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Review

Assessment of behavioural deficits following ischaemic stroke in the marmoset

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ABSTRACT

Stroke is a common and devastating disease worldwide. Over the last two decades, many therapeutic approaches to ameliorate ischaemic stroke have been promising in animal studies but failed when transferred to the clinical situation. One of the possible explanations for these failures is the widespread use of animal models of cerebral ischemia that do not mimic the pathology encountered in the clinic. Accordingly, many expert committees recommended the integration of higher order species such as non-human primates in pre-clinical stroke studies. The common marmoset (*Callithrix jacchus*), a small New World monkey, start to stand out in the neuroscience field as a good compromise between larger primates and rodents. In this review, we discuss the relevance of the use of the marmoset in stroke studies. We will focus on behavioural tests developed in this species to assess sensorimotor deficits and their recovery during acute and chronic stages of brain ischaemia. The aim of this appraisal is to provide a comprehensive overview of the existing approaches to induce stroke in the marmoset as well as the paradigms for behavioural testing in this species. The data summarized in this review should contribute to the improvement of future stoke studies in the marmoset and accordingly improve the translation of the results from bench to bed.

1. Introduction

Stroke is a common and devastating disease worldwide. Despite declining stroke mortality rates in developed countries, its global burden is increasing. Fifty percent of survivors of the acute phase remain physically or mentally impaired, which represent a major socio-economic burden. Ischaemic strokes, which represent 80–85% of all stroke subtypes, are caused by an abrupt occlusion of a major feeding vessel resulting in a decrease in local blood flow and hence delivery of oxygen and nutrients to the affected brain territory. Nowadays, the only approved treatments are thrombolysis with t-PA and mechanical thrombectomy, both of which are administered within the first hours after the initial onset of symptoms and therefore are limited to a minority of the patients that enter into this therapeutic window [1,2]. Consequently, further preclinical and clinical investigations are required to better understand the pathophysiology of stroke and to develop novel therapeutic interventions.

Over the last two decades, many therapeutic approaches to ameliorate ischaemic stroke have been proven efficient in animal studies but failed when transferred to the clinical situation [3]. One of the issues that has been put forward to explain these failures of translation between the bench and the bed is the inadequacy of the animal models of stroke [2–4]. Indeed, most of the available preclinical studies rely on the use of young and healthy rodents (mice and rats) due to their low cost compared to most other laboratory species and because rodents are generally considered as ethically more acceptable [4]. In an attempt to improve the translation of findings from bench to bed, many recommendations, such as those of the STAIR (Stroke Therapy Academic Industry Roundtable), have been published to emphasize the integration of higher order animals such as non-human primates in preclinical stroke research, due to their significant similarities (cerebrovascular, neuroanatomical and biomolecular) to man [3,5]. In this review, we address the interest of a new world primate, namely the common marmoset, as a reasonable and complementary animal model of stroke. We focus on an appraisal description of behavioural tests which are employed to assess functional deficits and their recovery following ischaemic stroke in the marmoset.

2. Interest of the marmoset for stroke's studies

Although momentous studies in respect to the pathophysiology of stroke have been performed in non-human primates such as baboons

Abbreviations: AChA, anterior choroidal artery; AChAO, anterior choroidal artery occlusion; ET-1, endothelin-1; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; tMCAO, transient middle cerebral artery occlusion

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and macaques [6,7], these species have been relatively abandoned over the last three decades. The reasons for this are mainly due to the high cost and the absence of technically simple stroke models in these species. Recently, there has been considerable interest in the common marmoset (*Callithrix jacchus*) for neuroscience research [8]. Indeed, the marmoset, a small new world primate of a non-endangered status, is closer to man than rodents in term of cerebrovascular system, brain metabolism, gray-to-white matter ratio and presents several advantages in matter of economies, size (close to rats), husbandry and ease of breeding [6,9]. Although marmosets are not fully gyrencephalic, they are closer in the phylogenetic tree to man. On the other hand, their relative lissencephaly provides advantages for the analysis of the cerebral cortex [8]. Marmosets also have a larger brain to body weight ratio than rodents since an adult marmoset has a brain approximately 4 times the size of the rat with similar body weight of 250–400 g. As a result of their small size, common marmosets are generally easy to manage and handle, which represent a great advantage for behavioural testing. Moreover, the common marmoset has a disease susceptibility profile that is similar to humans, making it a suitable model for drug development [10]. Furthermore, the complete genome of the common marmoset is currently being sequenced and the recent development of the first genetically-modified marmoset [11] has attracted attention for its potential in pre-clinical studies since genetic models of primates are with their long lifespan (*i.e.* more than 10 years), highly attractive [12].

Various fundamental research tools have been developed for marmosets [13]. Firstly, magnetic resonance imaging (MRI) techniques are largely employed in order to investigate internal body structure in details *in vivo*. Since this method is non-invasive, the animal does not have to be sacrificed which opens the possibility to perform long-term studies in the same subject. Among the MRI techniques, T2-weighted scanning sequence (T2-MRI) is commonly used to visualise the ischaemic lesion [14,15]. Nonetheless, as T2-MRI is based on the increased water content in the tissue, it cannot reveal the lesion until vasogeneic oedema develops (approximately 12 h following the insult). Diffusion-weighted imaging (DWI) is designed to detect random water molecules movement. As water enters rapidly the cells after stroke, its movement becomes restricted in the intracellular space in the ischaemic brain tissue, resulting in a bright signal on DWI. Thus, DWI is an extremely sensitive method for detecting the lesion very acutely [16]. Diffusion tensor imaging (DTI), another approach of diffusion imaging, is also used to visualise white matter features [17]. Since grey-to-white matter ratio is higher in marmosets than in rodents, they are more suited to address stroke-induced white matter alterations. Bihel et al. [16] have showed that DTI was able reveal disorganization of white matter, despite an absence of a visible lesion at the chronic stage of stroke in the marmoset. Voxel-based morphometry (VBM) is a method of analysis of T1-MRI, which allows the assessment of local tissue volume changes over the whole brain, independent of operator or region of interest [13]. This method has been employed in a model of Parkinson's disease in the marmoset, but not yet in stroke models [18]. Recently, functional mapping of the visual [19,20], auditory [21] and somatosensory cortex [22] of the marmoset's brain has been carried out using fMRI in the anaesthetised and awake marmoset (for review see [23]). A full 3D reconstitution of the entire marmoset cerebral cortex as well as a brain template are now available which will facilitate future MRI investigations in this species [24–26].

3. Models of ischaemic stroke in the marmoset

In order to benefit of the many advantages of this primate, several groups have developed various approaches to induce focal brain ischaemia (Fig. 1) [27]. Most of these approaches are adaptations of those performed in rodents and target mainly the middle cerebral artery (MCA), the vessel that is the most frequently affected in patients.

The first model of focal stroke in the marmoset was described by Marshall and Ridley [28]. In this paradigm, ischaemia was induced by unilateral electrocoagulation of the proximal portion of the MCA following a large fronto-temporal craniotomy (Fig. 1.1). The MCA occlusion (MCAO) produced an infarction in the frontal, temporal and parietal cortices, the underlying white matter and the caudate and putamen [29] (Fig. 2). Although, this model requires somewhat sophisticated surgical procedures, it had the merit to be the first in which behavioural deficits, be they sensorial or motor, were assessed with a battery of tests up to ten weeks after the induction of stroke [28]. Thus, many behavioural tests were described and validated in this model and formed a valuable base for the subsequent stroke studies in the same species. Nonetheless, in addition to the invasiveness of the surgical approach (lesion of the temporalis muscle, large craniotomy, dura dissection, brain retraction), this model of stroke has the disadvantage of the impossibility to reperfuse because of the electrocoagulation of the artery.

To overcome this issue, Virley et al. [30] reported a model of transient ischaemia in the marmoset through the use of the vasoconstrictor agent, endothelin-1 (ET-1) that was applied directly onto the distal segments of the MCA (Fig. 1.2). In this model, which also necessitates craniotomy and dissection of the dura mater, the duration of the occlusion cannot be efficiently controlled and, accordingly induces an increased inter-individual variability [30].

To reduce the invasiveness of the surgical approach and to permit the induction of both permanent and transient ischaemia in the marmoset, our group has adapted the intraluminal filament approach, which is widely employed in laboratory rodents and is considered to better replicate the clinical situation. To avoid craniotomy, the insertion of a calibrated nylon thread into the external carotid artery up to the origin of the MCA requires relatively minor cervical surgery and results in minimal morbidity and suffering [31] (Fig. 1.5). Through the use of multiple outcome analyses (neuroimaging, behavioural tests, along with post-mortem neuropathology), the evolution of brain damage and behavioural impairments in acute, subacute and chronic stages of ischaemia following permanent (pMCAO) or temporary MCAO (tMCAO) have been described [14]. The intraluminal occlusion of the MCA produced reproducible brain lesions that embraced both cortical and subcortical structures (Fig. 2) and persistent functional deficits up to 6 weeks following the induction of ischaemia [14]. These alterations were more severe in marmosets subjected to pMCAO in comparison to those in which reperfusion was applied 3 h after the occlusion (tMCAO). *Post-mortem* analyses revealed a widespread neuronal loss and associated astrogliosis in greater extent in permanently occluded animals. Interestingly, and thanks to the relative high white to grey matter ratio in the marmoset, which facilitates imaging of white matter lesions, intraluminal occlusion of the MCA has been showed to result in major alterations of the white matter bundles, analysed by DTI-MRI, that were correlated with functional deficits and could explain, at least in part, the persistence of stroke-induced functional deficits [14]. The profile of brain damage and functional deficits seen in the marmoset suggest that this model of intraluminal MCAO could be suitable to investigate the pathophysiology and the treatment of ischaemic stroke. Besides, the relevance of this reversible MCAO model has already been well demonstrated in rodents by stroke researchers [4,32,33]. While the rodent model can provide a rapid screening of new therapeutic molecules, the marmoset model allows a more detailed characterization of the drug therapy before a possible clinical adaptation.

Recently, a model of focal stroke by photothrombosis has been reported in the marmoset [34]. It is an adaptation of the approach initially described in the rat [35]. Following an exposition of the skull, the sensorimotor area of the cerebral cortex is irradiated with green light after intravenous injection of the photosensitive Rose Bengal dye [35] (Fig. 1.4). The thrombosis so induced results in circumscribed cortical lesion, the size of which is determined by the extent of the irradiated zone (Fig. 2). This technique does not require any craniotomy and is thus less invasive and also lead to small variations between individuals since the ischaemic area can be precisely defined.