REVIEW

Effect of Sex and Age Interactions on Functional Outcome after Stroke

Tae-Hee Kim^{1,2} & Raghu Vemuganti^{1,2}¹ Department of Neurological Surgery, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA² Neuroscience Training Program, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA**Keywords**

Aging; Cerebral ischemia; Hormones; microRNAs; Neuroprotection; Sex differences; Thrombolytic agents.

Correspondence

R. Vemuganti, Department of Neurological Surgery, University of Wisconsin, 600 Highland Ave. K4/870, Madison WI 53792, USA.

Tel.: +1-608-2658448;

Fax: +1-608-2631728;

E-mail: vemuganti@neurosurgery.wisc.edu

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SUMMARY

Stroke is one of the leading causes of death and disability worldwide. Experimental and clinical studies showed that sex and age play an important role in deciding the outcome after stroke. At younger ages, males were shown to have a higher risk for stroke than females. However, this trend reverses in older ages particularly when females reach menopause. Many preclinical studies indicate that steroid hormones modulate the age-dependent differential stroke outcome. In addition, patterns of cell death pathways activated following cerebral ischemia are distinct between males and females, but independent of steroid hormones. Recent studies also indicate that microRNAs play important roles in mediating sex-specific stroke outcome by regulating stroke-related genes. This review discusses the contribution of sex and age to outcome after stroke with particular emphasis on the experimental studies that examined the effects of steroid hormones, differential cell death pathways, and involvement of sex-specific microRNAs following cerebral ischemia. Current understanding of the role of thrombolytic agents in stroke therapy is also discussed.

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Introduction

Stroke causes extensive, irreversible brain damage in humans. Every year, ~795,000 people suffer a stroke in the United States and ~18% of them die. The survivors show severe, long-term disabilities such as loss of motor control, disturbed speech, and emotional problems [1]. In the United States alone, costs associated with stroke totals greater than \$36 billion annually, causing a significant burden on the healthcare system [2]. Despite such devastating disease outcome, there is only one FDA-approved option available for stroke therapy, the thrombolytic tissue plasminogen activator (t-PA) [3]. Though being beneficial for certain groups of patients, t-PA is not suitable for a large number of stroke patients given its short time window and serious side effects including intracerebral hemorrhage [4]. In an effort to replace t-PA with better therapeutic options, numerous molecular targets as well as neuroprotective agents have been identified using experimental stroke models [5]. However, all the neuroprotective candidates tested so far failed in the clinical trials, suggesting that there is a discrepancy between experimental rodent models of stroke and the human stroke patients. This poses questions such as: (1) Are we using the right experimental stroke models that best represent the clinical population? (2) Are we designing the clinical trials that best reflect the findings of experimental models? These are impor-

tant considerations to develop viable neuroprotective stroke agents.

Young adult male rodents are most commonly used in experimental stroke research as they exhibit minimal confounding variables when compared to other ages and sex. This is beneficial for identifying molecular mechanisms of ischemic brain damage, but it does not reflect the clinical population that shows comorbidities including hypertension and diabetes which are major risk factors for stroke. In addition, elderly are more prone to stroke. The brain undergoes numerous neurochemical and physiological changes as it ages resulting in the differential responsiveness to the therapies which also depend on the stage of the lifespan.

Aging leads to many changes in brain, and aged animals respond differentially to treatments. For example, brains of aged rats show decreased levels of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase that reflects higher oxidative stress, and decreased acetylcholinesterase activity that reflects impaired learning and memory [6]. In addition, melatonin (a potent antioxidant) protects nuclear and mitochondrial DNA and prevents apoptosis when chronically administered to older rats, but induces apoptosis when administered to younger rats [7]. These and other neurochemical and molecular changes in the aged brain might be responsible in part for the increased post-stroke brain damage in older individuals. Sex-dependent effects

are also seen in the incidence and outcome after stroke. The incidence of stroke onset seems to be higher in men at middle age than older age, whereas women show fewer stroke incidences during middle age that increases significantly at older age. Furthermore, poststroke motor disability, mental impairment and recurrence rate are higher in older females than older males [8,9]. Hence, sex and age should be taken into consideration when designing animal experiments that reflect clinical stroke population.

Effect of Sex and Age on Epidemiology of Stroke in the United States

Incidence, Prevalence, and Mortality

Data from Greater Cincinnati/Northern Kentucky Stroke Study and National Institutes of Neurological Disorders and Stroke show that ~55,000 more women have a stroke than men annually [10]. In general, women have lower stroke incidence than men before 75–80 years of age, while this trend reverses at >85 years of age [11,12]. Approximately 6.8 million Americans over 20 years or older had a stroke between 2007 and 2010, and the overall stroke prevalence in United States is ~2.8% per year [1]. In case of silent cerebral infarction, a higher prevalence was observed with increasing age [8,13,14]. It was observed that ~13 million people had silent strokes in 1998 [15,16]. A national cohort study reported that ~17.8% of the population over 45 years of age showed at least 1 stroke-related symptom [17]. It is predicted that stroke prevalence will increase among those aged 18 years and above in 2030 by >20% when compared to 2012. Although women overall tend to have less stroke incidence than men, mortality rate appears to be higher in women [18]. A decline in stroke mortality was also observed in men between 1981 and 2009 (1.11–1.05 male-to-female ratio), and this phenomenon was most salient in people in the age group of 45–64 [2]. The mean age at stroke death was ~79.6 years in 2002 with men having younger mean age than women [19]. As per United States Centers for Disease Control, the 30-day mortality rate between 1995 and 2002 was ~9% for the 65–74 years of age group (both males and females), while the 74–84 years age of group showed ~13.1% and the >85 years of age group showed ~23% [1].

Risk Factors

Blood pressure is the most important risk factor for stroke. Studies have shown that lower blood pressure can significantly reduce recurrent strokes as well as intracerebral hemorrhage [20]. A meta-analysis revealed that prehypertension is associated with stroke incidence part determinant of stroke risk. Similar to blood pressure, diabetes increases stroke incidence in middle age population (55–65 years) [21]. Data from a meta-analysis of randomized controlled clinical trials of interventions on prediabetic patients revealed ~24% reduction in fatal and nonfatal strokes [22]. Comorbidity of diabetes is also prominent in younger age group [23], and a longitudinal study on first-stroke patients (25–74 years old) showed a higher mortality rate in diabetic patients than nondiabetic patients, particularly in younger females [24].

Many other risk factors including disorders of heart rhythm [25,26], high blood cholesterol [27,28], smoking [29], physical inactivity [30], nutrition [31,32], and family history of stroke and genetics [33] have been implicated in stroke risk.

Sex-specific Pathologic Mechanisms

Estrogen and Stroke

As discussed above, females show less incidence of stroke when compared to the age-matched males at younger age, but that trend reverses at later age, particularly when females enter the postmenopausal stage. Hence, many studies evaluated the role of steroid hormones in the mechanisms of ischemic brain damage (Table 1). Consistent with the clinical observations, experimental studies showed that younger female rodents are more resistant to ischemic brain damage than younger male rodents [34–36], and estrogen and progesterone play important roles in providing neuroprotection following ischemia [37]. Estrogen administration also appears to be neuroprotective in males regardless of the male sex steroid availability, as both acute and chronic estrogen administration reduced infarct volume in normal as well as castrated male rats [38]. The efficacy of estrogens after stroke seems to be age sensitive in both sexes. Cai et al. reported that physiological doses of 17 β -estradiol (E₂) provide neuroprotection in young adult, but not in aged, females. This might be the consequence of decreased estrogen receptor density in the brains of aged females compared to the young adult females [39].

Several mechanisms were proposed for estrogen-mediated neuroprotection after cerebral ischemia. First, genomic effects of estrogen are modulated by the activation of estrogen response element (ERE) resulting in enhanced transcription of genes that contain ERE including *bcl-2* and *seladin-1* [40–42] (Figure 1). Once activated, these genes are implicated in cell proliferation and differentiation [43] as well as neuroprotection [44,45]. There is evidence that estrogen receptor ER- α plays a critical role in mediating the genomic effects of estrogen. ER- α mRNA and protein expression were shown to increase significantly in the cerebral cortex of female rodent brain following ischemia [46]. Moreover, in both male and female neuronal ER α knock-out mice, focal ischemia resulted in larger infarct volume even after E₂ treatment compared to the wild-type controls, indicating that the ER α is essential for the neuroprotective effects of estrogen after stroke [47]. ER α expression is regulated epigenetically. Westberry et al. [48] found that in ovariectomized female rats, ER α mRNA as well as protein are increased and ER α gene promoter undergoes demethylation by methyl CpG-binding protein 2 (MeCP2) dissociation following ischemia. Recent studies also implicated G protein-coupled estrogen receptor (GPER) in rapid nongenomic signaling events via the second messenger signaling cascades [49] such as phosphoinositide 3-kinase (PI3K) [50] and protein kinase C (PKC) [51], calcium mobilization [52], and ion channels [51,53] (Figure 1). Similar to classic estrogen receptor signaling, GPER activation is also associated with neuroprotection in brain injury after ischemia [54–56]. While GPER is present and widely distributed throughout the brain in both males and females, it was shown to be upregulated only in males after ischemia [57]. But surprisingly, adminis-

Table 1 Effects of sex steroids on ischemic brain damage

Steroid	Model	Species	Sex	Protection	Proposed mechanism	Ref.
Estrogen	tMCAO	Rat	F*	Yes	Reduction of infarct volume	[37]
	tMCAO	Rat	M	Yes	Reduction of infarct volume	[38]
	tMCAO	Mouse	F*	Yes	ER α -mediated	[39]
	pMCAO	Rat	F*	No	Increased infarct volume	[65]
	pMCAO	Rat, Mouse	F*	Yes	ER α -dependent	[46,47]
	pMCAO	Rat	F*	Yes	DNA methylation on ER α gene	[48]
	Global ischemia	Rat	F*	Yes	GPER-mediated	[54]
	tMCAO	Mouse	M; F; F*	N/A	GPER upregulated only in male	[57]
	tMCAO	Rat	F*	Yes	Enhances neurogenesis via SERM	[62]
Androgen	tMCAO	Rat	M	No	Increased lesion size	[70–72]
	tMCAO	Mouse	M	Mixed	Dose- and time-dependent	[78]
	tMCAO	Mouse	M [†]	No	PARP-1-mediated cell death pathway	[73]
	tMCAO	Rat	M [†]	No	Enhanced inflammation	[71]
	tMCAO	Rat	M [†]	Yes	Less astrocyte activation	[76]
	tMCAO	Rat	M [†]	Yes	Reduced oxidative stress, increased BDNF levels and neurogenesis	[77]

M, male; F, female; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; GPER, G protein-coupled estrogen receptor; SERM, selective estrogen receptor modulator; PARP-1, poly [ADP-ribose] polymerase 1; BDNF, brain-derived neurotrophic factor. *Ovariectomized. [†]Castrated.

tration of GPER agonist following focal ischemia worsened the functional outcome, increased the expression of the pro-apoptotic-cleaved caspase-3 in the penumbral neurons, and resulted in

Figure 1 Estrogen signals through multiple pathways. Endogenous estrogens (E_2) including 17 β -estradiol are nonselective activators of the known estrogen receptors (ERs) including ER α and G protein-coupled estrogen receptor (GPER). Classical genomic actions of estrogen involve the activation of nuclear ERs by binding of estrogen which induces receptor dimerization and binding of receptor dimers to the estrogen receptor elements (ERE) in the target gene promoters. Alternatively, activated ERs modulate the function of other classes of transcription factors (TF) through protein–protein interactions. Estrogen is also involved in rapid nongenomic signaling pathways via membrane-associated forms of ERs and the GPER. E_2 -ER complexes activate calcium mobilization and protein kinase cascades (e.g., MAPK, PI3K) leading to altered functions in the cytoplasm or gene regulation via phosphorylation of TFs.

bigger infarcts in male mice [58]. *In vitro* studies revealed that astrocytes also play an important role in the neuroprotective actions of estrogen by modulating the inflammatory responses via downregulation of pro-inflammatory transcription factor NF- κ B and the major pro-inflammatory cytokine interleukin-6 after cerebral ischemia [59,60]. The neuroprotection afforded by astrocytes was also thought to be due to their ability to regulate glutamate reuptake and thus mitigate excitotoxicity [61]. Estrogen acting via selective estrogen receptor modulators (SERMs) [62] promotes neurogenesis and angiogenesis, which play a significant role in poststroke plasticity [63,64].

Despite all the above studies showing the beneficial effects of estrogen, some studies suggested a possible detrimental effect of estrogen after cerebral ischemia. Gordon et al. [65] showed that a 2-week pretreatment with a high physiological dose of 17 β -estradiol increases the postischemic infarct volume in ovariectomized young adult female rats. Leon et al. [66] further showed worsened histological as well as functional outcome of estrogen therapy in aged female rats after transient focal ischemia. A meta-analysis of stroke/estrogen studies revealed that route and modality of estrogen administration are found to be the major factors that affect the outcome of the study. It tends to be exacerbation of brain damage when estrogen was delivered via high-dose, slow-release pellets and protection when estrogen was delivered via injection or silastic capsules [67]. Other factors including age or the duration of washout periods had negligible effects [67].

Androgens and Stroke

It is known that male sex is one of the major risk factors of stroke [68] and in addition, males show greater histologic and behavioral impairment after stroke than age-matched females [34,69]. However, the contribution of androgens to stroke outcome is unclear. Experimental evidence on the effects of

androgens on cerebral ischemia is seemingly contradictory (Table 1). Hawk et al. [70] showed that in castrated male rats, testosterone treatment reduces infarct size following transient focal ischemia compared to control groups treated with vehicle or estrogen. Other studies showed that castration provides protection against ischemic insult after transient ischemia and testosterone replacement therapy in castrated rats exacerbates ischemic brain damage by modulating the genes that regulate inflammation, apoptosis, and ionic imbalance [71–73]. In contrast, some studies showed that androgens have protective effects after cerebral ischemia. Testosterone treatment provided protection to cerebellar granule cells under *in vitro* conditions from oxidative stress [74] and primary hippocampal neurons from β -amyloid toxicity [75]. Pan et al. [76] showed that testosterone treatment in castrated male rats improves the functional recovery after transient focal ischemia. Similarly, Fanaei et al. [77] reported that treatment with testosterone reduces infarct volume and improves sensorimotor recovery presumably via increased BDNF levels and increased resistance to oxidative stress in castrated male rats following transient focal ischemia. Uchida et al. [78] made an interesting observation that in castrated male mice, lower doses of testosterone decreases, while the higher doses exacerbates, the infarct volume and motor dysfunction after transient focal ischemia. Thus, the effects of steroid hormones seem to be sex dependent, dose dependent, model dependent as well as age dependent.

Steroid Hormone-Independent Mechanisms

Although steroid hormones are mainly attributed as the causative factors, they do not fully account for the sex-specific outcome after stroke. One of the suggested steroid hormone-independent mechanisms for poststroke effects is the divergence of ischemic cell death pathways between males and females (Figure 2). In males, ischemic cell death seems to be dependent on the activation of poly (ADP-ribose) polymerase (PARP-1) and nuclear translocation of apoptosis-inducing factor (AIF), whereas in females, it is majorly mediated by caspase activation [79–81]. Overactivation of PARP-1 and nuclear translocation of AIF together promote DNA fragmentation and initiation of caspase-independent cell death [35,82]. Male mice are protected from perinatal ischemic brain injury when PARP-1 was knocked out, whereas the protection in female mice was minimal [83]. Furthermore, nitric oxide (NO) is an activator of PARP-1 and ischemic brain damage was observed to be exacerbated in female, but curtailed in male, neuronal NO synthase (nNOS) knockout mice [80]. Postsynaptic density-95 (PSD-95) is known to play a role in coupling NMDA receptor activity to NO-mediated toxicity [84]. Inhibition of PSD-95 was shown to significantly reduce ischemic brain damage in rat and primate models [85,86]. Treatment with PARP-1 inhibitor FR247304 was also shown to significantly decrease ischemic brain damage in male rats following transient focal ischemia [87]. In

Figure 2 Sex differences in cell death pathways following ischemic brain damage. Both young and aged males predominantly show caspase-independent cell death pathway after ischemic stroke mediated by PARP-1 activation and nuclear translocation of AIF, whereas female ischemic cell death pathway is mainly mediated by caspase activation. PARP-1 is a DNA repair enzyme that protects genome. During an ischemic insult, decreased oxygen and glucose supply leads to increase in NO production resulting in reactive oxygen species formation. PARP-1, in turn, induces release of AIF from mitochondria and promotes AIF translocation to nucleus. Overactivation of PARP-1 and nuclear translocation of AIF together promote DNA fragmentation and initiation of caspase-independent cell death pathway. In females of all ages, ischemia-induced oxidative stress leads to release of mitochondrial cytochrome C into cytosol, the major hallmark of caspase activation via induction of mitochondrial permeability transition pore (mPTP). In cytosol, cytochrome C interacts with apoptotic protease activating factor-1 (Apaf-1) to form an Apaf-1/Cyto-C complex in an ATP-dependent manner. This apoptosome complex cleaves pro-caspase-9 to active caspase-9 which induces activation of effector caspases including caspase-3. Caspase-3 is responsible for cleaving most apoptotic substrates including the inhibitor of caspase-activated DNase leading to DNA fragmentation. X-linked inhibitor of apoptosis (XIAP) is a known inhibitor of caspases in females that is regulated by miR-23a.

addition, minocycline that curtails PARP-1 activation protected male, but not ovariectomized female, mice following transient focal ischemia [88]. Studies have shown that focal ischemia leads to mitochondrial cytochrome C release and enhanced caspase activation in female rodents [89,90]. In support, treatment with the pan-caspase inhibitor quinolone-Val-Asp(Ome)-CH₂-phenoxyl (Q-VD-OPh) selectively decreased infarct volume and improved neurological deficits in females, but not in male mice [89,91].

Sex-specific microRNA (miRNA)-mediated Gene Regulation after Stroke

Focal ischemia was shown to induce extensive temporal changes in rat cerebral miRNAome [92,93]. Furthermore, several miRNAs were shown to modulate ischemic brain damage and plasticity [94–97]. A recent study demonstrated sexual dimorphism in the expression and function of miRNAs between male and female mice at 8 h following stroke [98]. Postischemic neurotoxic effects of estrogen in older female rats is partly due to loss of the insulin-like growth factor-1 (IGF-1) which is known to be neuroprotective [99,100]. Interestingly, the miRNA Let-7f that potently target IGF-1 mRNA was observed to be significantly upregulated in middle-aged (1 year old) female rats compared to young adults (7 months old) [101]. Furthermore, treatment with anti-Let-7f significantly decreased the ischemic brain damage and preserved sensorimotor function in female rats but neither in male rats nor in ovariectomized female rats indicating a sexual dimorphism in the actions of Let-7f [101]. Another sex-specific mediator in ischemic brain damage is X-linked inhibitor of apoptosis (XIAP) which might be a major contributor of sex differences in stroke. As discussed above, cell death after ischemic insult in female brain is primarily due to caspase-dependent mechanisms initiated by mitochondrial cytochrome C release. Consistent with this view, XIAP (endogenous inhibitor of caspase) was reported to be significantly downregulated in female, but not in male mice at 6 h after focal ischemia [102]. Furthermore, miR-23a, which is preferentially expressed in female, targets XIAP mRNA at 6 h following ischemia, and endogenous miR-23a induced after ischemia curtailed XIAP expression, thus providing evidence for sex-specific miRNA-mediated apoptotic mechanisms after stroke [102].

Many studies showed that circulating miRNAs are good biomarkers of stroke in rodents as well as humans [93,103,104]. Following stroke, blood levels of 5 miRNAs (miR-15a, miR-19b, miR-32, miR-136, and miR-199a-3p) were observed to be increased significantly in aged females but not in aged males or in middle-aged females or males [104]. Importantly, many predicted targets of these miRNAs target mRNAs that translate proteins involved in growth factor signaling, indicating that miRNAs are partially responsible for the age/sex-dependent outcome after stroke.

Sex and Age Interactions in Stroke Incidence and Severity

Neonates and young adults

Neonatal stroke, also referred to as perinatal cerebral injury of ischemic origin, is characterized by cerebrovascular injury

which occurs around birth [105]. There are two common subtypes: perinatal arterial ischemic stroke (PAIS) and cerebral sinovenous thrombosis (CSVT), with PAIS being the most extensively reported type [106]. Clinical studies had shown that the incidence of PAIS is more common in male than female neonates [107]. The outcome after perinatal stroke is devastating and often leads to lifelong neurological deficits ranging from motor syndrome to language and cognitive dysfunction [106]. Furthermore, these negative effects are higher in intensity in perinatal stroke sufferers than the later childhood stroke sufferers [106].

Similar mechanisms seen in adult stroke patients including sex hormone effects and sex-specific ischemic cell death pathways were observed in neonatal stroke victims [108] (Table 2). In a rat neonatal hypoxic/ischemic model, estrogen treatment provided neuroprotection to hippocampus, basal ganglia, and amygdala [109] and attenuated the white matter damage [110]. In addition to the hormone-dependent sex-specific outcomes in neonatal stroke, a differential modulation of cell death pathways was also observed. When P9 neonatal mice were subjected to unilateral hypoxic ischemia, there was no difference in the infarct volume between sexes, but males showed enhanced nuclear translocation of AIF while females showed increased caspase 3 activity indicating sexual dimorphism in the cell death pathways in the immature brain [111]. Glutamate receptor-mediated excitotoxicity also plays a major role in acute perinatal brain injury due to high density of NMDA receptors in infant brain leading to hypersensitivity to glutamate than adult brain and due to ischemia-induced impairment of ATP-dependent glutamate transporters resulting in increased glutamate levels in the synaptic cleft [112–114]. *In vitro* studies also showed that hypoxic and ischemic insults to brain exacerbate synaptic accumulation of glutamate by depolarizing the nerve terminals and reversing the operation of glutamate transporters [115]. Though it appears that glutamate-mediated excitotoxicity plays a pivotal role in postischemic neuronal death, less is known about how sexual dimorphism affects excitotoxicity after stroke. Future studies are needed to elucidate whether differential activation of glutamate receptors and transporters contributes to age- and sex-dependent ischemic brain damage.

In addition, treatment with the caspase inhibitor Q-VD-OPh increased survival time of P7 rats and improved neurological function after focal ischemia, but this effect was seen only in females implicating caspase-dependent cell death pathways in female neonatal brain injury [116]. Furthermore, many neuroprotective drugs like 2-iminobiotin [117] and erythropoietin (EPO) [118–120] were shown to induce neuroprotection after neonatal stroke. Proposed mechanisms responsible for the neuroprotective effects of these drugs include restoration of normal levels of antioxidant enzymes [121], reduction of excitatory amino acid and NO [122–124], antiapoptotic effect [125], and neurogenesis [126]. Combined treatment of xenon and hypothermia has also been reported to provide neuroprotection following neonatal hypoxic ischemia in both sexes presumably via inhibition of glutamate receptor channels including α -amino-3-hydroxy-5-methyl-4-isoxazolone propionate (AMPA) and kainite receptors, a general reduction in neurotransmitter release, and effects on other ion channels [127].

Table 2 Sex and age interactions on experimental stroke

Age	Model	Species	Sex	Protection	Mechanisms/results	Ref.
Neonate/young adult	HI	Rat	Both	Yes	Estrogen-mediated	[109]
	HI	Rat	M	Yes	Estrogen-mediated protection in white matter	[110]
	HI	Mouse	Both	N/A	M: AIF translocation, F: caspase-3 activation	[111]
	HI	Rat	F	Yes	Caspase inhibitor-mediated protection	[116]
	HI	Rat	F	Yes	2-IB reduced cytochrome c and caspase-3	[117]
	HI	Rat	Both	Yes	EPO reduced brain damage	[118–120]
	HI	Rat	Both	Yes	Xenon/Hypothermia provides neuroprotection	[127]
Middle/older	Global	Rat	F	Yes	Estrogen rescued HC CA1 cells	[128]
	Global	Rat	F	Yes	Estrogen increased CREB phosphorylation	[129]
	tMCAO	Rat	F	Mixed	Estrogen efficacy is time/age dependent	[130]
	tMCAO	Rat	M	Yes	Progesterone reduced inflammation	[131]
	tMCAO	Rat	M	Yes	Progesterone modulates GFAP, VEGF, MMP-9 expression	[132]
	tMCAO	Rat	F	N/A	Age-related Impairment in astrocytes	[133]
	tMCAO	Rat	M	Yes	Irradiation prevented glial proliferation and apoptosis	[134]
	tMCAO	Rat	M	Yes	IL-1 inhibitor antagonist reduces damage in corpulent rats	[135]
	tMCAO	Rat	M	Yes	Quercetin reduced ROS	[136]
	tMCAO	Rat	F	Yes	Bryostatin reduced α PKC and increased ϵ PKC levels	[137]
	pMCAO	Rat	M	Yes	Mild-sensory stimulation reduced damage	[138]
	tMCAO	Rat	M	Yes	H ₂ S-induced hypothermia increased Annexin 1 expression	[139]

M, male; F, female; P, postnatal; HI, hypoxia–ischemia; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; HC, hippocampus; AIF, apoptosis-inducing factor; 2-IB, 2-iodobiotin; EPO, erythropoietin; CREB, cAMP response element-binding protein; GFAP, glial fibrillary acidic protein; VEGF, vascular endothelial growth factor; MMP-9, matrix metalloproteinase 9; IL-1, interleukin 1; ROS, reactive oxygen species; α PKC, α protein kinase C; ϵ PKC, ϵ protein kinase C.

Middle Aged and Older Subjects

Age is one of the critical risk factors of stroke. In general, older patients are more prone to stroke and show more severe post-stroke neurological and behavioral deficits than younger patients [1]. In an effort to elucidate possible underlying age-dependent mechanisms, recent studies have examined various neuroprotective interventions (Table 2). It was shown that estrogen treatment significantly reduced infarct volume and enhanced hippocampal CA1 transmission in hormone-deprived aged female rats [128]. Such enhanced CA1 transmission could be possible via prevention of ischemia-induced decrease of nuclear phosphorylated CREB [129]. However, estrogen treatment appears to increase the severity of ischemic brain damage in the forebrain in aged female rats [130]. Progesterone treatment also appears to be neuroprotective in aged rats. For example, Yousuf et al. [131] showed that a delayed, prolonged systemic inflammation after ischemic insult is associated with the detrimental outcome in middle-aged male rats and this could be rescued by poststroke progesterone treatment, probably by regulating the BDNF levels. In a follow-up study, Yousuf et al. [132] showed that the mechanism of the neuroprotection induced by the delayed progesterone treatment in middle-aged male rats after stroke also involves decreased GFAP and matrix metalloproteinase-9 expression and increased vascular endothelial growth factor levels. In addition to hormones, other factors such as astrocyte activation play a role in mediating ischemic brain damage in aged rodents. Lewis et al. [133] showed that cultured astrocytes from middle-aged female ischemic rats are less capable of clearing glutamate and show increased cytokine production compared to astrocytes from young female ischemic rats.

Several therapies were examined in aged rodents subjected to ischemia. Titova et al. [134] showed that a single exposure to brain-only radiation before transient focal ischemic insult is effective enough to prevent glial proliferation resulting in decreased glial scar formation in aged male rats. Interleukin-1 receptor antagonist administration was also shown to be neuroprotective against ischemic brain damage even in comorbid corpulent aged rats [135]. Nanoencapsulated quercetin also induces neuroprotection in young and aged male rats after transient focal ischemia by downregulating iNOS and caspase-3, and by decreasing ROS production and increasing antioxidant enzyme expression [136]. Bryostatin improves survival and reduces brain damage in aged female rats after ischemia by regulating α -PKC and ϵ -PKC expression in neurons [137]. Mechanical interventions like single-whisker stimulation [138] and hypothermia [139] have also been reported as protective after cerebral ischemia in aged rats.

Sex and Age Differences in Functional Outcome in Stroke Treatment

Effect of thrombolytic agents

Although thrombolytic agent t-PA is the only FDA-approved treatment for stroke patients, due to its narrow therapeutic window and adverse side effects, thrombolytic treatment is currently limited to a few patients as rapid reperfusion following recanalization by thrombolysis causes secondary damage including blood–brain barrier (BBB) breakdown, inflammatory cell infiltration, and free radical formation as shown in rodent brain following focal ischemia [140–142]. In addition, it is not clear

how sex and age affect the efficacy of thrombolytic treatment. For example, coadministration of t-PA with a plasminogen activator inhibitor type 1 derived peptide EEIIMD showed little effect on infarct volume and functional outcome in aged rats after focal ischemia, whereas younger rats showed significantly improved outcome [143]. A quantitative MRI study also showed that t-PA-induced BBB permeability was compromised to a greater extent in aged rats compared to young rats following cerebral ischemia [144]. However, t-PA treatment combined with VELCADE attenuates adverse cerebral vascular thrombotic events, decreases infarct volume, and mitigates BBB disruption following ischemic insult in aged rats by activating toll-like receptor signaling pathway [145]. Combination therapy using t-PA and TAK-937 (a cannabinoid receptor agonist) in aged rats after focal ischemia also significantly reduced infarct volume even when TAK-937 administration was delayed by an hour after MCAO [146]. Another promising drug, 3K3A-activated protein C (APC), that can be combined with t-PA showed reduced infarct volume, prevented t-PA-induced hemorrhaging and prolonged protective effect up to 4 weeks after stroke in aged female mice [147]. The neuroprotective effects of human recombinant 3K3A-APC are being tested for ischemic stroke and successfully completed the phase I safety trial [148]. 3K3A-APC is currently undergoing phase II trial funded by NIH. Future studies are warranted to find more neuroprotective drugs that can be combined with t-PA in human stroke patients.

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Final Remarks

Most preclinical stroke studies in the past 3 decades preferentially used young adult male rodents. These studies advanced our understanding of the molecular mechanisms of stroke-induced neuronal death and helped in the identification of many promising neuroprotective drugs. However, accumulating evidence shows that sex and age are significant factors in modulating the functional outcome after stroke. For example, numerous preclinical studies had shown that estrogen treatment is beneficial after stroke, but its effects are age and sex dependent and in particular estrogen therapy might be detrimental in aged women. Hence, future experimental studies that use rodents of both sexes and different ages are warranted to identify better, tailor-made drugs for each group after stroke. Furthermore, the stroke clinical trials should include both male and female patients and cohorts of different age groups.

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Conflict of interest

The authors declare no conflict of interest.

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