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Research report

Effects of 17β-oestradiol on cerebral ischaemic damage and lipid peroxidation

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Abstract

Introduction: Numerous studies demonstrate oestrogen's neuroprotective effect in stroke models, although the mechanisms are unclear. Since oestrogen is an antioxidant, we tested the hypothesis that oestrogen reduces stroke-induced damage by reducing free radical damage, particularly lipid peroxidation.

Methods: Sprague–Dawley rats were ovariectomised and a 17β-oestradiol (0.25 mg, 21 day release) or placebo pellet implanted subcutaneously. Two weeks later, permanent middle cerebral artery occlusion (MCAO) was induced by intraluminal filament. At 2 and 24 h post-MCAO, neurological deficits were assessed. At the 24 h end point, plasma oestradiol was measured and brain sections stained with haematoxylin and eosin or lipid peroxidation marker, 4-hydroxynonenol (4-HNE) immunohistochemistry carried out to measure infarct volume and volume of tissue displaying oxidative damage, respectively.

Results: Plasma 17β-oestradiol in oestradiol and placebo groups was 72.6 ± 38.0 and 9.3 ± 7.4 pg/ml (mean ± SD), respectively. Infarct volume was significantly increased (118%) with oestradiol treatment (oestradiol = 124 ± 84.5, placebo = 57 ± 46.4 mm^3, mean ± SD, $P < 0.05$). The relationship between 4-HNE and infarct volume was significantly influenced by 17β-oestradiol. Neurological deficits were similar between groups (oestradiol median = 13, placebo = 14, max score = 33).

Conclusion: Two week pre-treatment with a high physiological dose of 17β-oestradiol increased infarct volume after permanent MCAO. Although contrary to our original hypothesis, this result demonstrates that oestrogen does have the capacity to promote detrimental actions in the stroke-injured brain. Given the wide use of oestrogen (contraception, osteoporosis and menopause), more research to clarify the influence of oestrogen on brain injury is urgently required.

Theme: Disorders of the nervous system
Topic: Ischaemia

Keywords: Oestrogen; Middle cerebral artery occlusion; Intraluminal thread; Oxidative damage; 4-Hydroxynonenol; Neurological assessment

1. Introduction

There are many reports of 17β-oestradiol providing neuroprotection in animal stroke studies. Both pre- and post-stroke treatment with 17β-oestradiol has been shown to significantly reduce infarct size following a transient middle cerebral artery occlusion (MCAO) [1,28,35].

The results of these animal studies have been reflected in some but not all clinical studies. In 1993, a report on the impact of postmenopausal hormone use (oestrogen) suggested that it was associated with a decrease in risk of stroke incidence and mortality in postmenopausal, white women [12]. However, a clinical trial in 2001 reported that oestrogen actually increased the risk of stroke recurrence and mortality in women with a previous stroke or transient ischaemic attack [32] and the recent results of the Women's Health Initiative have shown both combined oestrogen–progesterone replacement and oestrogen alone increase the risk of

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stroke [23,34]. This controversy in the literature suggests that 17β-oestradiol has the capacity to exacerbate or ameliorate cerebral ischaemic damage, stressing the need for greater mechanistic insight.

The mechanisms by which oestrogen may protect the injured brain are complex and not wholly understood. There are many possibilities including oestrogen receptor dependent and independent mechanisms and genomic and non-genomic actions. One important protective mechanism of oestradiol is as an antioxidant. In 1999, Culmsee and colleagues used chick embryo neuronal cultures to show that oestradiol could significantly attenuate levels of iron-induced reactive oxygen species [10]. Another in vitro study demonstrated that a high concentration of oestrogen is protective against oxidative stress-induced cellular damage and ultimately against cell death in a clonal hippocampal cell line [4]. Several members of the oestrogen family are potent antioxidants with the phenolic structure in the steroid A ring of these steroids being responsible for the inhibition of iron catalysed lipid peroxidation. There is also evidence that the hydroxyl group at the C3 position of the A ring confers their neuroprotective antioxidant activity. The literature suggests that a high physiological dose of 17β-oestradiol is required to reduce lipid peroxidation and oxidative stress [4,31] while low doses may not be effective. Therefore, a 17β-oestradiol dose (0.012 mg per day), equivalent to proestrous levels of oestrogen, has been used in this study to investigate the influence of 17β-oestradiol on infarct size and oxidative damage in the rodent brain after permanent MCAO. The mean concentration of 17β-oestradiol measured in the plasma of 17β-oestradiol-treated animals in the present study (72.6 pg/ml) is equivalent to previously reported concentrations of 17β-oestradiol during the proestrous stage of the oestrous cycle in female rats [7,19].

Oxidative damage has been assessed with 4-hydroxynonenol (4-HNE) immunohistochemistry. 4-HNE is a cytotoxic aldehyde, released from polyunsaturated fatty acid side chains as a product of lipid peroxidation. It is an electrophilic species that can bind to cytoskeletal proteins, such as neurofilaments, myelin associated proteins and glial fibrillary acidic proteins, with modification of these proteins leading to neuronal perikaryal, axonal and glial cell damage.

We have tested the hypothesis that oestrogen will reduce the size of the infarct, the volume of tissue immunopositive for 4-HNE and the severity of the neurological deficit after experimental stroke.

2. Material and methods

All experiments were carried out under license from the British Home Office and were subjected to the Animals (Scientific Procedures) Act, 1986. Female Sprague–Dawley rats (3 months, Harlan Olac, Bicester, UK) were group housed in conditions of a 12 h light–dark cycle with water ad libitum. During a 24 h period before surgery and for 5 days post-surgery, the rats were fed ad libitum. Outwith these times, the animals were maintained at 90% of their normal body weight as described in Toth and colleagues [29] and body weight was monitored daily. The animals weighed 200 g to 240 g when ovariectomised and 200 g to 262 g at the time of experimental stroke surgery.

2.1. Ovariectomy

The animals were anaesthetised with halothane (induction 5%; maintenance 0.75–1.5% by mechanical ventilation in a 30%:70% oxygen/nitrous oxide mix). Throughout the procedure, the animal's body temperature was maintained at 36.8 °C–37.5 °C using a thermal blanket and monitored using a rectal temperature probe.

Animals underwent bilateral ovariectomy and either a 17β-oestradiol (0.25 mg, 21 day release) (n = 10) or placebo (n = 13) pellet (Innovative Research America, Sarasota, USA) was implanted subcutaneously at the nape of the neck. The animal was administered 0.5 ml of saline subcutaneously to prevent dehydration during recovery from anaesthesia.

2.2. Permanent MCAO and laser Doppler flowmetry

Two weeks later, animals were anaesthetised as described above. The femoral artery was cannulated and physiological variables (temperature, arterial oxygen tension and blood pressure) were monitored and kept within physiological limits. With the animal held in a stereotaxic frame, a small craniectomy was made with a saline-cooled drill 1 mm posterior and 4 mm lateral, relative to Bregma, leaving only a thin layer of bone over the brain. The laser Doppler probe was calibrated using a perfusion flux standard (Moore Instruments Limited), placed on the bone at this site and the local cortical blood flow measured before, during and after induction of ischaemia. This confirmed the induction and severity of ischaemia in the MCA territory, indicating that the origin of the MCA had been successfully occluded. The method of occlusion involved an intraluminal filament and was a modified version of Longa and colleagues [17] using a 3.0 Dermalon monofilament with its tip rounded to a bulb of 293 μm diameter using heat. The filament was bent at a point 20 mm from the tip for judging the distance from insertion through the external carotid artery (ECA) to the bifurcation of the MCA. The filament was held in place using a 6.0 silk suture tied round the ECA below the entry point of the filament and electrocoagulation of the ECA at the entry point. The neck wound was sutured and the anaesthetic withdrawn. The animal was administered 2 ml saline to prevent dehydration. When fully conscious, the animal was moved to a recovery room where it was regularly monitored during the next 24 h.