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## The Effects of Estrogen in Ischemic Stroke

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### Abstract

Stroke is a leading cause of death and the most common cause of long-term disability in the USA. Women have a lower incidence of stroke compared with men throughout most of the lifespan which has been ascribed to protective effects of gonadal steroids, most notably estrogen. Due to the lower stroke incidence observed in pre-menopausal women and robust preclinical evidence of neuroprotective and anti-inflammatory properties of estrogen, researchers have focused on the potential benefits of hormones to reduce ischemic brain injury. However, as women age, they are disproportionately affected by stroke, coincident with the loss of estrogen with menopause. The risk of stroke in elderly women exceeds that of men and it is clear that in some settings estrogen can have pro-inflammatory effects. This review will focus on estrogen and inflammation and its interaction with aging.

### Keywords

Stroke; Aging; Sex; Estrogen

### Stroke

Stroke is the fourth leading cause of death in the USA and ranks as one of the leading causes of long-term disability [1]. Given the current population trend of increasing life expectancy in the USA with its concurrent expansion of the elderly population, the annual number of stroke patients is expected to increase with every given year. The most important nonmodifiable risk factor for stroke is aging. The majority of stroke patients are over 65 [2, 3], and since 75–89 % of strokes occur in individuals over the age of 65 [4], the aging population bears the major brunt of stroke-related mortality and disability. At these current trends, the increased prevalence of stroke victims suffering from disability will place serious burdens on both the families caring for stroke victims and the capacity and costs of the healthcare system.

Ischemic stroke accounts for 87 % of all stroke cases and offers the greatest potential for therapeutic prevention and treatment [1]. Currently, there are only two therapies available to patients who present with an acute ischemic stroke: thrombolytic agents and endovascular

interventions for acute clot retrieval. If the patient is examined and diagnosed within three hours of stroke onset and there are no contra-indications to lytic use, then intravenous tissue plasminogen activator (tPA) can be given [5, 6]. Treatment with tPA carries risks, including possible intracerebral hemorrhage as demonstrated in Fig. 1. In any case, less than 5 % of the nearly 800,000 patients who present with stroke annually are treated with tPA, mostly due to its narrow time window [1, 7, 8]. For patients that fall outside of this 3-h window, alternative treatments include mechanical clot removal or local application of lytic agents through an intravascular catheter. Even with these advanced treatments (that are often not available outside of large comprehensive stroke centers), many patients still fall outside the therapeutic treatment window [1, 7]. Thus, given the current population trends and the limited availability of treatments, it is imperative that focus turns to stroke prevention, new therapeutic options that can be utilized in clinically realistic time windows, and strategies that improve recovery once a stroke has occurred.

## Aging and Stroke

Aging is a complex process that leads to an altered innate immune environment in the brain [9]. Chronic low-grade inflammation is seen in older individuals, which contributes to the development of age-related diseases including stroke [10–13]. Aging leads to both higher baseline and stimulus-induced activation of central pro-inflammatory cytokines [1, 14–17] compared with the young brain. Systemic inflammation (such as can occur with infections) increases brain inflammation in both animals and in humans [18], but the detrimental effects are primarily seen in the aged. Peripheral or central LPS administration (the major component of the outer membrane of Gram-negative bacteria) dramatically increases brain cytokine synthesis (i.e., interleukin (IL)-1 $\beta$ , IL-18, and IFN- $\gamma$ ), and this is much more robust in the aged brain [15, 16, 19]. To make matters worse, the aging brain also loses endogenous anti-inflammatory and protective substances, such as IL-10, IL-4, and brain-derived neurotrophic factor, making it even more difficult to cope with ischemic stress [20, 21]. Aging primates and humans have been described as having higher microglial numbers (although differentiation from other myeloid cells with currently available markers can be difficult) with a more “activated” phenotype [22, 23]. As microglia are a major source of pro-inflammatory cytokines, this results in a heightened basal level of cytokines even in the absence of ischemic injury in aged brain [24]. The biochemical, structural, and metabolic changes that occur in the aging brain have only recently been recognized (see review by [25]). These are extremely important factors in the response to stroke, and recent epidemiology and preclinical evidence suggest that aging is a major contributor to the sex differences seen in stroke epidemiology and outcomes.

## Sex Differences in Stroke

### Clinical Epidemiology

Clinical evidence that suggests intrinsic sex differences play a role in the response to stroke comes from the extreme ends of the lifespan—in the very young and in the elderly. Clinically, if one collapses across all age groups, women are disproportionately more likely to be affected by stroke than men each year [1]. However, almost all of this excess risk is due to the dramatic increase in stroke incidence seen in elderly women, as throughout most

of adulthood, the overall incidence of stroke in men is estimated to be 33 % higher than that of women [26]. This “male-sensitive” phenotype is also observed in the perinatal, neonatal, and childhood population; males are at higher risk of both hemorrhagic and ischemic stroke [27–32]. A sex disparity in outcomes after ischemic injury also exists, with female infants faring better than males [33–37]. Considering that sex hormone levels are low in both females and males at this age, these differences in outcomes may be influenced by sex-specific hormone-independent factors (i.e., XX vs. XY) or by early “organizational” (perinatal) effects of gonadal steroid exposure (see below).

Importantly, the epidemiology of stroke changes as women age. After the age of 75, stroke rates begin to increase and vastly exceed that of age-matched men by the age of 80 [17, 38]. Elderly women have more severe strokes, poorer recovery, and greater long-term disability [26, 39–41] compared with age-matched men. There are numerous potential confounding factors that contribute to this change in clinical stroke epidemiology, including the older age at which women have strokes. The age of a first stroke in women is on average four years higher than men [42] and advanced age is a well-established risk factor for poor outcomes and increased stroke-related mortality [43–45]. Aged patients also have additional co-morbid illnesses, such as diabetes and hypertension, which can influence outcome [42]. In addition, differences in stroke etiology also may contribute to the poorer outcomes seen in elderly women, most notably in cardioembolic strokes secondary to atrial fibrillation (AF). The incidence of atrial fibrillation is greater among men at all ages but given that there are almost twice as many women aged >75 years with AF compared with men, the absolute number of women with AF is growing [46–48]. Women are more likely to experience a cardioembolic stroke secondary to AF than men and this is true regardless of anticoagulant use [49, 50]. Female sex is an independent risk factor for stroke in AF with a hazard ratio of 1.5, and this is especially notable in women over 75. Risk stratification schemes now take female sex into account when considering risk of ischemic stroke from AF [49]. In a recent analysis of the AFFIRM study, women with AF spent less time in the therapeutic range (an INR of 2–3) when treated with warfarin, which not surprisingly led to increased stroke rates [51]. Sex-specific differences in the pharmacodynamics and therapeutic efficacy of warfarin anticoagulation may exist and remain to be explored. This is a critical point as embolic strokes lead to large territorial infarcts (i.e., MCA) with high NIHSS scores and greater resulting disability [52] compared with small vessel and atheromatous etiologies. There are also several social factors that likely contribute to the poor outcomes in elderly women. Many have outlived their spouses and are less likely to be able to return home after a stroke occurs leading to significantly higher institutionalization rates for women after stroke [51]. However, even after controlling for these confounders, evidence for an age-related loss of intrinsic female protection remains. It remains to be determined if this is loss of a beneficial factor in women (i.e., estrogen), or loss of a detrimental factor with age (i.e., androgens) in males, which is the subject of a review elsewhere in this issue.

## Experimental Studies

Preclinical studies confirm that the effects of stroke vary based on both the sex and age of the animal examined. Although decreased ischemic damage occurs in adult female vs. male rodents in many models of induced global [53] and focal [48] cerebral ischemia (for

reviews, see [54, 55]), we have found that female mice are more susceptible to stroke damage than males [24] after the age of 15 months. This has also been seen by others [56, 57] and the loss of circulating E2 with gonadal senescence has been one accepted etiology of this sex difference [58]. However, as hormone levels are similar in males and females prior to puberty yet females show reduced ischemic damage, there must be an unknown interaction with age and hormone loss or an intrinsic sex difference mediated by chromosome complement and/or organizational effects of steroids (see recent reviews [25, 59]).

## Estrogen Mediated Neuroprotection

Estrogen, specifically 17 $\beta$ -estradiol, is a neuroprotective hormone in the vast majority of animal studies (see the review by [60]). Sex steroid hormones can act via organizational effects which irreversibly commit tissues to a male or female phenotype, or activational effects which are reversible and dependent on the continued presence of the hormone [61–63]. Sex differences in stroke incidence and outcome are present in neonatal populations, long before the onset of the activational effects of gonadal hormones at puberty. Thus, recent attention has been given to the possible organizational effects of steroids which occur during prenatal and early postnatal life [64]. Males convert testosterone to estradiol within the brain through the action of aromatase, while the organization of the female brain occurs due to the lack of this locally produced estradiol. In females, estradiol produced by the ovaries circulates bound to  $\alpha$ -fetoprotein, which prevents it from crossing the blood–brain barrier (BBB) [65]. These sex-specific organizational effects contribute to the respective sex-specific behaviors of males and females [66], as they appear to be permanent and are incapable of reversal via gonadectomy. Experimental evidence suggests that estradiol can protect the brain from stroke via activational mechanisms, as ovariectomy extinguishes this neuroprotective effect [24, 66]. These activational effects of acute estrogen exposure are also independent of chromosomal sex as evidenced by findings that estradiol administered at reperfusion also decreases infarct size in males [67]. However, very recent data suggest that ischemic sensitivity can also be patterned very early in development secondary to organizational effects of gonadal steroids [68]. Neonatal male rats were administered either exogenous testosterone propionate (TP), the non-aromatizable androgen, dihydrotestosterone (DHT), or vehicle for 5 days after birth. At 3 months of age, a focal stroke was induced. Testosterone treated rats (but not DHT-treated animals) had significantly smaller infarct volumes as well as increased estradiol levels compared with oil-treated animals. TP-injected males also had increased testicular aromatase (P450arom) levels compared with oil-treated males. This suggests that neonatal exposure to exogenous testosterone (an aromatizable androgen) chronically up-regulates aromatase expression in the testes of the male rats, leading to higher serum estradiol and neuroprotection against ischemic injury in adulthood [68]. Interestingly peri-natal exposure to testosterone also increased the adult expression of X-linked inhibitor of apoptosis (XIAP), an anti-apoptotic protein that may be especially important in reducing ischemic injury in the female brain [69]. This study suggests that early exposure to gonadal hormones can have dramatic effects on the response to adult cerebrovascular injury.

## Sex Differences in Inflammatory Signaling

Genetic differences leading to alterations in the inflammatory response have been noted in other models [70, 71], but very little is known regarding ischemic stroke. Differential expression by sex of autosomal [72] and X-chromosome genes [73] have been identified in blood samples of ischemic stroke patients, and differential expression of Y-chromosome genes have been identified in male stroke patients compared with male controls [74]. Animal models have recently been developed that may help dissect out the effects of chromosomal sex and hormone exposure including the “Four Core Genotype” and the “Y consomic rat” model, but each has limitations (see reviews [75, 76]). Each of these models involves manipulation of the Y chromosome or Y chromosome genes and are currently being evaluated in stroke models. Recent experimental studies assessed chromosome dosage effects in two separate strains of female mice with either an XX or XO chromosome complement, which had no differences in infarct following induced stroke, regardless of acute hormone status. This suggests that the effects of X-chromosomal dose on acute ischemic sensitivity are minimal, at least in a reperfusion model. However, these animals are difficult to breed, animal numbers were small, and further studies using these models are warranted [77]. There are likely to be complex interactions between genetic sex and hormonal contributions to ischemic sensitivity that lead to subtle differences in phenotype. More translationally relevant (yet costly) models, such as examining stroke sensitivity in aged XO mice, or examining spontaneously occurring strokes, may lead to very different results. Epigenetic mechanisms in stroke are also beginning to be explored and are reviewed extensively elsewhere (see [78–80]). Experimental challenges arise in identifying purely genetic, epigenetic, and hormonal effects as these all contribute to the complex inflammatory response to ischemic stroke (see Fig. 2).

## Sexual Dimorphism in Stroke-Induced Cell Death

### Males Preferentially Utilize Caspase-Independent Cell Death Mechanisms

Several divergent cell death pathways are activated by ischemic injury, and these represent signaling cascades that could differ based on sex or hormonal patterning. Laboratory evidence demonstrates that males and females utilize different cell death pathways when confronted chemically with an insult, illustrated in Fig. 3. Males tend to exhibit caspase-independent cell death [81, 82] via the induction of nNOS [83], the formation of peroxynitrite (ONOO<sup>-</sup>) with subsequent DNA damage through oxidation and nitration [84]. This DNA damage induces poly(ADP-ribose) polymerase-1 activation, translocation of apoptosis inducing factor from the mitochondrion to the nucleus [85, 86], and subsequent cell death. This has been supported by numerous experimental studies (see Table 1) and has been a subject of several recent reviews [18, 59, 87].

### Females Favor Caspase-Dependent Cell Death

In females, caspase-induced cell death predominates, illustrated in Fig. 3. The influx of Ca<sup>2+</sup> induces formation of the mitochondrial apoptosis-induced channel (MAC) which permeabilizes the mitochondrial membrane, prompting the release of cytochrome c into the cytosol activating the caspase cascade and subsequent cell death [88]. Importantly, this

differential sensitivity to specific cell death mechanisms contributes to differential responsiveness to neuroprotective agents. Although this preclinical work is in its infancy, the future translational relevance could be high as many potential neuroprotective drugs interfere with these cell death pathways.

### Clinical Evidence

Clinical studies are revealing clear sex differences in therapies utilized in the treatment of stroke patients, including thrombolytics and antiplatelet agents [15, 89]. For example, in the Women's Health Study, aspirin lowered the risk of stroke by 24 % in women *in direct contrast* to findings in the Physicians Health Study, a similar, male-only cohort, in which there was *no effect* of aspirin on stroke [90]. Similar sex-specific effects are also seen in infancy, implying that sex dimorphisms are established very early in development, either via organizational effects or chromosomal effects. For example, indomethacin significantly lowered the incidence and severity of intraventricular hemorrhage in preterm infants but did not improve later childhood cognitive performance. Subsequent reports in animals showed sex-specific effects of this drug, leading to a reanalysis of the clinical data. In boys, indomethacin reduced the incidence of IVH by half, and led to higher verbal IQ scores, but the drug had no effect in girls [37, 91, 92]. In pediatric stroke, when levels of androgens in males and estrogens in females are low, epidemiological findings indicate that boys have a higher incidence rate of stroke and worse outcomes than their corresponding female cohorts [2, 19, 30]. This indicates that hormones are not the only component of sexual dimorphism in ischemic stroke and that there may be a genetic or epigenetic component. Future studies of sex as an independent response variable are needed to optimize the development of future stroke therapies.

The observation that reproductive-aged, estrogen-producing women are at a much lower risk for stroke than their age- matched male and postmenopausal female counterparts suggests that estrogen replacement therapy (ERT) could have potential for preventative treatment against ischemic stroke. However, attempts to use estrogen to reduce stroke risk have been unsuccessful. In both the Women Estrogen Stroke Trial (WEST; conjugated equine estrogen, 0.625 mg/day) and the Women's Health Initiative (WHI) trial (estradiol, 1 mg/day) there was an increase in stroke incidence in women treated with estrogen [93, 94]. Current recommendations state that hormone replacement therapy (HRT) is not indicated for primary or secondary prevention of cardiovascular disease or dementia, nor for preventing deterioration of cognitive function in postmenopausal women [95]. However, several issues have been extensively discussed in the literature regarding the design of these trials, specifically the timing of initiation of ERT and the chosen dose. High doses of estrogen correlated with an elevated serum level of C-reactive protein (CRP) which is a marker of vascular risk [96–98]. Interestingly, in reproductive age women, estrogen fluctuations that occur during the menstrual cycle inversely correlate with CRP protein levels [96, 99]. This suggests that endogenous levels of estrogen produce an anti-inflammatory effect, however higher doses of estrogen predispose women to increased vascular risk.

Recent data emerging from the Kronos Early Estrogen Prevention Study also suggest that increasing age is, as suspected, a major contributing factor in the response to ERT. Low-

dose estrogen and cyclic monthly progesterone in healthy women initiated within 3 years after menopause did not increase the risk of myocardial infarction (MI), transient ischemic attacks, stroke, or venous thromboembolic disease ([http://www.keepstudy.org/news/pr\\_100312\\_a.cfm](http://www.keepstudy.org/news/pr_100312_a.cfm)). Consistent with these results, a European trial of women who were followed for over 10 years after randomization into hormone replacement therapy cohorts (triphasic estradiol and norethisterone acetate, or 2 mg estradiol a day in women with previous hysterectomy) initiated early after menopause found that treated women had a significantly reduced risk of mortality, heart failure, or MI, without any apparent increase in risk of cancer, venous thromboembolism, or stroke [100]. This is in contrast with results from the WEST and WHI trials, where ERT increased stroke incidence and severity when ERT was initiated after a prolonged period of hypogonadism. Importantly, these new findings correlate well with animal studies that demonstrated that estrogen can enhance the inflammatory response and worsen stroke damage if ERT was initiated after prolonged ovariectomy [101] or given months after cessation of ovarian cycling to aged female mice [102]. These studies highlight the importance of using appropriate preclinical animal paradigms to model human disease.

## Stroke Risk and Hormonal Variations

An early age of menarche (<12 years of age) has been associated with increased risk of cardiovascular disease events including stroke; however, this was associated with increased adiposity [103]. Adiposity is associated with increased levels of estrogen, as white adipose tissue is capable of producing endogenous estrogen [104, 105]. High levels of estrogen may be associated with stroke risk, especially when they are in the nonphysiological range, as evidenced by the increase in stroke risk seen with older “high-estrogen” birth control [106, 107]. Recent studies have suggested that higher serum estrogen levels are associated with stroke [59], independent of BMI. However, as serum levels were measured post-stroke, this could be a response to the injury or even an attempt to control or reduce damage.

Another well-known period of high risk for women is during pregnancy and the peripartum. During pregnancy estrogen levels steadily climb and increases production of clotting factors, which may account for the increased risk of stroke in women in the peripartum period [108, 109]. More research is necessary to understand the underlying mechanisms of how estradiol, or perhaps fluctuations or changes in estradiol, could predispose women to a higher risk of stroke.

## Effects of Estrogen on the Inflammatory Response in Stroke

Estrogen is a neuroprotective agent in most experimental studies, and this may be due in large part to a suppressive action of estrogen on inflammation [110–112]. Tumor necrosis factor (TNF) is involved in many neurodegenerative diseases including multiple sclerosis, Alzheimer's disease, and Parkinson's disease [113]. It has been known for some time that postmenopausal women have higher levels of circulating TNF [114]. Here, we will briefly discuss the role of estrogen on the TNF pathway and its downstream effects on nuclear factor- $\kappa$ B (NF $\kappa$ B) signaling in ischemic stroke.



## Estrogen and TNF

TNF is a pro-inflammatory cytokine that acts on receptors found on neuronal [115, 116], glial [117], and endothelial cell populations [118]. Growing evidence suggests that while TNF is intended to aid in injury repair, excessive TNF can be neurotoxic [119–121]. Levels of TNF can increase to 100- to 1,000-fold that of basal levels following stroke in rats [122, 123] and is significantly elevated in stroke patients [124]. Female rats with low estrogen levels tend to produce more TNF than female rats with higher estrogen levels [125]. However, chronic administration of estradiol in mice stimulates the secretion of a variety of pro-inflammatory cytokines, including TNF itself [126]. Physiologic levels of estrogen appear to attenuate TNF expression [125] while estrogen deficiency (as in menopause) represents a loss of this attenuation and excessive TNF expression, as illustrated in Fig. 4.

Several experiments suggest that targeting TNF may be a translationally relevant target for ischemic stroke therapy. Infliximab (Remicade) and adalimumab (Humira) are anti-TNF therapies that have been approved for use in other clinical conditions. As we are already in the era of “biological” therapies, it is possible that some of these existing therapies have the potential to be developed for emergency care. Recently, a fusion protein of the extracellular domain of human TNFR2 was fused to the C-terminus of the heavy chain mouse transferrin receptor (TfR), named cTfRMAb-TNFR [127]. Administration of this antibody 45 min after arterial occlusion led to a significant reduction in both infarct and neurobehavioral deficits, suggesting that these agents can cross the BBB and have significant therapeutic effects for estrogen-deficient postmenopausal women. However, the effect of this agent in aged mice and in females has not yet been examined. If the detrimental effects of TNF signaling are normally suppressed by estrogen, this could be a viable therapy for the majority of women with stroke (who are postmenopausal).

## Estrogen and NF $\kappa$ B

NF $\kappa$ B is an inducible transcription factor that functions to mediate inflammatory signaling in a variety of cell types, including neurons [128, 129], and is illustrated in Fig. 4. Hypoxic conditions [130] and oxidative stress [131] lead to the immediate activation of NF $\kappa$ B [132]. Contradictory views on the role of NF $\kappa$ B in stroke stem from its dual function: to induce pro-inflammatory cytokine expression [133–135] and to induce survival signaling [136–139]. The difference may reside within the extent and severity of the ischemia and is reviewed elsewhere [140, 141]; however, here we focus on estrogen effects on NF $\kappa$ B signaling.

In vitro experiments in rat aortic smooth muscle cells have shown that estradiol binds to intracellular estrogen receptor  $\beta$  (ER $\beta$ ) and promotes synthesis of inhibitor of  $\kappa$ B- $\alpha$  (I $\kappa$ B $\alpha$ ) [142]. This increase in I $\kappa$ B $\alpha$  levels is responsible for increased inhibition of NF $\kappa$ B signaling in the presence of estradiol [142]. A deficiency of I $\kappa$ B $\alpha$  in the brain predisposes the brain to a higher basal level of neuro-inflammation [143]. Pre-treatment with estradiol enhances binding of estradiol-ER $\beta$  complexes at promoters of vascular inflammatory genes *MCP-1* and *CINC-2 $\beta$*  and reduces binding of NF $\kappa$ B, thereby reducing their expression [142]. While neurons express NF $\kappa$ B, some argue that the primary cell type subject to NF $\kappa$ B regulation in the CNS is the astrocyte [143]. Thus, while the interplay of estrogen in the NF $\kappa$ B pathway is

being elucidated, more research needs to be done to understand the role of astrocytes in communicating responses to neurons following ischemic insult.

## Final Remarks

The mechanisms underlying ischemic sexual dimorphism are only beginning to be understood and investigated. Differences in the cell death pathways activated by an ischemic injury in males and females require therapeutic exploitation. We require a better understanding of the immune response to injury and how both sex and gonadal hormones can influence the post-stroke immune response. Biological agents targeting stroke-induced inflammatory cytokines will be developed and these agents will need to be tested in clinically relevant animal models prior to administration to stroke patients [127]. The role of genes, epigenetic regulation, and how hormones interact with these will require considerable research.

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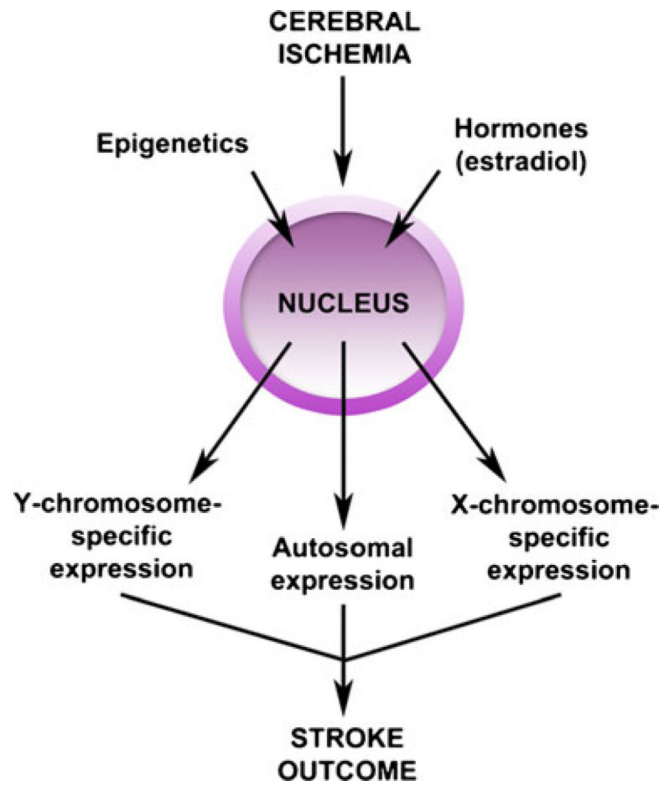


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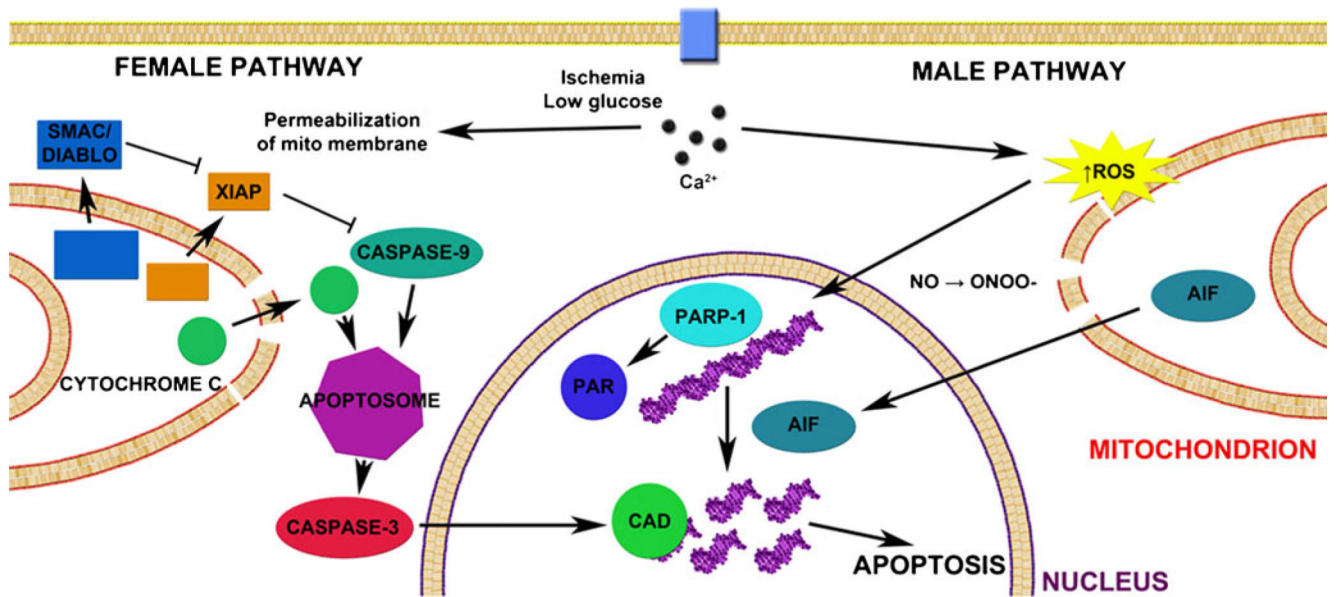
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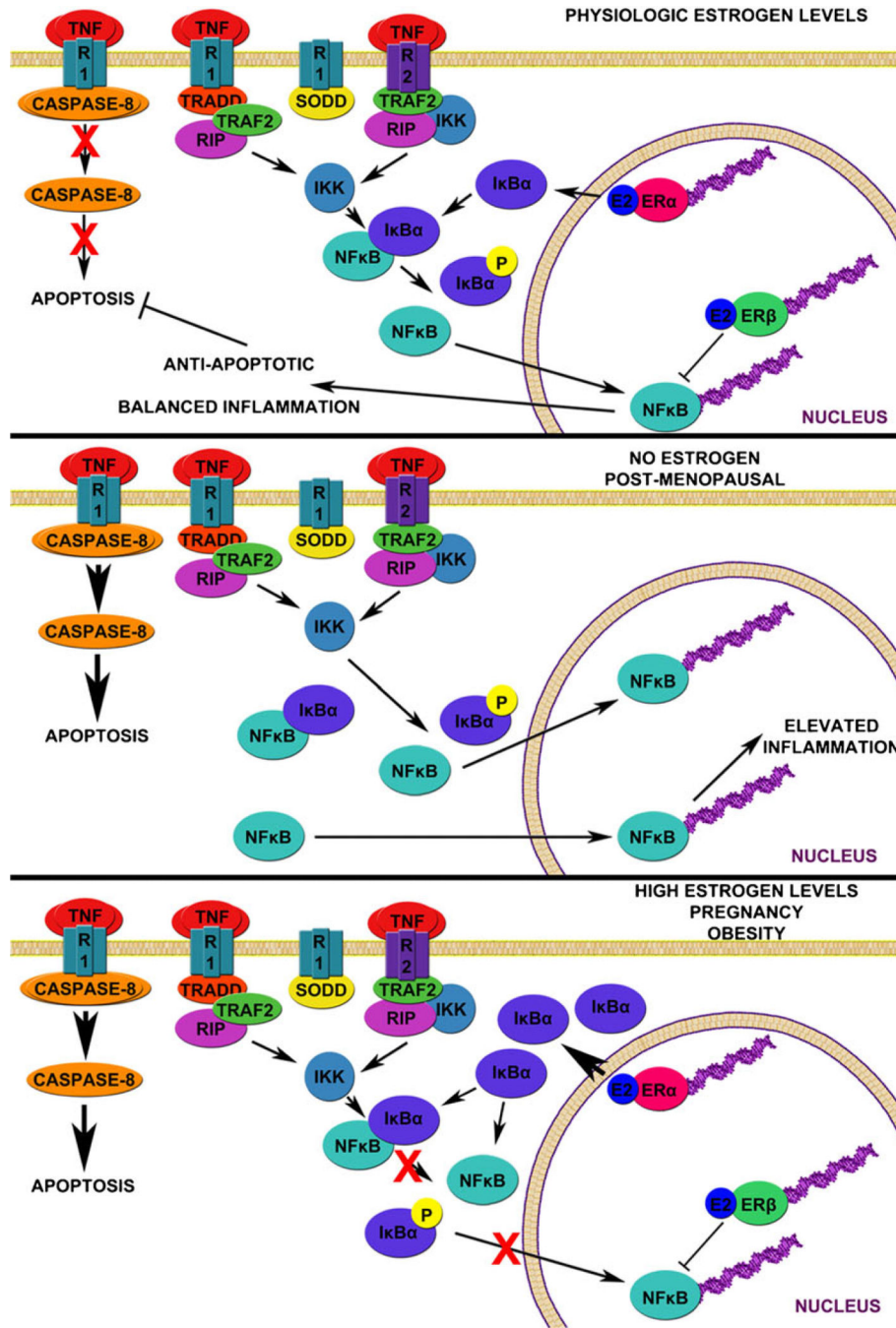
**Fig. 1.** Case of intraventricular and intracerebral hemorrhage following treatment with tPA 70-year-old man with left middle cerebral artery stroke who was administered tPA and subsequently worsened. Repeat CT scan showed intraventricular and intracerebral hemorrhage as well as midline shift. *Blue arrow* indicates ischemic infarct and *red arrow* indicates hemorrhage



**Fig. 2.**  
Influence of epigenetics and hormones on genetic expression and stroke outcome



**Fig. 3.** Sexual dimorphism in cell death following cerebral ischemia. Caspase-dependent death predominates in females, where the influx of  $\text{Ca}^{2+}$  induces formation of the MAC which permeabilizes the mitochondrial membrane. Cytochrome c, Smac/DIABLO, and X-linked inhibitor of apoptosis protein (*XIAP*) are released into the cytosol. Cytochrome c and caspase-9 facilitate formation of the apoptosome which in turn activates caspase-3. Caspase-3 cleaves the inhibitor of caspase-activated DNase to give caspase-activated DNase (*CAD*) which subsequently cleaves DNA and results in apoptosis. Caspase-independent cell death predominates in males, in which ischemia results in an increase in reactive oxygen species (*ROS*) which combine with nitric oxide (*NO*) to form peroxynitrite (*ONOO-*). *ONOO-* damages DNA via oxygenation and nitration which activates polymerase (ADP-ribose), polymerase-1 (*PARP-1*), and formation of poly(ADP-ribose) (*PAR*) polymers. Permeabilization of the mitochondrial membrane enables apoptosis-inducing factor (*AIF*) to translocate from the intermembrane space of the mitochondrion to the nucleus where it causes specific DNA fragmentation patterns and chromatin condensation, ultimately resulting in apoptosis.



**Fig. 4.** Hypothesized interaction between estradiol, TNF, and NFκB signaling At physiological levels of estradiol (*E2*), tumor necrosis factor (*TNF*) preferentially binds to TNF receptor 2 (*TNFR2*), denoted *R2*, leading to activation of nuclear factor-κ B (*NFκB*) which translocates to the nucleus. Balance between estrogen receptor-α (*ERα*) transcription of inhibitor of κ B-α (*IκBα*) and estrogen receptor-β (*ERβ*) inhibition of NFκB binding is present, resulting in balanced inflammation and net anti-apoptotic signaling. With physiological levels of *E2*, *TNF* levels are lower and there is reduced stimulation of TNF receptor 1 (*TNFR1*), denoted

R1, and subsequent caspase signaling. In postmenopausal women, E2 levels decline and there is a lack of ER $\alpha$ -induced I $\kappa$ B $\alpha$  expression and ER $\beta$ -mediated inhibition of NF $\kappa$ B. Higher circulating TNF levels stimulate both TNFR2 and TNFR1, leading to relatively uninhibited inflammation and induction of caspase-dependent apoptosis. With high levels of E2 as seen in pregnancy and obesity, there is increased expression of I $\kappa$ B $\alpha$  which inhibits NF $\kappa$ B. The NF $\kappa$ B that translocates to the nucleus is inhibited by ER $\beta$  binding, inhibiting expression of anti-apoptotic genes by NF $\kappa$ B. Lack of inhibition of TNFR1 signaling results in caspase-dependent apoptosis. See reviews [120, 144]

**Table 1**

Summary of experimental evidence suggestive of sexual dimorphism in cellular death following cerebral ischemia

	Experimental setting	Authors	Outcome on stroke severity	
			Males	Females
Caspase independent	PARP-1 <sup>-/-</sup>	[11, 145]	↓	↑
	PARP-1 inhibitor (minocycline)	[146]	↓	↑ <sup>1</sup>
		[147]	↓	↑ <sup>1</sup>
	nNOS <sup>-/-</sup>	[148]	↓	↑
	nNOS inhibitor	[67, 148]	↓	↑
	Harlequin (low AIF)	[87, 149]	↓	↔
Caspase dependent	Broad spectrum caspase inhibitor	[18]	↔	↓
	XIAP small molecule inhibitor	[69]	↔	↑

These results may be model dependent