Experimental Neurology 222 (2010) 13–24

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Sustained sensorimotor impairments after endothelin-1 induced focal cerebral ischemia (stroke) in aged rats

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ARTICLE INFO

Article history:
Received 27 August 2009
Revised 6 November 2009
Accepted 6 November 2009
Available online 12 November 2009

Keywords:
Aged
Endothelin-1
Stroke
Behavioural deficits
Plasticity
Preclinical models

ABSTRACT

Despite recent advances, stroke remains a leading cause of neurological disability with the vast majority of victims being the elderly, who exhibit more severe neurological deficits and a reduced capacity to recover from these disabilities in comparison to young stroke survivors. The objective of the present study was to develop a model of focal ischemic stroke in aged rats using endothelin-1 (ET-1) to produce low mortality rates as well as reliable, robust sensorimotor deficits that resemble functional impairments associated with stroke in humans. Here, we studied the functional and histological outcome following unilateral ET-1 infusions into the sensorimotor cortex of aged rats (20–23 months old). This procedure resulted in low mortality rates (13.3%) and no loss in body weight one week following surgery. Functional assessment was performed using a number of reliable behavioural tests: staircase test (fine motor function), horizontal ladder (skilled locomotion), bilateral tactile stimulation test (somatosensory function) and cylinder test (postural weight support). Following ET-1 induced stroke, all tests demonstrated large and sustained sensorimotor deficits in both forelimb and hindlimb function that failed to improve over the 28-day testing period. In addition, histological assessment revealed a substantial loss of retrogradely labelled corticospinal neurons in the ipsilesional hemisphere following stroke. Our results establish a model for the use of aged rats in future preclinical studies, which will enhance assessment of the long-term benefit of potential neural repair and regenerative strategies.

Introduction

Ischemic stroke represents a leading cause of long-term neurological disability that is strongly associated with age and affects the majority of the elderly population (Donnan et al., 2008; Kelly-Hayes et al., 2003). It has been reported in the United Kingdom that 98.5% of strokes occur in people over the age of 45 years, and 75% in people aged over 65 years (Carroll et al., 2001). Elderly stroke survivors are more likely to be disabled, with almost half possessing moderate to severe neurological deficits (Kelly-Hayes et al., 2003). Due to the increase in life expectancy in many countries (Kinsella, 2005), the number of individuals at risk from stroke is anticipated to rise, making the burden of stroke disability an even more serious public health-care concern that needs to be urgently addressed.

Experimental and human studies both demonstrate that neuroplasticity, neurophysiology and neurochemistry alter during aging and that the brain's response to insults changes (Badan et al., 2003; Buga et al., 2008; Cox, 1983; Davis et al., 1995; Futrell et al., 1991; Hachinski et al., 1992; Li and Carmichael, 2006; Markus et al., 2005; Sutherland et al., 1996; Ward, 2005). Consequently, the age of the animal when modelling the clinical disorder is critical, particularly when assessing ischemic damage and the extent of recovery. At present, the majority of experimental research is still conducted on young animals, despite recommendations by the STAIR (Stroke Therapy Academic Industry Roundtable) committee and the Stroke Progress Review Group suggesting that data from aged animals might be considered more appropriate in preclinical studies (Fisher et al., 2009). This may also elucidate reasons why most strategies and treatments demonstrating effectiveness in animal models have failed to show any clinical benefits in aged humans (Fisher and Ratan, 2003; Gladstone et al., 2002; Millikan, 1992).

Preclinical stroke models in aged rats have been established, with all based on permanent or temporary occlusion of the middle cerebral artery (MCAo) or photothrombosis, yet the use of aged animals for stroke research is generally limited because of their high price and high mortality rates following surgery compared to younger animals (Futrell et al., 1991; Hachinski et al., 1992; Lindner et al., 2003; Wang et al., 1995, 2003; Zhang et al., 2000). Another method increasingly being used to induce ischemic injuries is the potent vasoconstricting peptide, endothelin-1 (ET-1) (Yanagisawa et al., 1988). Unlike other models, the use of ET-1 is diverse in that it can be applied directly to the cortical surface (Adkins et al., 2004;

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doi:10.1016/j.expneurol.2009.11.007

Fuxe et al., 1997; Hsu and Jones, 2006), applied near cerebral arteries (Sharkey et al., 1994; Sharkey and Butcher, 1995; Yager et al., 2006) or microinjected into particular regions of the brain (Frost et al., 2006; Fuxe et al., 1992; Gilmour et al., 2004; Windle et al., 2006) to cause a reduction in cerebral blood flow and consequent ischemic damage.

Topical applications of ET-1 are most common, involving direct placement onto the cortical surface to produce a localised and dose-dependent ischemic injury to the sensorimotor cortex (Windle et al., 2006). Conversely, it is difficult to ensure the exact distribution of ET-1 once applied to the surface, as well as the extent of diffusion into the underlying cortical layers. One approach to minimise this variability and provide greater accuracy is to make intracortical injections of ET-1 into specific regions of the sensorimotor cortex (Gilmour et al., 2004; Tennant and Jones, 2009; Windle et al., 2006). Functional assessment after intracortical infusions of ET-1 demonstrate long lasting impairments in skilled forelimb use (Gilmour et al., 2004; Windle et al., 2006), yet to date all have been performed on young animals. It is known that co-ordinates need to be altered in relation to animal size and age (Paxinos et al., 1985), thus to provide more clinical relevance it would be valuable to modify this technique with co-ordinates adapted for the aged rat brain and to measure the functional deficits induced by this lesion in aged rats.

Therefore, the objectives of the present study were as follows: (1) to develop a model of focal sensorimotor cortical stroke in aged rats using endothelin-1 at specific co-ordinates to ensure greater reproducibility, (2) to obtain a low mortality rate and (3) to obtain long-lasting, robust functional disabilities that resemble the sensorimotor deficits associated with stroke victims. Such a model could potentially have considerable use in assessing the functional recovery and long-term benefit of prospective neural repair strategies.

Materials and methods

Subjects

Twenty-four male Wistar rats were obtained from Harlan, UK at approximately 18 months old (480–680 g) and used in the experiment between 20 and 23 months of age. Animals were housed 2–3 to a cage in standard laboratory conditions on a 12:12 h light/dark cycle. Rats were moderately food restricted (13–15 g per rat, per day) during behavioural training and only the day before post-surgery behavioural testing sessions.

Surgery

All procedures were in accordance with guidelines from the UK Home Office and Animals (Scientific Procedures) Act of 1986. Animals were anesthetized with isoflurane (4% in O_2 for induction) and then transferred to a stereotaxic frame (David Kopf Instruments, USA) where anaesthesia was maintained at 1.5–2% in O_2 delivered via a facemask. Body temperature was monitored via a rectal thermometer and maintained at approximately 36 °C with a heating pad.

Prior to surgery, rats were allocated to sham or stroke group in a counterbalanced fashion ensuring that there was no difference in group mean preoperative performance of the preferred forepaw on the staircase test. Surgery was performed in a randomised block design. Unilateral lesions were performed in the hemisphere contralateral to the dominant forelimb, as determined by staircase behavioural test. In stroke rats ($n=15$), a midline incision was made and the sensorimotor cortex was exposed by craniotomy the following co-ordinates (defined as anterioposterior (AP), mediolateral (ML), dorsoventral (DV)): AP 4 mm to −2 mm, ML 2 mm to 4 mm, relative to bregma. The dura mater was removed using a 25-gauge needle. Due to variations in skull thickness between aged rats, a craniotomy was performed to enable accurate depth placement of ET-1 intracortical injections.

Four 2 -μl injections of ET-1 (CalBioChem; 200 pmol/μl; 0.5 μg/μl dissolved in sterile saline) was delivered via a glass micropipette connected to a syringe (Hamilton), with the first 1 μl administered at a depth of 1 mm from the brain surface and the subsequent 1 μl applied to the surface of the cortex at the following co-ordinates:

(1) AP +3.5 mm, ML 2.8 mm, DV −1.0 mm/0 mm;
(2) AP +2 mm, ML 2.8 mm, DV −1.0 mm/0 mm;
(3) AP +0.5 mm, ML 2.8 mm, DV −1.0 mm/0 mm;
(4) AP −1 mm, ML 2.8 mm, DV −1.0 mm/0 mm, from bregma, midline and brain surface respectively.

For each injection, ET-1 was delivered at a rate of 0.5 μl/min with a 1-min interval between each microlitre. The dose and delivery of ET-1 was determined from a preliminary study using aged rats (unpublished data). Prior to suturing, the animal was left undisturbed for 5 min and the skull fragment was replaced. Sham-operated rats ($n=9$) received all procedures up to, but not including, craniotomy.

Animals were allowed to recover from anaesthesia in a recovery box until fully conscious and buprenorphine (0.01 mg/kg, s.c.) was administered for postoperative pain relief. During the early post-operative period, the health of each animal was carefully monitored and supplementary food (sugared-wet mash) was given when necessary.

Retrograde tracing of CST neurons

To differentiate corticospinal neurons (CSNs) from other neurons in layer V of the cortex and determine the number of surviving CSNs after injury, animals (sham $n=3$; stroke $n=3$) were given bilateral spinal injections of the retrogradely transported fluorescent tracer Fast Blue (FB; Sigma; 2% in PBS; 0.25 μl on each side) after all postoperative behaviour was completed. Animals were anesthetized as previously described and the cervical spinal cord at level C4 was exposed via laminectomy, a glass micropipette was positioned into the dorsal columns and FB was delivered at a rate of 0.5 μl/min. Animals were subsequently left for 10 days before being perfused.

Behavioural testing

A variety of behavioural tests previously found to be effective in assessing sensory and motor deficits were included within this study. Animals were handled every day for 3 weeks before the onset of the experiment and trained for 4 weeks on the staircase task to identify forepaw preference. All behavioural testing was carried out by an experimenter blinded to group membership by recoding animals after surgery. Baseline values were recorded 3 days before surgery on all behavioural tasks and animals were assessed every week on all tests until the fourth postoperative week. Animals that failed to meet the minimum criteria for the staircase test were excluded from behavioural analysis and used in a further study ($n=5$).

Staircase test and training

The staircase test was used to assess reaching performance; this provides a sensitive measure of skilled forepaw motor function (Montoya et al., 1991). The staircase apparatus (Campden Instruments Ltd., UK) consists of a chamber with a central platform for the rat to climb onto and a set of seven steps is located on either side. Each step holds three sucrose pellets (45 mg, Research Diets Inc, New Brunswick, NJ). To reduce initial neophobic responses animals were given sucrose pellets in their home cage prior to training sessions. The animals were pre-trained twice per day on weekdays over a 4-week period; they were first introduced to the apparatus in their home cage with their cagemate, and then afterwards alone for 15 min with sucrose pellets placed along the central platform and three pellets on each step of the staircase.