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Short communication

Mouse model of focal cerebral ischemia using endothelin-1

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ABSTRACT

Intracerebral injection of the vasoconstrictor peptide, endothelin-1 (ET-1), has been used as a method to induce focal ischemia in rats. The relative technical simplicity of this model makes it attractive for use in mice. However, the effect of ET-1 on mouse brains has not been firmly established. In this study, we determined the ability of ET-1 to induce focal cerebral ischemia in four different mouse strains (CD1, C57/BL6, NOD/SCID, and FVB). In contrast to rats, intracerebral injection of ET-1 did not produce a lesion in any mouse strain tested. A combination of ET-1 injection with either CCA occlusion or *N*(*G*)-nitro-L-arginine methyl ester (L-NAME) injection produced only a small infarct and its size was strain-dependent. A triple combination of CCA occlusion with co-injection of ET-1 and L-NAME produced a lesion in all mouse strains tested, and this resulted in a significant motor deficit. However, lesion size was still relatively small and strain-dependent. This study shows that ET-1 has a much less potent effect for producing an infarct in mice than rats.

1. Introduction

The majority of research on cerebral ischemia has been done using rat models of stroke. However, the use of mice in stroke research offers certain advantages. For example, using knock-out mice offers the potential to ask more mechanistic questions about the molecules involved in stroke pathophysiology. Furthermore, with the advent of research into cell transplantation therapy for stroke, various immunodeficient mouse strains (e.g. NOD/SCID mice) are beneficial as they can support survival of xenogenic cell grafts without the need for daily immunosuppression regimes (Pflumio et al., 1996; Cashman et al., 1997; Cummings et al., 2005).

There are a number of rodent focal ischemia models, although the more commonly used models (suture model and distal middle cerebral artery occlusion) are technically challenging in mice given their small size. An alternative and simpler model is intracerebral injection of the vasoconstrictor peptide, endothelin-1 (ET-1). This has been reported as a simple and reproducible method of focal ischemia in rats (Fuxe et al., 1989; Sharkey and Butcher, 1995; Gilmour et al., 2004; Frost et al., 2006; Windle et al., 2006), with the lesions caused by a drop in blood flow in the injected area (Kurosawa et al., 1991). However, the vasoconstrictive effect of ET-1 in the mouse brain has not been fully reported. In this study, we examined the ability of ET-1 to induce focal cerebral ischemia in different mouse strains as it offers the potential of a technically simple stroke model for mice.

2. Animals and methods

2.1. Animals

All experimental protocols performed on animals were approved by the Stanford University Administrative Panel on Laboratory Animal care. Adult male Sprague–Dawley rats (Charles-River, USA, 320–340 g), CD-1 mice (Charles-River, USA, 22–25 g), C57/BL6 mice (Jackson, USA, 20–25 g), NOD/SCID mice (Stanford Colony and Taconic, USA, 20–25 g), and FVB mice (Jackson, USA, 20–25 g) were used in this study.

2.2. Surgical procedure

All animals were anesthetized with 2–3% isoflurane plus oxygen and air (ratio: 0.2/0.8 L/min) by facemask, and maintained with 1.5–2% isoflurane. Temperature was maintained at 37 °C throughout the surgery using a self-regulating heating blanket. ET-1 (Calbiochem, USA) was dissolved in sterile saline at various

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concentrations (see Results) and different doses were delivered into the cortex or striatum by stereotaxic injection. ET-1 was injected at 0.3 μl/min by an infusion pump, and the needle left *in situ* for 5 min post-injection before being slowly removed. The stereotaxic coordinates (listed below) were determined from the Paxinos/Watson rat atlas and Paxinos/Franklin mouse atlas (Paxinos and Watson, 1998; Paxinos and Franklin, 2004); all stereotaxic measurements are relative to bregma and with the depth determined from the brain surface:

(1) Rat: cortex and striatum (triple injection): AP 0, ML +2.5, DV −2.3; AP +2.3, ML +2.5, DV −2.3; AP +0.7, ML +3.8, DV −7.0
(2) Rat: cortex (triple injection): AP 0, ML +2.5, DV −2.3; AP +2.3, ML +2.5, DV −2.3; AP +0.7, ML +3.8, DV −2.3
(3) Mice: cortex (single injection): AP +1.0, ML +1.0, DV −1.0
(4) Mice: cortex (double injection): AP +1.0, ML +1.0, DV −1.0; AP 0, ML +1.0, DV −1.0
(5) Mice: cortex (triple injection): AP +2.0, ML +2.0, DV −0.7; AP +1.5, ML +1.0, DV −0.7; AP +1.0, ML +2.0, DV −0.7
(6) Mice: striatum (single injection): AP +1.0, ML +1.0, DV −3.0
(7) Mice: striatum (double injection): AP 0, ML +2.0, DV −3.0; AP +1.0, ML +1.0, DV −3.0

For some mice, the ipsilateral common carotid artery (CCAo) was permanently occluded just prior to the ET-1 injection in order to further reduce the cerebral blood flow. Some mice also received *N*(*G*)-nitro-L-arginine methyl ester (L-NAME; Sigma, USA), a nitric oxide synthase (NOS)-inhibitor, via intracerebral injection (2.7 μg/μl) with ET-1.

2.3. Behavioral testing

Motor behavior was tested at 2 days post-surgery using the cylinder test to measure forelimb use during vertical exploration (Schallert et al., 2000). Mice were placed in a plexiglas cylinder, and the number of times the mouse reared and touched the cylinder in a weight-bearing fashion with the left, right, or both forelimbs was counted. Approximately 20 of these limb-use movements were counted per trial. The behavior score was calculated using the equation (affected limb use + 'both' limb use)/(unaffected limb use + 'both' limb use) which gives a ratio of affected to unaffected limb use. Statistical analyses were performed using Tukey post hoc test following two way ANOVA (SigmaStat, SYSTAT, California, USA).

2.4. Sacrifice and 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining

Two days after the surgery, the animals were anaesthetized with isoflurane and decapitated. The brains were immediately taken for TTC staining. Four equidistant sets of coronal sections (2 mm thickness) were prepared. The sections were incubated in distilled water containing 2% TTC (Sigma) at 37 °C for 20 min. The infarct size was defined as: small (<20% of the hemisphere); medium (20–60% of the hemisphere) and large (>60% of the hemisphere).

3. Results

We first tested ET-1 in rats since it is known to produce lesions in rats (Windle et al., 2006). Intracerebral injection of ET-1 (1 or

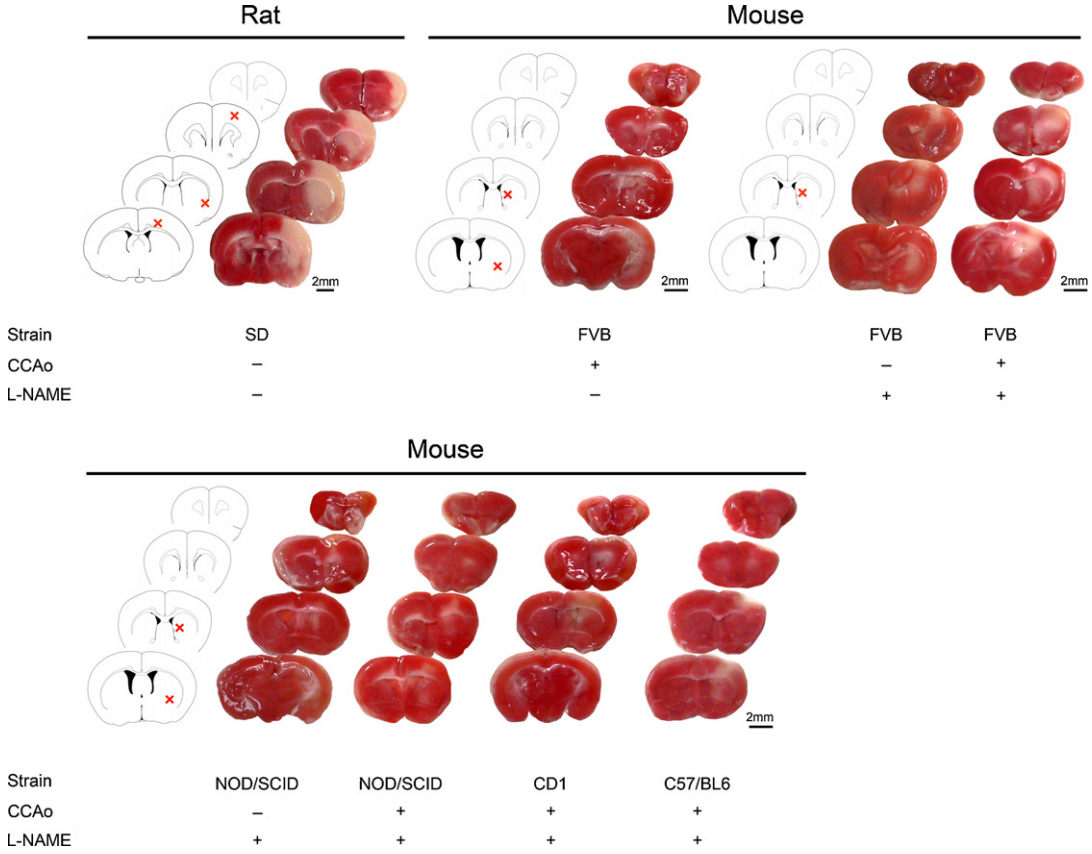

Fig. 1. Summary of ET-1 induced lesions. TTC staining 2 days after ET-1 injection (±L-NAME and CCAo as indicated) shows the variability in lesion sizes between Sprague–Dawley rats and the various mouse strains tested. The dose per injection site for ET-1 was 1.0 μg and 2.7 μg for L-NAME. The schematic illustrates the injection coordinates used. The infarcts were reproducible within each group.