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Research report

Morphological and neuro-behavioral parallels in the rat model of stroke

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ABSTRACT

Middle cerebral artery occlusion (MCAO) is widely used as a rat model of focal brain ischemia. Evaluation of brain damage often includes the morphological analysis of the injury area, MRI, and various scales which depend on functional tests, commonly known as neurological severity score (NSS). We determined the optimal number of NSS tests and assessed their capacity for non-invasive evaluation of brain ischemic injury in the rat MCAO model. 275 male Sprague-Dawley rats were randomly divided into five groups, given either permanent (p) MCAO or transient (t) MCAO using an uncoated 4-0 monofilament catheter or a silicone-coated monofilament. The rats' neurological status was examined before and at 1 and 24 h following MCAO. The size of brain injury was then measured histologically and the extent of right cerebral hemisphere edema was calculated. We established a correlation between these tests and morphological data for brain injury. Adjusted R^2 of the prediction of total histology score was 0.7. The Hosmer–Lemeshow p-value of this model was 0.812 for total brain histology. For the brain edema the adjusted R^2 of the prediction model was 0.48. The Hosmer–Lemeshow p-value of this model was 0.558 for brain edema. Our methods of estimating infarct size produces reliable and well correlated results at 24 h and demonstrates to be an easy and quick way to assess infarct size soon after ischemic injury has occurred. The described method for neurological assessment could ultimately aid in assessing various treatment modalities in the early hours following stroke.

1. Introduction

Acute brain ischemia (stroke) is the third most common cause of death worldwide, after heart disease and cancer [1]. Stroke is furthermore the leading cause of adult disability, as nearly one third of patients who survive more than 6 months are left dependent on others [2]. Ischemic stroke accounts for approximately 87% of all strokes and results from a thrombotic or embolic occlusion of a major cerebral artery (most often middle cerebral artery, MCA) or its branches [1].

Animal models of cerebral ischemia represent an important contribution to both our understanding of the pathophysiology of stroke and the development of new therapies. Baseline, intermediate and final assessments of neuroprotection in vivo utilize motor and behavior scores as well as morphological examination. The majority of visualization tools necessitates sacrificing of animals for histological examination or utilizes computed tomography (CT) or magnetic resonance imaging (MRI) that makes those tests expensive, time consumptive and ethically problematic. An ideal test for the assessment of neurological impairment should be non-invasive, non-expensive, easy to perform, time-saving and reliable. An assessment of motor and behavioral tests which closely correlate with volume of brain lesion and brain edema after ischemic stroke offers such an opportunity. Additionally, early neurological assessment of animals after the stroke could provide an ability to divide the rats into treatment groups based equally on the severity of the injury, providing homogeneity of the groups.

Many experimental models have been developed in order to investigate the mechanisms of ischemic brain injury and test different neuroprotective strategies [3,4]. Amongst all current animal models of stroke, the one used most frequently is a rat model of

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focal brain ischemia caused by a middle cerebral artery occlusion (MCAO) [5–9]. The MCAO technique minimizes the collateral circulation to the intended ischemic area and therefore has the benefit of obtaining high homogeneity and reproducibility of the results [5,7,8]. The existing techniques of MCAO produce brain injury areas of varying sizes, ranging from ~6% to ~55% of hemisphere, depending on the duration of arterial occlusion and the type of occluding filaments being used [7,8,10]. Since 1984, when MCAO in the rat was preformed via an open technique [11], the number of publications on this subject has been steadily increasing from a few works in the 1980s, to 448 publications in 2009 (Pubmed search – keywords – middle cerebral artery occlusion; rats); and various new techniques of producing MCAO have since then been introduced [5,12–15].

In focal cerebral ischemia, there may be a complete lack of blood flow in the very central core of the ischemia, but usually there is some flow that reaches the area via a collateral circulation. The amount of collateral flow is insufficient to maintain cerebral metabolism and function, resulting in neural cell death via necrosis or apoptosis [3]. The methods used most often for evaluation of brain damage include the morphological analysis of the injury area (using histology and requiring sacrifice of the animal), MRI, and various scales which depend on functional tests, commonly known as neurological severity score (NSS) [6–10]. The NSS is based on the assumption of a correlation between size and progression of the brain injury, as well as neurological deficit [16].

It is worth noting that functional and behavioral changes precede morphological changes. NSS is generally a simple, rapid, non-invasive, and inexpensive test that does not require complicated and expensive equipment. Given the severity of the underlying pathology and the necessity to start treatment as quick as possible, the NSS tests should be easy to perform, should not aggravate the animal's condition and should be predictive of the size of infarct, even before it is fully formed histologically. Several proposed NSS regimens have been used most widely for purpose of brain ischemia verification, consisting of a wide range of functional tests (from 1 [17] to 26 tests [18]) and score points (from 3 [17] to 70 score points [19]). Therefore, developing an optimal NSS which complies with the above mentioned criteria would be highly valuable for a non-invasive evaluation of stroke injury and for assessing possible treatments. The purpose of this study was to determine the optimal number of NSS tests and their capacity for non-invasive evaluation of brain ischemic injury in the rat MCAO model. Ultimately, this information can lead to the prediction of brain injury by these NSS tests.

2. Materials and methods

The experiments were conducted according to the recommendations of the Declarations of Helsinki and Tokyo and to the Guidelines for the Use of Experimental Animals of the European Community. The experiments were approved by the Animal Care Committee of Ben-Gurion University of the Negev, Israel.

2.1. Animals

In our current study we used total of 275 male Sprague-Dawley rats (Harlan Laboratories, Israel) with no overt pathology, weighing 300–380 g. The rats were kept in cages, three rats per cage for at least three days after arrival to allow adaptation. Free access to water and chew was allowed throughout the entire experiment.

The rats were randomly divided into five groups and in each group we performed either permanently (p) MCAO or transiently (t) MCAO using an uncoated 4-0 monofilament catheter or a silicone-coated monofilament [10]. This variety of the groups was chosen in order to produce a wide spectrum of the severity of brain injury.

Rats were randomized into following groups and subjected to corresponding interventions. The amount of animals in each group is presented in Table 1.

Group 1 was used as a control sham-operated group without MCAO, and was only subjected to a skin incision and anesthesia. Group 2 was subjected to permanent MCAO group using an uncoated 4-0 monofilament catheter. Group 3 was subjected to transient MCAO group using an uncoated 4-0 monofilament catheter. Group 4 was subjected to permanent MCAO group using a silicone-coated monofilament. Group 5 was subjected to transient MCAO group using a silicone-coated monofilament.

Rats with subarachnoid hemorrhage caused by rupture of the intracranial ICA and rats without neurological deficits 1 h following MCAO were excluded from further analysis.

2.2. Surgery

The MCAO was performed according to the methods described by Boyko et al. [5] with minimal modification as described below. The rats were anesthetized with mixture of isoflurane (5% for induction, 2% for surgery, 1.3% for maintenance) in 100% oxygen (1.5 L/min) without tracheotomy and allowed to breathe spontaneously. The right CCA was exposed through a midline neck incision and carefully dissected from surrounding tissues, from its bifurcation to the base of the skull. The occipital artery branches of the ECA were then isolated, their branches were dissected and coagulated. The ECA was further dissected distally and coagulated along with the terminal lingual and maxillary artery branches. The ICA was isolated and carefully separated

Table 1
The volume of brain injury for each group, including total infarct volume, infarcted volume in the striatum, infarcted volume in the cortex and brain edema. Values are expressed as mean ± standard. The total number of rats in each group, as well as the number of rats included and excluded in each group is also presented. Rats were excluded due to mortality, intracranial hemorrhage, or unreliable data which would suggest recording errors.

Groups	Brain Injury	Volume of Injury (Mean ± SD)	Total amount of the rats	Rats excluded	ICH caused	Mortality	Amount of rats included
Permanent MCAO Silicon	*Infarcted Volume Total*	22.4% ± 7.9	48	2	5	5	36
	Infarcted Striatum	10% ± 3.5					
	Infarcted Cortex	12.4% ± 4.9					
	Brain Edema	15.4% ± 7.6					
Permanent MCAO Monofilament	*Infarcted Volume Total*	17.7% ± 5.8	58	4	5	5	44
	Infarcted Striatum	8.2% ± 2.9					
	Infarcted Cortex	9.3% ± 3.7					
	Brain Edema	12.6% ± 5.5					
Transient MCAO Silicon	*Infarcted Volume Total*	11.6% ± 6	59	3	6	4	44
	Infarcted Striatum	6.3% ± 3.3					
	Infarcted Cortex	7.3% ± 4.1					
	Brain Edema	9.5% ± 5.4					
Transient MCAO Monofilament	*Infarcted Volume Total*	3.9% ± 5.8	54	3	7	6	38
	Infarcted Striatum	2.3% ± 2.5					
	Infarcted Cortex	1.5% ± 4					
	Brain Edema	4.3% ± 4.8					
Control	*Infarcted Volume Total*	0.3% ± 0.4	44	0	0	0	44
	Infarcted Striatum	0.3% ± 0.3					
	Infarcted Cortex	0.1% ± 0.2					
	Brain Edema	2.3% ± 2.1					
Total			263				208

MCAO—middle cerebral artery occlusion; ICH—intracranial hemorrhage.