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Research report

Optimization of behavioural tests for the prediction of outcomes in mouse models of focal middle cerebral artery occlusion

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ABSTRACT

Intraluminal middle cerebral artery occlusion (MCAO) is the most widely used model of stroke. We aimed to predict the outcome of MCAO using a combination of fine behavioural tests for the prediction of unsuccessful surgery in mice leading to no infarction, haemorrhage and unexpected death. MCAO was performed on adult mice under the guidance of laser-Doppler flowmetry (LDF) to warrant a decrease in regional cerebral blood flow (rCBF) in the MCA territory. Four outcomes of MCAO were defined according to histological analysis: infarction, no infarction, haemorrhage and unexpected death (death within 24 h post-surgery). Fine behavioural tests including the rotarod, modified neurological severity score (mNSS), Clark general and Clark focal tests were performed separately at 6 h, 12 h and 24 h post-stroke. A total of 94 mice were included in the analysis. The infarction rate associated with MCAO was 58.5% (55/94). After optimization of the timing and behavioural tests, we found that higher Clark focal (>17.5) or higher mNSS scores (>10) were markedly related to early death, whereas a lower mNSS score (<3.5) was indicative of a tendency to show no infarction at 6 h post-stroke. After 24 h post-stroke, there was a positive correlation between the infarct volume and Clark focal results. Behavioural tests could help to predict the outcomes in the MCAO mouse model.

1. Introduction

Stroke is one of the leading causes of adult mortality and disability (Hankey, 2014). Increasing numbers of studies are being performed to explore effective therapeutics for stroke. Stroke animal models play an essential role in the investigation of mechanisms and drug development (Strom et al., 2013). Among these models, middle cerebral artery occlusion (MCAO) using an intraluminal filament (Koizumi et al., 1986) has been the most widely used because it achieves minimally invasive injury, is easy to perform and results in reproducible infarct lesions. However, the infarction rate associated with intraluminal MCAO is heterogeneous among different individual studies, which might cause some bias.

Laser-Doppler flowmetry (LDF) has been introduced to guide filament insertion via monitoring of ipsilateral blood flow. When the regional cerebral blood flow (rCBF) of the middle cerebral artery (MCA) decreases to 20–30% of baseline, the surgery is defined as successful and could cause cortical infarction (Bodhankar et al., 2013; Fujioka et al., 2010). However, reduction of rCBF is not equal to histological infarction based on our observations as well as those of others (Taninishi et al., 2014). Many other methods are used to verify infarction, including magnetic resonance imaging (Bihel et al., 2010), laser speckle imaging (Akamatsu et al., 2012) and positron emission tomography (Martin et al., 2012). However, these advanced imaging techniques are time consuming and resource intensive. Some animals die within several hours of MCAO, which cannot be predicted, even with the aid of imaging modalities. It is accepted that functional and behavioural changes precede morphological changes. A simple and economic method for predicting the outcome of the intraluminal filament MCAO model remains elusive.

Behavioural tests are routine procedures performed in experimental stroke research to evaluate neurological function in the

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MCAO model (Balkaya et al., 2013b). Many behavioural tests, such as the rotarod, adhesive removal and mNSS tests, have been developed to evaluate neurological function (Balkaya et al., 2013a; Ferrara et al., 2009; Zhang et al., 2002). In the present study, a battery of behavioural tests addressing motor, sensory and vestibular functions were used to provide a more accurate prediction of the outcomes in the MCAO model.

2. Results

MCAO surgery was performed on a total of 107 animals. Four mice died before reperfusion, and 6 mice failed to exhibit a decline (>70%) of baseline rCBF during ischemia and were therefore excluded (LDF-guided success 90.6%, 97/107). Twenty-eight animals died post-reperfusion, among which 3, 11 and 14 animals died within 6 h, 6–12 h and 12–24 h post-stroke, respectively. The three animals that died within 6 h before any behavioural tests were performed were not included in the analysis. A total of 55, 5, 9 and 25 animals exhibited infarction (58.5%, 55/94), no infarction (5.3%, 5/94), haemorrhage (9.6%, 9/94) and unexpected death (26.6%, 25/94), respectively (Fig. 1B). The brains of all animals that experienced unexpected death were removed via autopsy, and it was found that among the 25 unexpected deaths, nine animals showed obvious blood clots around the Willis circle. The remaining 16 animals that experienced unexpected death exhibited infarcted areas that could be observed with the naked eye or through TTC staining.

2.1. rCBF levels are highest in the no-infarction group

As the suture was inserted at the right position, there was a prompt decline in CBF, which decreased below 30% of baseline (Fig. 1A). A significant increase in CBF was observed in the no infarction group at 10 min and 100 min after MCA occlusion relative to the other groups. At the same time, a moderate increase in CBF was also observed in the successful group at 100 min after MCA occlusion.

2.2. Classification and distribution of infarction

The infarct volume assessed via TTC staining ranged from 1.45% to 33.96% of the bilateral hemisphere (mean 16.89% ± 8.78; n = 55). According to the infarction volume (IV), we divided the infarctions into three groups: minor stroke (IV < 10%), mild stroke (10% < IV < 20%), and severe stroke (IV > 20%). Representative morphological characteristics of the three groups are shown as Fig. 1D. The percentages of mice with various degrees of infarction were as follows: minor stroke 25.5% (14/55), mild stroke 38.2% (21/55), severe stroke 36.4% (20/55). The neocortex, striatum, hippocampus and thalamus were the most frequently affected areas of the brain. In animals showing different degrees of infarction, the infarct lesions were variable (S Fig. 1B). The neocortex was the most affected area overall; the striatum was the second most affected area in severe and mild stroke; and the hippocampus was the most affected area in minor stroke.

2.3. Combined behavioural test predicted outcomes of MCAO

2.3.1. Correlation between infarction volume and behavioural tests

We considered the relationship between the infarct volume percentage and changes in the behavioural tests at 6 h, 12 h, and 24 h post-stroke. Only one positive correlation was found between the infarct volume percentage and the Clark focal score at 24 h post-stroke (r = 0.308, $P = 0.022$; Fig. 1C).

2.3.2. Early prediction of death through behavioural tests

The scores of the death group were markedly higher than those of the other three groups in many of the applied behavioural tests (S2–S11 Figs). To identify the most sensitive behavioural test for differentiating early death, ROC analysis was applied to assess the sensitivity and specificity of each behavioural test to identify the test with the greatest early diagnostic value. As shown in Table 1, a time on the rotarod>0 s, an mNSS>10 and a Clark focal score>17.5 were of medium diagnostic accuracy for excluding mice that would die according to the AUC curve, among which a Clark focal score>17.5 presented the highest Youden's index (Fig. 2A).

2.3.3. Optimization of timing and behavioural tests for predicting no infarction

We evaluated different groups at distinct times, as shown in Tables 2–4. At 6 h post-stroke, an mNSS<3.5, a Clark general score<4.5 and a Clark focal score<8.5 were of medium diagnostic accuracy for excluding mice with no infarction. According to Youden's index, mNSS<3.5 was the best behavioural test for distinguishing no infarction. At 12 h post-stroke, mNSS<5.5, Clark general<3.5 and Clark focal<10.5 were of medium diagnostic accuracy for excluding mice with no infarction, and taking Youden's index into account, Clark general<3.5 was the best behavioural test for distinguishing no infarction. Finally, at 24 h post-stroke, time on the rotarod>8 s, mNSS<5.25, Clark general<4.5 and Clark focal>7.5 was of medium diagnostic accuracy for excluding mice with no infarction while the mNSS<5.25 was the best behavioural test for distinguishing no infarction. Based on comparison with AUC and Youden's index, mNSS<3.5 at 6 h post-stroke was the best behavioural test for distinguishing no infarction (Fig. 2B).

3. Discussion

High mortality and high heterogeneity have been major obstacles in suture MCAO models (Aspey et al., 2000; Takano et al., 1997), resulting in a waste of animals and medicine. Many behavioural tests focus only on successful models, without regard for unsuccessful models. Our study first employed the most commonly used behavioural tests to differentiate animals dying within 24 h post-stroke and animals presenting neurological deficits with no infarct. In our study, we compared the relationship between the infarct volume percentage and behavioural tests and demonstrated that the Clark focal score was closely related to the infarct volume percentage.

Many imaging modalities employed to replace autopsy have been used to visualize brain infarction (Akamatsu et al., 2012; Bihel et al., 2010; Martin et al., 2012). These imaging methodologies are costly, generally showing high acquisition and running costs, and lack a sufficient specificity, imaging speed and spatial resolution in small animal models (Nagy et al., 2013). Thus, a simple, effective and economical tool for identifying the outcome of MCAO is urgently needed. Behavioural testing is a routine procedure for evaluating the neurological function of MCAO models (Hunter et al., 2000a).

Rogers et al. (1997) demonstrated a significant correlation between infarct volume and post-stroke behavioural tests. However, many studies have found a poor correlation between infarct volume and post-stroke behavioural tests (Johansson, 1996; Hunter et al., 2000b). In our study, only the Clark focal score presented a relatively high relationship with the infarct volume percentage. The reasons underlying the lack of a close association may involve functional compensation or recovery. In addition to histological infarction in the model, there are other potential outcomes of MCAO, such as no infarction, haemorrhage post-surgery and unexpected death within 24 h post-surgery. The occurrence