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Review

Window of opportunity: Estrogen as a treatment for ischemic stroke

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ABSTRACT

The neuroprotection research in the last 2 decades has witnessed a growing interest in the functions of estrogens as neuroprotectants against neurodegenerative diseases including stroke. The neuroprotective action of estrogens has been well demonstrated in both in vitro and in vivo models of ischemic stroke. However, the major conducted clinical trials so far have raised concern for the protective effect of estrogen replacement therapy in postmenopausal women. The discrepancy could be partly due to the mistranslation between the experimental stroke research and clinical trials. While predominant experimental studies tested the protective action of estrogens on ischemic stroke using acute treatment paradigm, the clinical trials have mainly focused on the effect of estrogen replacement therapy on the primary and secondary stroke prevention which has not been adequately addressed in the experimental stroke study. Although the major conducted clinical trials have indicated that estrogen replacement therapy has an adverse effect and raise concern for long term estrogen replacement therapy for stroke prevention, these are not appropriate for assessing the potential effects of acute estrogen treatment on stroke protection. The well established action of estrogen in the neurovascular unit and its potential interaction with recombinant tissue Plasminogen Activator (rtPA) makes it a candidate for the combined therapy with rtPA for the acute treatment of ischemic stroke. On the other hand, the "critical period" and newly emerged "biomarkers window" hypotheses have indicated that many clinical relevant factors have been underestimated in the experimental ischemic stroke research. The development and application of ischemic stroke models that replicate the clinical condition is essential for further evaluation of acute estrogen treatment on ischemic stroke which might provide critical information for future clinical trials.

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Contents

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1. Basic research: estrogen as neuroprotection for ischemic stroke

Stroke is one of the leading causes of death and morbidity worldwide. Approximately, 1 in 6 people will have a stroke in their lifetime. In the United States, stroke currently ranks as the first leading cause of disability and the fourth leading cause of death. The epidemiologic data suggest that the decline in both stroke incidence and mortality reached a nadir in the early 1990s and is now rising because of the aging population (Stapf and Mohr, 2002). Stroke patients must not only survive the acute stages of infarction, but must then cope with significant mental, physical, and economic stresses associated with neurological impairment. Considering the cost in loss of life, physical and mental disability and subsequent loss of productivity, the need for effective therapeutic interventions is obvious.

Ischemic stroke is by far the most frequent type of stroke, accounting for over 80% of all stroke cases (Gillum, 2002). Two primary therapeutic approaches have been intensively studied for the treatment of acute cerebral ischemia: (i) vascular approach that targets re-opening occluded blood vessel and (ii) cellular approach to interfere with the pathophysiological cascades leading to ischemic damage (Schaller and Graf, 2004). The vascular approach is based on the fact that ischemic stroke is a cerebral vasculature event, initiated by occlusion of a cerebral artery and results in substantial brain tissue damage. Interruption of blood flow to the brain results in ischemia and deprives neurons and surrounding cells of crucial substrates. Unless the supply of these substrates can be restored, the cells in the region will ultimately die. Given the high energy demand and vulenrability of brain to ischemic damage, the vascular approach focuses on limitation of cerebral ischemia by early reperfusion after cerebral ischemia. The effort to develop effective vascular therapy for acute ischemic stroke achieved several important successes in 1990s. Based on the results from the National Institute of Neurological Disease and Stroke (NINDS) trial in 1995, intravenous recombinant tissue Plasminogen Activator (rtPA) is recommended for selected patients within 3 h of ischemic stroke onset (Wardlaw et al., 2003). The use of rtPA is now the only established stroke treatment for those patients presenting within 3 h of ischemic stroke onset. Beyond this time window, systemic rtPA does not appear to be as beneficial and increases the risk of serious side effects. Therefore, thrombolytic therapy has been severely limited by the need for hyper-acute administration with less than 5% of the potentially eligible patients receive the treatment (Zivin, 1999). Overall, rtPA and all other thrombolytic therapies, has less medical impact for the stroke patients.

In the last 2 decades, tremendous effort has been invested to develop new neuroprotective agents that aim to prevent the progression of ischemic cascades and reduce brain damage. Various neuroprotective strategies have been developed and tested for nearly all components of the ischemic cascades, including free radical scavengers, anti-excitotoxic agents, apoptosis inhibitors, anti-inflammatory agents, metal ion chelators, ion channel modulators, gene therapy and stem cells transplantation. The premise of neuroprotection has originally focused on the prevention of neuron from death. With recent research emphasizing ways to reduce tissue damage by both vascular and cellular mechanisms, the spotlight has been shifting towards the study of neurovascular interaction. The neurovascular unit provides a conceptual model comprised of cerebral endothelial cells, glia, and neurons, along with an extracellular matrix that maintains the integrity of brain tissues. This modular concept emphasizes the dynamics of vascular, cellular and matrix signaling in the brain (Del Zoppo, 2013; Lo et al., 2004). In addition, as a cerebral vascular event, ischemic stroke induces not only a complex array of pathogenic cellular cascades in brain parenchyma, but also impairment of autoregulation in brain vasculature at both acute and chronic stages (Cipolla et al., 1997; Cipolla and Curry, 2002; Jimenez-Altayo et al., 2007; Winters et al., 2012). Therefore, therapeutic intervention targets neurovascular unit and cerebral vasculatures might provide a more integrative framework that could guide the discovery of treatment for ischemic stroke (Zhang et al., 2012).

Estrogens were first described by Frank et al. (1925) as a component of the ovary needed for reproductive function. Later, estradiol was isolated and crystallized by Doisy et al. (1930). Among estrogens, 17β-estradiol is the most potent natured occurring estrogen. It is now clear that estrogens are pleiotropic hormones that functions beyond the scope of the reproductive system. The neuroprotection research in the last 2 decades has witnessed a growing interest in the functions of estrogens as neuroprotectants against neurodegenerative diseases, including stroke. The first experimental evidence to implicate possible role of endogenous female hormones as being neuroprotective against global cerebral ischemia-reperfusion injury showed that intact adult female rodents sustain less neuronal damage as compared to age-matched males (Hall et al., 1991). Later, estrogen was demonstrated a potent neuroprotectant in vitro (Bishop and Simpkins, 1994) and are very effective against ischemia-induced brain damage (Alkayed et al., 1998; Dubal et al., 1998; Simpkins et al., 1997). There is now abundant evidence for neuroprotection by estrogens both in vitro and in vivo (McCullough and Hurn, 2003). Importantly, the potency and efficacy of estrogens have

been well demonstrated for a variety of cell types in the neurovascular unit against ischemia/reperfusion injury (Yang et al., 2005). The protective effects of estrogens have been shown in astroglia, microglia and oligodendrocytes (Garcia-Segura et al., 1999; Mor et al., 1999; Takao et al., 2004). In addition, it is known that estrogens regulate cerebral autoregulation by enhancing basal release of Nitric Oxide (NO) and so reducing pressure-induced myogenic constriction (Geary et al., 1998; Skarsgard et al., 1997). There is also evidence suggests that a portion of the vasoprotective effect of estrogens is through increased vessel compliance (De Meersman et al., 1998; Nagai et al., 1999). The effects of estrogens on cerebral vasculature homeostasis and endothelium could contribute the protective effects on disruption of BBB induced by ishcemic stroke. Consistently, estrogens have been demonstrated to protect BBB disruption induced by transient focal cerebral ischemia (Liu et al., 2005).

The neuroprotective effects of estrogens have been demonstrated in a variety of stroke models. These include transient and permanent middle cerebral artery occlusion models (Alkayed et al., 1998; Dubal et al., 1998; Simpkins et al., 1997), global forebrain ischemia models (Sudo et al., 1997), photothrombotic focal ischemia models (Fukuda et al., 2000), and glutamate-induced focal cerebral ischemia models (Mendelowitsch et al., 2001). The protective effects of estrogens have been described in rats, mice and gerbils (Chen et al., 2001; Culmsee et al., 1999; Simpkins et al., 1997). Estrogen-induced neuroprotection has been demonstrated in adult female, middle-aged female as well as reproductively senescent female rats (Wise et al., 2001). Similarly, these effects of estrogens have been shown despite the presence of diabetes and hypertension (Carswell et al., 2000; Toung et al., 2000). The neuroprotective effects of estrogens have been demonstrated against subarachnoid hemorrhage, a highly prevalent form of stroke in females (Yang et al., 2001). Further, the neuroprotective action of estrogens is not limited to the female, inasmuch as estrogen protection is also seen in males (Hawk et al., 1998; Toung et al., 1998). Finally, the protective effects of estrogens are not limited in the pretreatment paradigms as 17β-estradiol exerts dose-dependent protective effects against ischemic stroke in post-treatment paradigms (McCullough et al., 2001; Yang et al., 2000a; Yang et al., 2003). Collectively, these results indicate that estrogens could be valuable candidates for brain protection during acute stroke.

2. Clinical trials: estrogen replacement therapy fails to prevent primary and secondary stroke

Overwhelming evidence from basic research suggests neuroprotective effects of estrogens against ischemic stroke (Yang et al., 2005). There are also considerable epidemiological studies suggesting gender differences exist in the incidence of stroke between pre-menopausal women and man (Rosamond et al., 2008; Turtzo and McCullough, 2008). This has prompted clinical trials to examine the effect of estrogen on cardiovascular diseases and stroke. Most of the trials, if not all, were designed to evaluate the effect of long-term estrogen replacement therapy or estrogen plus progestin replacement therapy on the incidence of cardiovascular diseases and stroke which has not been adequately addressed in experimental studies. The Women's Health Initiative (WHI) trail was the largest trail designed to examine the effects of estrogen alone and estrogen plus progestin on breast cancer and the incidence of cardiovascular disease in post-menopausal women. The estrogen and progestin therapy trail was terminated prematurely in 2002 due to a significantly higher incidence of stroke, cardiovascular disease, venous thrombosis, and breast cancer in the estrogen plus progestin-treated group (Wassertheil-Smoller et al., 2003). The estrogen-alone study was stopped in 2004 due to the increase risk of stroke for postmenopausal women in the treatment group (Espeland et al., 2004). Similar results have also been found in Heart and Estrogen-progestin Replacement Study (HERS) (Simon et al., 2001). Further, estrogen replacement therapy did not reduce mortality or the recurrence of stroke in postmenopausal women with cerebrovascular disease (Viscoli et al., 2001). Conjugated equine estrogen alone also increases the risk of ischemic stroke in generally healthy postmenopausal women (Hendrix et al., 2006). Interestingly, these trials of exogenous estrogen replacement were contradicted by the observatory study that indicated that longer lifetime exposure to ovarian estrogens may protect against noncardioembolic ischemic stroke (de Lecinana et al., 2007). Nonetheless, the WHI and HERS trails are not appropriate for assessing the potential effects of acute estrogens treatment in stroke protection. On the other hand, the WHI and other clinical trials did raise concern for long term estrogen replacement therapy for stroke patients as the conjugated equine estrogen was indicated to have an adverse effect on cognition, especially among women with lower cognitive function at initiation of treatment (Coker et al., 2010; Espeland et al., 2004).

3. The mistranslation between bench side and bedside

As aforedescribed, a clearly difference appears in the estrogen treatment paradigm between experimental studies and conducted clinical trials. Predominate experimental studies tested the protective action of estrogen on ischemic stroke using acute treatment paradigm. On the other hand, the clinical trials have been mainly focusing on the effect of long term estrogen replacement therapy on the primary and secondary stroke prevention. No clinical study has been conducted to test the protective action of acute estrogen treatment in ischemic stroke which was predominately reported in the experimental studies. While the major clinical trials have suggested that estrogen replacement therapy should not be initiated to prevent vascular disease among postmenopausal women, the available clinical data do not adequately argue against acute estrogen treatment for ischemic stroke. Clearly, given the safety of short-term of estrogen treatment and knowledge obtained from decades of basic research, there is a need of new clinical trials to determine the effects of acute estrogen treatment on ischemic stroke.

As the emerging negative results from clinical trials, more and more experimental studies have been conducted to re-evaluate the effect of estrogen on ischemic stroke with the involvement of more and more clinical relevant circumstance. Detrimental effects of estrogen have been demonstrated in

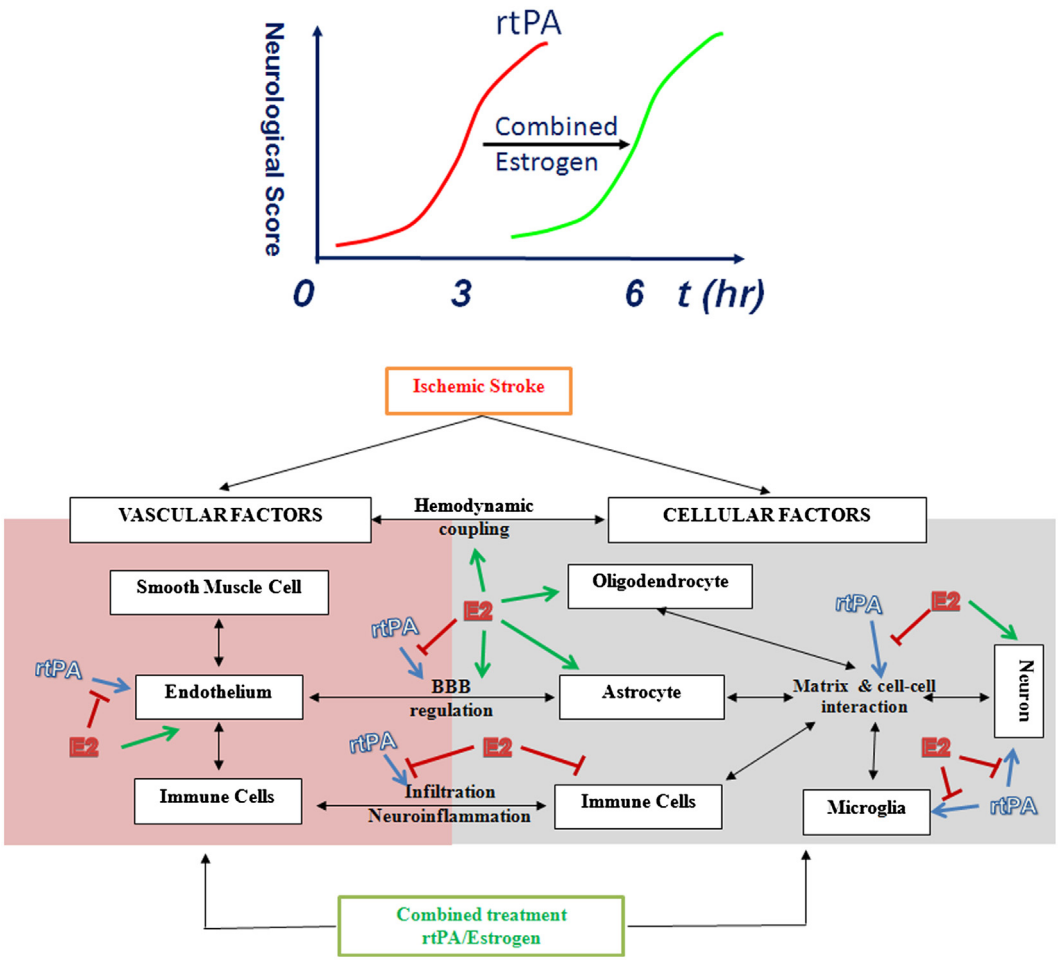

Fig. 1 – Schematic presentation of the potential mechanisms underlying the combined rtPA/estrogen treatment for ischemic stroke. Upper panel: combined estrogen treatment expands therapeutic window of rtPA for the treatment of ischemic stroke. Lower panel: the neurovascular unit neurovascular unit showing functional interactions between the vascular and cellular components the integrative response of brain to ischemia. Ultimately, strategies that view the neurovascular unit as an integrative unit whose compromise contributes to brain damage after ischemic stroke could guide the future of the treatment for ischemic stroke. As indicated, estrogens have profound effects on both vascular and cellular components of neurovascular unit under ischemia insult. Estrogen might not only provide protection but also antagonize many detrimental action of rtPA.

ischemic stroke models upon chronic treatment (Carswell et al., 2004; Harukuni et al., 2001). In aged females, estrogen replacement has been shown to exaggerate ischemic stroke potentially associated with age-dependent loss of insulin-like growth factor-1 (Selvamani and Sohrabji, 2010a). There are also many factors that might have been underestimated in the experimental studies. For example, most, if not all, of the experimental stroke studies to determine the effect of estrogen on ischemic stroke used transient endovascular suture middle cerebral artery occlusion models. Even for the permanent middle cerebral artery occlusion, normally a smaller 4-0 suture was introduced which only lead to about 50% reduction of cerebral blood flow in the ischemic territory (Dubal et al., 1998; Yang et al., 2000a). Therefore, the experimental design of these studies guaranteed the subjects equal duration of ischemia followed by reperfusion or fairly amount of residual cerebral blood flow during ischemia. On the other hand, ischemic stroke induced by thromboemoblism in stroke patients might render very different severity in ischemic damage depending on the thromboembolic states which could be affected by estrogen replacement therapy.

Oral contraceptives including estrogen and progestogen components, are known to affect the metabolism of most proteins involved in blood coagulation although the underlying mechanism is till poorly elucidated. In type 2 diabetic patients, estrogen replacement therapy has been shown to increase the coagulation and fibrinolytic potency via increasing of Factor VII and prothrombin fragment 1+2 and decreasing Plasminogen Activator Inhibitor 1 (PAI1) (Brussaard et al., 2002). It has been indicated that thrombotic risk of hormone replacement therapy may particularly affect women with prothromobitc mutations, specially factor V Leiden (Rosendaal et al., 2002). Although it might not account for all thromboembolic events, hormone replacement therapy may be associated with a significant increase of venous thromboembolism in both pre- and post-menopausal women

(Society, 2012). Therefore, the increase of incidence of ischemic stroke and stroke severity might be attributed to the potential thrombotic action of estrogen replacement therapy. Take this into account, thromboembolic stroke models might be more appropriate to test the effect of estrogen on ischemic stroke.

4. Window of opportunity: estrogen as treatment for ischemic stroke

4.1. *Combined estrogen treatment to expand the therapeutic window of rtPA*

There is increasing evidence indicates a potential clinical application of combination therapy of estrogens with thrombolysis for the treatment of ischemic stroke. Without rtPA thrombolysis derived reperfusion, neuroprotectant may not be effective simply because not enough neuroprotectant could reach the ischemic area, or it might not antagonize the extensive damage as a result of prolonged arterial occlusion. As indicated above, both vascular and cellular factors are needed to be considered for the ischemic stroke therapy. As such, combination of a protective agent with thrombolytic agent might be able to further improve the outcome of stroke patients. In principle, treatments using a combined neuroprotective drug could delay the irreversible cell damage induced by ischemic stroke, hence, extend the short therapeutic window of rtPA. Synergistic or additive effects have been reported when thrombolysis was used in conjunction with neuroprotectants such as oxygen radical scavengers (Asahi et al., 2000), AMPA (Meden et al., 1993) and NMDA receptor antagonists (Zivin and Mazzarella, 1991), MMP inhibitor (Pfefferkorn and Rosenberg, 2003; Sumii and Lo, 2002), citicoline (Andersen et al., 1999), topiramate (Yang et al., 2000b), anti-leukocytic adhesion antibodies and anti-thrombotics (Bowes et al., 1995). Combination therapies might decrease dosages for each agent, thereby reducing the occurrence of adverse events. Encouragingly, two clinical trials reported the feasibility and safety of treatment with rtPA followed by the neuroprotectants, clomethiaxole (Lyden et al., 2001) or lubeluzole (Grotta, 2001). Ultimately, what is required of combination therapies is that they target the entire neurovascular unit, promote cell survival mechanisms, and extend the therapeutic time-window for reperfusion therapy (Lo et al., 2004).

The major deterrent to using rtPA in acute ischemic stroke is the fear of inducing intracerebral hemorrhage. The hemorrhage rate increase to around 6.4% in rtPA treated patients from 0.6% in control group (Wardlaw et al., 2003). However, there was no clear-cut increase in those patients treated in the 3–6 h window compared to those being treated under 3 h (Hacke et al., 2004). Although the risks of hemorrhage do not appear to increase aross time, the chances of acheiving a reperfusion-induced recovery deteriorate rapidly. Collectively, the 3 h therapeutic window of rtPA treatment indicated that the recovery threshold of brain tissue is around 3 h. Beyond this threshord, beneficial effects of thrombolysis induced reperfusion by rtPA or other agents will be overshaded by the deleterious effects through reperfusion injury mechanims *pe se*. Combined therapies are needed to cope with the reperfusion injury induced by thrombolytic approach. We envision such a role for estrogens. The potency and efficacy of estrogens have been well demonstrated for a variety of cell types in the neurovascular unit against ischemia/reperfusion injury (Yang et al., 2005). Given the potent protective effects of estrogens on the whole neurovascular unit and cerebral vasculature, estrogens could be one of the best candicates for the combination therapy. Combination therapies with estrogens might not only extend the therapeutic window for thrombolytic therapy, but might also decrease the dose of rtPA, thereby reducing the occurrence of adverse events. Recently, a number of studies have determined the effect of estrogen on ischemic stroke using embolic stroke models. In young adult female rats, both acute and chronic 17β-estradiol treatment exerts protective effect in an embolic middle cerebral artery occlusion model when combined with rtPA therapy (Leon et al., 2012; Liu et al., 2010). However, long-term 17β-estradiol treatment before stroke worsened ischemic brain injury in aged female rats using the embolic stroke model (Leon et al., 2012), which parallel the finding of the WHI trial (Espeland et al., 2004). Further studies are warranted to determine if acute estrogen treatment could expand the short therapeutic window of rtPA in aged female using embolic stroke models (Fig. 1).

4.2. *From the critical period hypothesis to biomarker window of estrogen on neuroprotection*

A critical period hypothesis has emerged from reconciling the beneficial effects of estrogen in experimental stroke models with the findings of clinical trials (Barrett-Connor, 2007; Maki, 2006; Sherwin, 2009). This hypothesis proposes that hormone therapy is more benign for stroke when taken by younger women or during the peri-menopausal or early post-menopausal period, but deleterious when taken by women significantly past the menopause. The timing window hypothesis was supported by abundant animal studies as well as some clinical studies (Scott et al., 2012). However, further analysis of WHI data argues against the critical timing hypothesis in that overall net harm for combined estrogen-progestin and the lack of a net benefit for estrogen-only therapy in women initiating hormone therapy soon after menopause (Banks and Canfell, 2009; Prentice et al., 2009). Therefore, there might be additional factors that contribute to the discrepancy between the experimental studies and clinical trials. More recently, a biomarker window hypothesis was introduced as the loss of endocrine factors as Vitamin D and IGF-1 might alters the effects of hormone therapy with the postmenopausal demographic in the context of stroke (Selvamani and Sohrabji, 2010a; Selvamani and Sohrabji, 2010b; Selvamani et al., 2012; Sohrabji et al., 2012). In addition, the beneficial action of estrogen might be counter balanced by its action on coagulation and fibrinolysis, which might further affect the incidence of stroke and cardiovascular event and the progression of vascular dementia.

It has been proposed that the controversial effects of hormone replacement therapy on cardiovascular events between the animal studies and clinical might be due to the stage of their atherosclerosis at the time of initiation of hormone replacement therapy in postmenopausal women (Koh and Sakuma, 2004). Development of intimal-medial thickening and atherosclerotic

plaque are relatively common in middle-aged women with the appearance of new carotid plaques in up to 47.5% of healthy women (Bonithon-Kopp et al., 1993). Atherosclerotic condition in the postmenopausal women might affect the outcome of estrogen treatment through thrombogenic as well as inflammatory mechanism. The disruption of atherosclerotic plaque exposes the lipid core to platelets and circulating factor that mediate thrombosis (Penz et al., 2005), which might interact with the pro-thrombotic action of estrogen and lead to the detrimental action of estrogen replacement therapy in the increase of heart attack, stroke, and deep vein thrombosis. Therefore, development and application of ischemic stroke models that replicate the clinical condition is essential to evaluate the effect of estrogen on ischemic stroke. Further evaluation of the effect of acute estrogen treatment on ischemic stroke using more clinical relevant models might provide critical information for future clinical trials that may lead to the ultimate discovery of an effective therapy for ischemic stroke.

5. Conclusion

Estrogen is one of the most extensively studied neuroprotectants for the treatment of ischemic stroke in the last 2 decades. However, the overwhelming beneficial effects conferred by estrogens in the experimental stroke studies are contradicted with the findings in the major clinical trials. This discrepancy might be partly due to the mistranslation between the experimental stroke research and clinical trials. While predominant experimental studies tested the protective action of estrogens on ischemic stroke using acute treatment paradigm, the clinical trials have mainly focused on the effect of estrogen replacement therapy on the primary and secondary stroke prevention which has not been adequately addressed in experimental stroke study. Therefore, the clinical trials conducted so far are not appropriate for assessing the potential effects of acute estrogen treatment on stroke protection. On the other hand, the well established action of estrogen in the neurovascular unit and its potential interaction with rtPA makes it a candidate for the combined therapy with rtPA for the acute treatment of ischemic stroke. The "critical timing window" and the newly emerged "biomarker window" hypotheses have also raised concerns of the underestimated clinical relevant factors in the experimental stroke studies. The critical period hypothesis and the biomarker window hypothesis might be inseparably intertwined as aging process is likely associate with change of the biomarkers that could affect the outcome of estrogen treatment for ischemic stroke. The development and using of ischemic stroke models that replicate the clinical condition is essential for further evaluation of acute estrogen treatment on ischemic stroke which might provide critical information for future clinical trials.

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