Pharmacology, Biochemistry and Behavior 99 (2011) 468–474

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Chronic ibuprofen treatment does not affect the secondary pathology in the thalamus or improve behavioral outcome in middle cerebral artery occlusion rats

Anu Lipsanen, Mikko Hiltunen, Jukka Jolkkonen *

Institute of Clinical Medicine, Neurology, University of Eastern Finland, Kuopio, Finland

ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 13 April 2011
Accepted 24 April 2011
Available online 30 April 2011

Keywords:
β-amyloid
Calcium
Ibuprofen
Inflammation
Middle cerebral artery occlusion
Thalamus

ABSTRACT

Anti-inflammatory drug ibuprofen decreases the β-amyloid (Aβ) deposition and associated inflammation in transgenic Alzheimer disease mice. Based on this, we studied whether ibuprofen could modulate the secondary pathology described in the thalamus of middle cerebral artery occlusion (MCAO) rats. Our hypothesis was that ibuprofen could decrease inflammatory reaction and Aβ load in the thalamus of MCAO rats, which in turn is reflected in improved behavioral outcome. Forty male Wistar rats (250–340 g) were subjected to sham-operation or transient occlusion of the right middle cerebral artery (120 min). Ibuprofen (40 mg/kg/day, *per os*) was administrated for 27 days beginning the treatment on post-operative day 2. MCAO controls were given vehicle. Sensorimotor impairment was assessed using the limb-placing, tapered ledged beam-walking and cylinder tests during the follow-up. The rats were perfused for histology on postoperative day 29. Histological data showed that ibuprofen did not affect Aβ or calcium load in the thalamus of MCAO rats. In addition, behavioral tests did not show significant difference between vehicle- and ibuprofen-treated MCAO rats. The present data do not support the idea that ibuprofen reduces the secondary Aβ/calcium pathology in the thalamus or associated sensorimotor impairment following cerebral ischemia.

1. Introduction

Inflammation in the brain after injury is a complex process characterized by astrogliosis, migration of macrophages, activation of microglia and increased release of immune mediators and cytokines such as interleukins, tumor necrosis factor, and prostaglandins in the brain (Akiyama et al., 2000; Hurley et al., 2002; Stewart et al., 1997). Inflammatory changes are also observed in remote areas with synaptic connections to the primary lesion such as the thalamus (Block et al., 2005). Astroglial and microglial activation is detected in the thalamus already 24 h after focal cerebral ischemia (Rupalla et al., 1998) persisting up to 6 months (Watanabe et al., 1998). Temporally this is associated with anterograde and retrograde degeneration (Sorensen et al., 1996), which leads eventually to a severe shrinkage of the thalamus. In addition, we recently demonstrated overlapping aggregation of amyloid precursor protein (APP), β-amyloid (Aβ) and calcium in the thalamus of rats subjected to transient middle cerebral artery occlusion (MCAO) (Mäkinen et al., 2008; van Groen et al., 2005). Interestingly, the deposits were surrounded by astrocytes and microglia indicating an intriguingly similar pathology between cerebral ischemia and Alzheimer's disease (AD) (Itagaki et al., 1989; Willuweit et al., 2009).

Increasing evidence shows that chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen may provide neuroprotection against excitotoxic neuronal injury *in vitro* (Iwata et al., 2010) and ischemic damage *in vivo* (Antezana et al., 2003; Cole et al., 1993). Ibuprofen also reduces the formation of Aβ plaques in the transgenic AD mice and improves functional outcome in association with a reduction of inflammatory mediators (Lim et al., 2000, 2001; Yan et al., 2003). Eriksen et al. (2003) showed a significant correlation between reduced $A\beta_{42}$ and ibuprofen in cell cultures. It is also suggested that non-steroidal anti-inflammatory drugs lower amyloid pathology by a shift in γ-secretase activity (Lleo et al., 2004; Weggen et al., 2001). In addition, inhibition of mitochondrial Ca^{2+} overload by NSAIDs may prevent Aβ toxicity (Sanz-Blasco et al., 2008). Another study suggests that ibuprofen protects ischemia-induced neuronal injury via up-regulating interleukin-1 receptor antagonist expression (Park et al., 2005).

Pharmacological intervention reducing inflammation and/or secondary neurodegeneration in the thalamus leads to functional improvement (Freret et al., 2006; Gopez et al., 2005; Zhang et al., 2011). Given the strong inflammatory reaction in the thalamus following cerebral ischemia, we studied whether chronic ibuprofen treatment prevents the thalamic pathology in MCAO rats and whether this in turn is reflected in improved sensorimotor recovery.

* Corresponding author at: Institute of Clinical Medicine, Neurology, University of Eastern Finland, Yliopistonranta 1 C, 70210 Kuopio, Finland. Fax: +358 17 162048.
E-mail address: jukka.jolkkonen@uef.fi (J. Jolkkonen).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.04.019

2. Material and methods

2.1. Animals

Male Wistar rats (Laboratory Animal Centre, Kuopio, Finland) weighting 251–359 g were used in this study. The rats had free access to food (2016S, Teklad) and water throughout the experiment and were housed under 12:12 h light/dark conditions in a temperature-controlled environment (20 ± 1 °C). All animal procedures were approved by the Animal Ethics Committee (Hämeenlinna, Finland) and conducted in accordance with the guidelines set by the European Community Council Directives 86/609/EEC. All efforts were made to minimize the number of animals used and to ensure their welfare.

2.2. Middle cerebral artery occlusion

Focal cerebral ischemia was induced by the intraluminal filament technique (Longa et al., 1989). Anesthesia was induced in a chamber using 5% halothane in 30% O_2/70% N_2O. A surgical depth of anesthesia was maintained throughout the operation with 0.9–1.3% halothane delivered through a nose mask. Body temperature was monitored and maintained at 37 °C using a heating pad connected to a rectal probe (Harvard Homeothermic Blanket Control Unit). The right common carotid artery was exposed through a midline cervical incision under a surgical microscope and gently separated from the nerves. The external carotid artery was closed with a suture and cut with microscissors and electrocoagulated. A heparinized nylon filament (diameter 0.25 mm, rounded tip) was inserted into the stump of the external carotid artery. The filament was advanced 1.8–2.1 cm into the internal carotid artery until resistance was felt. The filament was held in that place by tightening the suture around the internal carotid artery and placing a microvascular clip around the artery. After 120 min of occlusion, the filament was removed and the external carotid artery was permanently closed by electrocoagulation. The sham-operated rats were treated in a similar manner, except the filament was not placed into the internal carotid artery. Buprenorfin (0.03 mg/kg, s.c.) was used to relieve postoperative pain. In addition, supplemental 0.9% NaCl (i.p.) and softened food pellets were given to prevent weight loss.

2.3. Study design and drug treatment

Two days after surgery, a modified version of the limb-placing test (Jolkkonen et al., 2000; Puurunen et al., 2001) was used to verify successful ischemia (see Fig. 1). Only those MCAO rats with total score less than 10 and thus severe corticostriatal damage (Jolkkonen et al., 2000; Puurunen et al., 2001) were included in the study. Based on the limb-placing scores the animals were assigned to behaviorally equivalent experimental groups: MCAO + ibuprofen (n = 8), MCAO + vehicle (n = 11), SHAM + vehicle (n = 5) and SHAM + ibuprofen (n = 4). Ibuprofen (Cayman Chemical Company, MI, USA) was dissolved in polyethylene glycol (Macrogol 400, Fargon) and administrated at a dose of 40 mg/kg (10 ml/kg, p.o.) once a day. The selected dose has been shown to attenuate inflammatory reaction following various brain insults without gastrointestinal effects (Lim et al., 2000, 2001; Richardson et al., 2002). Vehicle controls were administered at identical volume of Macrogol 400 (10 ml/kg). The drug treatment started on postoperative day 2 to avoid interference of the maturation of acute ischemic damage.

2.4. Behavior assessment

Behavior tests selected for the study are sensitive to detect treatment effects and not affected by repeated testing. In MCAO rats, a spontaneous recovery of function is typically seen in the limb-placing test whereas the sensorimotor impairment is more permanent when beam-walking and cylinder tests are applied. All behavioral tests and analyses were carried out in blind manner.

2.4.1. Limb-placing test

The modified version of the limb-placing test was used to assess forelimb and hindlimb responses to tactile and proprioceptive stimulation. The rats were habituated for handling and tested before operation and on postoperative days 2 to 5 and 7, 10, 14, 19, 21 and 28 (Fig. 1). The test consisted of seven limb-placing tasks which assess forelimb and hindlimb responses to tactile and proprioceptive stimulation. Tactile stimulation was elicited by contacting the limb being tested with a table surface and proprioceptive stimulation elicited by pulling down the limb being tested. The following scores were used: 2 points, normal response; 1 point, delayed and/or incomplete response; 0 points, no response. The maximum score was 14. Both sides of the body were tested.

2.4.2. Beam-walking test

Sensorimotor functions of hindlimbs were tested using a tapered/ledged beam (Zhao et al., 2005). The rats were pretrained for 3 days to traverse the beam before operation. The beam-walking apparatus consists of a tapered beam with underhanging ledges on each side to permit foot faults without falling. The end of the beam is connected to a black box (20.5 × 25 × 25 cm) with a platform at the starting point. A bright light is placed above the start point to motivate the rats to traverse the beam. Each rat's performance was videotaped and later analyzed by calculating the slip ratio for the impaired (contralateral to lesion) forelimb and hindlimb. The more slips indicates a greater degree of impairment. Steps onto the ledge were scored as a full slip and a half slip was given if the limb touched the side of the beam. The slip ratio was

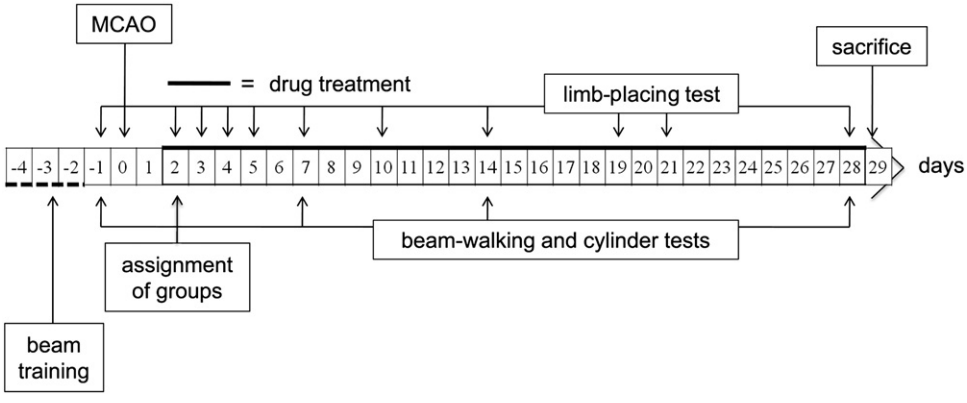

Fig. 1. Study design. Before operation, animals were trained to perform the tapered/ledged beam-walking test. Behavioral assessment was carried out at baseline and at selected time points during the follow-up. Vehicle or ibuprofen (40 mg/kg/day) treatment started on postoperative day 2. At the end of the follow-up, rats were sacrificed for histology.

calculated as: [(full slips + 1/2 × half slips)/total steps] ×100%. The mean of three trials was used for statistical analyses.

2.4.3. Cylinder test

The cylinder test was used to assess the imbalance between the impaired and non-impaired forelimb use (Karhunen et al., 2003). For the test, the rat was placed in a transparent cylinder (Ø 20 cm) and videotaped during the light part of the light/dark cycle. A mirror was placed at 45° angle beneath the cylinder so that behavior could be filmed from below the cylinder. Exploratory activity for 1 to 3 min was analyzed by using a recorder with slow motion capabilities. The number of contacts by both forelimbs and by either impaired or unimpaired forelimb was counted. Cylinder score for impaired forelimb was calculated as: [(contralateral contacts + 1/2 × bilateral contacts) /total contacts] ×100%.

2.5. Histology

Rats were perfused transcardially on postoperative day 29 with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4. The brains were removed from the skulls, postfixed, and cryoprotected. Frozen sections (40 μm) were cut with a sliding microtome and stored in a cryoprotectant tissue collection solution at −20 °C. Glial fibrillary acidic protein (GFAP) was stained as a marker for astrocytes (mouse anti-GFAP, Sigma, St. Louis, USA) and OX-42 as a marker of microglia (mouse anti-rat CD11b, Serotec, Oxford, UK). Thalamic accumulation of Aβ was examined using a rodent-specific antibody (rabbit anti-rodent Aβ3-16, #9151; Covance, USA). The sections for Aβ staining were pretreated for 30 min with hot (85 °C) citrate buffer. Then sections were transferred to a solution containing the primary antibody (GFAP at 1:1000, OX-42 at 1:4000 or Aβ at 1:5000) and Tris buffered saline with 0.5% Triton X-100 (TBS-T). After incubation in this solution overnight on a shaker table at room temperature (20 °C) in the dark, the sections were rinsed three times in TBS-T and transferred to a solution containing the secondary antibody (sheep anti-mouse Ig*biotin; Serotech or goat anti-mouse*-biotin; Sigma or goat anti-rabbit Ig*biotin, Chemicon). After 2 h, the sections were rinsed three times with TBS-T and transferred to a solution containing mouse ExtrAvidins (Sigma), and then incubated for approximately 3 min with diaminobenzidine (van Groen et al., 2005).

Calcium was stained with Alizarin red method (Mäkinen et al., 2008). Sections were mounted on gelatinized glass and immersed in 2% (w/v, distilled water, pH 4.1 to 4.3) Alizarin Red (Merck, Darmstadt, Germany) for 30 s followed by a rinse in distilled water. Sections were quickly dehydrated with acetone and xylene and mounted in Depex.

2.6. Quantification of inflammation markers and Aβ and calcium load in the thalamus after MCAO

An image analysis system (MCID) and a DAGE MTI CCD-72 series camera were used to quantify activated astrocyte (GFAP) and microglia (OX-42) and Aβ and calcium load in the thalamus. The digitized image was displayed on a video screen and converted to gray scale, after which the thalamus was manually outlined and optical densities above the threshold levels (background taken from contralateral thalamus) were recognized automatically by the image analysis system. The areal value was taken for statistical analysis.

2.7. Assessment of infarct volumes

Infarct volumes were measured using an image analysis system (MCID) from Nissl-stained sections collected at 0.4 mm intervals. The image of each section was taken as 1280 × 1024 matrix of calibrated pixel units. The digital image was displayed on a video screen and areas of surviving gray matter in the cortex and striatum were outlined with a mouse-controlled cursor separately for each hemisphere. The difference between the size of an outlined area in the contralateral hemisphere and the respective residual area in the ipsilateral hemisphere was taken as the infarcted area. The total infarct volume was calculated by multiplying the infarct area by the distance between the sections and summing together the volumes for each brain.

2.8. Statistics

SPSS software was used in statistical analysis. Differences in the infarct volumes, calcium staining and immunohistochemistry between vehicle controls and ibuprofen-treated MCAO rats were analyzed using one-way analysis (ANOVA, LSD). Differences in the limb-placing scores between experimental groups were analyzed by Mann–Whitney *U*-test. There was no significant difference between sham-operated rats treated with vehicle or ibuprofen thus these rats were pooled for statistical analysis. Body weight, beam-walking and cylinder data for the overall group effect were analyzed using analysis of variance for repeated measures (MANOVA). Comparisons between groups were then made using one-way ANOVA followed by a post hoc test (LSD).

3. Results

3.1. Mortality and adverse effects

From 40 operated rats, 11 died. Seven rats died within 48 h after operation, with the main reason being hemorrhage and severe edema. Three MCAO rats treated with ibuprofen had to be euthanized because of severe weight loss. One sham-operated animal died because of a *per os* administration related complication. One MCAO rat was excluded from the study because the animal did not show sufficient behavioral deficit in the limb-placing test (more than 10 points).

During the course of the study animals were daily observed and weight was measured (Fig. 2). MCAO rats lost weight (20%) during the first postoperative days. In addition, at later time points ibuprofen treatment seemed to decrease body weight both in sham-operated as well in MCAO rats (4–8%).

3.2. Infarct volumes and inflammatory response in the thalamus following MCAO

Infarct size in the cortex and striatum was measured from Nissl-stained sections in MCAO rats after the 29-day follow-up. There was no significant difference in infarct size between vehicle and

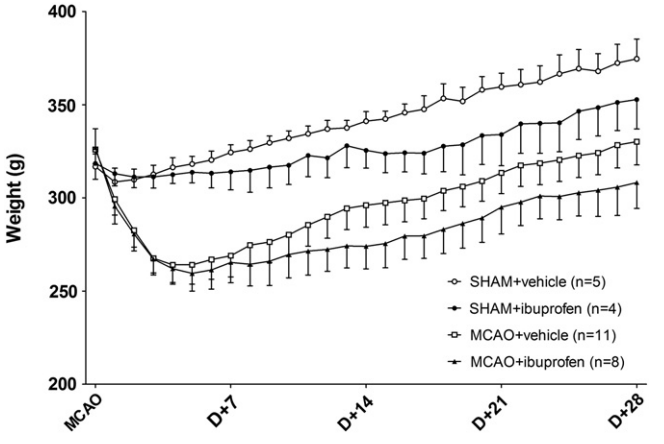

Fig. 2. Body weight following middle cerebral artery occlusion (MCAO) in rats. MCAO rats showed a severe weight loss ($p<0.001$, MANOVA). Ibuprofen treatment seemed to decrease body weight both in sham-operated and MCAO rats.

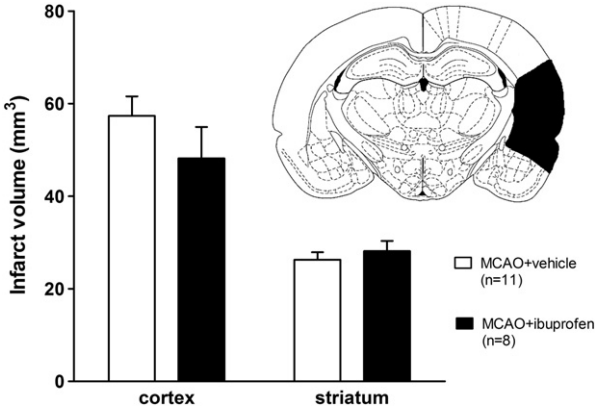

Fig. 3. Infarct volumes following middle cerebral artery occlusion (MCAO) in rats. Infarct volumes as assessed from Nissl-stained sections were not different between the treatment groups. A maximal infarct size at the coronal level taken for histology is illustrated (Paxinos and Watson, 1997).

ibuprofen-treated rats (Fig. 3A). Note that the thalamus was spared from acute ischemic damage because of its blood supply through the posterior cerebral artery.

Astrocyte and microglia activation in the thalamus was measured from GFAP and OX-42 stained sections, respectively. A robust increase in both GFAP (Fig. 4) and OX-42 (Fig. 5) staining was evident in the ipsilateral thalamus and areas adjacent to the infarct. Ibuprofen treatment (40 mg/kg/day) seemed to increase OX-42 staining ($p<0.05$) compared to vehicle treatment.

3.3. Aβ deposits in the thalamus following MCAO

Rodent specific Aβ antibody was used to reveal the amyloid deposits in the thalamus. Aβ staining was observed only in the ipsilateral ventromedial (VM), ventrolateral (VL), ventroposteriomedial (VPM), and ventroposteriolateral (VPL) nuclei in MCAO rats (Fig. 6). There was a statistically significant difference between the sham-operated and MCAO animals ($p<0.05$), but not between vehicle-treated and ibuprofen-treated MCAO animals.

3.4. Calcium accumulation in the thalamus following MCAO

Consistent with our previous studies (Hiltunen et al., 2009; Mäkinen et al., 2008), a significant increase of calcium staining was seen in the thalamus after MCAO (Fig. 7). There was only a statistically significant difference between sham-operated and MCAO animals ($p<0.05$).

3.5. Behavioral data following MCAO

3.5.1. Limb-placing test

The limb-placing test showed that MCAO rats were initially severely impaired followed by partial recovery (Fig. 8). A significant overall group effect was explained by the difference between sham-operated and MCAO rats throughout the follow-up ($p<0.01$). MCAO groups did not differ from each other.

3.5.2. Beam-walking test

Tapered/ledged beam-walking test was used to assess hindlimb function. A significant overall group effect was a consequence of MCAO rats making significantly more slips compared to sham-operated rats ($p<0.05$) (Fig. 9). There was no difference between vehicle-treated and ibuprofen-treated MCAO rats.

3.5.3. Cylinder test

Spontaneous forelimb use was assessed by the cylinder test at baseline, and 7, 14 and 28 days after MCAO. There was a significant overall group effect in spontaneous forelimb use (Fig. 10). This was due to a severe impairment of MCAO rats ($p<0.05$). Vehicle-treated and ibuprofen-treated MCAO rats did not differ from each other.

4. Discussion

Focal cerebral ischemia in the cortex evokes inflammatory responses in the perilesional areas. The thalamus is spared from acute ischemic damage because of blood supply through the posterior cerebral artery. However, because of its synaptic connections, delayed damage to various thalamic nuclei occurs. This secondary damage is associated with anterograde and retrograde degeneration and robust inflammatory response, which are followed by accumulation of Aβ, impaired calcium homeostasis and sensorimotor deficits. In the present study we investigated whether chronic treatment with non-steroidal anti-inflammatory drug, ibuprofen, prevents the thalamic pathology in MCAO rats and whether this is reflected in sensorimotor behavior.

4.1. Effects of ibuprofen on infarct volumes and inflammatory response in the thalamus following MCAO

Ibuprofen reduces infarct volumes in rats subjected to transient MCAO (Antezana et al., 2003; Cole et al., 1993). In the present study, the drug treatment was started on postoperative day 2 to avoid interference of the maturation of acute ischemic damage and to target the delayed secondary pathology. Thus, the infarct volumes in the

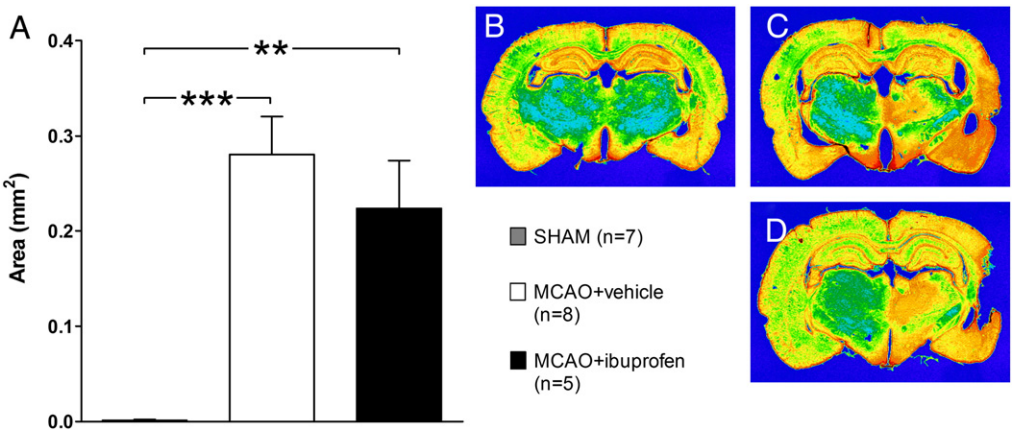

Fig. 4. GFAP staining as a marker of glial activation following middle cerebral artery occlusion (MCAO) in rats. A) The area of staining in the thalamus was measured by image analysis (mean ± SEM; **$p<0.01$; ***$p<0.001$). A robust GFAP staining in the ipsilateral thalamus and cortex adjacent to the infarct was observed in MCAO rats. Representative pseudocolor images from B) a sham-operated rat, C) an MCAO rat treated with vehicle and D) an MCAO rat treated with ibuprofen.

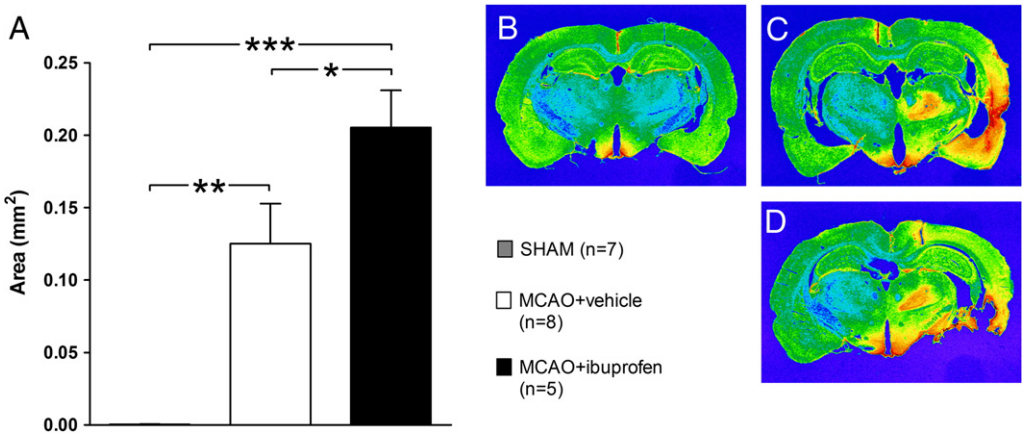

Fig. 5. OX-42 staining as a marker of microglia activation following middle cerebral artery occlusion (MCAO) in rats. A) The area of staining in the thalamus was measured by image analysis (mean ± SEM; *p<0.05; **p<0.01; ***p<0.001). B) Representative pseudocolor images from a sham-operated rat, C) an MCAO rat treated with vehicle and D) an MCAO rat treated with ibuprofen.

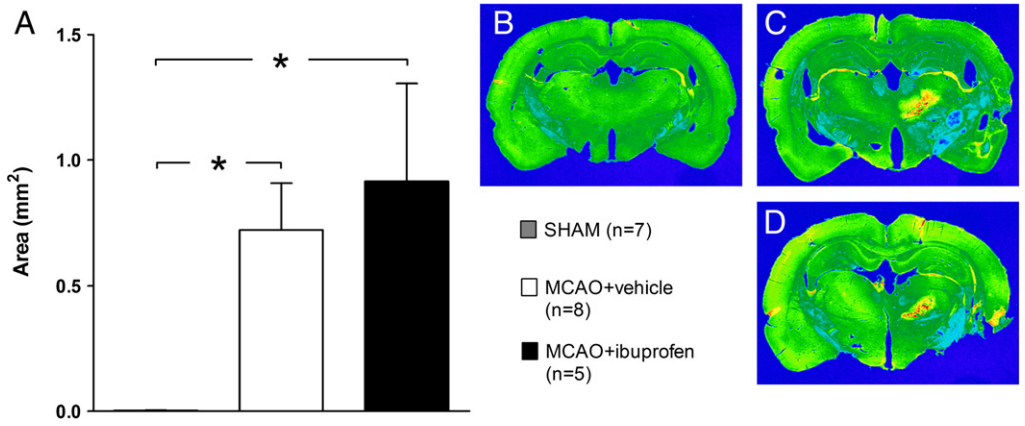

Fig. 6. Aβ accumulation in the thalamus after middle cerebral artery occlusion (MCAO) in rats. A) The area of staining in the thalamus was measured by image analysis (mean ± SEM). Statistical analysis showed a significant increase of Aβ immunoreactivity in the ipsilateral thalamus 29 days after MCAO in both vehicle-treated and ibuprofen-treated MCAO rats compared to sham-operated rats (*p<0.05). Representative pseudocolor images are from B) a sham-operated rat, C) an MCAO rat treated with vehicle and D) an MCAO rat treated with ibuprofen.

cortex and striatum were not different between MCAO rats treated with vehicle or ibuprofen.

The inflammatory response in the thalamus is delayed compared to that in perilesional areas in rats subjected to MCAO (van Groen et al., 2005). Early signs of astroglia and microglia activation are seen 3 days after MCAO (Loos et al., 2003; Rupalla et al., 1998; Sorensen et al., 1996). The degeneration of corticothalamic axon terminals is thought to trigger an inflammatory response (Acarin et al., 1999). The number of activated

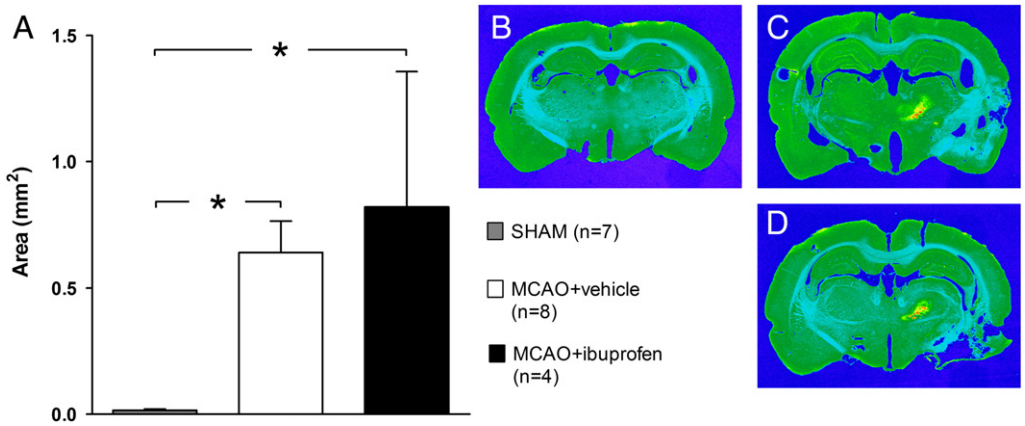

Fig. 7. Calcium accumulation in the thalamus after middle cerebral artery occlusion (MCAO) in rats. A) The area of staining in the thalamus was measured by image analysis (mean ± SEM). Alizarin red staining 29 days after MCAO showed a significant increase in calcium accumulation in the ipsilateral thalamus in both vehicle-treated and ibuprofen-treated MCAO rats compared to sham-operated rats (*p<0.05). Representative pseudocolor images from B) a sham-operated rat, C) an MCAO rat treated with vehicle and D) an MCAO rat treated with ibuprofen.

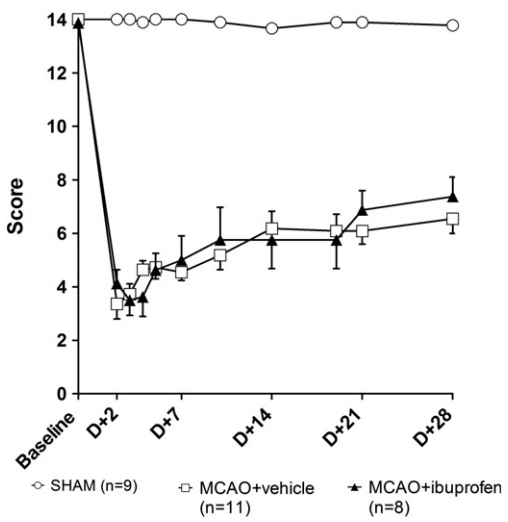

Fig. 8. The limb-placing data following middle cerebral artery occlusion (MCAO) in rats showed initial severe impairment that followed by a partial recovery. There was no statistical difference between the MCAO groups. Maximum score was 14. The values are mean ± SEM.

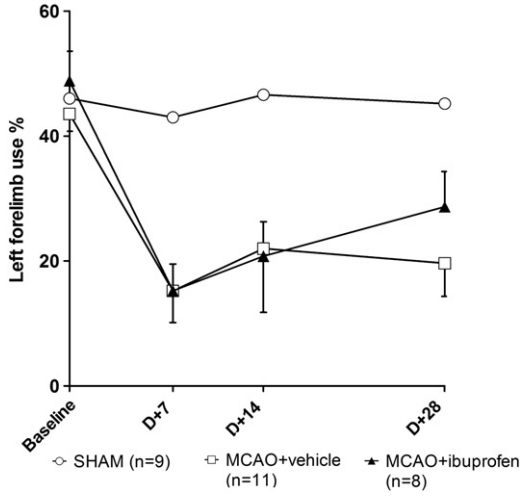

Fig. 10. The cylinder data following middle cerebral artery occlusion (MCAO) in rats showed initial severe impairment that was followed by a partial recovery of function. There were no statistical differences between the MCAO groups. The values are mean ± SEM.

microglial cell peaks between 15 and 30 days after MCAO (Rupalla et al., 1998) while the number of glial cells shows a continuous increase up to 3 months after MCAO (Watanabe et al., 1998). Here we showed that both GFAP and OX-42 staining were increased in the ipsilateral thalamus 29 days following MCAO.

Ibuprofen treatment has been shown to decrease the number of astroglial and microglial cells after various brain insults (Browne et al., 2006; Richardson et al., 2002, 2005). Thus, we expected to see a similar attenuation of inflammatory response in MCAO rats. The extent or location of inflammatory response in the ipsilateral thalamus was, however, not affected by ibuprofen. It may be that the degree of inflammation produced by MCAO in the thalamus was too pronounced to respond to ibuprofen treatment with the dose applied. Another technical explanation is that the massive inflammatory response may mask minor changes due to saturation of immunoreactivity measured by image analysis.

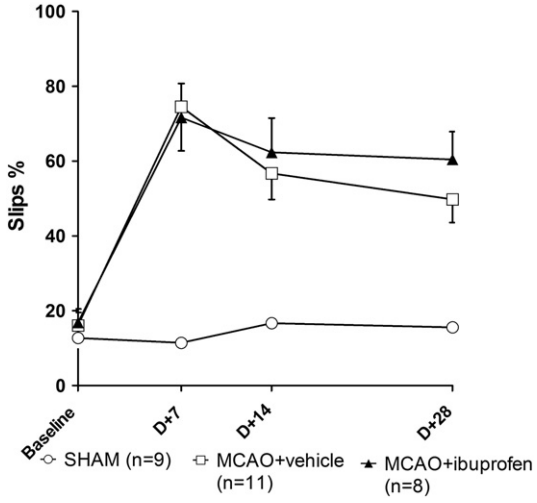

Fig. 9. The beam-walking data following middle cerebral artery occlusion (MCAO) in rats showed initial severe behavioral impairment that was followed by partial recovery. There was no statistical difference between the MCAO groups. The values are mean ± SEM.

4.2. Effects of ibuprofen on Aβ/calcium pathology in the thalamus following MCAO

Previous studies have shown that ibuprofen reduces the formation of Aβ plaques in the transgenic AD mice and improves functional outcome in association with a reduction of inflammatory mediators (Choi et al., 2010; Lim et al., 2000, 2001; Yan et al., 2003). In addition, NSAIDs including ibuprofen lowers Aβ42 *in vitro* independently from cyclooxygenase (COX) activity possibly by altering γ-secretase activity (Weggen et al., 2001). Here we could not demonstrate a significant effect of ibuprofen on Aβ pathology in the thalamus as assessed by immunohistochemistry. One possibility is that, although the thalamic Aβ pathology following MCAO seems to be intriguingly similar to that in AD, the underlying primary mechanisms are different. Particularly axonal damage following MCAO that is associated with inflammatory reaction is not seen in AD.

We also measured whether ibuprofen had effect on calcium accumulation that has overlapping distribution with Aβ in the thalamus (Mäkinen et al., 2008). It has been suggested that excessive released Aβ incorporates into neuronal membranes, forming calcium-permeable channels (Bhatia et al., 2000; Kawahara and Kuroda, 2000) that leads to the massive entry of Ca^{2+} into cells. Mitochondrial Ca^{2+} overload is suggested to underlie Aβ neurotoxicity in cell cultures and NSAIDs, including ibuprofen prevent this (Sanz-Blasco et al., 2008). Our data showed, however, that calcium accumulation is not affected by ibuprofen. It may be that calcium accumulation in the thalamus following MCAO leads to secondary degeneration through other mechanisms such as inappropriate activation of proteases rather than mitochondrial dysfunction.

4.3. Effects of ibuprofen on sensorimotor behavior following MCAO

Consistent with the histological data, we showed that ibuprofen did not improve performance in limb-placing, beam-walking or cylinder tests after MCAO. Previous evidence support that the pathology in the thalamus has detrimental functional consequences. Thalamic atrophy in MCAO rats correlates with the late sensory deficit shown through the adhesive-removal test and tests measuring skilled forelimb function (Freret et al., 2006). Sensorimotor outcome is also more impaired in hAPP transgenic rats compared to wild type littermates following cerebral ischemia and this occurs in parallel with excessive Aβ load in the thalamus (Clarke et al., 2007). In addition, pharmacological studies (Freret et al., 2006; Gopez et al., 2005; Heneka et al., 2005; Kumon et al., 1996; Yamada et al., 1991;

Zhang et al., 2011) show that delayed administration of various drugs prevents ischemia-induced pathology in the thalamus, which in turn is reflected in improved sensorimotor or cognitive functions.

5. Conclusion

Degeneration of corticothalamic and thalamocortical connections seems to trigger complex secondary pathology in the thalamus following MCAO. The present data with ibuprofen do not support the idea that inflammation or COX independent mechanisms contribute to the $A\beta/Ca^{2+}$ aggregation in the thalamus or associated sensorimotor impairment following cerebral ischemia.

Acknowledgements

We thank Nanna Huuskonen and Laura Tolppanen for their expert technical help. The study was supported by the Päivikki and Sakari Sohlberg Foundation and the Health Research Council of the Academy of Finland.

References

Acarin L, Gonzalez B, Castro AJ, Castellano B. Primary cortical glial reaction versus secondary thalamic glial response in the excitotoxically injured young brain: microglial/macrophage response and major histocompatibility complex class I and II expression. Neuroscience 1999;89:549–65.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.

Antezana DF, Clatterbuck RE, Alkayed NJ, Murphy SJ, Anderson LG, Frazier J, et al. High-dose ibuprofen for reduction of striatal infarcts during middle cerebral artery occlusion in rats. J Neurosurg 2003;98:860–6.

Bhatia R, Lin H, Lal R. Fresh and globular amyloid beta protein (1–42) induces rapid cellular degeneration: evidence for AbetaP channel-mediated cellular toxicity. FASEB J 2000;14:1233–43.

Block F, Dihne M, Loos M. Inflammation in areas of remote changes following focal brain lesion. Prog Neurobiol 2005;75:342–65.

Browne KD, Iwata A, Putt ME, Smith DH. Chronic ibuprofen administration worsens cognitive outcome following traumatic brain injury in rats. Exp Neurol 2006;201:301–7.

Choi JK, Jenkins BG, Carreras I, Kaymakcalan S, Cormier K, Kowall NW, et al. Anti-inflammatory treatment in AD mice protects against neuronal pathology. Exp Neurol 2010;223:377–84.

Clarke J, Thornell A, Corbett D, Soininen H, Hiltunen M, Jolkkonen J. Overexpression of APP provides neuroprotection in the absence of functional benefit following middle cerebral artery occlusion in rats. Eur J Neurosci 2007;26:1845–52.

Cole D, Patel P, Reynolds L, Drummond J, Marcantonio S. Temporary focal cerebral ischemia in spontaneously hypertensive rats: the effect of ibuprofen on infarct volume. J Pharmacol Exp Ther 1993;266:1713–7.

Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, et al. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. J Clin Invest 2003;112:440–9.

Freret T, Chazalviel L, Roussel S, Bernaudin M, Schumann-Bard P, Boulouard M. Long-term functional outcome following transient middle cerebral artery occlusion in the rat: correlation between brain damage and behavioral impairment. Behav Neurosci 2006;120:1285–98.

Gopez JJ, Yue H, Vasudevan R, Malik AS, Fogelsanger LN, Lewis S, et al. Cyclooxygenase-2-specific inhibitor improves functional outcomes, provides neuroprotection, and reduces inflammation in a rat model of traumatic brain injury. Neurosurgery 2005;56:590–604.

Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, Kuiperi C, et al. Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. Brain 2005;128:1442–53.

Hiltunen M, Mäkinen P, Peräniemi S, Sivenius J, van Groen T, Soininen H, et al. Focal cerebral ischemia in rats alters APP processing and expression of Abeta peptide degrading enzymes in the thalamus. Neurobiol Dis 2009;35:103–13.

Hurley SD, Olschowka JA, O'Banion MK. Cyclooxygenase inhibition as a strategy to ameliorate brain injury. J Neurotrauma 2002;19:1-15.

Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 1989;24:173–82.

Iwata Y, Nicole O, Zurakowski D, Okamura T, Jonas RA. Ibuprofen for neuroprotection after cerebral ischemia. J Thorac Cardiovasc Surg 2010;139:489–93.

Jolkkonen J, Puurunen K, Rantakömi S, Härkonen A, Haapalinna A, Sivenius J. Behavioral effects of the alpha(2)-adrenoceptor antagonist, atipamezole, after focal cerebral ischemia in rats. Eur J Pharmacol 2000;400:211–9.

Karhunen H, Virtanen T, Schallert T, Sivenius J, Jolkkonen J. Forelimb use after focal cerebral ischemia in rats treated with an alpha(2)-adrenoceptor antagonist. Pharmacol Biochem Behav 2003;74:663–9.

Kawahara M, Kuroda Y. Molecular mechanism of neurodegeneration induced by Alzheimer's beta-amyloid protein: channel formation and disruption of calcium homeostasis. Brain Res Bull 2000;53:389–97.

Kumon Y, Sakaki S, Watanabe H, Nakano K, Ohta S, Matsuda S, et al. Ciliary neurotrophic factor attenuates spatial cognition impairment, cortical infarction and thalamic degeneration in spontaneously hypertensive rats with focal cerebral ischemia. Neurosci Lett 1996;206:141–4.

Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 2000;20:5709–14.

Lim GP, Yang F, Chu T, Gahtan E, Ubeda O, Beech W, et al. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. Neurobiol Aging 2001;22:983–91.

Lleo A, Berezovska O, Herl L, Raju S, Deng A, Bacskai BJ, et al. Nonsteroidal anti-inflammatory drugs lower Abeta42 and change presenilin 1 conformation. Nat Med 2004;10:1065–6.

Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989;20:84–91.

Loos M, Dihne M, Block F. Tumor necrosis factor-alpha expression in areas of remote degeneration following middle cerebral artery occlusion of the rat. Neuroscience 2003;122:373–80.

Mäkinen S, van Groen T, Clarke J, Thornell A, Corbett D, Hiltunen M, et al. Coaccumulation of calcium and beta-amyloid in the thalamus after transient middle cerebral artery occlusion in rats. J Cereb Blood Flow Metab 2008;28:263–8.

Park E, Cho B, Volpe B, Cruz M, Joh T, Cho S. Ibuprofen protects ischemia-induced neuronal injury via up-regulating interleukin-1 receptor antagonist expression. Neuroscience 2005;132:625–31.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 3 rd ed. USA: Academic Press Inc.; 1997.

Puurunen K, Jolkkonen J, Sirviö J, Haapalinna A, Sivenius J. An alpha(2)-adrenergic antagonist, atipamezole, facilitates behavioral recovery after focal cerebral ischemia in rats. Neuropharmacology 2001;40:597–606.

Richardson RL, Kim EM, Shephard RA, Gardiner T, Cleary J, O'Hare E. Behavioural and histopathological analyses of ibuprofen treatment on the effect of aggregated Abeta (1–42) injections in the rat. Brain Res 2002;954:1-10.

Richardson RL, Kim EM, Gardiner T, O'Hare E. Chronic intracerebroventricular infusion of lipopolysaccharide: effects of ibuprofen treatment and behavioural and histopathological correlates. Behav Pharmacol 2005;16:531–41.

Rupalla K, Allegrini PR, Sauer D, Wiessner C. Time course of microglia activation and apoptosis in various brain regions after permanent focal cerebral ischemia in mice. Acta Neuropathol 1998;96:172–8.

Sanz-Blasco S, Valero RA, Rodriguez-Crespo I, Villalobos C, Nunez L. Mitochondrial Ca2+ overload underlies Abeta oligomers neurotoxicity providing an unexpected mechanism of neuroprotection by NSAIDs. PLoS One 2008;3:e2718.

Sorensen JC, Dalmau I, Zimmer J, Finsen B. Microglial reactions to retrograde degeneration of tracer-identified thalamic neurons after frontal sensorimotor cortex lesions in adult rats. Exp Brain Res 1996;112:203–12.

Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997;48:626–32.

van Groen T, Puurunen K, Mäki HM, Sivenius J, Jolkkonen J. Transformation of diffuse beta-amyloid precursor protein and beta-amyloid deposits to plaques in the thalamus after transient occlusion of the middle cerebral artery in rats. Stroke 2005;36:1551–6.

Watanabe H, Kumon Y, Ohta S, Sakaki S, Matsuda S, Sakanaka M. Changes in protein synthesis and calcium homeostasis in the thalamus of spontaneously hypertensive rats with focal cerebral ischemia. J Cereb Blood Flow Metab 1998;18:686–96.

Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature 2001;414:212–6.

Willuweit A, Velden J, Godemann R, Manook A, Jetzek F, Tintrup H, et al. Early-onset and robust amyloid pathology in a new homozygous mouse model of Alzheimer's disease. PLoS One 2009;4:e7931.

Yamada K, Kinoshita A, Kohmura E, Sakaguchi T, Taguchi J, Kataoka K, et al. Basic fibroblast growth factor prevents thalamic degeneration after cortical infarction. J Cereb Blood Flow Metab 1991;11:472–8.

Yan Q, Zhang J, Liu H, Babu-Khan S, Vassar R, Biere AL, et al. Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. J Neurosci 2003;23:7504–9.

Zhang Y, Xing S, Zhang J, Li J, Li C, Pei Z, et al. Reduction of beta-amyloid deposits by gamma-secretase inhibitor is associated with the attenuation of secondary damage in the ipsilateral thalamus and sensory functional improvement after focal cortical infarction in hypertensive rats. J Cereb Blood Flow Metab 2011;31:572–9.

Zhao CS, Puurunen K, Schallert T, Sivenius J, Jolkkonen J. Effect of cholinergic medication, before and after focal photothrombotic ischemic cortical injury, on histological and functional outcome in aged and young adult rats. Behav Brain Res 2005;156:85–94.