Progesterone is neuroprotective following cerebral ischaemia in reproductively ageing female mice

Claire L. Gibson, Ben Coomber and Sean P. Murphy

1 School of Psychology, University of Leicester, Leicester LE1 9HN, UK
2 Department of Neurological Surgery, University of Washington School of Medicine, Seattle, WA 98105, USA

Correspondence to: Dr Claire L. Gibson,
School of Psychology,
University of Leicester,
Henry Wellcome Building,
Lancaster Road,
Leicester LE1 9HN, UK
E-mail: cg95@le.ac.uk

Gender differences in both vulnerability to stroke and outcome following cerebral ischaemia have frequently been observed and attributed to the action of steroid hormones. Progesterone is a candidate neuroprotective factor for stroke; however, studies are lacking which: (i) study those groups representing high risk i.e. postmenopausal females; (ii) administer progesterone solely post-ischaemia; and (iii) combine histopathological and functional assessments. Postmenopausal females, along with males, represent the group at highest risk of cerebral stroke and can be modelled using aged or ovariectomized animals. In the current study, we aimed to determine the neuroprotective effects of progesterone administration following cerebral ischaemia in aged and ovariectomized mice. Following transient middle cerebral artery occlusion, progesterone was administered at 1, 6 and 24 h post-ischaemia to aged female mice. At 48 h post-ischaemia, progesterone significantly reduced the lesion volume (P < 0.05) but had no effect on neurological outcome in aged female mice. Whereas in ovariectomized mice, at 48 h post-ischaemia, progesterone treatment had no effect on the amount of lesion volume present but did significantly improve neurological outcome. In a further study of ovariectomized mice, allowed to survive for 7 days post-ischaemia, progesterone treatment significantly improved motor outcome as assessed using both the rotarod and grid test. In fact, by 7 days post-ischaemia, progesterone-treated ovariectomized mice did not differ significantly in performance compared with shams, whereas vehicle-treated ovariectomized mice displayed a significant functional impairment following ischaemia. The current study has demonstrated that progesterone has different neuroprotective effects whether it is administered to aged or ovariectomized female mice and emphasizes the need to combine histopathological and functional outcomes within the same study. In addition, as progesterone-only treatment may not improve all outcomes in all groups, therapies that combine progesterone with other neuroprotective candidates should be investigated to maximize benefit following stroke.

Keywords: ageing; hormones; stroke; neuroprotective agents
Abbreviations: MCAO = middle cerebral artery occlusion

Introduction

Cerebral ischaemia is a major cause of death and disability in the Western world resulting in extensive cell death and loss of function (van der Worp and van Gijn, 2007). Although significant advances have occurred in understanding the pathophysiology underlying cerebral ischaemia, current treatments are limited in terms of their effectiveness (e.g. aspirin) and utility...
(e.g. thrombolysis). There is a need for basic research to investigate potential neuroprotective candidates in order to determine whether clinical investigation is warranted.

Female steroid hormones, such as progesterone and oestrogens, may provide females with an endogenous protection against cerebrovascular events as there are clear gender differences both in vulnerability to stroke and, indeed, outcome (Murphy et al., 2004). Prior to the menopause, females have a lower risk of stroke and suffer less damage, relative to males of the same age (Turtzo and McCullough, 2008). However, stroke incidence rapidly increases after the menopause coincident with a decline in circulating steroid hormones including progesterone and oestrogens.

There is a wealth of experimental evidence supporting the neuroprotective role of progesterone following various models of CNS injury including traumatic brain injury, cerebral ischaemia and spinal cord injury. In fact, the first randomized clinical trial for acute traumatic brain injury (ProTECT) was successfully completed in 2007 and reported that the use of progesterone following traumatic brain injury was well tolerated in terms of safety and resulted in a lower 30-day mortality rate compared with the placebo group (Wright et al., 2007). This was further supported by a subsequent clinical trial that showed improved neurological outcome in progesterone-treated patients with traumatic brain injury (Xiao et al., 2008) and the ProTECT II study is currently underway to further assess the benefits of progesterone following traumatic brain injury.

In terms of focal cerebral ischaemia, progesterone has been shown to be effective at reducing lesion volume (Jiang et al., 1996; Chen et al., 1999; Murphy et al., 2002) and improving functional outcome (Gibson and Murphy, 2004; Sayeed et al., 2007; Cai et al., 2008). Some previous studies have investigated whether progesterone in terms of protecting against ischaemic injury and therefore commenced treatment prior to ischaemic injury. However, most studies have administered progesterone post-ischaemia, in order to investigate the reparative ability of progesterone, but have mainly done so using young adult males. Thus, as highlighted in a recent systematic review (Gibson et al., 2008), there is an insufficient number of studies examining the neuroprotective ability of progesterone in various other groups such as ovariectomized females and aged animals, with particular reference to post-ischaemic treatment. In addition, relatively few studies measure functional outcomes that are important determinants, in combination with histopathological measures, of outcome (STAIR, 1999).

The current study aimed to determine whether progesterone, when administered solely post-ischaemia, is neuroprotective in aged or ovariectomized female mice. In order to determine whether progesterone was neuroprotective we combined measures of lesion volume and functional outcome. In addition, previous studies examining the effects of progesterone following ischaemia in aged animals (Wang et al., 2010) have used rats, whereas, in the current study, mice were used. It is important to assess the effectiveness of progesterone following ischaemia in aged and ovariectomized mice, as this will allow us, in future studies, to utilize the range of genetic variants available that may aid our understanding of progesterone’s mechanism(s) of action.

## Materials and methods

### Animals and hormone treatment

This study was conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986 (Project License 80/2015) and NIH guidelines. Mice were either young (12 weeks old) adult C57 BL6 ovariectomized female mice (n = 40, Charles River, UK) or ageing (12-month old) C57 BL6 female mice (n = 15, Harlan, WA, USA). A total of 55 female mice were used in the current study; eight mice (five ovariectomized, three aged) died following middle cerebral artery occlusion (MCAO) and two ovariectomized mice were excluded due to inadequate occlusion/reperfusion. In the aged group, all included mice (n = 12) were allowed to survive for 48 h post-MCAO. In the ovariectomized group, mice were either included in the short-term study (n = 15) and allowed to survive for 48 h post-MCAO or the long-term study and allowed to survive for 7 days following MCAO (n = 13) or sham surgery (n = 5). Mice were subjected to bilateral ovariectomy under isoflurane anaesthesia (induction 4%: maintenance 1.5% in a N2O/O2 70/30% mixture) and allowed to recover for at least 14 days prior to any further surgery. Group sizes for analysis were determined by previous research (Gibson and Murphy, 2004), which calculated that group sizes of six were sufficient to give 90% power to detect, at 5% significance, a 30% difference in lesion volume.

### Animal model and treatment

Anaesthesia was induced by inhalation of 4% isoflurane and maintained by inhalation of 1.5% isoflurane (in a N2O/O2 70/30% mixture). Body temperature was monitored throughout surgery (via a rectal probe) and maintained at 37.0 ± 0.6°C using a heating blanket (Harvard Apparatus Ltd). Cerebral blood flow was monitored for 5 min prior and 5 min following MCAO, and immediately before and after reperfusion. A small incision was made in the skin overlying the temporals muscle and a 0.7-mm flexible laser Doppler probe (model P10) was positioned on the superior portion of the temporal bone (6-mm lateral and 2-mm posterior from Bregma), secured with SuperGlue (Loctite). Focal cerebral ischaemia was induced for 60 min by occlusion of the right middle cerebral artery, and cerebral blood flow was monitored as described previously (Gibson and Murphy, 2004). Following 60 min of MCAO, mice were re-anaesthetized and the occluding filament was withdrawn gently back into the common carotid artery in order to allow reperfusion to take place. Relative cortical cerebral blood flow had to rise to at least 50% of pre-ischaemic values for the mice to be included in the study and subject to further analyses. Relative cerebral blood flow was monitored for a further 5 min prior to the wound being sutured and mice were allowed to recover from the anaesthesia. Sham-operated mice underwent the same surgical procedure, except that the filament was not advanced far enough to occlude the middle cerebral artery. During the 60-min period of MCAO, drug treatment was randomly assigned as either progesterone or vehicle treatment. The progesterone group received progesterone (USP, Sigma) dissolved in 50% dimethyl sulphoxide (16 mg/ml, Sigma) and 50% saline, which was injected intraperitoneally at 8 mg/kg at the onset of reperfusion, i.e. 1 h post-MCAO. Additional injections of progesterone (all 8 mg/kg) were administered at 6 and 24 h post-MCAO.
Dose and duration of progesterone treatment were consistent with previous research in young and aged animals (Gibson and Murphy, 2004; Wang et al., 2010). Mice in the vehicle group underwent the same experimental protocol, except that they received the same volume/weight of vehicle only. The experimenter was blinded to the treatment the mice had received prior to all subsequent analyses.

Indicators of general well-being
Mice were weighed at 24 and 48 h post-surgery for the short-term study and daily for 7 days in the long-term study as an indicator of their general well-being. Body weights are presented as a percentage change compared with values recorded immediately prior to undergoing MCAO. Survival rates of shams, progesterone- and vehicle-treated animals are also presented as a percentage compared with the number of animals undergoing surgery.

Neurological score
In the short-term study, the neurological status of each mouse was evaluated at 24 and 48 h post-MCAO by a blinded observer using a scoring system originally developed for rats (Bederson et al., 1986a) and later adapted for mice (Hayakawa et al., 2008). Mice were assigned a score between 0 and 5 (most severe): 0, normal motor function; 1, flexion of torso and the contralateral forelimb upon lifting of the animal by the tail; 2, circling to the ipsilateral side, but normal posture at rest; 3, circling to the ipsilateral side; 4, rolling to the ipsilateral side; and 5, leaning to the ipsilateral side with no spontaneous motor activity.

Assessment of infarct volume
All mice in the short-term study were sacrificed at 48 h following transient MCAO for lesion volume analysis. Following cervical dislocation, brains were removed and sectioned into 10 × 1 mm coronal slices using a mouse brain matrix (ASI Instruments). To quantify ischaemic damage, slices were stained with 2% 2,3,5-triphenyltetrazolium chloride (Sigma) in saline for 30 min at room temperature in the dark. They were then stored at 4°C in 10% formalin prior to analysis. 2,3,5-triphenyltetrazolium chloride is a marker for mitochondrial function and has been shown to be a reliable indicator of ischaemic areas for up to 3 days after ischaemia (Bederson et al., 1986b). Digital photographs of all stained slices were taken and the unstained area of infarction was measured on the posterior surface of each coronal section using Scion Image software. Infarct areas were calculated as previously described (Loehl et al., 1999), using an indirect method whereby overestimation of the infarct area due to the contribution of oedema is avoided.

Assessment of motor function
In the long-term study, motor function was assessed using the rotarod and grid test. For the rotarod test, an accelerating rotarod (Letica Scientific Instruments) was used as described previously (Gibson and Murphy, 2004). Briefly, mice were placed on the rotating rungs (diameter 29 mm), which accelerated in speed from 4 to 40 rpm over a 5-min period. A trial ended if the mouse fell off the rungs, or gripped and spun around for a complete revolution. Mice were acclimatized to the rotarod for five trials, the mean of which was used as the pre-surgery control value for each animal. After surgery, each mouse underwent testing on Day 2 and Day 7 and on each testing day mice underwent five trials on the rotarod, with an intertrial interval of 1 min.

The data are expressed as the percentage of mean duration per day compared with the pre-surgery control value. For the grid test on Days 2 and 7 post-surgery, mice were placed on an elevated grid surface (30 × 35 × 31 cm) with grid openings of 2.5 cm². During locomotion on the grid, the number of foot faults made by the ipsilateral and contralateral limbs was counted. Each test consisted of three trials lasting 1 min each, with an intertrial interval of 1 min. The foot faults are expressed as the number of errors made by the contralateral limbs as a percentage of the total errors made.

Statistical analysis
All data are expressed as mean ± standard deviation of the mean apart from neurological score data which are presented as median ± range. Survival data were analysed by applying the Kaplan–Meier curve, followed by the Mantel–Haenszel log-rank test to identify differences between the curves. Neurological score data were analysed using the non-parametric Mann–Whitney test. Experiments conducted over a series of time points (i.e. body weight, rotarod, grid test) were analysed by two-way analysis of variance (ANOVA) for differences according to time point and treatment group. Lesion volume data were analysed using a two-tailed Student’s t-test. The data were analysed using GraphPad Prism Version 5.0 for Windows (GraphPad Software). The criterion for statistical significance was P < 0.05.

Results
Cerebral blood flow measurements
Doppler monitoring showed that, in all mice subjected to MCAO, cortical cerebral blood flow was reduced by at least 70% of pre-ischaemic values within 5 min of advancing the filament and induction of MCAO. Analysis of the Doppler data revealed that there were no significant differences in cortical cerebral blood flow after occlusion between ageing female mice that had received progesterone or vehicle treatment or ovariectomized female mice that had received progesterone or vehicle treatment. There were no significant differences in the increase in relative cerebral blood flow when comparing progesterone-treated to vehicle-treated mice in either the ageing female or ovariectomized female experimental groups, at least for the 5 min following withdrawal of the filament.

Indicators of general well-being over the first 48 h
The survival data are presented by applying the Kaplan–Meier curve (Fig. 1) and analysed using the Mantel–Haenszel log-rank test, which revealed no significant differences in survival rate between experimental groups whether animals were ovariectomized females (χ² = 0.048, P = 0.83) or aged females (χ² = 0.25, P = 0.62). All experimental groups lost weight over the first 2 days following MCAO (Fig. 2). Progesterone treatment in either ovariectomized (P = 0.84, Fig. 2A) or aged (P = 0.67, Fig. 2B) females did not alter the rate of weight loss over the first 48 h following MCAO.
Progesterone treatment improved neurological score in ovariectomized mice

Mice were assessed using a 6-point neurological score at 24 and 48 h post-MCAO (Fig. 3). In the ovariectomized group, mice that had received progesterone treatment performed significantly better at 48 h post-ischaemia than mice that had received vehicle treatment (P = 0.042), whereas in the aged mice, progesterone treatment did not alter the neurological score following MCAO (P = 0.67).

Progesterone treatment reduced lesion volume in aged mice

Lesion volume was calculated by measuring the ischaemic damage i.e. loss of viable tissue represented by a lack of staining, in coronal sections stained with 2,3,5-triphenyltetrazolium chloride. In the ovariectomized group, there were no significant differences (P = 0.89) in lesion volume following progesterone (23.90 ± 9.27 mm³) or vehicle (23.36 ± 5.75 mm³) treatment. However, in aged mice progesterone treatment significantly (P = 0.02) reduced the amount of lesion volume present (18.89 ± 3.57 mm³) compared with vehicle treatment (26.41 ± 5.59 mm³) (Fig. 4).

Indicators of general well-being over 7 days

In the longer-term study, where mice were allowed to survive for 7 days post-MCAO or post-sham surgery, analysis of the survival data (Fig. 5A) revealed no significant differences in survival between the experimental groups (P = 0.42). As predicted from the short-term study, both MCAO groups i.e. progesterone- and vehicle-treated, lost weight over the first 2 days post-MCAO after which they began to gain weight. However, both progesterone-treated [F(1,11) = 79.66, P < 0.0001] and vehicle-treated [F(1,10) = 167.7, P < 0.0001] animals gained weight at a significantly slower rate than shams.
Progesterone treatment improved functional outcome

Motor function, as assessed using the rotarod (Fig. 6A), revealed that vehicle-treatment following MCAO resulted in a functional deficit compared with shams \(F(1,10) = 5.25, \quad P = 0.03\). Such a functional deficit, in terms of time spent on the rotarod, was prevented following progesterone treatment as these mice did not differ significantly from shams \(P = 0.65\).

Unilateral foot faults were expressed by the number of foot faults as a percentage of the total errors made (Fig. 6B), and a value of 50% represents an equal number of errors made by both sides. In comparison with shams, following MCAO, there was a significant increase in the number of contralateral errors made in the vehicle-treated group \(F(1,10) = 5.07, \quad P = 0.037\). However, progesterone treatment prevented this functional deficit as there were no significant differences in the number of contralateral errors between progesterone-treated animals and shams \(P = 0.07\).

Discussion

The current study demonstrated that progesterone treatment was beneficial following transient MCAO in aged and ovariectomized female mice. In aged mice, progesterone treatment significantly reduced the lesion volume present but had no effect on neurological outcome. Whereas in ovariectomized females, progesterone treatment significantly improved neurological outcome at 48 h post-ischaemia but did not have any effect on the amount of ischaemic damage presented. Due to the observation of improved neurological outcome following progesterone treatment in ovariectomized females, a further group of ovariectomized females were tested for motor function for 7 days following ischaemia and demonstrated enhanced functional recovery following...
ischaemia and progesterone treatment. In terms of physiological parameters, there was no effect, in the current study, of progesterone treatment on relative cerebral blood flow following reperfusion, and others have reported no effects of a variety of progesterone doses on pH, partial pressure of CO₂ and O₂, haematocrit, blood glucose, heart rate and mean arterial pressure (Jiang et al., 1996; Chen et al., 1999; Kumon et al., 2000).

The neuroprotective potential of progesterone was initially indicated by gender differences in recovery following traumatic brain injury (Attella et al., 1987; Stein, 2001) and later observations that recovery following traumatic brain injury was highest in pseudo-pregnant (i.e. high endogenous progesterone levels) females (Roof et al., 1993). The evidence for the neuroprotective potential of progesterone following traumatic brain injury is extensive, resulting in the initiation of clinical trials (e.g. ProTECT I, II). In terms of cerebral ischaemia, previous experimental studies have also demonstrated the neuroprotective ability of progesterone. This has been demonstrated using both pre- and post-ischaemic administration of progesterone in models of both transient (Jiang et al., 1996; Chen et al., 1999; Murphy et al., 2002; Gibson and Murphy, 2004; Cai et al., 2008) and permanent ischaemia (Gibson et al., 2005; Sayeed et al., 2006; Ishrat et al., 2010) ischaemia. Most of these previous studies have reported the beneficial effects of progesterone on morphological outcomes largely in young healthy male rats or mice. However, a few studies have also reported improvement in functional outcomes following progesterone treatment (Gibson and Murphy 2004; Sayeed et al., 2007; Cai et al., 2008; Wang et al., 2010). As highlighted in a recent systematic review (Gibson et al., 2008), there are few studies that have explored the neuroprotective ability of progesterone in experimental groups such as ovariectomized females and aged females even though, along with males, post-menopausal females represent the group at highest risk of cerebral stroke.

Reproductive senescence in rodents has been used to study neuroendocrine changes associated with menopause in females. In rodents, reproductive senescence is typically induced surgically, via ovariectomy, which removes the gonadal supply of oestrogens and progesterone. Previous work by ourselves (Coomber and...
Gibson, 2010), and others (Yahata et al., 1996), have shown that ovariectomy in mice reduces progesterone serum levels to ~0.2 ng/ml comparable with levels in reproductively senescent females (Wise et al., 1991). Although cerebral stroke is considered a disease of ageing, experimental studies using aged animals are rarely undertaken due to financial and logistical costs.

In rodents, ageing is associated with progressive changes in the reproductive system and reproductive success. Laboratory rodents typically show a marked decline in fertility from about 6–8 months characterized by decreased litter size and a decreased ability of the uterus to maintain implanted embryos (Parkening et al., 1978). This is accompanied by declining hormone levels such as progesterone, prolactin and luteinizing hormone (Fox et al., 1996). In mice, mid-life (10–12 months of age) is typified by a transition period consisting of chronic high oestriadiol levels and increased length of oestrous cycles (Nelson, 1988) followed by more moderate levels of oestriadiol accompanied by a marked decline in progesterone levels to almost zero (Wise et al., 1991). Thus, in laboratory rodents, from ~8 months of age, there is increasing cycle irregularity that is comparable with the irregular cycles of human perimenopause. The current study deliberately chose middle-aged reproductively senescent mice to enable us to model the effects of natural sex steroid loss during mid-life.

Previous research, in terms of ovariectomized females, has demonstrated that progesterone actually exacerbated the lesion volume present when progesterone was administered prior to the onset of cerebral ischaemia (Murphy et al., 2000). However, the same researchers also demonstrated that progesterone treatment reduced the lesion volume present when it was administered both pre- and post-ischaemia (Murphy et al., 2002). In the current study, post-ischaemic administration of progesterone did not affect the amount of lesion volume present, but did have a beneficial effect on functional outcome both short-term (i.e. 48 h), and longer-term (i.e. 7 days), following ischaemia. However, the measurement of lesion volume may be underpowered in that an increased number of ovariectomized animals may have produced a significant effect of progesterone on lesion volume. This seems unlikely as smaller group sizes, in the aged animals, were sufficient to detect a significant effect of progesterone treatment on lesion volume. In addition, minimum group sizes in the current study were based on previous research (Gibson and Murphy, 2004), which was sufficiently powered to detect a significant difference in lesion volume as a consequence of progesterone treatment and such a reduction in lesion volume was sufficient to improve functional outcome. However, the current study only measured lesion volume at 48 h post-ischaemia and it may be that progesterone treatment, in ovariectomized animals, had a delayed effect on structural outcome in comparison with aged animals. In addition, measurement of lesion volume by 2,3,5-triphenyltetrazolium chloride is rather a gross measure and other pathological changes may have occurred that this current study did not evaluate. From the data obtained in the current study, longer term studies are warranted to further assess the functional impact of progesterone treatment in ovariectomized females.

In one of the very few studies that have utilized aged animals, Toung et al. (2004) demonstrated that progesterone treatment, initiated 30 min prior to the onset of ischaemia, did not have any effect on lesion volume. Whereas Alkayed et al. (2000) showed that progesterone treatment significantly reduced lesion volume in aged rats when progesterone was delivered, via implants, for 7 days prior to ischaemia. To date, only one study, has administered progesterone post-ischaemia to aged rats, and observed that at 3 days following ischaemia, progesterone treatment had resulted in reduced lesion volume and improved neurological outcome (Wang et al., 2010). In addition, traumatic brain injury studies utilizing aged male rats, have demonstrated a positive effect of progesterone on both morphological and functional outcomes (Wali et al., 2011). However, the current study did not observe any effect of progesterone treatment, initiated post-ischaemia, on neurological outcome in aged mice, although we did observe a significant improvement in lesion volume at 48 h following ischaemia. In addition, we cannot exclude the possibility that other functional outcomes, which we did not measure in the aged animals, were not affected by progesterone treatment.

In the current study, we combined histopathological outcomes with functional assessments. Lesion volume calculation is a limited way of investigating whether a treatment is beneficial and although useful, interpretation of changes in lesion size must be interpreted with caution when investigating potential therapies, as they give little indication of how affected an individual is after stroke. Whereas some studies argue that infarct size correlates well with certain neurological deficits (Rogers et al., 1997) this is certainly not always the case (Wahl et al., 1992; Hattori et al., 2000; Reglodi et al., 2003). In the current study, we observed different benefits of progesterone treatment, in terms of reducing lesion volume or improving functional outcome, depending on whether it had been administered to aged animals or ovariectomized animals. Some previous studies have combined histopathological and functional measures following ischaemia, and the current study highlights the need, within studies, to combine such measures as focusing on only one may not reveal any neuroprotective potential for the therapeutic treatment under investigation. Future studies should include additional measurements of pathological markers and functional outcomes to gain more insight into the potential protective actions of putative therapies such as progesterone.

Historically there has been a failure in the translation of positive results obtained in experimental research to putative neuroprotective agents entering clinical practice. Such failures contributed to the publication of standards for preclinical neuroprotective drug development (STAIR, 1999) in order to improve experimental design and reduce experimental bias. Although it is over 10 years since they were first introduced, and there is still no clinically effective neuroprotective drug for stroke, a review of the data available for 1026 candidate neuroprotective therapies found that the majority of studies did not adhere very closely to the STAIR guidelines (Macleod et al., 2009). The current study does adhere to a number of the STAIR guidelines including: stating exclusion and inclusion criteria (e.g. pre-specified drop in cerebral blood flow), randomization to treatment group, blinding assessment of outcome and reporting of animals excluded from analysis. However, a recent review (Kilkenny et al., 2009) highlighted serious omissions in the way research using animals is reported leading to the development of the Animal Research: Reporting of...
In Vivo Experiments (ARRIVE) guidelines for reporting animal research (Kilkenny et al., 2010). Such guidelines are based on the assumption that improved reporting of animal experiments will increase the output from animal research by optimizing the information that is provided in terms of the design, conduct and analysis of the experiments. Although the current study does comply with many of the ARRIVE guidelines, incorporation of them into future studies, and scrutiny for them via the publication review process, will ultimately improve the quality of stroke research and increase the potential for effective translation, from experimental to clinical studies, of putative neuroprotective therapies.

This study has reported, for the first time, the neuroprotective ability of post-ischaemic administration of progesterone in aged and ovariectomized mice. It is important to assess potential neuroprotective candidates, such as progesterone, in experimental groups representing those at greatest risk of stroke. Although this study focused on examining outcomes in aged and ovariectomized females, further studies are warranted to include longer term assessment of outcome and also to assess outcome in aged males. The current study adds further evidence to support progesterone as a potential neuroprotective treatment following cerebral ischaemia. However, the current study did demonstrate that progesterone affected lesion volume and functional outcome differently depending on whether it was administered to aged or ovariectomized mice, thus suggesting that progesterone treatment alone, in either group, is not ideal. Treatment strategies for ischaemic stroke need to target both the histopathological damage and the functional impairments. Thus, the limitations of treatments that only improve one outcome e.g. lesion volume, may be overcome by designing combination therapies consisting of drugs that together improve a variety of outcomes.

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