In rodents, reduction of infarct volume may be limited to males	• Varying efficacy in human trials without examination of sex differentiation • In rodents, ineffective in females, both normal and ovariectomized
Cell Survival		
IGF-1	• Improves stroke outcome	• Improves stroke outcome • Can reverse estrogenic toxicity
anti-Let7F	• No Effect	• Improves outcome, only in gonadally intact

Therapy	**Males**	**Females**
Cell Death Inhibition		
PARP-1 Inhibition	• Required for minocycline effectivity • Can decrease infarct volume and ameliorate neurological deficits	• Can decrease infarct volume and ameliorate neurological deficits
Nitric Oxide Reduction	• Neuroprotective	• Harmful – can lead to worse outcome
Caspase Inhibition	• No effects	• Neuroprotective