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Research Paper

Montelukast, a cysteinyl leukotriene receptor-1 antagonist, attenuates chronic brain injury after focal cerebral ischaemia in mice and rats

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Abstract

Objectives Previously we demonstrated the neuroprotective effect of montelukast, a cysteinyl leukotriene receptor-1 ($CysLT_1$) antagonist, on acute brain injury after focal cerebral ischaemia in mice. In this study, we have determined its effect on chronic brain injury after focal cerebral ischaemia in mice and rats.

Methods After transient focal cerebral ischaemia was induced by middle cerebral artery occlusion, montelukast was intraperitoneally injected in mice or orally administered to rats for five days. Behavioural dysfunction, brain infarct volume, brain atrophy and neuron loss were determined to evaluate brain lesions.

Key findings Montelukast (0.1 mg/kg) attenuated behavioural dysfunction, brain infarct volume, brain atrophy and neuron loss in mice, which was similar to pranlukast, another $CysLT_1$ receptor antagonist. Oral montelukast (0.5 mg/kg) was effective in rats and was more effective than edaravone, a free radical scavenger.

Conclusion Montelukast protected mice and rats against chronic brain injury after focal cerebral ischaemia, supporting the therapeutic potential of $CysLT_1$ receptor antagonists.

Keywords antagonist; cysteinyl leukotriene receptor 1; focal cerebral ischaemia; montelukast; neuroprotection

Introduction

Cysteinyl leukotrienes (CysLTs), the 5-lipoxygenase metabolites of arachidonic acid, are potent inflammatory mediators.[1] It is well known that CysLTs play a regulatory role in inflammatory diseases in the periphery, such as bronchial asthma and allergic rhinitis.[2,3] Recently, it has been reported that CysLTs and their receptors ($CysLT_1$ and $CysLT_2$ receptors) are involved in post-ischaemic inflammation in brain injury after ischaemic stroke.[4–8] We have reported that both production of CysLTs and $CysLT_1$ receptor expression increased in the ischaemic brain after focal cerebral ischaemia in animal models, and $CysLT_1$ receptor antagonists exerted protective effects on ischaemic injury.[4,6–11]

At present, $CysLT_1$ receptor antagonists are clinically available for the treatment of allergic and inflammatory diseases.[1–3,12,13] We have demonstrated that the selective $CysLT_1$ receptor antagonist pranlukast (ONO-1078) inhibited acute, subacute and chronic ischaemic injury in the brains of mice and rats after focal cerebral ischaemia.[4,9,10,14] Post-ischaemic treatment with pranlukast (0.1 mg/kg) for five consecutive days had a long-term protective effect after focal cerebral ischaemia in mice; it did not reduce mortality but significantly improved neurological deficits and promoted sensorimotor recovery during 70 days.[9] However, whether other $CysLT_1$ receptor antagonists have the same protective effect on chronic injury after cerebral ischaemia needs clarification.

Montelukast, 1-((1(*R*)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)-3-[(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thiol]-methyl)cyclopropane acetate, is another selective $CysLT_1$ receptor antagonist that is structurally different from pranlukast.[5,15] Montelukast is the most prescribed $CysLT_1$ receptor antagonist in Europe and the USA, whereas pranlukast is only marketed in Japan and other Asian countries.[1] We have reported that montelukast is as effective as pranlukast on acute brain injury in mice with focal

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cerebral ischaemia, but its long-term effect on chronic injury is unknown.[11] Determination of drug effects on chronic brain injury after cerebral ischaemia is important for evaluating the therapeutic potential of neuroprotective agents, so it is necessary to investigate the long-term effect of montelukast on chronic ischaemic injury.[16]

On the other hand, both montelukast and pranlukast were intraperitoneally injected in our previous studies, but they are orally active agents.[4,9–11,14] Thus, it was necessary to assess the effect of montelukast after oral administration. Therefore, in this study we have determined the effect of montelukast on chronic brain injury after focal cerebral ischaemia to further reveal its therapeutic potential. We performed two series of experiments, one to compare its effect with pranlukast after intraperitoneal administration in mice and the other to confirm its effect after oral administration in rats.

Materials and Methods

Measurements of physiological variables

Male ICR mice (25–30 g) and male Sprague–Dawley rats (250–300 g) were purchased from the Experimental Animal Center, Zhejiang Academy of Medical Sciences (specific pathogen free, certificate no. 2008–0033). Mice and rats were housed under a controlled temperature (22 ± 2°C), 12 h light/12 h dark cycle and allowed free access to food and water. All experimental protocols were approved by the Animal Care Committee of Zhejiang University School of Medicine and all experiments conformed to the Guiding Principles for Research Involving Animals of Zhejiang University School of Medicine.

Animals were anaesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg). A polyethylene tube was inserted into the right femoral artery for continuous monitoring of blood pressure using a computer-assisted system (PC-Lab, Kelong Inc., Nanjing, China), as well as for measuring the partial pressure of oxygen and carbon dioxide in arterial blood ($Pa O_2$ and $Pa CO_2$, respectively), and arterial pH (Blood Gas Analyzer ABL 330, Leidu Inc., Copenhagen, Denmark). Blood glucose was monitored with a one touch basic blood glucose monitoring system (Lifescan Inc., Milpitas, USA). Rectal (core) temperature was monitored and maintained at 37.0 ± 0.5°C with a heating pad and a heating lamp during surgery.

Induction of focal cerebral ischaemia

After anaesthesia with chloral hydrate (400 mg/kg, i.p.), transient focal cerebral ischaemia was induced by middle cerebral artery occlusion (MCAO) as described previously.[9,14,17,18] Briefly, after anaesthesia, a midline incision was made in the neck. The right external carotid artery (ECA) and the right internal carotid artery (ICA) were carefully exposed and dissected, and a 6–0 (mice) or 3–0 (rats) monofilament nylon suture was inserted from the ECA into the ICA to occlude the origin of the right MCA. After 30-min occlusion, the suture was withdrawn to allow reperfusion, the ECA was ligated and the incision was closed. Sham-operated mice and rats underwent identical surgery, except that the intraluminal filament was not inserted. After surgery, mice and rats were kept in a warm box heated by lamps for approximately 2 h to maintain body temperature.

Drug administration

In mouse experiments, the effects of the $CysLT_1$ receptor antagonists, montelukast and pranlukast were compared, using the same dosage regimen as reported previously.[9] Montelukast (0.01 or 0.1 mg/kg), pranlukast (0.1 mg/kg) or saline (5 ml/kg) was intraperitoneally injected 30 min, 4 h, and 12 h after MCAO on the operation day, and then once every 12 h for four consecutive days from the next day (five days in total). In rat experiments, the effect of oral montelukast was compared with edaravone as control. Montelukast (0.1 or 0.5 mg/kg) or saline (5 ml/kg) was intragastrically administered 30 min and 4 h after MCAO, and then twice daily for four days (five days in total). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a free radical scavenger with neuroprotective effects on cerebral ischaemia; it was used as a control for oral montelukast in rats using a reported dosage regimen, namely 10 mg/kg (i.p.), immediately and 4 h after ischaemia then once daily for four days (five days in total).[19,20]

Montelukast was a kind gift from Merck Pharmaceutical Co, Wilmington, USA. Pranlukast (ONO-1078) was from Dr Masami Tsuboshima (Ono Pharmaceutical Co. Ltd, Osaka, Japan). Edaravone was provided by Conba Pharmaceutical Co., Hangzhou, China.

Neurological deficit scoring

Neurological deficit scores were evaluated in mice and rats 1, 3, 7, 14, 21 and 28 days after MCAO: 0, no deficit; 1, flexion of contralateral forelimb upon lifting the whole animal by the tail; 2, circling to the contralateral side; 3, falling to the contralateral side; 4, no spontaneous motor activity.[9,14]

Corner test

The corner test was carried out in mice according to the reported method.[21] The mouse was placed between two cardboard pieces (30 × 20 × 1 cm) and encouraged to enter into a corner of 30° with a small opening along the joint between the two boards. When the mouse entered into the deep part of the corner, both sides of the vibrissae were stimulated together by the two boards. The mouse would rear forward and upward, and turn back to face the open end. Ten trials were performed for each mouse and the number of right turns was recorded. Only turns involving full rearing along either board were recorded. This task detected integrated sensorimotor function as it involved both stimulation of the vibrissae (sensory/neglect) and rearing (motor response), and was performed 1, 3, 7, 14, 21 and 28 days after MCAO.

Skilled reaching test

This test examined both gross ability of the forelimb to retrieve pellets and reaching accuracy, because forelimb reaching requires fine motor skill of both proximal and distal musculature as well as sensation to successfully retrieve food pellets. We carried out this test in rats according to the reported method with modifications.[22,23] Briefly, rats were gradually food deprived to 85% of their original body weight. They were trained for two weeks to reach through a 1.1-cm-wide vertical slot to obtain a food pellet (45 mg)

situated in a well (on a 2-cm-high shelf) 2 cm from the front of a Plexiglas box. For each test session, rats reached for 20 pellets (presented one at a time). Reaches were only considered successful if the pellet was eaten after one reach, whereas failed attempts included all limb advances resulting in a missed, displaced, or dropped pellet. Rats that did not learn the reaching test were excluded from statistical analyses. In the task, rats were successively presented with 20 food pellets, and success rate was calculated using the formula: success rate = number of pellets retrieved/20 × 100%. Rats were trained every day to reach with their left forelimb for two weeks before MCAO, and tested 1, 3, 7, 14, 21 and 28 days after MCAO.

All the behavioural examinations described above were performed by researchers who were blind to the treatments.

Histological examination

Four weeks after MCAO, mice and rats were anaesthetized and perfused transcardially with 4% paraformaldehyde after pre-flushing with saline. Brains were removed, quickly photographed with a digital camera (FinePix S602 Zoom, Fuji, Japan), fixed in 4% paraformaldehyde overnight, and then transferred to 30% sucrose for 1–3 days. Six serial coronal slices were prepared at 1-mm (mice) or 2-mm (rats) intervals from the frontal pole. Two sets of sections (8- and 20-μm thick) were then cut by cryomicrotomy (CM1900, Leica, Germany) from the slices. The 8-μm sections were a microphotographic estimation of neuron density, and the 20-μm sections for gross photographic examination after being stained with 1% toluidine blue. The lesion area of brain tissue was defined as an area with reduced Nissl staining and was confirmed by light microscopy to have dark pyknotic-necrotic cell bodies. The lesion areas were determined as the difference of apparently normal tissue between contralateral and ipsilateral sides of the sections using the ImageTool 2.0 analysis program (University of Texas Health Science Center, Texas, USA). The lesion volume of each section was calculated as: lesion area × slice thickness (1 or 2 mm). Total lesion volume was the summation of the lesion volumes of all sections.

In the 8-μm sections, neurons in temporoparietal cortical layers III and IV adjacent to the ischaemic core (0.2–0.4 mm (mice) or 1.8–2 mm (rats) caudal to bregma) were immunostained with a monoclonal antibody against neuronal nuclei (NeuN, 1:200, Chemicon, USA), a specific marker of neurons. Neurons were randomly counted in three 200 μm^2 squares at upper, middle and lower sites of the boundary zone adjacent to the ischaemic core by a researcher who was blind to the treatments.

Statistical analysis

Data are reported as the mean ± SEM. The differences between groups were analysed by one-way analysis of variance followed by Student-Newman-Keuls *t*-test, except that the neurological deficit score was analysed by non-parametric Mann-Whitney *U*-test (SPSS 13.0 for Windows 2004, SPSS Inc., Chicago, Illinois, USA). $P < 0.05$ was considered statistically significant.

Results

Physiological variables

Mean blood pressure, arterial blood pH, PaO_2, $PaCO_2$ and glucose did not change 30 min before or after surgery. There were no differences between sham operation, ischaemia and drug treatment groups (Table 1).

Effects of montelukast and pranlukast in ischaemic mice

In mice, the death rate (43–57%, all deaths occurred within seven days after MCAO) was not significantly different between the four ischaemic groups with or without drug treatment; no deaths occurred in the sham-operated group. Neurological deficit scores were recorded during the 28 days of observation after MCAO. Montelukast 0.1 mg/kg reduced the scores from 3 to 28 days after MCAO, and a statistically significant reduction was found at 3, 21 and 28 days; while pranlukast (0.1 mg/kg), another $CysLT_1$ receptor antagonist, also significantly reduced the scores from 3 to 28 days (Table 2). In the corner test, the saline-treated ischaemic mice showed a significant increase in the right turns throughout the 28 days after MCAO, indicating asymmetry (Table 2). Montelukast (0.1 mg/kg) significantly attenuated the asymmetry in the corner test at 7–28 days as the right turns were reduced; while pranlukast was effective at 1–28 days (Table 2). However, montelukast 0.01 mg/kg was ineffective on both neurological deficits and asymmetry.

At the end of 28 days observation, the gross photographs of whole brains and coronal sections showed moderate brain atrophy in the ischaemic mice. Montelukast (0.1 mg/kg) and pranlukast reduced both lesion volume and brain atrophy (reduced ipsilateral/contralateral hemisphere ratio), but montelukast at 0.01 mg/kg was ineffective (Figure 1). At 28 days after ischaemia, neurons were largely lost in the temporoparietal cortcial layers III and IV adjacent to the ischaemic core. Montelukast 0.1 mg/kg, but not 0.01 mg/kg, and pranlukast (0.1 mg/kg) significantly attenuated the loss of neurons (Figure 2).

Effects of oral montelukast and edaravone in ischaemic rats

In rats, the death rate (38–50%, the deaths mainly occurred within seven days after MCAO) was not significantly different between the four ischaemic groups with or without drug treatment; no deaths occurred in the sham-operated group. The maximal neurological deficit scores were recorded at 1 day after MCAO and then gradually recovered. Oral administration of 0.5 mg/kg montelukast significantly reduced the scores from 3 to 21 days after MCAO, but did not at 28 days, which might have been due to the apparent functional recovery at this time point. Montelukast at 0.1 mg/kg was substantially ineffective, although a significant difference was found at 14 days. The control drug edaravone (10 mg/kg) did not reduce neurological deficit scores significantly (Table 3). In the single-pellet reaching test, the success rate was 60–70% in sham-operated rats, but no successful reaching was found in ischaemic control rats. Montelukast 0.5 mg/kg increased the success rate from 1 to 28 days, while 0.1 mg/kg montelukast

Table 1 Summary of selected physiological parameters 30 min before and after surgery

Variable		Sham operation	Ischaemia	Montelukast (0.1 mg/kg)	Pranlukast (0.1 mg/kg)
Mice		$n = 7$	$n = 7$	$n = 7$	$n = 6$
MABP (mmHg)	Before	88.7 ± 5.43	85.9 ± 3.90	85.8 ± 4.21	89.0 ± 3.83
	After	84.0 ± 5.67	90.3 ± 4.78	84.0 ± 4.95	84.8 ± 3.42
PaO_2 (mmHg)	Before	120 ± 4.56	125 ± 2.74	123 ± 3.69	119 ± 6.90
	After	125 ± 2.42	121 ± 4.65	119 ± 5.89	122 ± 6.03
$PaCO_2$ (mmHg)	Before	37.2 ± 0.97	36.4 ± 1.12	35.8 ± 1.73	38.0 ± 2.02
	After	39.0 ± 1.86	38.1 ± 1.65	37.3 ± 2.00	36.7 ± 1.78
Glucose (mmol/l)	Before	6.43 ± 0.90	5.97 ± 0.69	6.26 ± 0.67	6.14 ± 0.84
	After	5.89 ± 0.93	5.67 ± 0.80	5.94 ± 0.56	5.97 ± 0.90
pH	Before	7.36 ± 0.02	7.35 ± 0.04	7.39 ± 0.02	7.38 ± 0.03
	After	7.35 ± 0.03	7.34 ± 0.05	7.36 ± 0.03	7.35 ± 0.02
Rats		**Sham operation $n = 6$**	**Ischaemia $n = 6$**	**Montelukast (0.5 mg/kg) $n = 6$**	**Edaravone (10 mg/kg) $n = 6$**
MABP (mmHg)	Before	118 ± 21	121 ± 19	125 ± 20	115 ± 25
	After	121 ± 19	115 ± 23	120 ± 27	119 ± 21
PaO_2 (mmHg)	Before	92 ± 12	95 ± 15	93 ± 13	96 ± 18
	After	89 ± 16	92 ± 21	88 ± 19	90 ± 22
$PaCO_2$ (mmHg)	Before	42.3 ± 2.9	41.1 ± 3.8	43.8 ± 3.9	42.7 ± 4.2
	After	42.1 ± 3.3	40.2 ± 4.5	46.3 ± 5.2	40.9 ± 4.8
Glucose (mmol/l)	Before	6.33 ± 2.02	6.14 ± 1.92	6.40 ± 2.47	6.01 ± 2.48
	After	6.11 ± 2.28	6.22 ± 1.59	6.29 ± 1.97	6.25 ± 2.54
pH	Before	7.38 ± 0.05	7.36 ± 0.07	7.38 ± 0.06	7.37 ± 0.06
	After	7.35 ± 0.08	7.32 ± 0.07	7.37 ± 0.08	7.35 ± 0.08

MABP, mean arterial blood pressure; PaO_2, partial pressure of oxygen in arterial blood; $PaCO_2$, partial pressure of carbon dioxide in arterial blood.

Table 2 Effect of montelukast on behavioural dysfunction within 28 days after focal cerebral ischaemia in mice

Group	*n*	Days after middle cerebral artery occlusion					
		1	3	7	14	21	28
Neurological deficit score							
Sham	10	0	0	0	0	0	0
Ischaemia	14	1.47 ± 0.25	1.47 ± 0.25	1.13 ± 0.23	1.00 ± 0.23	1.12 ± 0.26	1.21 ± 0.20
Montelukast 0.01 mg/kg	13	1.70 ± 0.29	1.52 ± 0.30	1.17 ± 0.20	1.03 ± 0.10	1.08 ± 0.09	1.08 ± 0.09
Montelukast 0.1 mg/kg	13	1.42 ± 0.29	0.91 ± 0.06$^{\#}$	0.93 ± 0.15	0.95 ± 0.25	0.73 ± 0.20$^{\#}$	0.70 ± 0.21$^{\#}$
Pranlukast 0.1 mg/kg	10	1.40 ± 0.27	0.80 ± 0.21$^{\#}$	0.60 ± 0.20$^{\#}$	0.50 ± 0.21$^{\#}$	0.50 ± 0.21$^{\#}$	0.40 ± 0.16$^{\#}$
Right turn (%)							
Sham	10	52.3 ± 5.4	48.3 ± 6.0	46.5 ± 5.8	60.0 ± 9.8	53.5 ± 8.0	51.1 ± 9.7
Ischaemia	14	98.1 ± 6.0**	95.6 ± 6.7**	92.0 ± 8.5**	87.2 ± 7.5**	85.1 ± 9.0**	83.0 ± 9.3**
Montelukast 0.01 mg/kg	13	71.7 ± 15.2	84.2 ± 7.8**	96.0 ± 5.3**	96.1 ± 5.3**	87.0 ± 8.0**	91.0 ± 8.1**
Montelukast 0.1 mg/kg	13	78.9 ± 12.8	69.5 ± 11.0	63.1 ± 10.2$^{\#}$	72.8 ± 15.2$^{\#}$	60.1 ± 13.7$^{\#}$	59.8 ± 12.9$^{\#}$
Pranlukast 0.1 mg/kg	10	64.0 ± 16.1$^{\#}$	58.0 ± 14.6$^{\#}$	56.0 ± 13.8$^{\#}$	53.0 ± 11.9$^{\#}$	41.0 ± 15.2$^{\#}$	44.0 ± 10.7$^{\#}$

Mean ± SEM. **$P < 0.01$ vs sham operation, $^{\#}P < 0.05$ vs ischaemic control.

showed only a tendency to increase but no significant changes at 14–28 days. Edaravone (10 mg/kg) only increased the success rate at 28 days (Table 3).

The lesion volume and brain atrophy in rats 28 days after MCAO were reduced by montelukast (0.5 mg/kg but not 0.1 mg/kg) and edaravone (10 mg/kg) (Figure 3). The neuron density was significantly reduced from 1.31 ± 0.16 ($\times 10^3$ cells/mm^2) of the control level to 0.49 ± 0.08 ($P < 0.01$) 28 days after ischaemia. The reduced density was ameliorated by montelukast at 0.5 mg/kg (0.87 ± 0.12, $P < 0.01$ vs ischaemic control) and edaravone (0.81 ± 0.10, $P < 0.01$), but not by 0.1 mg/kg montelukast (0.56 ± 0.17, $P > 0.05$).

Discussion

In this study, we found that montelukast exerted a protective effect on chronic brain injury after focal cerebral ischaemia similar to pranlukast in mice. Montelukast was also effective after oral administration in rats. Although montelukast is structurally different from pranlukast and shows some differ-

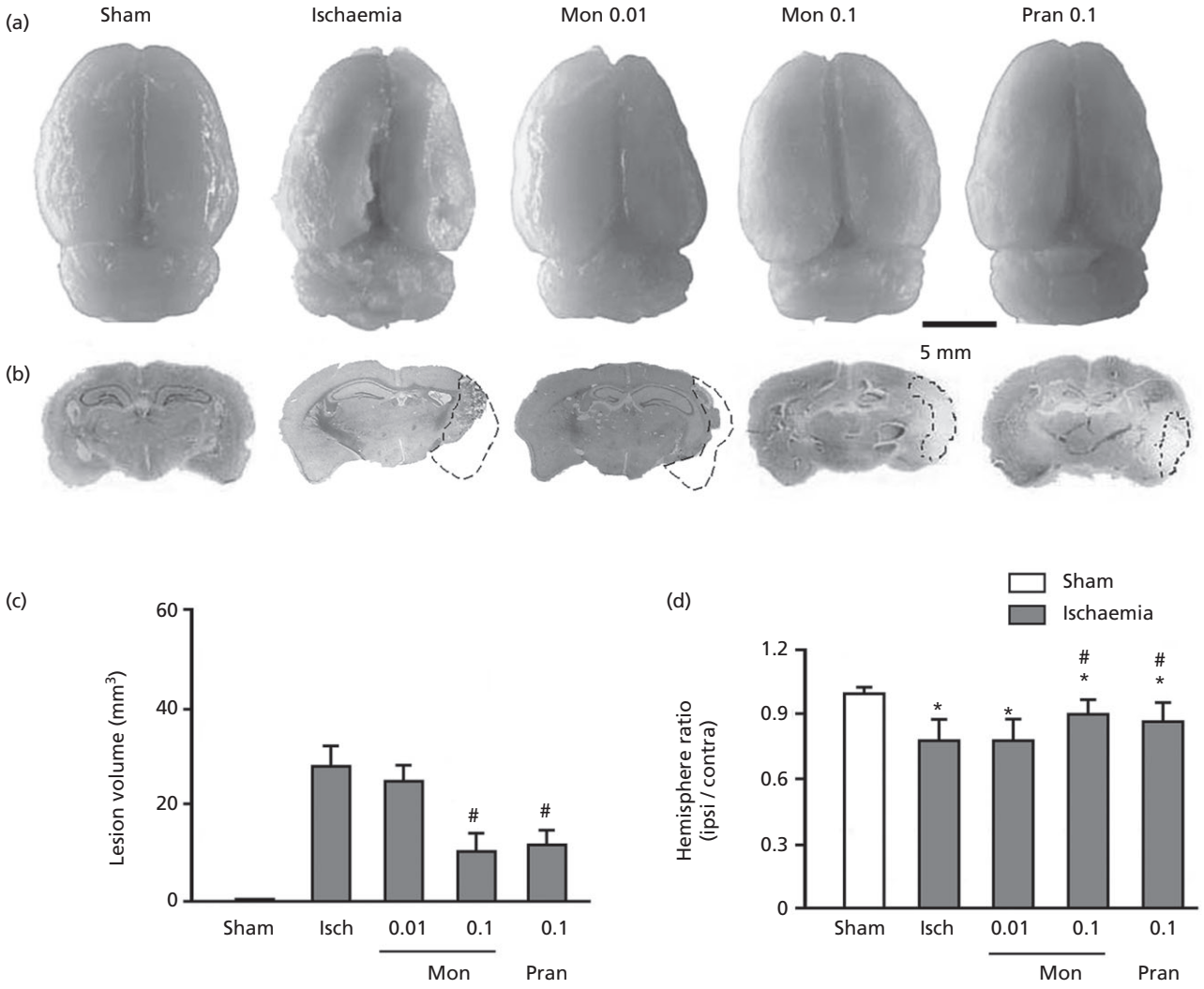

Figure 1 Effect of montelukast on ischaemic infarct and brain atrophy 28 days after focal cerebral ischaemia in mice. Brain photographs (a) and coronary sections stained with 1% toluidine blue (b) indicated that montelukast (Mon: 0.01 or 0.1 mg/kg) and pranlukast (Pran 0.1 mg/kg) attenuated brain lesion volume (c) and brain atrophy (d). Data are reported as mean ± SEM; $n = 10–14$ mice per group; $*P < 0.05$ vs sham operation, $\#P < 0.05$ vs ischaemic (Isch) control.

ences in its pharmacological properties, it has the same property of $CysLT_1$ receptor antagonism and the same effects on acute cerebral ischaemia as pranlukast.[11,24–26] Thus, these findings confirmed that long-term neuroprotection is a common effect of $CysLT_1$ receptor antagonists, at least including montelukast in addition to pranlukast as previously reported.[9] We demonstrated the orally active property of montelukast, as it is an oral preparation for the treatment of bronchial asthma in the clinic.[13,27]

In mouse experiments, we compared the long-term effect of montelukast with that of pranlukast. The latter has been reported to have a long-term protective effect on chronic injury after focal cerebral ischaemia in mice.[9] Montelukast and pranlukast did not reduce the death rate after ischaemia as we reported in the acute and chronic phases of focal cerebral ischaemia in mice.[9,11] However, they attenuated chronic brain injury in the surviving ischaemic mice. Generally, the effective dose of montelukast was similar to pranlukast, i.e. 0.1 mg/kg was effective for both drugs but 0.01 mg/kg montelukast was not effective. The effect of the higher dose was demonstrated in acute or subacute injury after focal cerebral ischaemia.[4,6,14] However, the effect of pranlukast on behavioural dysfunction seemed to be better than that of montelukast, because pranlukast reduced the right turn percentage earlier (from one day after ischaemia) than montelukast (from seven days), and persistently attenuated the neurological deficits (3–28 days, Table 2). Since pranlukast possesses $CysLT_1$ receptor-independent effects, such as antioxidative and nuclear factor-κB (NF-κB)-inhibiting effects, this may potentiate its effects on ischaemic brain injury.[28–31]

In rat experiments, we compared the effect of oral montelukast on chronic ischaemic injury with that of edaravone, a well-tested drug with protective effects on cerebral ischaemia in both clinical and experimental studies.[20,32] Montelukast attenuated chronic brain injury but did not reduce the death rate. The oral effective dose of montelukast (0.5 mg/kg, twice

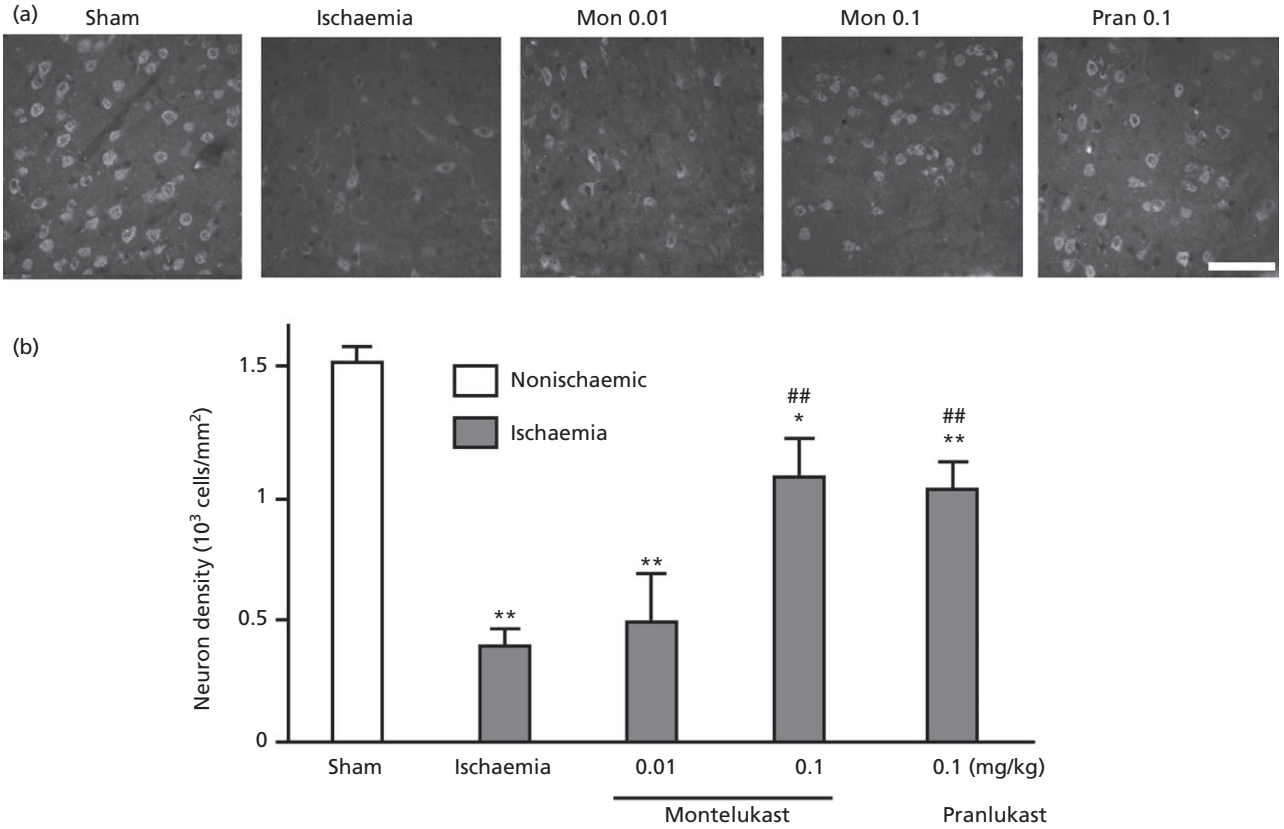

Figure 2 Effect of montelukast on neuron density 28 days after focal cerebral ischaemia in mice. Microphotographs indicated that montelukast (Mon: 0.01 or 0.1 mg/kg) and pranlukast (Pran 0.1 mg/kg) attenuated the reduced density of neuronal nuclei-positive neurons in the boundary zone of the ischaemic core (a). Data are reported as mean ± SEM; $n = 10$–14 mice per group; $^*P < 0.05$ and $^{**}P < 0.01$ vs sham operation, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs ischaemic control. Scale bar = 50 μM.

Table 3 Effect of montelukast on behavioural dysfunction within 28 days after focal cerebral ischemia in rats

Group	Days after MCAO					
	1	3	7	14	21	28
Neurological deficit score						
Sham	0	0	0	0	0	0
Ischaemia	3.00 ± 0	2.90 ± 0.1	2.47 ± 0.22	1.61 ± 0.36	1.30 ± 0.28	0.75 ± 29
Montelukast 0.1 mg/kg	2.89 ± 0.11	2.25 ± 0.40	1.50 ± 0.52	0.71 ± 0.32$^{\#}$	0.71 ± 0.32	0.50 ± 0.27
Montelukast 0.5 mg/kg	3.00 ± 0	2.00 ± 0.37$^{\#}$	0.68 ± 0.32$^{\#}$	0.82 ± 0.20$^{\#}$	0.11 ± 0.11$^{\#}$	0.36 ± 0.27
Edaravone 10 mg/kg	2.89 ± 0.11	2.00 ± 0.45	1.82 ± 0.58	1.05 ± 0.37	1.08 ± 0.00	0.52 ± 0.36
Success rate (%)						
Sham	70.0 ± 5.7	60.0 ± 6.7	61.0 ± 6.0	68.0 ± 2.3	72.0 ± 5.0	70.0 ± 5.2
Ischaemia	3.0 ± 0.2*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Montelukast 0.1 mg/kg	0 ± 0*	0 ± 0*	0 ± 0*	22.5 ± 18.1*	19.8 ± 17.9*	25.3 ± 19.8*
Montelukast 0.5 mg/kg	28.1 ± 9.8*$^{\#}$	35.2 ± 17.2*$^{\#}$	58.0 ± 21.5$^{\#}$	61.7 ± 23.6$^{\#}$	48.5 ± 21.0$^{\#}$	52.1 ± 20.2$^{\#}$
Edaravone 10 mg/kg	0 ± 0*	0 ± 0*	8.1 ± 7.2*	19.2 ± 17.8*	24.3 ± 18.2*	26.0 ± 19.0*$^{\#}$

Mean ± SEM. $n = 8$ rats per group; $^*P < 0.05$ vs sham operation, $^{\#}P < 0.05$ vs ischaemic control.

daily) in rat experiments was larger than its intraperitoneal dose in mice. This may have been due to the bioavailability of oral montelukast, which is 58–66% in humans but is unknown in rats.[33,34] Compared with edaravone, oral montelukast attenuated both the behavioural and morphological changes after chronic ischaemic injury. In addition to neurological deficits, oral montelukast improved the forelimb reaching ability in the skilled reaching test (Table 3), indicating an effect on impairment of complex behaviour after focal cerebral ischaemia.[22,23] However, edaravone showed a similar but somewhat weaker effect on morphological changes, while it was almost ineffective on behavioural dysfunction (except an

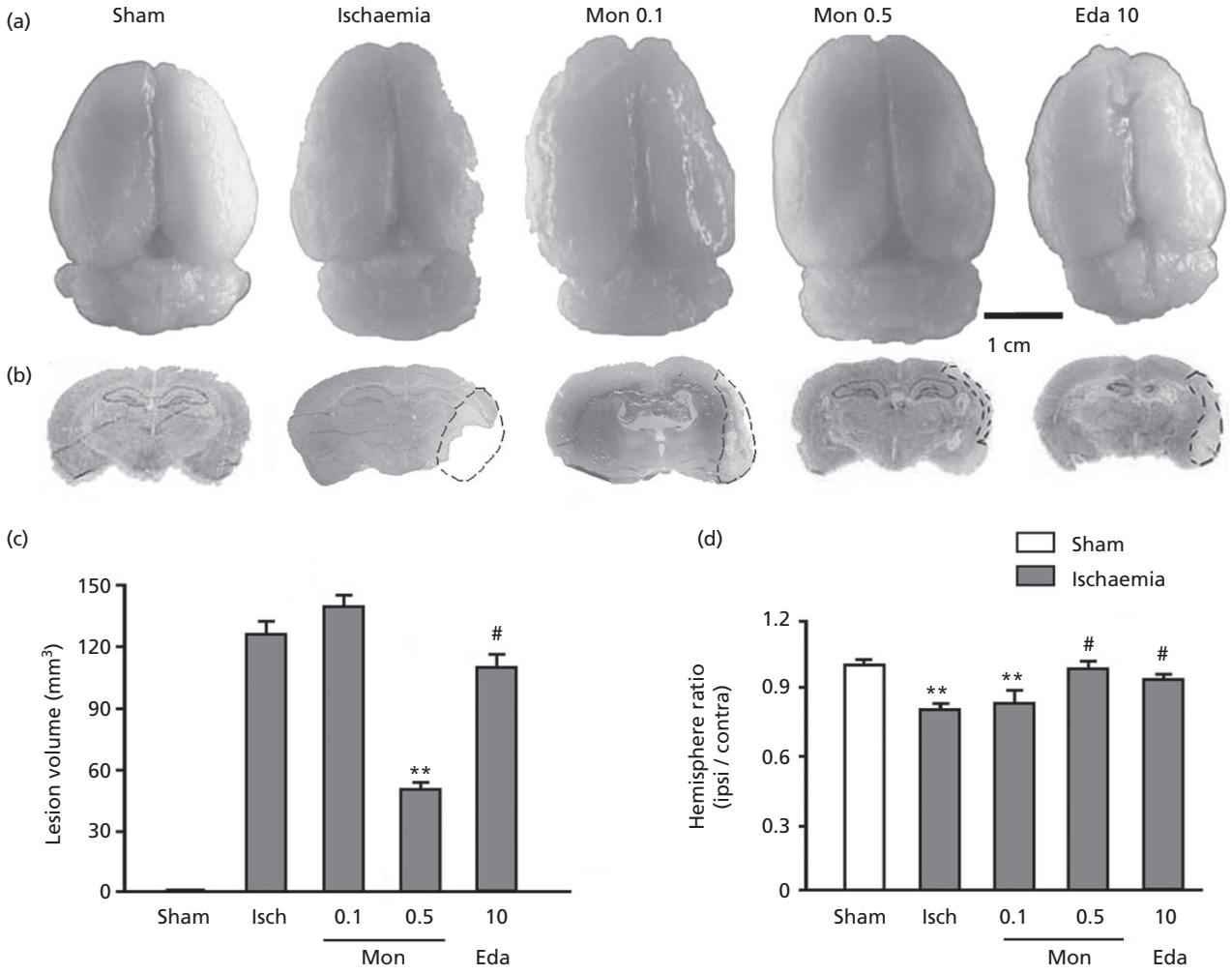

Figure 3 Effect of montelukast on ischaemic infarct and brain atrophy 28 days after focal cerebral ischaemia in rats. Brain photographs (a) and coronary sections stained with 1% toluidine blue (b) indicated that montelukast (Mon: 0.1 or 0.5 mg/kg) and edaravone (Eda 10 mg/kg) attenuated brain lesion volume (c) and brain atrophy (d). Data are reported as mean ± SEM; $n = 8$ rats per group; $^{**}P < 0.01$ vs sham operation, $\#P < 0.05$ vs ischaemic (Isch) control.

improvement of forelimb reaching 28 days after ischaemia). The relatively weaker effect of edaravone was also found in acute brain injury after focal cerebral ischaemia in mice.[11] Consistently, it was reported that short-term administration of edaravone (for two days) was more effective than long-term administration (for five or 10 days) on chronic brain injury after hypoxic-ischaemia in younger rats.[19] This may be one reason for the weaker effects of edaravone, but the mechanisms need to be investigated.

The mechanisms underlying the long-term protective effect of montelukast are unknown. However, the inhibition of glial scar formation by the $CysLT_1$ receptor antagonist pranlukast in the chronic phase of brain ischaemia may be one mechanism. $CysLT_1$ receptors mediate astrocyte proliferation, which is the basis of post-ischaemic astrocytosis and glial scar formation.[8,35] $CysLT_1$ receptor antagonists can inhibit ischaemia-like injury-induced astrocytosis, thereby inhibiting glial scar formation.[9] Since the glial scar, a barrier for neuronal regeneration, impairs functional recovery, $CysLT_1$ receptor antagonists may be beneficial to neural functional recovery in the chronic phase of ischaemic stroke.[36,37] However, the effect of montelukast on glial scar formation remains to be investigated.

Conclusion

The $CysLT_1$ receptor antagonist montelukast was effective on chronic brain injury after focal cerebral ischaemia in mice; it was also effective after oral administration in rats. Our findings further support the therapeutic potential of $CysLT_1$ receptor antagonists in the treatment of cerebral ischaemia.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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