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Research report

Long-term functional recovery and compensation after cerebral ischemia in rats

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HIGHLIGHTS

- Skilled reaching task allowed to differentiate between compensation and recovery.
- Infarct size correlated with long-term motor function, not with social behavior.
- Acute IL-1Ra led to recovery while compensation is seen in vehicle-treated animals.

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ABSTRACT

Cerebral ischemia is one of the most common causes of disabilities in adults and leads to long-term motor and cognitive impairments with limited therapeutic possibilities. Treatment options have proven efficient in preclinical models of cerebral ischemia but have failed in the clinical setting. This limited translation may be due to the suitability of models used and outcomes measured as most studies have focused on the early period after injury with gross motor scales, which have limited correlation to the clinical situation. The aim of this study was to determine long-term functional outcomes after cerebral ischemia in rats, focusing on fine motor function, social and depressive behavior as clinically relevant measures. A secondary objective was to evaluate the effects of an anti-inflammatory treatment (interleukin-1 receptor antagonist (IL-1Ra)) on functional recovery and compensation. Infarct volume was correlated with long-term (25 days) impairments in fine motor skills, but not with emotional components of behavior. Motor impairments could not be detected using conventional neurological tests and only detailed analysis allowed differentiation between recovery and compensation. Acute systemic administration of IL-1Ra (at reperfusion) led to a faster and more complete recovery, but delayed (24 h) IL-1Ra treatment had no effect. In summary functional assessment after brain injury requires detailed motor tests in order to address long-term impairments and compensation processes that are mediated by intact tissues. Functional deficits in skilled movement after brain injury represent ideal predictors of long-term outcomes and should become standard measures in the assessment of preclinical animal models.

1. Introduction

Acute brain injury, due to cerebral ischemia and head trauma, is one of the leading causes of death in industrialized countries and leads to long-term motor and cognitive impairments in survivors [1]. Although research has made tremendous advances in our understanding of the mechanisms leading to initial brain injury, damage progression and the implication of inflammatory processes in damage evolution, there are still no widely applicable treatments [2,3]. In part this may be due to difficulties in correlating data from pre-clinical studies with the clinical setting as a result of the heterogeneity of cerebral ischemia [4]. Although the predictive value of animal models of stroke to the human pathology is limited, recent advances in determining behavioral homologies between rat and human movements is making correlation of functional outcomes between rodents and humans feasible and more accurate [5,6].

Abbreviations: IL-1Ra, interleukin-1 receptor antagonist; MRI, magnetic resonance imaging; MBP, myelin basic protein; SR, skilled reaching; SW, skilled walking; tMCAo, transient middle cerebral artery occlusion.

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Most preclinical studies have focused on acute outcomes after injury, mainly through the analysis of the size of the infarct at 24 h combined with basic neurological scores as a measure of acute motor impairments. Early behavioral and histological outcomes are of value although there have been controversial reports on the possible correlation between infarct size and long-term functional assessments and their predictive value for long-term outcome [7–11]. Furthermore, animals usually show rapid functional improvement after brain injury, based on their neurological score, making it more difficult to assess permanent impairments. In contrast to gross motor assessment some tests, such as the staircase test and skilled pellet reaching tasks, have proven efficient and allow detection of fine long-term impairments and can differentiate between genuine recovery and functional compensation by adopting alternate strategies [9,12–14]. The latter assessment is particularly important for a meaningful evaluation of therapeutic efficacy of treatments, and to give insight on possible roles in rehabilitation.

While motor function is of particular concern after brain injury, global patient recovery and wellness is also dependent on the presence of depression and anxiety [15–17]. The inflammatory processes that arise after brain injury are a likely cause of depression since inflammation has been shown to affect emotional behavior, including sociability, independently of the infarct itself [18–22]. Furthermore inflammatory cytokines, and particularly interleukin (IL)-1, can directly alter mood and cognition through their actions on the brain [23]. This can be transient, known as "sickness behaviour", as well as chronic such as in case of stress-induced depression [21] or depressive disorders [24]. Since post-stroke depression affects approx. 40% of survivors and will greatly affect motor recovery and global wellbeing, it is important to include its assessment in preclinical animal models of stroke to allow comprehensive evaluation of potential therapeutics.

The objective of this study was to determine long-term functional outcomes after brain injury induced by transient middle cerebral artery occlusion (tMCAo) in rat, with special focus on fine motor skills as well as depression, sociability and anxiety. Furthermore, we assessed the long-term effects of protection against initial infarct using the anti-inflammatory interleukin-1 receptor antagonist (IL-1Ra), and whether delayed administration of IL-1Ra is also beneficial. Using a fine motor task, we showed that impairments are clearly visible 1 month after injury, despite partial infarct resolution, and that functional recovery can be separated from compensation. Ischemic injury induced both long-lasting sociability impairments and depressive-like behavior. Skilled reaching success correlated with infarct volume although there was no correlation with either sociability or depression. Systemic administration of IL-1Ra at occlusion decreased initial infarct size, enhanced recovery and provided protection against defects in sociability and depressive-like state.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (obtained at 8 weeks of age, approx. 200 g; Charles River, Kent, UK) were used. All experiments were conducted under UK Animals (Scientific Procedures) Act, 1986. The animals were kept in a 12 h light–dark cycle and acclimatized for 1 week with free access to food and water prior to any manipulation.

2.2. Experimental design and skilled reaching training

Animals were trained in the skilled reaching (SR) task daily for 2 weeks. To stimulate motivation, the animals were placed on a restricted diet to maintain 90% of their free feeding body weight (weight monitored twice daily). Restricted diet was used only for the first week of training after which animals were allowed free access to food and water. Four days prior to brain injury induced by tMCAo the animals were subjected to behavioral testing (as described below) in order to acquire baseline and ensure that all animals performed similarly (Fig. 1). After training animals were allowed to rest for a day prior to surgery (Fig. 1). Full behavioral testing was performed at an early time point after surgery (day (D) 6–9) and after 1 month (D25–28) (Fig. 1).

2.3. Focal cerebral ischemia

Brain injury was induced by 60 min tMCAo as previously described [25]. Briefly, animals (approx. 12 weeks of age) were anesthetized with isoflurane (4% induction and 1.5% maintenance in 70:30 N_2O/O_2) and body temperature maintained at 37 °C. A 350 μm silicon-coated monofilament (Doccol, CA, USA) was introduced in the external carotid artery and advanced through the internal carotid artery to occlude the MCA. After 60 min, the filament was retracted to allow reperfusion. Lidocaine (EMLA cream, AstraZeneca, Luton, UK) was applied locally to the wound and buprenorphine administered (Reckitt Benckiser Healthcare, Hull, UK, 0.025 mg/kg, s.c.) once, after reperfusion, to minimize pain. Sham animals received the same surgical procedure without occlusion. IL-1Ra (100 mg/kg, Biovitrum, Sweden) or vehicle (saline) were administered subcutaneously (s.c.) either acutely (at the time of occlusion) or in a delayed manner (24 h after occlusion). The experimenter was blinded to treatment until completion of all assessments and analysis. The dose and timing of administration of IL-1Ra was selected based on previous work [25]. Animals were randomly allocated to the following experimental groups: Sham + Veh ($n = 6$); Sham + IL-1Ra ($n = 6$); tMCAo + Veh ($n = 10$); tMCAo + IL-1Ra ($n = 10$; acute treatment, $n = 5$ and delayed treatment, $n = 5$). All sham animals were combined for analysis, as there was no significant difference between the subgroups groups. tMCAo animals treated with vehicle, either acute or delayed, did not differ significantly and were therefore combined for analysis. Gross neurological score was assessed daily as previously described [26]. Briefly, neurological deficits was scored by an observer blinded to the experimental conditions as follow: 0 = no observable deficit, 1 = flexion to the side contralateral to the ischemia whilst elevated, 2 = spontaneous circling to the contralateral side, 3 = falling to the contralateral side and 4 = no spontaneous movement.

2.4. Magnetic resonance imaging (MRI)

Brain infarct was evaluated early, 48 h after injury, by magnetic resonance imaging (MRI). Anesthetized animals (isoflurane: 4% induction, 1.5% maintenance) were scanned using a Magnex 7-Tesla horizontal-bore magnet (Agilent Technologies, UK) interfaced to a Bruker Advance III console (Bruker Biospin, UK) with a separate volume-transmit surface-receive radiofrequency head surface coil. T_2-weighted images were acquired using a fast spin-echo pulse sequence (repetition time/effective echo time = 4800/60 ms, 8 echoes, field of view: 40 × 40 mm, matrix: 256 × 256, 2 averages, 25 slices of 1 mm). Infarct size and edema were determined using Image J (NIH Image, US) by an observer blinded to the experimental conditions.

2.5. Skilled reaching

Skilled forelimb reaching (SR) was assessed as previously described [27] with slight modifications. Training and testing sessions consisted of each rat reaching for 20 food pellets (40 mg, LBS Biotechnology, Surrey, UK). Training was considered completed