Oestrogen and stroke: the potential for harm as well as benefit

I.M. Macrae and H.V. Carswell
Division of Clinical Neuroscience, Wellcome Surgical Institute, University of Glasgow, Garscube Estate, Glasgow G61 1QH, U.K.

Abstract
Epidemiological studies point to a beneficial influence of the female reproductive hormones on stroke risk in that women have a lower incidence of stroke prior to the menopause compared with men, but this difference weakens with age and stroke risk in women rises after the menopause. However, recent Women’s Health Initiative trials in post-menopausal women report an increased stroke risk on hormone replacement therapy. An influence of gender is also apparent on stroke outcome in animal models: female rats exposed to transient MCA (middle cerebral artery) occlusion sustain less brain damage than age-matched males, with loss of protection following ovariectomy. The major hormone thought to be responsible for beneficial influences on stroke incidence and outcome is oestrogen, and a large preclinical literature now exists where exogenously administered oestrogen has been studied in male and ovariectomized female rats using a range of stroke models and outcome measures. Most of these studies administer oestrogen prior to the stroke, use a model of transient ischaemia followed by reperfusion and report a significant oestrogen-induced neuroprotection. However, in some studies where the MCA is permanently occluded, oestrogen pre-treatment in ovariectomized female rats has been shown to significantly exacerbate ischaemic damage. Therefore preclinical results demonstrate harmful as well as beneficial influences of oestrogen on the ischaemic brain, highlighting the need for further study to elucidate the mechanisms responsible for both detrimental and beneficial influences. Ultimately, this could lead to the development of new classes of oestrogenic compounds with improved risk/benefit profiles, designed to selectively activate pathways inducing only the beneficial effects of oestrogen in vivo.

Introduction and clinical background
Oestrogen is a pluripotent steroid hormone that has influences throughout the body, not only on the reproductive organs but also on bone, heart, blood, vasculature and brain. Consequently, the oestrogen deficiency associated with the menopause is accompanied by many physical changes including vasomotor instability, changes in lipid metabolism, increased risk of cardiovascular and cerebrovascular diseases, loss of bone density and effects on cognition and mood [1,2].

The concept of ORT (oestrogen replacement therapy) was developed to alleviate the symptoms of the menopause and oestrogen was hailed as the ‘miracle medication that could keep women young and feminine forever’ [3]. Progestins were subsequently combined with oestrogen [giving rise to HRT (hormone replacement therapy)] to limit its growth-stimulating effects on the endometrium and to prevent endometrial hyperplasia and cancer in women with an intact uterus. By the mid 1990s, millions of post-menopausal women were using HRT and accumulated evidence, collected over some 25 years, along with multiple observational studies suggested benefits of oestrogen therapy in reducing risk of heart disease, bone fracture and dementia, but also raised concerns about increasing the risk of thromboembolic events and breast cancer. Subsequently, a number of controlled clinical trials of HRT and ORT were conducted to address these issues. The largest of these, the WHI (Women’s Health Initiative) Hormone Trials, were multicentre, randomized, double-blind, placebo-controlled trials conducted in predominantly healthy post-menopausal women. The trials were designed to test the ability of conjugated equine oestrogens, with (for women with an intact uterus) or without (for women who had had a hysterectomy) progestin, to prevent heart disease and bone fracture and to reveal whether breast cancer incidence was increased. Both arms of the WHI trial (oestrogen alone, and oestrogen plus progestin) were stopped prematurely because of disease risks and the failure to demonstrate an overall benefit for the health of post-menopausal women [4,5]. The clinical trial of oestrogen plus progestin revealed an increased risk of breast cancer and cardiovascular disease, including stroke: 5.6 years of follow up revealed an annualized ischaemic stroke event rate of 26 per 10000 women in the HRT group versus 18 per 10000 in the placebo group, representing a 44% increase in ischaemic stroke risk compared with placebo [6]. The oestrogen alone trial, which ran for 6.8 years, reported no long-term effect on coronary heart disease or increased risk of breast cancer but an increased ischaemic stroke risk, with annualized stroke rates...
of 38 per 10 000 for oestrogen treatment versus 25 for placebo [7]. The similarity in the results in the two independent trials substantially strengthened the evidence that ischaemic stroke risk was elevated, and implicated oestrogen (as opposed to progestin) as the more likely cause of stroke. There was no significant effect of treatment on stroke severity, assessed by the Glasgow Outcome Score, in either the oestrogen alone or oestrogen plus progestin arm of the WHI trial [6,7].

**Rodent stroke models**

Animal stroke studies have focused on the influence of gender or the acute effects of exogenous oestrogen on stroke outcome rather than the influence of the steroid on stroke risk. Most studies have been conducted in rats or mice with the stroke induced by permanent or transient MCAO [MCA (middle cerebral artery) occlusion], the cerebral artery that is most commonly affected in human occlusive stroke. Different methods have been developed to occlude the MCA, which results in focal ischaemia confined to the MCA territory. In some models, a craniectomy is performed to expose the MCA, which is then permanently occluded by electrocoagulation and subsequently cut to confirm complete occlusion (Figure 1a). Alternatively, an occluding device, such as a micro-aneurysm clip or suture, is applied permanently or transiently, for a specified period (normally 90–120 min). For studies involving transient focal ischaemia, removal of the occluding device after the specified period leads to reperfusion of the ischaemic tissue, allowing the study of any influence oestrogen might have on restoration of cerebral blood flow or reperfusion-related injuries. Most focal ischaemia studies now employ an alternative method of MCAO, which does not require craniectomy and direct exposure of the artery but involves introducing an intraluminal filament into the external carotid artery and advancing it along the internal carotid artery to block the origin of the MCA (Figure 1b). This model can be used to induce either permanent or transient focal ischaemia. Outcome measures in animal models include quantifying ischaemia [e.g. with cerebral blood flow autoradiography (Figure 1c), laser Doppler flowmetry, or MRI (magnetic resonance imaging) techniques], extent of brain damage (infarct volume measured by histology or MRI; Figures 1d–1f) and behavioural assessments of the neurological deficit produced by MCAO.

**Influence of gender on experimental stroke**

In rodent strains carrying known risk factors for stroke such as the SHRSP (spontaneously hypertensive stroke-prone) rat, the incidence of spontaneous strokes and mortality is reported to be lower in female rodents compared with males [8]. When strokes are experimentally induced in the SHRSP rat, the influence of gender on stroke outcome is more complex. Different results are generated depending on the type of stroke and the stage of the oestrus cycle when the stroke is induced. Using the intraluminal filament model to induce transient focal ischaemia followed by a period of reperfusion (Figure 1b), female SHRSP rats, randomly selected for stage in cycle, sustain smaller infarct volumes than males, and this protection is lost in ovariectomized females [10]. In models where the MCA is permanently occluded by electrocoagulation (Figure 1a), female SHRSP rats in met-oestrus (low endogenous oestrogen) sustain significantly greater infarct volumes than males [9]. When SHRSP rats were exposed to permanent MCAO at different stages of the oestrus cycle, smaller infarct volumes were induced when the stroke was induced in pro-oestrus (high endogenous oestrogen) compared with met-oestrus (low endogenous oestrogen) [11].
A similar picture emerges in WKY (Wistar-Kyoto) rats, the normotensive reference strain from which the SHRSP rats were derived by selective breeding [12]. Following permanent MCAO by electrocoagulation, infarct volumes were significantly higher in WKY female rats during met-oestrus compared with males [9], while after transient MCAO, infarct volumes were smaller in females, randomly selected for cycle, compared with males, and this protection was lost after ovariectomy [10]. However, there was no significant difference in infarct volume between met- and pro-oestrus in WKY rats [11].

**Influence of oestrogen on experimental stroke**

The effect of oestrogen administration on stroke-induced brain injury has been extensively studied in rodent models with more than 30 focal ischaemia studies published to date plus several studies in models of global cerebral ischaemia (used to model brain damage due to cardiac arrest) (reviewed in [13–15]). Most of the studies corroborate the protective effects of oestrogen reported in the in vitro literature [13–16], and have resulted in a universal view that oestrogen treatment is beneficial in animal models of cerebral ischaemia. Most in vivo stroke studies have employed the intraluminal filament model (Figure 1b) to induce transient focal ischaemia with reperfusion and have administered 17β-oestradiol (the most potent oestrogen) prior to the stroke. Several studies have been conducted in intact male rats and report that 17β-oestradiol pre-treatment reduces infarct volume and functional deficits after transient focal ischaemia with reperfusion (detailed in [13–15]). Regarding females, most of the studies have been conducted in ovariectomized rats and, again, the results overwhelmingly demonstrate significant neuroprotection in transient MCAO models with 17β-oestradiol pre-treatment. However, a recent systematic review of the literature, which rated study quality, revealed that the effectiveness of oestrogen decreased with increasing quality scores in transient ischaemia studies, with no benefit recorded in the highest scoring studies [15]. Closer scrutiny of the literature reveals that under certain ischaemic conditions, oestrogen treatment lacks efficacy and, in some cases, can be detrimental [17–21]. One study that employed a prolonged and severe 3 h ischaemic period (combining MCAO with bilateral carotid occlusion) found no influence of either low- or high-dose 17β-oestradiol given acutely or chronically as a pre-treatment in male, intact female or ovariectomized female rats [17], while a study employing 1 h MCAO with reperfusion reported oestrogen-mediated increases in infarct size [18]. Many studies also report oestrogen-mediated neuroprotection in intraluminal filament models of permanent MCAO [22,23].

However, studies from our own laboratory have consistently shown oestrogen-mediated exacerbation of brain damage after permanent MCAO. Low- and high-dose oestrogen pre-treatment failed to show neuroprotective efficacy in the ovariectomized SHRSP rat, and exacerbated ischaemic damage in WKY rats, Lister Hooded and Sprague-Dawley rats when the MCA was permanently occluded by electrocoagulation or intraluminal filament [19–21]. Oestrogen has also been reported to lack neuroprotective efficacy or to exacerbate damage in global ischaemia studies in normal and diabetic rodents [24,25].

Examining the literature as a whole, it appears that the influence of oestrogen is in some way linked to the severity and duration of the ischaemic insult, and possibly the dose and duration of oestrogen treatment. Oestrogen appears to mediate most benefit where transient periods of moderate ischaemia are followed by reperfusion. Its known neurotrophic, vasodilatory, anti-inflammatory and antioxidative effects could contribute to improved outcome by supporting neuronal survival [26], promoting reperfusion, limiting post-ischaemic hypoperfusion [27,28] and reducing oxidative stress [29]. As stroke severity and duration increase, the ability of oestrogen to mediate neuroprotection may be lost and alternative deleterious oestrogenic mechanisms may be unmasked that exacerbate brain injury. Mechanisms responsible for exacerbating injury are less clear but may involve excitotoxicity, since oestrogen has been shown to up-regulate glutamate NMDA (N-methyl-D-aspartate) receptors [30], enhance NMDA receptor-mediated currents, induce seizure activity [31] and inhibit glutamate uptake by astrocytes [32]. Several studies provide evidence that as oestrogen dose is increased to supraphysiological levels, neuroprotective effects are lost [33,34] or detrimental effects increase [19,20]. Therefore, depending on the ischaemic conditions present and the dose administered, oestrogen has the ability to harm as well as protect ischaemically compromised brain tissue. Future research is therefore required to identify the mechanisms responsible for the detrimental effects of oestrogen in addition to those that mediate benefit, and to determine whether they are receptor-dependent [and the ER (oestrogen receptor) responsible] or receptor-independent. Once this information is known, it may be possible to develop new oestrogenic compounds [e.g. selective ER agonists, SERMs (selective ER modulators) or oestrogenic compounds lacking ER activity] with improved pharmacological profiles.

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**References**


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