BRAIN RESEARCH 1629 (2015) 318–328

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Brain Research

Research Report

Experimental model of small subcortical infarcts in mice with long-lasting functional disabilities

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ARTICLE INFO

Article history:
Accepted 22 October 2015
Available online 29 October 2015

Keywords:
Small subcortical infarcts
Animal models
Brain ischemia
Behavior (rodent)
White matter disease

ABSTRACT

Small subcortical infarcts account for 25% of all ischemic strokes. Although once considered to be a small vessel disease with a favorable outcome, recent studies have reported relatively poor long-term prognoses following small subcortical infarcts. Limited pre-clinical modeling has hampered understanding of the etiology and development of treatments for this disease. Therefore, we attempted to develop a new experimental model of small subcortical infarcts in mice to investigate pathophysiological changes in the corticospinal tract and assess long-term behavioral performance. The vasoconstrictor peptide, endothlin-1 (ET-1), in combination with the nitric oxide synthase inhibitor, N(G)-nitro-L-arginine methyl ester (L-NAME), were injected into the internal capsule of mice. Histological and behavioral tests were performed 0–8 weeks after the injection. The ET-1/L-NAME injection resulted in severe neurological deficits that continued for up to 8 weeks. The loss of axons and myelin surrounded by reactive gliosis was identified in the region of the injection, in which the vasoconstriction of microvessels was also observed. Moreover, a tract-tracing study revealed an interruption in axonal flow at the internal capsule. The present model of small subcortical infarcts is unique and novel due to the reproduction of neurological deficits that continue for a long period, up to 8 weeks, as well as the use of mice as experimental animals. The reproducibility, simplicity, and easy adoptability make

Abbreviations: AP, Anterior–posterior; DAPI, 4′,6 diamidino-2-phenylindole; DV, Dorsal–ventral; ET-1, Endothelin-1; GFAP, Glial fibrillary acidic protein; GST-pi, Glutathione S-transferase Pi; Iba1, Ionized calcium binding adaptor molecule 1; PBS, Phosphate buffered saline; PECAM, Platelet endothelial cell adhesion molecule; ML, Medial–lateral; MRI, Magnetic resonance imaging; L-NAME, N(G)-nitro-L-arginine methyl ester; SCID, Severe combined immunodeficiency.

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http://dx.doi.org/10.1016/j.brainres.2015.10.039

the present model highly appealing for use in further pre-clinical studies on small subcortical infarcts.

1. Introduction

Small subcortical infarcts account for 25% of all ischemic strokes (Sudlow and Warlow, 1997). Small subcortical infarcts (<15 mm diameter) are normally located in the basal ganglia, thalamus, internal capsule, corona radiata, and brainstem, and have been attributed to the occlusion of a single perforating artery (Fisher, 1982). Although patients with small subcortical infarcts have a more favorable functional outcome than patients with other subtypes of stroke, damage to the white matter including the corticospinal tract contributes to severe disabilities in spite of the small lesion size. Recent studies demonstrated that the long-term prognosis after small subcortical infarcts was not benign (Jackson and Sudlow, 2005; Norrving, 2003). The percentage of patients with small subcortical infarcts that are dependent is as high as 18–33% at 1 year, 36% at 2 years, and 42% at 3 years (Norrving, 2003). Thus, preclinical studies using animal models that mimic human small subcortical infarcts would undoubtedly be valuable to improve understanding of the disease in humans and develop treatments.

In contrast to large artery occlusive ischemic stroke, the lack of information on the pathophysiological events associated with small subcortical infarcts makes this stroke subtype particularly difficult to model (Sozmen et al., 2012). Although several clinically relevant models have been suggested such as the injection of a vasoconstrictor peptide (Frost et al., 2006; Sozmen et al., 2009), the anterior choroidal artery occlusion method (He et al., 1999; Tanaka et al., 2008), or systemic hypertension model (Fredriksson et al., 1985), these methods only partly mimic the features of human small subcortical infarcts (Bailey et al., 2009). In addition, long-lasting neurological deficits, which are mandatory to assess the therapeutic effects of novel treatments, are lacking in previous studies (Bailey et al., 2009). Moreover, few studies have used mice as experimental animals for small subcortical infarcts in spite of the availability of sequence data, antibodies, and genetically-modified strains (Sozmen et al., 2012). The limitations associated with small subcortical infarct models in previous studies indicate that a more useful and simpler model may be needed for further investigations on small subcortical infarcts.

Endothlin-1 (ET-1) is a naturally produced vascular endothelial cell peptide that exhibits strong vasoconstrictive effects (Yanagisawa et al., 1988). On the other hand, N(G)-nitro-L-arginine methyl ester (L-NAME) is known to reduce the effects of injury-induced nitric oxide synthase, which causes vasodilation (Chataigneau et al., 1999; Horie et al., 2008). This study investigated whether an injection of ET-1 in combination with L-NAME into the internal capsule, in which descending fibers from the motor cortex and ascending fibers to the somatosensory cortex exist, caused focal white matter strokes in mice. This model was used for a comprehensive analysis of small subcortical infarcts, including long-term neurological assessments and histological, immunohistochemical, and physiological evaluations of corticospinal tract deformations to clarify the characteristic features of white matter strokes.

2. Results

2.1. Physiological parameters

No significant differences were observed in the physiological parameters between the phosphate buffered saline (PBS)-injected control and ET-1/L-NAME-injected groups (Table 1). Body weights did not significantly change during the 8 weeks after the injection in either of the two groups (Fig. 1A). No premature death occurred during and after the surgical procedures.

2.2. Long-lasting deterioration of behavioral performance induced by the ET-1/L-NAME injection

We first investigated the effects of the ET-1/L-NAME injection on behavioral performance. We injected PBS or ET-1/L-NAME into the posterior limb of the internal capsule, in which descending fibers from the motor cortex and ascending fibers to the somatosensory cortex exist (Fig. 1B). Neurological performance was monitored for 8 weeks post-injection using the corner test and cylinder test to assess sensorimotor function (Fig. 1C, D). The PBS injection induced no neurological deficits at any time points in either test. On the other hand, ET1/L-NAME injection significantly deteriorated the behavioral scores in the corner test from 4.5 h to 8 weeks after the injection. Furthermore, the cylinder test revealed that behavioral scores were significantly lower in the ET-1/L-NAME-injected group than in the PBS-injected control group 4.5 h, 1 day, and 1, 4 and 8 weeks post-injection.

Taken together, these results indicated that the injection of ET-1 in combination with L-NAME in the posterior limb of the internal capsule resulted in long-lasting significant neurological deficits in mice.

Table 1 – Physiological parameters.

	ET-1/L-NAME ($n=3$)	PBS ($n=3$)
Pre-injection		
pH	7.36±0.04	7.36±0.03
pCO_2 (mmHg)	34.4±1.9	36.8±2.9
pO_2 (mmHg)	92.3±6.6	95±1.4
SBP (mmHg)	83.3±7.1	85.7±6.6
DBP (mmHg)	59.3±10.4	44±9.2
10 min post-injection		
pH	7.36±0.03	7.35±0.03
pCO_2 (mmHg)	33.8±2.2	33.7±1.9
pO_2 (mmHg)	95.3±2.1	96±4.1
SBP (mmHg)	84.7±10.8	87.7±4.8
DBP (mmHg)	50.7±4.5	47±8.5

SBP=Systolic blood pressure, DBP=Diastolic blood pressure.