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Interactions between Age, Sex, and Hormones in Experimental Ischemic Stroke

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Abstract

Age, sex, and gonadal hormones have profound effects on ischemic stroke outcomes, although how these factors impact basic stroke pathophysiology remains unclear. There is a plethora of inconsistent data reported throughout the literature, primarily due to differences in the species examined, the timing and methods used to evaluate injury, the models used, and confusion regarding differences in stroke incidence as seen in clinical populations versus effects on acute neuroprotection or neurorepair in experimental stroke models. Sex and gonadal hormone exposure have considerable independent impact on stroke outcome, but these factors also interact with each other, and the contribution of each differs throughout the lifespan. The contribution of sex and hormones to experimental stroke will be the focus of this review. Recent advances and our current understanding of age, sex, and hormone interactions in ischemic stroke with a focus on inflammation will be discussed.

1. Introduction

Stroke is a major cause of mortality and the leading cause of long-term disability in the USA. Ischemic stroke accounts for 87% of all strokes [1, 2]. To date only one FDA approved therapy is available for patients with acute ischemic stroke, the thrombolytic tissue plasminogen activator (tPA) [3]. Unfortunately, despite our best efforts, only a small number of patients are eligible for tPA therapy. Thrombolytic therapy, although extremely efficacious, has a very short time window of treatment, ranging to 3 hours in the USA to 4.5 hours in Europe [4] after which decreasing efficacy and increased risk of hemorrhagic complications occur. Basic scientists have identified numerous potential “neuroprotective” agents that reduce stroke injury in experimental models, but attempts to bring these therapies into the clinic has met with limited success. Numerous promising agents have failed to show protective effects in clinical trials [5]. This has led to the questions 1) are we utilizing the most appropriate animal models in our preclinical studies? and 2) are we designing our clinical trials with guidance from emerging experimental data? These concepts must be considered if we hope to develop efficacious neuroprotective candidates.

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Young, male animals are the invariable favorite for use by most stroke researchers in experimental studies. However, clinical stroke is a disease that mainly affects the elderly [1, 2]. In fact, age is the most important independent risk factor for stroke [6] with stroke rates doubling every decade after the age of 55 [1]. The aged brain undergoes numerous neurochemical and physiological changes over the lifespan that change the responsiveness to a variety of therapies compared with young brains [7]. Acetylcholinesterase (AChE) and Na^+K^+ATPase activity in synaptosomes decreased with age resulting in neuronal vulnerability changes to excitotoxic insults [8]. Simvastatin, a hypolipidemic drug for the treatment of dyslipidemia, fully restored short- and long-term memory in adult, but not in aged mice of Alzheimer's disease model [9]. Sex differences are also seen in the epidemiology of ischemic stroke [10]. In neonates, male sex is a risk factor for poor outcome, whereas increased incidence and morbidity is seen in elderly females. Childhood ischemic stroke appears to be more common in boys regardless of age, stroke subtype, or history of trauma [11, 12]. Elderly women not only have higher stroke incidence than age-matched men, but also have poorer recovery, higher morbidity and mortality once a stroke occurs [13-17]. These clinical data remind us that age and sex are important impact factors which should be fully taken into account in experimental stroke studies. This review discusses age- and sex-related differences in stroke phenotypes and the possible underlying mechanisms, aiming to shed light on the discrepancies between experimental and clinical data of stroke studies.

2. Impact of age and sex on normal brains

2.1 Aging effects on the brain

Both global and regionally specific changes in brain tissue volume occur with aging [18] (Table 1). Studies with magnetic resonance imaging (MRI) revealed that in the elderly white matter volume loss predominates over that seen in the gray matter [19-21]. The frontal lobes show the greatest decline in the volume with age (approximately 12%), followed by the temporal lobe (9%) and the occipital and parietal lobes which only showed modest change [22]. Apart from the age related changes in the brain volume, neurochemical and physiological changes also occur with aging [7] (Table 1). Cerebral blood-flow (CBF) is regionally reduced with aging in the fronto- and temporocortical area and in the subcortical region, and age negatively correlates with perfusion in both the left and right fronto-cortical regions [23]. Recent studies showed that in aging tissues from female and male animals, markers of oxidative stress increase due to decreased activity of antioxidant enzymes. In addition, proteolysis increases due to decreased activity of aminotransferase [24, 25]. Significant alterations in neurotransmitters and enzyme activity are seen with advancing age in the non-demented elderly. These include well documented changes in levels of choline acetyltransferase, and an increase in vasoactive intestinal peptide immunoreactivity [26]. Age-related changes are also seen by the relatively robust decrease in brain nutritional factors, including carbohydrates, proteins, and fat [27], and by the loss of key enzymes of the respiratory chain involving cytochrome oxidase and succinic dehydrogenase [28].

2.2 Sex differences in the brain

Numerous sex differences in human brain structure have been described demonstrating that the brain is a sexually dimorphic organ (Table 2). *In vivo* imaging and postmortem studies report that the cerebrum is larger in men than women by 8-10% [29-33], a finding that is not wholly attributed to body size. Regionally specific sex differences relative to size of cerebrum have also been reported. Goldstein et al [34] reported that sexual dimorphisms of adult brain volumes were more evident in the cortex, with women having larger volumes, relative to cerebrum size, particularly in frontal and medial paralimbic cortices. Men had larger volumes, relative to cerebrum size, in the frontomedial cortex, the amygdala and

hypothalamus. Other studies showed that relative to cerebrum size, women have larger volumes in cortical gray matter [35], in regions associated with language functions, e.g. Broca's area [36], in the hippocampus, caudate, and thalamic nuclei [29, 37, 38] than men. In contrast, men have been found to have larger volumes, relative to cerebrum size, in hypothalamus [39-41], paracingulate gyrus [42], and greater cerebrospinal fluid [35, 43, 44].

Normal sexual dimorphism exists not only in the brain structure, but also in brain function and neurochemistry (Table 2). Compared with men, healthy women have higher 5-HT transporter availability in the striatum, diencephalon and brainstem [45]. Greater dopamine release in the right globus pallidus and inferior frontal gyrus was seen in females than males [46]. Sex differences were even reported in some receptor systems, such as cholinergic system [47, 48], GABAergic system [49, 50], and opioid system [51, 52]. Sex steroids exert significant impacts on neurotransmission and cerebral blood flow (CBF). For example, women received combined estrodiol and progestorone administration had higher 5-HT(2A)R binding potential throughout the cerebral cortex relative to baseline [45]. Estrogens inhibit the dopaminergic supersensitivity induced by neuroleptics [53]. In premenopausal women, the menstrual cycle has a significant impact on physiological and neurochemical parameters in the brain. Accumulating data showed that women have higher global cerebral blood flow (CBF) compared with men during rest [54, 55] and cognitive activity [55-59]; consistent with this, cerebral metabolic rate of glucose utilization tends to be higher in women versus men [60]. Corresponding to high plasmic concentration of 17β-estrodial (E2), increased CBF was found in women between days 10 and 15 of the cycle followed by an established elevation in the luteal phase [61, 62]. The increased CBF is caused mainly by a decrease in vascular resistance in the brain, presumably due to the direct dilating effect of estrogen on cerebral vessels [63, 64]. Fluctuating hormone levels during the menstrual cycle also have acute effects on brain glucose metabolism [65] and D2 dopamine receptor density[66]. These studies highlighted the importance that studies on sex differences in the brain should consider, or if necessary, control the effects of sex steroids.

3. Effect of Age on Ischemic Stroke

3.1 Clinical data

Aging is not only the single most important independent risk factor for the incidence and the prevalence of stroke [67], but also a significant predictor of outcome independent of stroke severity, etiology, efficacy of thrombolysis, sex, other vascular risk factors, and stroke complications [68, 69]. For each successive 10 years after the age of 55, stroke rates more than double in both men and women [1]. Neurologists also see constant reminders of the fact that older patients do not do as well after a stroke compared to younger counterparts indicating that stroke in older patients has different characteristics than that seen in the young. A clinical investigation revealed that in the old, stroke outcome data such as 30-day case fatality rate and disability are far poorer than for young patients, and hospital management is often less active in this age group [70]. The increased mortality seen in older individuals may not be related to the stroke per se, but rather an effect of aging and advanced co-morbid diseases. Results from mixed-sex clinical studies showed stroke severity and location did not differ between young and old stroke patients, but the latter seem more vulnerable to infectious complications, such as pneumonia and urinary tract infection [71]. This mirrors what we have shown in our studies [72, 73]: aging female mice had larger infarct and males had paradoxically smaller strokes, but both of them had higher mortality than young mice.

3.2 Experimental data

Only a few experimental studies exist in the literature that have examined aging animals and have led to somewhat inconsistent results. The studies during the recent two decades have been summarized in Table 3. Intriguingly, experiments performed on animals of different sexes yielded different results. Studies using females consistently showed aging females have worsened stroke outcomes than their young counterparts regardless of strains and model types employed [72, 74-76], except those in which ovariectomized(Ovx) females [77, 78] were used, that exhibited no difference in outcomes most likely due to effects of acute ovary removal. However, studies using male animals have yielded very inconsistent data, with 5 studies in which aging males have larger strokes [79-83], 6 studies that demonstrated smaller infarcts in aging males [72, 73, 84-87], and 3 studies that found equivalent infarct size between young and aging animals [88-90]. Male animals gain more body weight than females over the life span [72, 91, 92], which may complicate surgical procedures used in experimental studies and thus leading to larger variability in histological stroke outcomes in different labs using different stroke models. Further studies on stroke in aging animal are needed to determine the effect of aging on stroke, including detailed assessment of both histological and behavioral phenotypes. In spite of these disagreements on the histological effects of aging on infarct volume, invariably significantly higher mortality rates and more severe neurological impairments were found in the older animals, which is consistent with clinical data [70, 71] and data from our own lab which showed both aging male and female mice have exacerbated neurological deficits compared to young animals independent of infarct volumes [72].

In an attempt to understand the strikingly different stroke phenotypes seen in animals of different ages, our own laboratory recently explored several specific neurochemical markers closely related to ischemia and revealed interesting results. Phosphorylated adenosine monophosphate-activated protein kinase (pAMPK), an evolutionary conserved energy sensor sensitive to changes in cellular AMP/ATP ratio [93], exhibits a muted response to stroke in aged brain, perhaps due to the fact that aging independently increased baseline brain pAMPK levels [87]. Compared to young mice, aging (15-16 months) C57BL6 mice had decreased expression of the Na^+-K^+-Cl^- co-transporter (NKCC) in the brain after stroke, and concurrently, stroke-induced edema formation is also significantly less robust, suggesting NKCC expression and edema formation are age dependent after ischemic stroke [73]. Another recent study reported that concentrations of brain-derived neurotrophic factor (BDNF) and bFGF were significantly lower in both the cortex and striatum of old mice compared with young and middle-aged mice after stroke [94]. Neuron-specific intermediate filaments, neurofilaments (NFs) that play critical roles in establishment of new synaptic contacts with other nerve cells, also exhibited significantly lower level of gene expression in the brain of aged rats compared with young animals after ischemia [95]. Although limited data are currently available about aging effect on neurochemical markers in the ischemic brain, all the studies suggested that aging down-regulates the activity of biochemical mediators that respond to ischemia. One notable exception is that the N- and C-terminal amyloid precursor protein beta was more rapidly increased in both the peri-infarct area and the infarct core in aging compared to that of young brains after stroke [96]. All these data seem to support the hypothesis that stroke outcomes are exacerbated with aging; however, the inconsistent histological outcomes imply that the molecular signaling pathways underlying ischemic infarction with aging are quite complex and need further investigation.

4. Effect of Hormones on Ischemic Stroke

4.1 Estrogen and stroke

Clinically women have lower age-adjusted stroke incidence than men [97]; and numerous experimental studies have also shown that young adult female animals are guarded from stroke relative to their counterpart males [98]. This “male-sensitive” phenomenon in stroke has been attributed to the protective effect of ovarian hormone exposure [99]. Observational clinical studies have demonstrated the potential protective effects of circulating ovarian hormones in coronary heart disease (CHD) [100], vascular disease [100-102] and stroke [103]. Experimental studies also showed young male animals have larger infarct size after induced strokes compared to young females, and E2 treatment at physiological relevant concentrations reduces infarction after stroke in ovariectomized female animals [72] as well as in males [104, 105], even when given after stroke [106]. All these studies indicate that estrogens have protective effect in stroke, at least in young adults.

Estrogen’s neuroprotective effects on ischemic stroke are regulated by multiple signaling pathways [107]. These include genomic effects mediated by estrogen-responsive elements (EAE) leading to enhanced transcription, in addition to non-genomic effects executed by rapid activation of second messenger cascades [99]. Several estrogen receptors (ER) such as ERα, ERβ, or ERx, play important roles in mediating these effects. E2 modulates the expression of a variety of genes in the ischemic brain, including those that influence the balance between cell death and cell survival such as the Bcl-2 family of genes [108] and caspases [109]. Through ERs, E2 can also exert anti-inflammatory actions by inhibiting nuclear factor (NF)-κB, a transcription factor that regulates expression of many pro-inflammatory molecules [110]. E2 is a vasodilator and promotes blood flow in the brain recovering from an acute insult [111], a non-genomic effect induced by ERα-mediated activation of tyrosone kinase-MAPK and Akt/protein kinase B signaling, leading to increases in endothelial nitric oxide synthase (eNOS) activity [99, 112]. In addition, at pharmacological concentrations (the micromolar range) E2 can act as directly as an anti-oxidant [113, 114]. E2 has also been reported to enhance both angiogenesis [115] and neurogenesis [116] following ischemic damage.

4.2 Hormone replacement therapy (HRT) in aging

Recent studies revealed that aging not only affects the brain response to ischemic insults, but also alters the neuroprotective effects of estrogen on the ischemic brain [117-119]. Although numerous experimental studies have demonstrated that E2 has neuroprotective effects on ischemic injury in young animals, two well-known clinical trials of HRT in aged women, Women’s Health Initiative (WHI) and Women Estrogen Stroke Trial (WEST), have concluded that E2 increases the incidence of stroke and can lead to enhanced damage and increased rates of fatal stroke [120, 121]. The trials have triggered an ongoing debate in the literature as to how the beneficial effects of E2 seen in pre-clinical studies could be translated into a feasible and effective clinical application. A hypothesis of the importance of the timing of initiation of replacement therapy has therefore been put forward that suggests that ERT should be initiated immediately after menopause in women to achieve its protective effect [122]. In both the WHI and WEST trials the participants were well beyond menopause when they received ERT which might explain why detrimental effects occurred. Utilizing young ovariectomized (Ovx) mice, ERT was given to female mice either immediately after Ovx or delayed for 10 weeks. It was found that only the immediate E2 treatment group exhibited beneficial effect of ERT which was secondary to a profound decrease in stroke-induced inflammation [119]. In a more recent study designed to mimic the clinical population that is a target for ERT [117], aged female mice (17months; an age post natural gonadal senescence in mice) were examined. ERT initiated at 17 months

(chronic ERT) led to improved infarct damage when the animals were subjected to stroke 3 months later, at 20 months of age. However ERT given to mice of 20 months (acute ERT), for only two weeks prior to the onset of ischemia, was no longer protective and exacerbated tissue damage and markers of inflammation. Intriguingly an independent effect of sex was seen in this model as aged males benefited from both chronic and acute ERT, regardless of the timing and duration of administration. Of note, differentiated levels of NF-κB translocation after stroke were seen between chronic and acute ERT groups [117]. These studies suggest that timing effect of E2 therapy may be mediated by different inflammatory responses following ischemia. One recent study indicated that persistently elevated levels of ERβ2 may be the molecular basis for the diminished effectiveness of ERT in late post-menopausal women [118] and this remains to be investigated in pre-clinical models.

4.3 Androgens and stroke

Although male sex is an acknowledged risk factor for stroke, data from experimental studies of cerebral ischemia testing effects of androgens are surprisingly few and contradictory [123]. Hawk et al [124] found that administration of testosterone led to increased infarct size after MCAO in male rats. In young adult animals, castration significantly reduces ischaemic injury. Supplementation with testosterone or its non-aromatisable metabolite dihydrotestosterone (DHT) restored infarct volumes to levels seen in intact males [123]. However other studies reported that testosterone or DHT treatment reduces the number of pyknotic cells in the dentate gyrus after adrenalectomy [125]; similar protective effects of testosterone was also found in in vitro studies of oxidative stress, β-amyloid toxicity, and serum deprivation [126-129]. How androgens impact on sex differences in ischemic injury remains elusive.

Interestingly the effect of androgens on ischemia seems to mirror that seen with E2, and show a strong age dependent effect. Clinical studies have reported that high testosterone levels are associated with an increased risk of thromboembolic events in pediatric populations [130], but that low circulating testosterone levels are associated with higher stroke incidence and poorer functional outcomes in elderly men [131, 132]. However, despite the fact that low testosterone levels are associated with increased stroke incidence in older males [131-133], attempts to replace testosterone led to an increase in vascular risk in elderly men [134]. This mirrors what was seen in the WEST and WHI trials with ERT in aging women, suggesting gonadal hormones have important and distinct effects on the vasculature in aging populations. Further studies in the aging vasculature are needed if we hope to optimize any potential neuroprotective or vasculoprotective effects of hormone replacement therapy.

Sex differences in ischemic sensitivity over the life span

5.1 From neonates to young adults

Both clinical and experimental data indicate that stroke is sexually dimorphic throughout the life span. A male predominance in childhood ischemic stroke has been seen in multiple centers worldwide [11, 135-138]. One recent study revealed that boys comprise a significantly higher proportion (57-63%) of both arterial ischemic stroke (AIS) and cerebral sinovenous thrombosis [11]; another center reported similar findings, a higher number of males among children with AIS (63.8 in boys vs. 36.2% in girls), with the exception of transient ischemic attack (42.9 in boys vs. 57.1% in girls) [138]. This male “ischemia-sensitive” phenotype persists after stratification by ischemic stroke subtype or by other etiologies [11]. Risk-taking behaviors may partly explain a gender disparity in childhood stroke; however, the increased prevalence seen in males persisted when cases of ischemia due to trauma were excluded [11, 139, 140]. The male predominance in childhood ischemic

stroke continues throughout the neonatal period into adolescence [11, 137], and then switches to a female predominance until the age of 30 [137, 141, 142]. This phenomenon is associated with the wide use of oral contraceptives, frequency of migraine, and reproductive activity in young females of this age group and may also occur in conjunction with complex, poorly understood, genetic and environmental interactions [143]. After the age of 30, male predominance again occurs [141, 142], which may be secondary to the protective effect of estrogens in women [99]. These clinical reports have been recapitulated in experimental studies, as PND 3-11 male mice show more brain volume loss than age matched females after experimentally induced hypoxic ischemic encephalopathy (HIE) [144]. Importantly for translation, these sex differences extend to the response to therapies designed to protect the injured brain. A study performed in a model of HIE in P7 rats showed that hypothermia protects females, but not males, from histological damage and sensorimotor deficits [145].

5.2 Aging populations

Evidence of sexual dimorphism in aging populations also exists. As women age, stroke rates increase and surpass that of men, coincident with diminished circulating levels of E2 and progesterone [97, 98]. Despite the higher overall lifetime incidence of stroke in men, aged women have more severe strokes, poorer recovery and greater long-term disability [14-17] compared to age-matched men. There is even a persistent sex disparity in midlife stroke prevalence in the United States [146]. According to the National Health and Nutrition Examination Surveys (NHANES) 1999-2004, women aged 45 to 54 years were more than twice as likely to have had a stroke than men [147]. The trend of female predominance in stroke in midlife extended as the NHANES 2005-2006 reported women aged 35-64 years had three times the odds of prior stroke compared with men, and the significant difference was also in group of 45-54 years [146]. The exact reasons behind this persistent sex difference are difficult to decipher precisely; however, obesity, the hormonal and inflammatory milieu in midlife and middle aged women may conceivably play important roles [146]. So far very few experimental studies have been performed to mimic this clinical phenomenon. The only available data is from our own lab, which showed either aged (20 months) or middle aged (15-16 months) female mice had larger infarcts than their male counterparts after induced ischemia, and this was related to more robust inflammatory responses seen in females [72, 117]. More research is required to understand sex differences in the pathophysiological mechanisms of stroke in aging populations.

5. Effects of age, sex, and hormones on inflammatory responses following stroke

6.1 Effect of age and sex on inflammatory responses

Inflammatory processes have a fundamental role in the pathophysiology of ischemic injury. Brain ischemia is a powerful stimulus that triggers a series of events that lead to vasodilatation, increased permeability of local blood vessels, and mobilization and infiltration of circulating leukocytes, a process modulated by many inflammatory cell adhesion molecules and cytokines [148-151]. Inflammatory responses following ischemia have different characteristics depending on age and sex, and are also regulated by hormone levels. Recent studies revealed that ischemia induced inflammatory responses are compromised in the aged brain, probably due to the immunosenescence that occurs with aging [152]. For example, the response of pro-inflammatory cytokines (TNF and IL-1β) and the level of chemokines (Mip-1α and MCP-1) were strongly diminished in the aged post-ischemic brain tissue, and IL-6 showed the strongest age-dependent decrease in its post-ischemic expression profile [85]. Neutrophils isolated from elderly individuals exhibit attenuated chemotaxis, oxidant release, and phagocytosis, and it has been suggested that these deficiencies are related to an age-associated increase in glucocorticoid production and

oxidative stress [153]. Aging also has a detrimental effect on progenitor populations, for example oligodendrocyte progenitor cells (OPCs) showed more proliferative activity and process branching in young brains compared to aged after induced ischemia [154]. Interestingly evidence of immunosenescence has been primarily obtained from males [155, 156]; aged females appear to exhibit a more robust inflammatory response than young females. One recent study [157] reported that astrocyte-conditioned media from middle-aged female astrocytes induced greater migration of peripheral blood monocyte cells and neural progenitor cells, and expressed higher levels of the chemoattractant macrophage inflammatory protein-1 (MIP-1) compared with young female astrocytes; however, no age-related impairment was observed in astrocyte function in males. An enhanced immunological response in aged females is also seen in other organ systems. The pulmonary inflammatory response is greater in aged female mice (18-20 months) which showed a six-fold higher neutrophil infiltration and three-fold higher level of myeloperoxidase activity in the lung compared to young females upon lipopolysaccharide (LPS) exposure [158].

Biological sex has a distinct impact on many inflammatory pathways. One example is the pro-inflammatory signaling pathway mediated by NF-κB. NF-κB is a transcription factor that regulates the expression of multiple inflammatory molecules, such as IL-6, tumor necrosis factor-α (TNF-α), mitogen-activated protein kinase 1 (MAPK-1), etc. [159, 160] all of which are activated following ischemic insults. Female fibroblasts cultured without E2 exhibited higher level of NF-κB than male cells after hypoxia [161]. P2 female rats had evidence of high levels of NF-κB in the anteroventral periventricular nucleus (AVPV), whereas NF-κB signaling was repressed in male neonates, forming the basis of a new model of sex differentiation of the AVPV that may apply to the development of other sexually dimorphic nuclei [162]. Aged female mice (20 months old) had significantly higher NF-κB expression than their counterpart males when subjected to 90 minute MCAO and corresponding elevations in serum levels of inflammatory markers regulated by NF-κB were also seen [72]. Intriguingly the up-regulated activity of NF-κB signaling correlated with exacerbated stroke injuries in aging females [72, 117], suggesting NF-κB is a key protein in mediating sex differences in ischemic injury. The sexual dimorphism in NF-κB pro-inflammatory signaling may be related to specific X-chromosome linked genes (see below).

6.2 Effects of hormones on inflammatory responses

The effects of hormones on the inflammatory responses are complicated and no conclusive studies currently available. In general, estrogens have anti-inflammatory effects in the brain [163]; however, this effect is modified by multiple other factors, such as 1) the immune stimulus; 2) the cell type involved during different phases of the disease; 3) timing and duration of hormone exposure; 4) the concentration of estrogens; 5) the variability in expression of ERs, etc. [164]. The protective, anti-inflammatory effect of E2 on cerebral blood vessels that is observed in young adults may be attenuated in aged animals, which exhibit a greater overall cerebrovascular response to inflammatory stimuli [165]. However, estrogen's anti-inflammatory effect in aged animals seem to be demarcated by both biologic sex and timing of administration, as discussed earlier [117]. ERT suppressed plasma level of IL-6, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and IL-5 in MCAO mice if given 10 weeks after Ovx, but not if administered immediately after Ovx [119]. Data regarding of progesterone or testosterone effects on inflammatory responses after stroke are virtually absent from the literature. However, several studies reported that either progesterone or testosterone augmented cerebrovascular inflammation in young adult rats induced by intraperitoneal LPS injection, opposite to 17β-estradiol's suppressive effect [165, 166]. Nevertheless, one recent study indicates that testosterone may exert anti-inflammatory effects by reducing TNF-α expression in macrophages obtained from healthy

older men and postmenopausal women [167]. Most likely the effects of progesterone or testosterone are also modified by age, sex, and timing of administration, etc.

6. X-chromosome contribution to ischemic sensitivity

As at both neonatal and post-menopausal ages, hormone levels are relatively equivalent between the sexes, sex differences in ischemic sensitivity seen at both ends of the age spectrum may be influenced by biologic sex (XX vs. XY) in addition to the hormonal milieu [168-171]. Males and females differ in sex chromosome compliment (XY vs. XX) and X chromosome imbalance is tolerated because of dosage compensation by X-chromosome inactivation (XCI) [172-175]. As a result of XCI, both males and females are functionally monosomic for most X-linked genes. A recent study from this lab confirmed X-chromosome dosage has no effect on the degree of cerebral infarction after experimental stroke in female mice [176]. However, non-random XCI [177] or genes escaping from XCI with aging may be associated with X-linked diseases [178, 179] and may lead to the imbalance of X-linked genes expression between sexes. Several X-chromosome linked genes have been recently recognized as contributors to disease. These genes regulate several molecular pathways, including that of NFκB [180-182]. Three important X-linked genes have been identified in the toll-like receptor (TLR) signaling family [182] and are intimately involved in the immune response via activation of NF-κB inflammatory signaling [182-184]. These include interleukin 1 receptor-associated kinase 1 (IRAK-1), NF-κB essential modulator (NEMO), and Bruton tyrosine kinase (BTK). However, no literature is available as to how these three X-linked proteins are involved in stroke. To add to this complexity, NF-κB may also have anti-apoptotic functions by inhibiting JNK and caspase activity, a process primarily mediated via up-regulation of another X-chromosome gene, X-linked inhibitor of apoptosis protein (XIAP) [185-187] (Fig. 1). It is presently unclear how the seemingly paradoxical NF-κB pro-inflammatory and anti-apoptotic signaling can affect ischemic insults; however, the intimate interaction between NF-κB and X-linked genes makes it possible that the balance could be perturbed or dysregulated due to the instability in XCI with aging [177, 188]. Emerging data have suggested that different cell death pathways predominate respectively in male and female neurons subjected to ischemic insult [10]; nevertheless, whether the ischemic sexual dimorphism is mediated by chromosomal complements remains undetermined.

In summary, multiple factors impact the pathophysiological changes that occur in ischemic stroke throughout the lifespan. Age, sex, and hormone exposure are three independent yet interacting parameters that mediate the response to ischemic stroke (Fig.2). Different mechanisms may underlie age and sex specific responses to cerebral ischemia. Due to the small amount of literature available, further investigation is needed to fully address the complicated relationship between age, sex, and hormones in the setting of stroke. Given that most experimental stroke studies are performed in young male animals, it seems an important priority for pre-clinical researchers to examine these interactions. Hopefully this will lead to identification of novel targets and to the development of therapies to treat this devastating disease.

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Abbreviations

AChE Acetylcholinesterase

AIF	apoptosis inducing factor
AMP	adenosine monophosphate
ATP	adenosine-5′-triphosphate
AVPV	anteroventral periventricular nucleus
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
BTK	Bruton tyrosine kinase
CBF	cerebral blood flow
CSF	cerebrospinal fluid
CHD	coronary heart disease
DAPI	4′,6-diamidino-2-phenylindole
DHT	dihydrotestosterone
eNOS	endothelial nitric oxide synthase
E2	17β-estrodial
ERT	estrogen replacement therapy
ER	estrogen receptor
ERE	estrogen-responsive element
GABA	γ-Aminobutyric acid
GM-CSF	granulocyte-macrophage colony-stimulating factor
HIE	hypoxic-ischemic encephalopathy
HRT	hormone replacement therapy
5-HT	5-hydroxytryptamine
IL	interleukin
IRAK-1	interleukin 1 receptor-associated kinase 1
JNK	c-Jun N-terminal kinase
LPS	lipopolysaccharide
MIP-1	macrophage inflammatory protein-1
MCP-1	monocyte chemotactic protein-1
MCAO	middle cerebral artery occlusion
MRI	magnetic resonance imaging
MAPK-1	mitogen-activated protein kinase 1
NADPH	nicotinamide adenine dinucleotide phosphate Neuronal Nucleus protein (NeuN)
NHANES	National Health and Nutrition Examination Surveys
NKCC	Na^+-K^+-Cl^- co-transporter
NFs	neurofilaments

NF-κB	nuclear factor-κB
NEMO	NF-κB essential modulator
OPC	oligodendrocyte progenitor cell
Ovx	ovariectomy
pAMPK	phosphorylated adenosine monophosphate-activated protein kinase
PARP	poly (ADP-ribose) polymerase
TNF	tumor necrosis factor
tPA	thrombolytic tissue plasminogen activator
WHI	Women's Health Initiative
WEST	Women Estrogen Stroke Trial
XCI	X-chromosome inactivation
XIAP	X-linked inhibitor apoptosis protein

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Highlights

- Age, sex, and gonadal hormones impact stroke outcomes
- Literature on the effects of age and sex on stroke outcome is inconsistent
- Sex and gonadal hormone exposure have a major impact on stroke outcome
- The contribution of sex and hormone varies throughout the lifespan
- Age and hormones are major factors in the inflammatory response to stroke

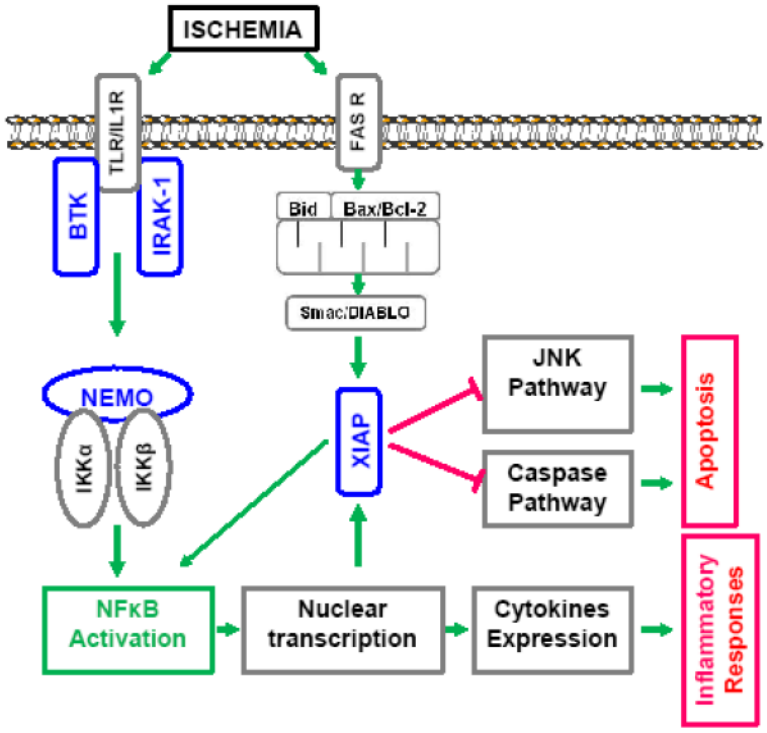

Figure 1.

Diagram of proposed mechanisms underlying the dual roles of NF-κB signaling in ischemic stroke. Mediated by three X-chromosome linked genes (BTK, IRAK-1, and NEMO) upon ischemic insults, NF-κB translocates into the nucleus and regulates the expression of pro-inflammatory cytokines. NF-κB also interacts with XIAP to exert inhibitory effect on JNK and caspase mediated apoptosis.

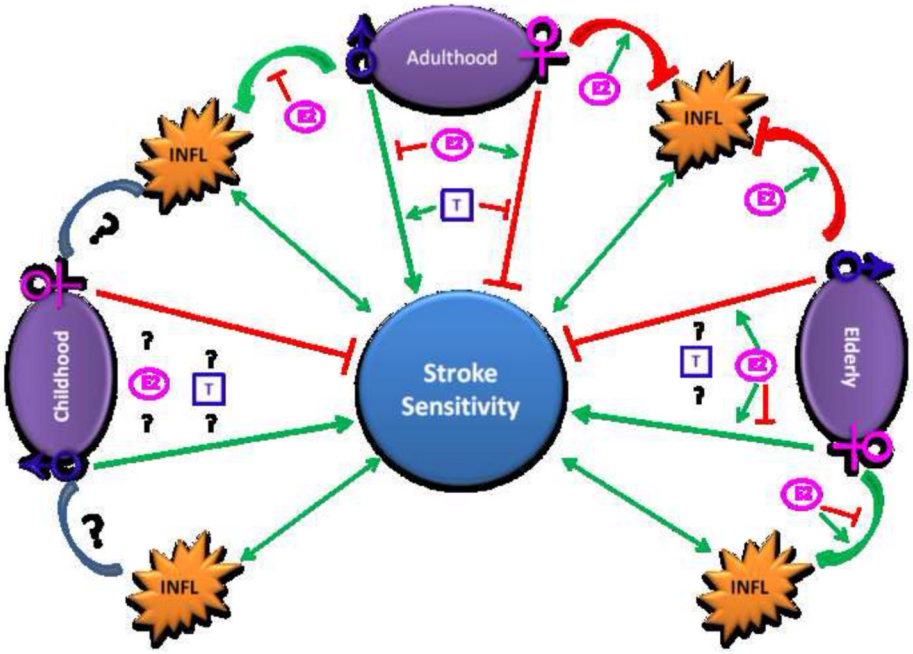

Figure 2.
Schematic summary of effects of age, sex, and hormones on stroke sensitivity. Green colors stand for stimulating effects; red colors stand for inhibitory effects. INFL: inflammation; E2: estrogen; T: testosterone.

Table 1

Impact of aging on brain anatomy and physiology.

	Parameters	Changes with aging	Species	Refs
Tissue Volume	Amount of CSF	Increase	Human	[189, 190]
	Overall brain volumes	Decrease	Human	[189, 190]
	White matter	Decrease	Human	[19-21]
	Gray matter	Small decrease	Human	[19-21]
	Frontal lobe	Decrease	Human	[22]
	Temporal lobe	Decrease	Human	[22]
	Occipital and parietal lobe	Decrease	Human	[22]
	Hippocampus	Decrease	Human	[191]
Physiology and Neurochemistry	Oxidative stress	Increase	Rat	[24, 25]
	Glutathione peroxidase and NADPH generation	Increase	Rat	[192]
	Glucose-6-phosphate dehydrogenase activity	Increase	Rat	[193]
	Malic enzyme	Increase	Rat	[193]
	Vasoactive intestinal peptide immunoreactivity	Increase	Human	[26]
	CBF	Decrease	Dog	[23]
	ATP citrate-lyase	Decrease	Rat	[193]
	Acetyl Co-A carboxylase	Decrease	Rat	[193]
	Fatty acid synthetase	Decrease	Rat	[193]
	Carbohydrates	Decrease	Human	[27]
	Tryptophan and tyrosine	Decrease	Human	[27]
	Unsaturated fatty acids	Decrease	Human	[27]
	GABA	Decrease	Rat	[194]

Table 2

Sex differences in brain anatomy and physiology.

	Parameters	Women(F) vs.Men(M)	Species	Refs
Tissue Volume	Gray matter	F>M	Human	[35]
	Broca's area,hippocampus,caudate and thalamic nuclei	F>M		[36]
	Frontal and medial paralimbic cortices	F>M	Human	[34]
	Frontomedial cortex,amygdala and hypothalamus	F<M	Human	[34]
	Amount of CSF	F<M	Human	[35, 43, 44]
	Cerebrum size	F<M	Human	[29-33]
	White matter	F<M	Human	[35]
	Hypothalamus,paracingulate gyrus	F<M	Human	[39-42]
	Number of neurons	Inconsistency	Human	[32, 36, 195, 196]
Physiology and Neurochemistry	Global CBF	F>M	Human	[54, 55]
	5-HT transporter availability	F>M	Human	[45]
	Dopamine release	F>M	Human	[46]
	Cortical GABA level	F>M	Human	[49]
	Cortical muscarinic acetylcholine receptors	F>M	Human	[48]
	Cortical mu-opioid binding	F>M	Human	[51]
	N-acetyl aspartate	F>M	Human	[197]
	Receptor affinity of glucocorticoids	F<M	Human	[198]
	Glutathione/reduced glutathione ratio	F<M	SHR rat	[199]
	Cerebral metabolic rate of glucose utilization	Inconsistent	Human	[60, 200-203]

Table 3

Effect of aging on experimental ischemia.

Strain/Species	Sex	Age		Model	Aging effect on infarct volume	Refs
		Young	Aging			
F344 rats	M	2-3 m	26-28 m	pMCAO	24h =; 7d ↑	[79]
Wistar rats	M	4 m	20,27m	100 min tMCAO	7d ↑	[80]
SD rats	M	3 m	6,12,18 m	20 min tMCAO	2w ↑	[81]
C57BL6 mice	M	4 w,4 m	11,18 m	60 min tMCAO	1,3d =; 7d ↑	[82]
Wistar rats	M	4 m	22-24 m	pMCAO	1 m ↓	[84]
C57BL6 mice	M	2 m	9,15,24 m	30 min tMCAO	2h, 2d, 7d ↓	[85]
C57BL6 mice	M	4 m	9,20 m	pMCAO	24h ↓	[86]
C57BL6 mice	M	2-3 m	15-16 m	90 min tMCAO	24h ↓	[72]
C57BL6 mice	M	3 m	15-16 m	90 min tMCAO	24h ↓	[73]
C57BL6 mice	M	2-3 m	16-18 m	91 min tMCAO	24h,30d ↓	[87]
F344 rats	M	3 m	24 m	60 min tMCAO	3d =	[88]
SD rats	M	3-4 m	22-24 m	60 min tMCAO	24h,28d =	[89]
SD rats	M	3-4 m	22-24 m	60 min tMCAO	1w =	[90]
SD rats	F	3m	9,18 m	2h tMCAO	24h ↑	[83]
SD rats	F	3-4 m	18-20 m	2h tMCAO	24h ↑	[74]
SD rats	F	3-4 m	18-20 m	2h tMCAO	24h ↑	[75]
SD rats	F	3-4 m	17-18 m	2h tMCAO	24h ↑	[76]
C57BL6 mice	F	2-3 m	15-16 m	90 min tMCAO	24h ↑	[72]
Wistar rats	Ovx F	3 m	15 m	60 min tMCAO	7w =	[77]
SD rats	Ovx F	3-4 m	9-12 m	pMCAO	24h =	[78]

M,male; F, female; Ovx, ovariectomy; min, minute; h, hour; d, day; w, week; m, month; p, permanent; t, transient; =, no change; ↑, increase; ↓, decrease.