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Brief Communication

Lack of secondary pathology in the thalamus after focal cerebral ischemia in nonhuman primates

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ABSTRACT

Remote regions such as the thalamus undergo secondary degeneration after cerebral ischemia. In rodents, the pathology in the thalamus is characterized by a robust inflammatory reaction, β-amyloid (Aβ) accumulation and calcification. Here we studied whether nonhuman primates subjected to middle cerebral artery occlusion (MCAO) display a similar pathology. Common marmosets ($n = 4$) were subjected to transient MCAO for 3 h. Two sham-operated animals served as controls. All animals underwent MRI examination (T2) on postoperative day 7 to assess the location of the infarct. After a 45-day follow-up period, the animals were perfused for histology to evaluate β-amyloid and calcium load in the peri-infarct regions and the thalamus. There was no Aβ or calcium staining in the sham-operated marmosets. The contralateral hemisphere was devoid of Aβ and calcium staining in MCAO animals, except calcium staining in one animal. In the ipsilateral cortex, patchy groups of Aβ-positive cells were observed. Occasional calcium staining was observed in the peri-infarct regions, lesion core, and remote regions such as the substantia nigra. The most important, the thalamus was devoid of any sign of Aβ and calcium aggregation in MCAO animals. Staining for glial fibrillary acidic protein (GFAP) showed marked astrogliosis in the ipsilateral cortex and thalamus. In conclusion, our preliminary study in marmosets did not identify Aβ and calcium pathology in the thalamus following cerebral ischemia as shown in rodents.

Introduction

The middle cerebral artery (MCA) is the most commonly affected vessel in ischemic stroke. In most cases, occlusion is followed by spontaneous reperfusion resulting in variable primary damage to the cortex and subcortical structures (Zanette et al., 1995). In addition, remote areas connected to the cortical infarct are affected due to delayed anterograde and retrograde degeneration (Ross and Ebner, 1990). Secondary neurodegeneration in areas such as the thalamus and substantia nigra is associated with an inflammatory reaction (Block et al., 2005; Lipsanen et al., 2011) and eventually a severe shrinkage of these nuclei occurs (Fujie et al., 1990; Tamura et al., 1991).

Recent studies in rats subjected to cerebral ischemia suggest a much more complex pathology in the thalamus (Hiltunen and Jolkkonen, 2011). It seems that β-amyloid precursor protein (APP) and its cleavage product β-amyloid (Aβ) or their fragments accumulate in dense, plaque-like deposits in the ventroposterior lateral and ventroposterior medial nuclei (VPL/VPM) after middle cerebral artery occlusion (MCAO) (van Groen et al., 2005; Zhang et al., 2011). This coincides with robust calcium aggregation (Mäkinen et al., 2008), altered APP processing, and the expression of Aβ-degrading enzymes in the overlapping areas (Hiltunen et al., 2009a; Sarajärvi et al., 2012). Secondary pathology occurs with a delayed time frame of 1–4 weeks after the initial ischemic insult (van Groen et al., 2005).

Since the thalamus is the place where sensorimotor pathways are organized and integrated (Briggs and Usrey, 2008), the described delayed pathology is suggested to provide a novel therapeutic target for stroke management (Zhang et al., 2012). Given the number of clinical failures with neuroprotective drugs in stroke, there is an urgent need to confirm experimental rodent data in higher species such as nonhuman primates (Stroke Therapy Academic Industry Roundtable, 1999; Fisher et al., 2009). In nonhuman primates, however, the availability of appropriate stroke models has been limited until recently (Bihel et al., 2010, 2011; Freret et al., 2008). Here we had a unique opportunity to study Aβ and calcium pathology in the thalamus in nonhuman primates

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45 days after transient MCAO. The data are discussed and compared in light of those from MCAO rodents.

Materials and methods

MCAO in marmosets

Six male and female marmosets (*Callithrix jacchus*) from the breeding center at the University of CAEN were used (age 22–30 months, weight 280–360 g). All procedures were performed according to the European Directive (86/609/EEC) and approved by the Regional Ethics Committee (# N/02-04-10/11/04-13). Focal cerebral ischemia was induced using the intraluminal filament technique as described previously (Bihel et al., 2010, 2011; Freret et al., 2008). Briefly, a nylon thread was inserted into the external carotid artery and gently advanced up to the origin of the MCA. The thread was removed after 3 h in MCAO animals ($n = 4$, 3 males/1 female) or immediately in sham-operated animals ($n = 2$, 2 females). During the surgical procedure, arterial blood pressure, heart rate and body temperature remained stable within physiological ranges (Table 1).

Each marmoset underwent MRI (T2) examinations on postoperative day 7 (7 T; Pharmascan, Bruker, France) (Bihel et al., 2011). To identify the lesion, MR images were thresholded at the mean + twice the standard deviation of the contralateral gray matter values (without the ventricles) to display a hypersignal on T2-MRI.

Histology

On postoperative day 45 the marmosets were deeply anesthetized with isoflurane (5% during 10 min) and transcardially perfused with a heparinized solution of saline followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed from the skull and postfixed for 4 h in the same fixative. Then the brains were cut in the coronal plane.

Sections (50 μm) from the four anterioposterior levels (A4, A5.5 — for the MD, A8 — for the VPL/VPM and P3) were used for staining (Stephan et al., 1980). The Aβ staining was examined using a human-specific antibody (mouse monoclonal anti-hAPP, 6E10, Covance, Emeryville, USA). Citrate buffer pretreated sections (30 min, 85 °C) were incubated with the primary antibody (1:1000) and Tris buffered saline with 0.5% Triton X-100 (TBS-T) overnight at room temperature (20 °C). The sections were then rinsed three times in TBS-T and transferred to a solution containing the secondary antibody (goat anti-mouse*biotin 1:400; Sigma). After 2 h, sections were rinsed and transferred to a solution containing mouse ExtrAvidin (Sigma-Aldrich, USA) and then incubated for approximately 3 min with diaminobenzidine (DAB) (van Groen et al., 2005). Adjacent sections were stained for glial fibrillary acidic protein (GFAP) (rabbit anti-GFAP 1:5000, Dako, Trappes, France) (Bihel et al., 2010). The used antibody showed Aβ deposits in brain sections from transgenic Alzheimer's disease mice and patients, which were used as a positive staining control.

Calcium was stained with the Alizarin red method (Mäkinen et al., 2008). Sections were mounted on gelatinized glass and immersed in 2% Alizarin Red (Merck, Germany) for 30 s followed by a rinse in distilled water. Sections were quickly dehydrated with acetone and xylene, and then mounted in Depex.

Histological analysis

The distributions of 6E10-positive cells and calcium positive (Alizarin Red) areas were manually mapped using Stereo Investigator software (MicroBrightField, Inc., VT, USA). The integrated hardware–software setup consisted of a PC computer system connected to an ECLIPSE E600 microscope (Nikon, Japan) via a 3-Chip CCD color video camera (QImaging, Canada). A motorized stage with a microcator (Heidenhain EXE 610C) attachment (providing a 0.1 μm resolution in the *Z* axis) was mounted on the microscope. The brain sections were first outlined using a CFI Plan Achro 2× objective (N.A. 0.06, W.D. 7.5). Thereafter, a CFI Plan Achro 10× objective (N.A. 0.45, W.D. 4.5) was used to map cells and areas of interest. The shrinkage of the thalamus was assessed from stained sections by separately outlining the ipsilateral and contralateral thalamus at the level A8 and then calculating the % change.

Results

The volume (median 284.1 mm^3, interquartile range 355.8) and location of infarct at day 7 post-occlusion varied greatly between MCAO animals: small cortical lesion in the MCA territory (#9142), striatocapsular infarct partly affecting cortical areas and minor anterior circulation infarction (#9103), subcortical infarct (#9187) and massive infarct in the MCA territory and cortical infarction affecting the anterior circulation (#9325) (Fig. 1). This was associated with shrinkage of the thalamus as measured from stained sections (−21.8 ± 7.4%, mean ± S.E.M.) as compared to the contralateral side. MRI did not show neurodegeneration in the thalamus whereas a hyperintense signal in the substantia nigra was found in all animals with subcortical infarcts.

There was no Aβ or calcium positive staining in the sham-operated marmosets in the four anterioposterior levels examined.

Total number of Aβ-positive neurons (30–2397 cells/ischemic hemisphere) and calcium staining (0–6.359 mm^2/ischemic hemisphere) were mapped and quantified at one coronal level in MCAO animals. The results did not seem to depend on lesion size or location. Patchy groups of Aβ-positive neurons were observed in the ipsilateral cortex around the infarct (Fig. 1A). In one animal (#9142), a high number of Aβ-positive cells was observed as a descending tract next to the white matter. Occasional calcium staining was observed, varying from possible intracellular accumulation in the superficial layer of the cortex (Fig. 1B) to diffuse, granular aggregations in the lesion core. More importantly, the thalamus was devoid of any sign of diffuse or granular Aβ or calcium staining in MCAO animals.

The contralateral hemisphere was devoid of Aβ staining and calcium staining in MCAO animals, except calcium staining was detected in one animal (Fig. 1, #9142). Remote regions such as the substantia nigra and hippocampus were occasionally stained for calcium. GFAP immunostaining revealed a marked astroglial reaction in the ipsilateral cortex and thalamus (Figs. 1C, D).

Table 1
Physiological data in marmosets subjected to transient middle cerebral artery occlusion (MCAO) or sham-operation (SHAM).

	MAP (mmHg)		HR (min^{-1})		Temperature (°C)	
	MCAO	SHAM	MCAO	SHAM	MCAO	SHAM
Before MCAO	66 ± 6	66 ± 8	197 ± 21	185 ± 18	37.4 ± 0.1	37.6 ± 0.4
During MCAO	62 ± 4	63 ± 5	207 ± 14	207 ± 9	37.1 ± 0.4	37.5 ± 0.2
After reperfusion	67 ± 5	66 ± 14	208 ± 22	233 ± 4	36.9 ± 0.3	37.8 ± 0.1

MAP: mean arterial blood pressure HR: heart rate. Data are mean ± S.E.M. (MCAO, n = 4: SHAM, n = 2).