Decreased spasticity of Baishaoluoshi Decoction through the BDNF/TrKB-KCC2 pathway on poststroke spasticity rats

Le Xie[a], Yao Xie[a], Guo Mao[a], Sijia Cao[b], Rui Fang[c], Shen Zhou[a], Junlin Jiang[a], Ting Yao[a], Jianhu Fan[a], Dong Liu[d], Dahua Wu[a] and Jinwen Ge[c]

Objective **K^+–Cl^- cotransporter-2 (KCC2), which primarily extrudes chloride in mature neurons, triggers hemiplegia limb spasticity after ischemic stroke by affecting neuronal excitability. Our previous study revealed that the Chinese herb Baishaoluoshi Decoction decreases hemiplegia limb spasticity in poststroke spasticity (PSS) patients. This study aimed at elucidating on the effects of Baishaoluoshi Decoction on the BDNF/TrKB-KCC2 pathway in PSS rat models.**

Methods **Middle cerebral artery occlusion (MCAO) was adopted for the establishment of PSS rat models. Muscle tension was evaluated by Modified Ashworth Scale. Nissl staining and transmission electron microscopy were used to measure the protective effects of Baishaoluoshi Decoction on ischemic injury-induced neuronal damage due to MCAO. Expression levels of BDNF, TrKB, and KCC2 in brain tissues around the infarct and brainstem were detected by immunohistochemical staining.**

Results **It was found that Baishaoluoshi Decoction suppressed hemiplegia limb spasticity and alleviated the damage in neurons and synapses in PSS rat models. Importantly, the expression of BDNF, TrKB, and KCC2 in brain tissues around the infarct and brainstem were significantly upregulated after treatment with low-dose and high-dose Baishaoluoshi Decoction.**

Conclusion **Suppression of spasticity by Baishaoluoshi Decoction in PSS rat models may be correlated with upregulated BDNF/TrKB-KCC2 pathway, which may be a complementary therapeutic strategy for PSS. *NeuroReport* 32: 1183–1191 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.**

NeuroReport 2021, **32**:1183–1191

Keywords: stroke, spasticity, KCC2, BDNF, traditional Chinese medicine

[a]Neurology Department, Hunan Academy of Chinese Medicine Affiliated Hospital, [b]Foreign Languages Department, School of Humanities and Management, Hunan University of Chinese Medicine, [c]Chinese Pharmacy Teaching and Research Department, College of Integrated Traditional Chinese and Western Medicine, Hunan University of Chinese Medicine, Changsha, Hunan and [d]Hunan Key Laboratory of Prevention and Treatment in Cardiovascular Disease, College of Pharmacy, Guiyang University of Chinese Medicine, Guiyang, Guizhou, China

Correspondence to Dahua Wu, PhD, Neurology Department, Hunan Academy of Chinese Medicine Affiliated Hospital, No. 58, Lushan Road, Changsha 410006, China
Tel: +86 13508491768; fax: +86 0731 85920368; e-mail: 893049352@qq.com

Received 15 April 2021 **Accepted** 11 June 2021

Introduction

Globally, stroke is the leading cause of adult disabilities and mortalities, and the economic costs of stroke management as well as poststroke care are enormous. For instance, in 2016, there were 13.7 million new stroke cases and 5.5 million stroke-associated mortalities [1]. Despite the clinical benefits of intravenous thrombolysis and percutaneous mechanical thrombectomy, spasticity of hemiplegia limb after stroke, which is called poststroke spasticity (PSS) has been reported in 30–80% of individuals [2,3]. Stroke patients with damaged internal capsule, superior corona radiata, thalamus, and putamen are more prone to developing spasticity [4]. People with PSS frequently suffer from dyskinesia, depression, pain, muscle stiffness, and poor quality of life, resulting in heavy economic and nursing burden [5].

PSS, which is characterized by velocity-dependent enhancement in the stretch reflex, results from increased excitability of α motoneurons due to stroke-associated brain injury. Hyperexcitability of motoneurons is the main pathology of PSS; however, its mechanisms have not been established [6,7]. Chloride homeostasis is associated with maintenance if motoneuron excitability, and the K^+–Cl^- cotransporter-2 (KCC2) is key in maintaining chloride homeostasis [8]. The main function of KCC2 is to extrude Cl^- from motoneurons in order to maintain low Cl^- levels, which are essential for hyperpolarizing the inhibitory and balance between inhibition and excitation of neuronal circuits [9,10]. Furthermore, KCC2 is involved in maintenance of spinal morphology, dendritic outgrowth, synaptogenesis, and synaptic plasticity [11]. Regulation of KCC2 is dependent on TrkB activation by BDNF. Therefore, elucidating on the BDNF/TrKB-KCC2 pathway mechanisms of motoneuron hyperexcitability will inform on PSS treatment and management [12].

It has been reported that Baishaoluoshi Decoction can decrease spasticity and enhance motor functions in PSS, thereby overcoming the limitations of muscle relaxants in

0959-4965 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/WNR.0000000000001709

reducing muscle strength [13]. In this study, we established rat PSS models through middle cerebral artery occlusion (MCAO), to further elucidate on the BDNF/TrKB-KCC2 pathway-associated mechanisms of Baishaoluoshi Decoction on PSS. We found that Baishaoluoshi Decoction decreased spasticity of hemiplegia limb in PSS rat models, which may be relevant to the BDNF/TrKB-KCC2 pathway.

Materials and methods

Materials and reagents

Baclofen tablets (positive reference drug) were purchased from Fuan Pharmaceutical (Group) Co., Ltd (Ningbo, China). Paraformaldehyde (4%) was obtained from Biosharp Co., Ltd (Hefei, China). Choral hydrate was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Nissl staining solution, in which the active ingredient is cresyl violet, was purchased from Beyotime Company (Shanghai, China). Glutaraldehyde, osmium acid, acetone, epoxy resin, uranium acetate, and lead citrate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Anti-BDNF antibody, anti-TrkB antibody, and anti-KCC2 antibody were obtained from Abcam (Cambridge, UK). Horseradish enzyme labeled chain affinity and diaminobenzidine were purchased from ZSGB-BIO Co., Ltd (Beijing, China). The inverted fluorescence microscope (TH4-200) was obtained from Olympus Corporation (Tokyo, Japan).

Preparation of the Baishaoluoshi Decoction

Baishao (*Paeoniae radix* Alba) and Luoshiteng (*Trachelospermi caulis* et folium), the two main components of Baishaoluoshi Decoction, were mixed at a ratio of 1:1. Formula granules of Baishao (no. 16050101) and Luoshiteng (no. 16082053) were obtained from Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd (Chengdu, China).

Animals

Adult male Sprague–Dawley rats weighing 250–280 g were used in this study. They were purchased from Hunan SJA Laboratory Animal Co., Ltd (No. 43004700027199; Changsha, Hunan, China) and housed in groups of 3–5 in polypropylene cages under a regular 12:12 h light/dark cycle. Ethical approval for this study was obtained from the Laboratory Animal Welfare and ethics committee of Hunan Normal University (No. 14-XL 1; Changsha, Hunan, China).

Quality analysis of Baishaoluoshi Decoction

High-performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) was used to establish the Baishaoluoshi Decoction chromatograms. The WondaSil C18 for herbal medicine (250 × 4.6 mm × 5 μm) (Shimadzu Corporation, Japan) and the mobile phase consisted of 0.1% phosphate aqueous solution and acetonitrile, which were used for gradient elution at a flow rate of 1.0 mL/min and a wavelength of 230 nm. Reference substances, albiflorin (PR141015-1) and paeoniflorin (110736), the two main components of Baishao, were purchased from ChromaDex Co. (California, USA) and the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), respectively. The reference substance, tracheloside (111858), the main component of Luoshiteng, was purchased from the National Institute for the Control of Pharmaceutical and Biological Products.

Establishing poststroke spasticity rat models

Establishment of PSS rat models through MCAO was performed as previously described [14]. Briefly, rats were anesthetized with 10% chloral hydrate (0.35 mL/100 g) after which the right common carotid artery (CCA), internal carotid artery, and external carotid artery (ECA) were cautiously exposed. The ECA was clamped and the origin of the right middle cerebral artery (MCA) blocked with a monofilament of diameter (0.26 mm; Beijing Cinontech Biotech Co. Ltd., Beijing, China) by inserting it from the CCA. Then, the surgical wound was disinfected and the skin sutured after pruning the monofilament. Temperatures were maintained at 37°C until the animal regained consciousness. Rats in the sham group were subjected to the same procedures, except that the MCA was not blocked by the monofilament. Rats in the control group were not subjected to any surgical procedures.

Three days after surgery, animals were randomly allocated into six groups: (1) high-dose Baishaoluoshi Decoction group (H), administered with Baishaoluoshi Decoction (10.8 g/kg) through gavage every day for 4 weeks; (2) low-dose Baishaoluoshi Decoction group (L), administered with Baishaoluoshi Decoction (5.4 g/kg) through gavage every day for 4 weeks; (3) Baclofen group (B) (positive reference drug), administered with baclofen (5.4 mg/kg) every day for 4 weeks; (4) Model group (M); (5) Sham-operated group (S), and (6) Control group (C). Rats in M, S, and C groups were gavaged with normal saline every day for 4 weeks.

Spasticity evaluation

The Modified Ashworth Scale (MAS) was used to evaluate spasticity of the hemiplegia limb, which was scored as: 0, no spasticity; I, mild increase in muscular tension at the end of passive movement in hemiplegia limb; I^+, a mild increase in muscular tension less than half of passive movement in hemiplegia limb; II, an obvious increase in muscular tension in most of the passive movement in hemiplegia limb; III, an evident increase in muscular tension, leading to dyskinesias; and IV, stiff limb [15,16].

Nissl staining

Brain tissues around the infarct were paraffin embedded and sliced (5 μm). After being dewaxed with xylene and dehydrated in graded alcohol, tissues were stained using the Nissl staining solution, after which they were dehydrated in graded alcohol. Tissue sections were observed using a TH4-200 inverted microscope (Olympus Corporation). Nissl body contents were quantitatively evaluated using integral optical density.

Ultrastructure detection by transmission electron microscopy

Immediately after the rats had been sacrificed ($n=5$), brain tissues around the infarct were fixed in 2.5% glutaraldehyde for 2–4 h. Then, brain tissues were washed using 0.2% phosphate buffered saline, fixed in 1% osmium acid for 2 h, and sequentially dehydrated using graded alcohol and acetone. Subsequently, brain tissues were sliced (60–80 nm) after being embedded in epoxy resin, and double stained using uranium acetate and lead citrate, respectively, for 15 min. The tissues were finally scanned using transmission electron microscopy (TEM; HITACHI Corporation, Tokyo, Japan).

Immunohistochemical staining

Rats were anesthetized, their brains harvested and fixed in 4% paraformaldehyde. Brain sections (5 μm thick) were prepared using a cryostat and dewaxed using dimethylbenzene and ethanol. Then, brain sections were incubated with 0.3% Triton X-100 and 5% normal bovine serum in phosphate buffer saline for 30 min. The sections were consecutively incubated with primary antibodies (anti-BDNF antibody, 1:750 dilution, Abcam; anti-KCC2 antibody, 1:800 dilution, Abcam; anti-TrkB antibody, 1:1000 dilution; Abcam) for 1 h and goat anti-rabbit IgG/HRP (1:500 dilution; ZSGB-BIO Co., Ltd) for 30 min. Horseradish enzyme labeled chain affinity and diaminobenzidine were subsequently used according to the manufacturer's instructions. Image-Pro Plus 6.0 (Media Cybernetics, Inc., Maryland, USA.) was used for quantitative analysis.

Statistical analysis

Statistical analyses were performed using SPSS v. 20.0 (IBM SPSS; Armonk, New York, USA). Comparisons among groups were evaluated using one-way analysis of variance for normally distributed quantitative data. Ranked data of MAS scores were compared using Kruskal–Wallis H followed by Mann–Whitney U test. $P \leq 0.05$ was considered statistically different.

Results

Characteristics of the high-performance liquid chromatography fingerprint of Baishaoluoshi Decoction

HPLC fingerprints of Baishaoluoshi Decoction showed that peak 1 (albiflorin) and peak 2 (paeoniflorin) belonged to Baishao while peak 3 (tracheloside) belonged to Luoshiteng (Fig. 1a). On the basis of the HPLC fingerprint, we established a precise, and stable procedure for quality control of Baishaoluoshi Decoction.

Baishaoluoshi Decoction decreased spasticity on poststroke spasticity rats

MAS scores, which are grade data, do not conform to normal distribution, therefore, Kruskal–Wallis H test was used for between group comparisons, followed by Mann–Whitney U test to evaluate differences between every two groups. Two weeks after intervention, there were no significant differences in MAS scores between groups ($P>0.05$). However, 4 weeks after interventions, the differences were found to be significant ($P<0.05$), as determined by Kruskal–Wallis H. Furthermore, Mann–Whitney U was used to evaluate differences in MAS scores between every two groups, 4 weeks after intervention. It was found that baclofen, as well as low-dose and high-dose Baishaoluoshi Decoction decreased spasticity of the hemiplegia limb, when compared to model rats ($P<0.05$) (Table 1).

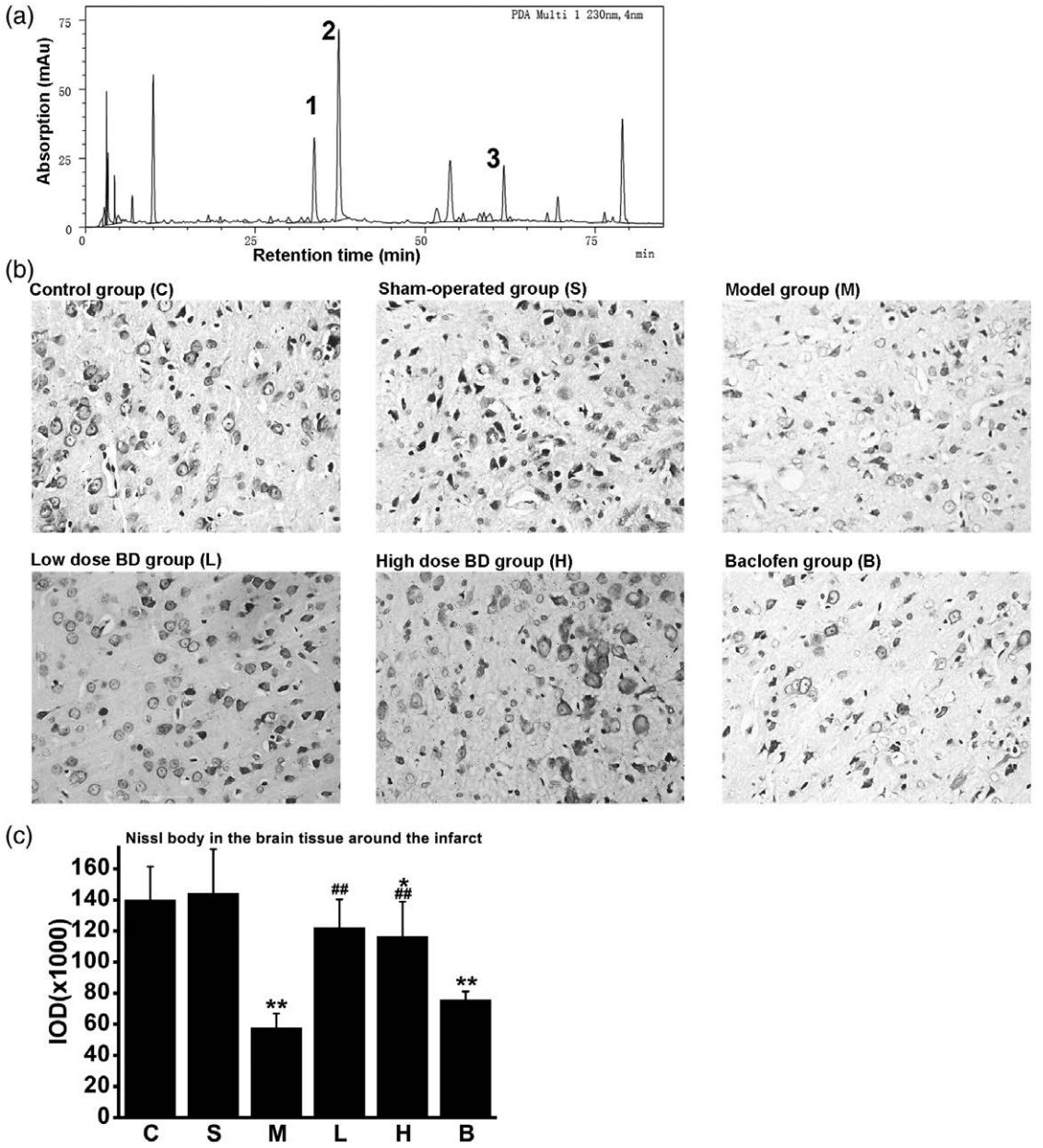
Protective effects of Baishaoluoshi Decoction on neuronal damage

Nissl's staining by cresyl violet revealed that Nissl body contents of the baclofen and model groups were significantly reduced when compared to the sham-operated and control groups ($P<0.01$). However, in the low-dose and high-dose Baishaoluoshi Decoction groups, the content of Nissl bodies was relatively mildly decreased (Fig. 1b and c). Moreover, TEM showed that there were a lot of damaged neurons and glial cells in the baclofen and model groups, the cytoplasm was concentrated, the mitochondria were swollen, there was nuclear condensation and cleavage while the number of synapses were decreased. However, the damage was relatively light in the high-dose and low-dose Baishaoluoshi Decoction groups (Fig. 2).

The BDNF/TrKB-KCC2 pathway was upregulated in poststroke spasticity rats

Subsequently, we measured the expression levels of BDNF, TrKB, and KCC2 in the brain tissue around the infarct 2, 4 weeks after intervention by immunohistochemical staining. Reticulospinal tract (RST) excitability in the brainstem was considered to be a significant pathophysiological factor for PSS [6]. Therefore, the expression levels of BDNF, TrKB, and KCC2 in the dorsal regions of the brainstem were detected. It was found that, after 2 weeks, the expression levels of BDNF were significantly upregulated, and gradually returned to normal levels in the brain tissue around the infarct. Moreover, BDNF was found to be significantly elevated in the low-dose and high-dose Baishaoluoshi Decoction groups, at 2 and 4 weeks ($P<0.05$) (Fig. 3a, b, and e). But in the brainstem, expression levels of BDNF in the PSS model group ($P>0.05$) were low, and the low-dose as well as high-dose Baishaoluoshi Decoction elevated the expression of BDNF to levels comparable to those of model group rats, especially at 2 weeks ($P<0.05$) (Fig. 3c, d, and f). Equally, low-dose and high-dose Baishaoluoshi Decoction elevated the expression levels of TrKB both in the brain tissues around the infarct and in the brainstem at 2 and 4 weeks ($P<0.05$) (Fig. 4a–f). Interestingly, expression levels of KCC2 in the brain tissue around the

Fig. 1



(a) The HPLC fingerprint of Baishaoluoshi Decoction. Peak 1 (albiflorin), peak 2 (paeoniflorin), and peak 3 (tracheloside) are the main contents of Baishaoluoshi Decoction. (b) Protective effect of Baishaoluoshi Decoction on neuronal damage. Middle cerebral artery occlusion (MCAO) was used to establish the PSS rat models. Intervention was initiated 3 days after surgery and continued for 4 weeks. (i) Nissl body in the brain tissue around the infarct. Nissl staining solution combines with ribosomes in the rough endoplasmic reticulum and RNA/DNA in the nucleus to form Nissl bodies, which is a characteristic structure of neurons. (ii) Integral optical density (IOD) was used to determine the expression levels of positive cells. C, control group (normal saline); S, sham-operated group (normal saline); M, model group (normal saline); L, low-dose Baishaoluoshi Decoction group (5.4 g/kg); H, high-dose Baishaoluoshi Decoction group (10.8 g/kg), and B, baclofen group (5.4 mg/kg). $^{*}P<0.05$ and $^{**}P<0.01$ compared to control group, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to model group. ($n=5$ for every group). HPLC, high-performance liquid chromatography; PSS, poststroke spasticity.

infarct and the brainstem were found to be suppressed at 2 weeks, but rebounded at 4 weeks. After being treated with low-dose and high-dose Baishaoluoshi Decoction, KCC2 did not decrease, but was significantly elevated in the brain tissue around the infarct and the brainstem at 2 and 4 weeks ($P<0.05$) (Fig. 5a–f).

Discussion

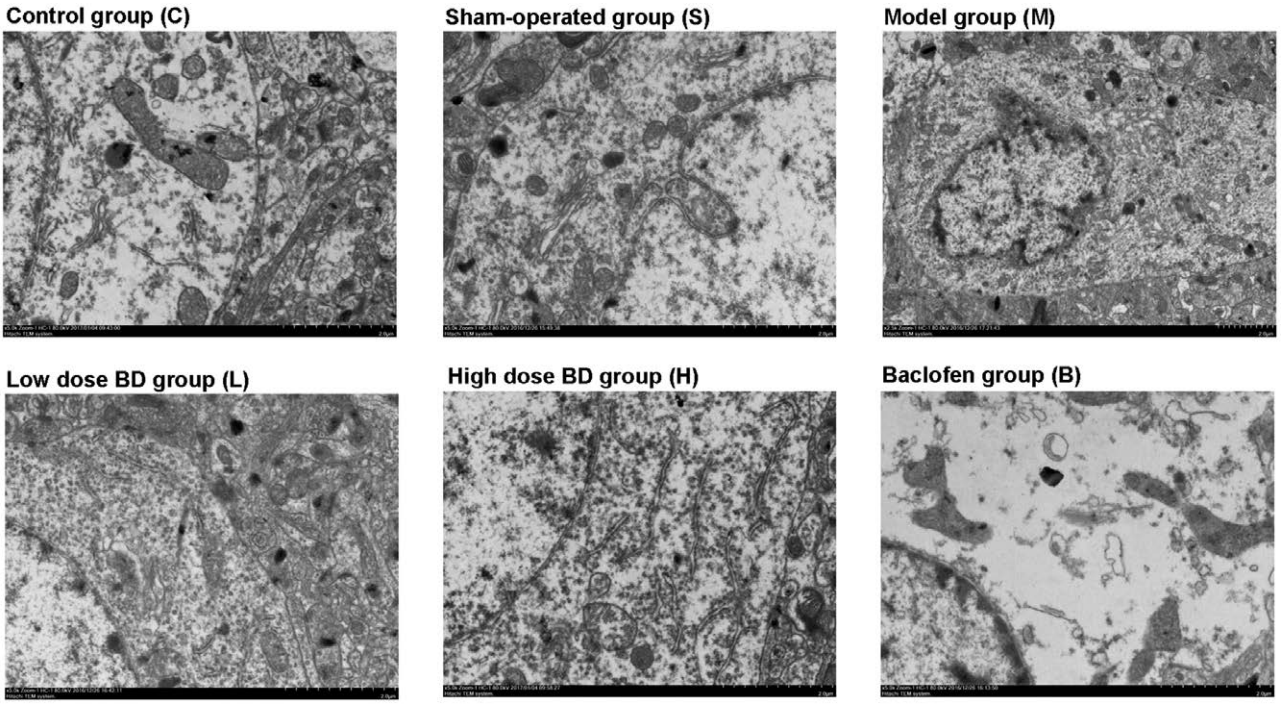
KCC2, a cation–chloride cotransporter that is exclusively expressed along the plasma membrane of neuron soma and dendrite, was discovered by Payne *et al.* in 1996 [17,18]. Membrane distribution of KCC2 manifests as punctate, decorating the neuron body, dendritic shafts and spines. In

Table 1 **Comparison of Modified Ashworth Scale in every group**

Time	Baseline				2 Weeks				4 Weeks				
MAS group	0	I	I^+	II	0	I	I^+	II	0	I	I^+	II	Mann–Whitney *U* (*P*)
High-doses group	8	3	0	0	1	3	5	2	7	4	0	0	0.015
Low-doses group	10	2	0	0	1	3	5	3	7	5	0	0	0.033
Baclofen group	8	3	0	0	1	5	4	1	7	4	0	0	0.026
Model group	9	3	0	0	0	3	3	6	3	5	4	0	
Kruskal–Wallis *H*	χ^2=0.480, *P*=0.923				χ^2=4.908, *P*=0.179				χ^2=8.069, *P*=0.045				

Kruskal–wallis *H* was used for comparison between groups, Mann–Whitney *U* was used for comparison with model group.

Fig. 2



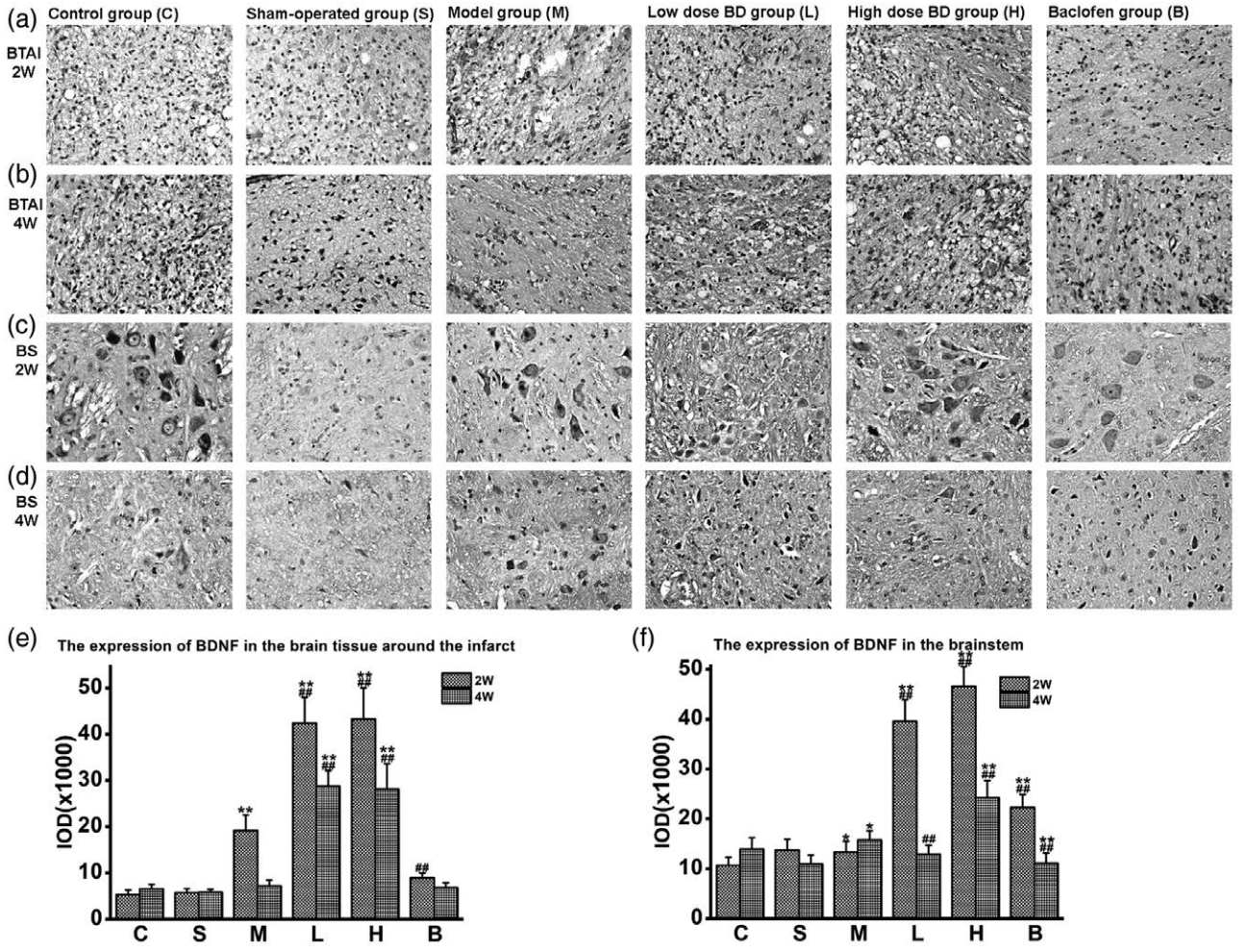
Effects of Baishaoluoshi Decoction on brain ultrastructure in PSS rat models. The ultrastructure of brain tissues around the infarct were detected by transmission electron microscopy (TEM) 4 weeks after intervention (×5000). PSS, poststroke spasticity.

the membrane pool, KCC2 is almost totally replaced every 10 min in a physiological manner, which presents a high turnround rate [19]. Synaptic localization of KCC2 is within the perisynaptic region of the postsynaptic neuron, in line with the transporter in γ-aminobutyric acid (GABA) signaling and chloride homeostasis, but generally not expressed in the presynaptic boutons [20]. KCC2 is primarily clustered in mushroom-type spines and stubby spines [21].

KCC2 is essential in synaptic formation [22]. Enhancing the expression levels of KCC2 in immature hippocampal neurons can increase the density of GABAergic terminals, the number of $GABA_AR$ in the postsynaptic membrane, and the frequency as well as amplitude of inhibitory postsynaptic potential induced by $GABA_AR$ [23]. Downregulation of the expression of KCC2 reduces the frequency and amplitude of inhibitory postsynaptic potential [24]. We found that damages in neurons and synapses were relatively minor after treatment with high-dose and low-dose Baishaoluoshi Decoction, when compared to the model group.

KCC2 serves as a trigger of spasticity after brain and spinal cord injuries by affecting neuronal excitability [25–27]. In mature central nervous systems, increasing the expression of KCC2 suppresses the concentration of intracellular chloride by extruding chloride to the extracellular domain. Consequently, engaging $GABA_AR$ results in an influx of chloride into neuronal cells, leading to neuronal inhibition [12]. Ischemic stroke-induced brain injury decreases KCC2 levels, resulting in an increase in the levels of intracellular

Fig. 3

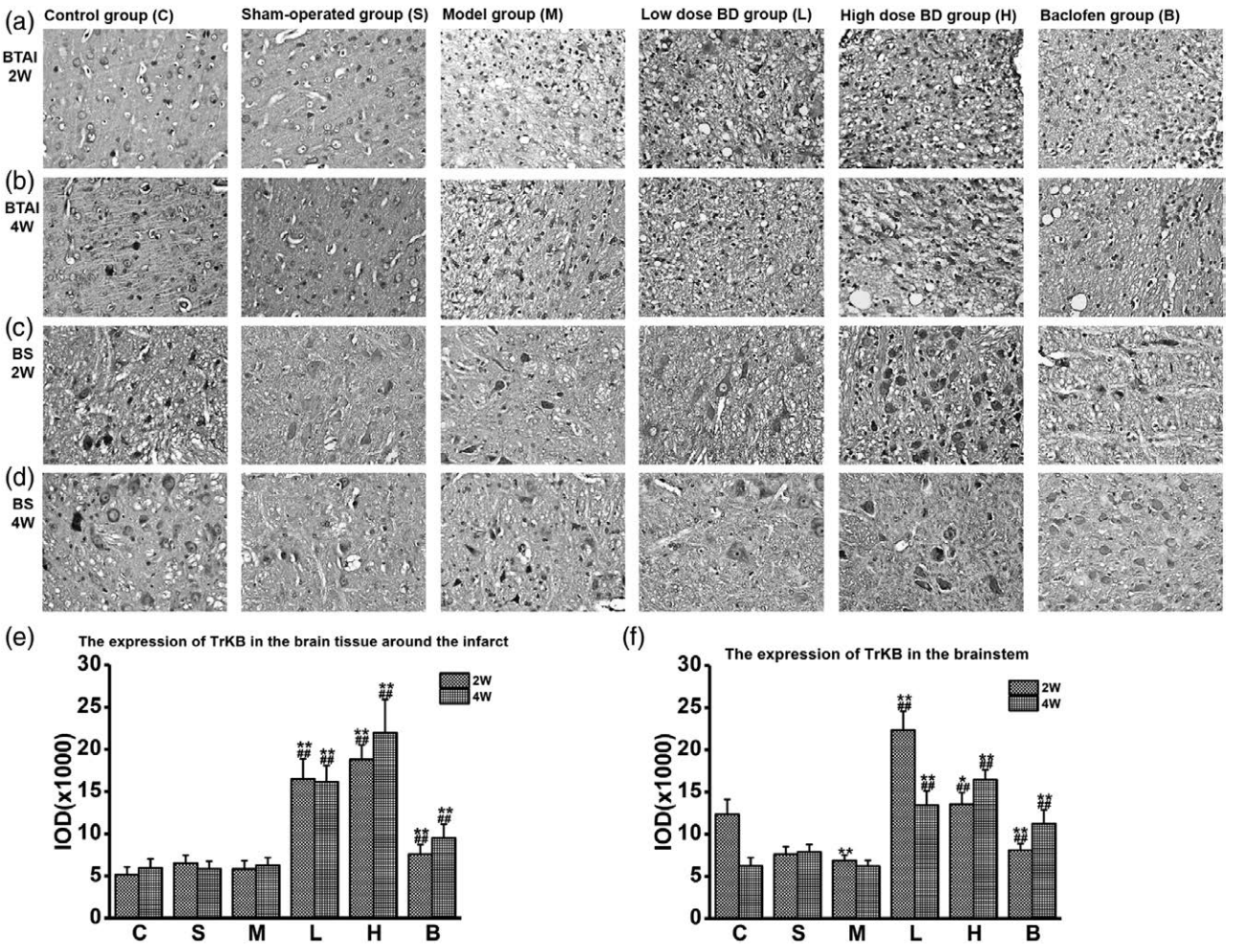


Effects of Baishaoluoshi Decoction on BDNF expression in PSS rat models. Protein expression levels of BDNF in brain tissues around the infarct (BTAI) and brainstem (BS) (2 and 4 weeks after intervention) were detected by immunohistochemical staining (×400). IOD was adopted evaluate the expression levels of positive cells. C, control group (normal saline); S, sham-operated group (normal saline); M, model group (normal saline); L, low-dose Baishaoluoshi Decoction group (5.4 g/kg); H, high-dose Baishaoluoshi Decoction group (10.8 g/kg); and B, baclofen group (5.4 mg/kg). $^{*}P<0.05$ and $^{**}P<0.01$ compared to the control group, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to the model group. ($n=5$ for every group). PSS, poststroke spasticity.

chloride. Engaging $GABA_AR$ leads to an efflux of chloride into the extracellular domain, leading to neuronal excitement [28,29]. Excitatory motoneurons lead to hyper-stretch reflexes, leading to hemiplegia limb spasticity [30]. Henrike Beverungen and colleagues [25] found that rehabilitation decreases spasticity through the BDNF-KCC2 pathway in spinal cord injury (SCI) rat models. After complete SCI, the SCI rat models were subjected to 60 min bicycling session for 4 weeks from day 5. Bicycling rehabilitation increased BDNF and KCC2 expression levels in the motoneuronal membrane, which was associated with decreased hyperreflexia and spasticity. However, blocking BDNF or KCC2 activity during bicycling rehabilitation reversed this effect. KCC2 levels in brain tissues around the infarct and brainstem in PSS rat models were also decreased at 2 weeks, but rebounded at 4 weeks.

Maladaptive synaptic plasticity-associated RST hyperexcitability in the brainstem is a significant pathophysiological factor for PSS. Cortical reticular tract was damaged after ischemic stroke induced by MCAO. Subsequently, there was an increase in the excitability of brainstem reticular system and its declining RST, due to loss of descending inhibition from cortical reticular tract [6]. Therefore, we detected the expression levels of KCC2 in the dorsal region of the brainstem. KCC2 expression was shown to be decreased at 2 weeks but rebounded at 4 weeks in the brainstem. Low-dose and high-dose Baishaoluoshi Decoction restored KCC2 expression. Previously, KCC2

Fig. 4



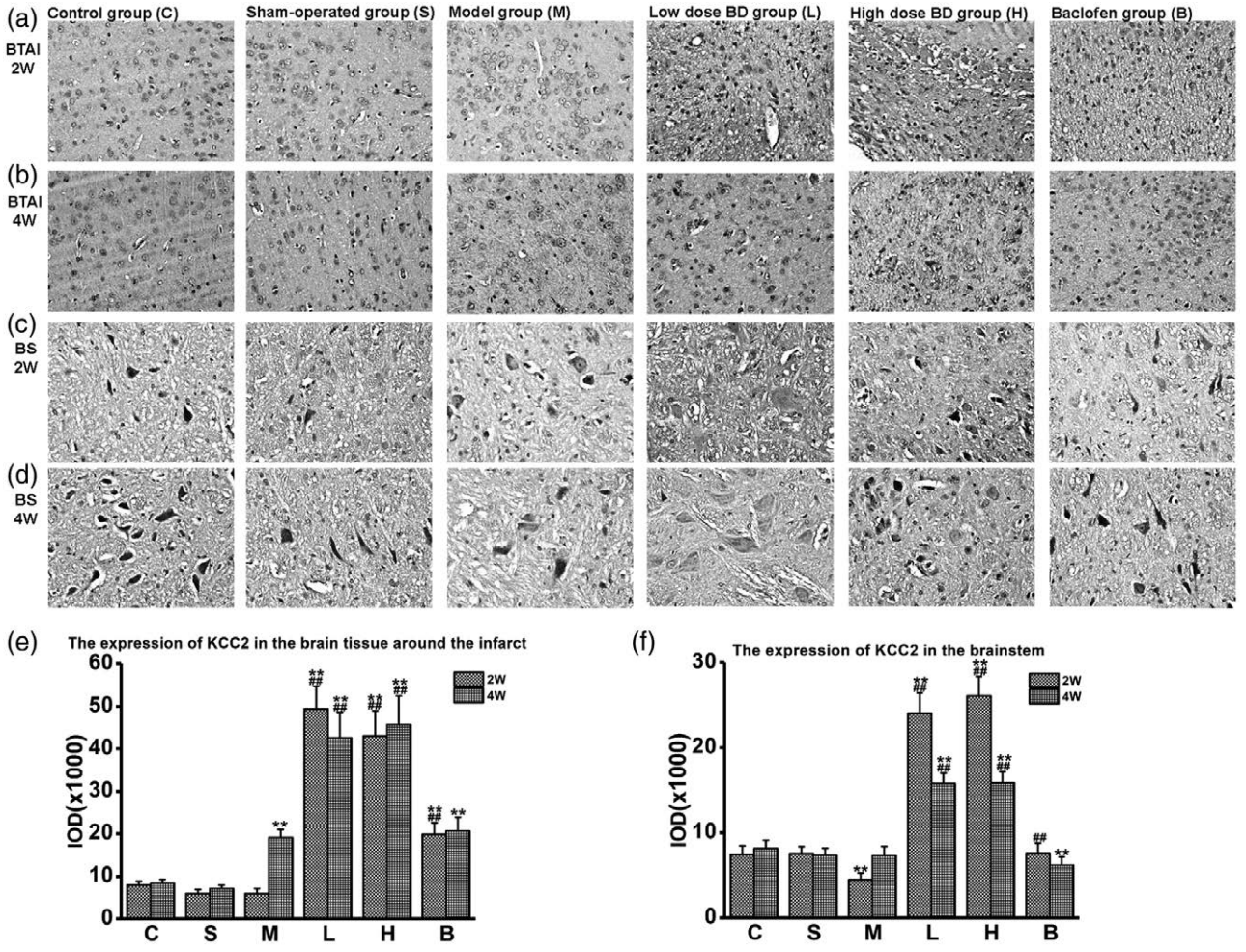
Effects of Baishaoluoshi Decoction on expression levels of TrKB in PSS rat models. Protein expression levels of TrKB in brain tissues around the infarct (BTAI) and brainstem (BS) (2 and 4 weeks after intervention) were detected by immunohistochemical staining (×400). IOD was adopted to evaluate the expression levels of positive cells. C, control group (normal saline); S, sham-operated group (normal saline); M, model group (normal saline); L, low-dose Baishaoluoshi Decoction group (5.4 g/kg); H, high-dose Baishaoluoshi Decoction group (10.8 g/kg), and B, baclofen group (5.4 mg/kg). $^{*}P<0.05$ and $^{**}P<0.01$ compared to control group, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to the model group. ($n=5$ for every group). PSS, poststroke spasticity.

was shown to regulate neuronal excitability and ameliorate synaptic plasticity. Therefore, we postulated that KCC2 is strongly involved in the hyperexcitability of motoneuron and RST in brain tissues around the infarct and brainstem, leading to hyperreflexia and spasticity.

Regulation of KCC2 is dependent on TrkB activation by BDNF. The overexpression of BDNF was shown to significantly elevate KCC2 mRNA expression levels *in vivo* [31]. Gene knockout of the BDNF receptor, TrkB, strongly suppresses the KCC2 mRNA expression levels [32]. Boulenguez *et al.* [26] found that the expression of KCC2 in motor neurons was reduced by 84% after SCI. Subsequently, $GABA_AR$ was reversed to depolarization from hyperpolarization, which enhanced motor neuron excitability and hemiplegia limb spasticity. Intrathecal BDNF injection downregulated KCC2 and alleviated spasticity. Beverungen *et al.* [25] found that rehabilitation inhibited spasticity in SCI rat models through the BDNF/TrKB-KCC2 pathway. In this study, KCC2 expression levels were decreased at 2 weeks, but rebounded at 4 weeks in PSS rat models. After treatment with low-dose and high-dose Baishaoluoshi Decoction, KCC2 levels did not decrease, but were significantly elevated in the brain tissue around the infarct and brainstem.

In conclusion, Baishaoluoshi Decoction relieved neuronal as well as synaptic damage and ameliorated hemiplegia limb spasticity in PSS rat models by upregulating the BDNF/TrKB-KCC2 pathway. Therefore, Baishaoluoshi Decoction may be a potential therapeutic agent for PSS.

Fig. 5



Effects of Baishaoluoshi Decoction on expression levels of KCC2 in PSS rat models. Protein expression levels of KCC2 in brain tissues around the infarct (BTAI) and brainstem (BS) (2 and 4 weeks after intervention) were detected by immunohistochemical staining (×400). IOD was adopted to evaluate the expression levels of positive cells. C, control group (normal saline); S, sham-operated group (normal saline); M, model group (normal saline); L, low-dose Baishaoluoshi Decoction group (5.4 g/kg); H, high-dose Baishaoluoshi Decoction group (10.8 g/kg); and B, baclofen group (5.4 mg/kg). $^{*}P<0.05$ and $^{**}P<0.01$ compared to the control group, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to the model group ($n=5$ for every group). PSS, poststroke spasticity.

Acknowledgements

This study was financially supported by the China Postdoctoral Science Foundation (grant number 2020M682571); Natural Science Foundation of Hunan Province China (grant number 2019JJ50343); The Scientific Research Project of Education Department of Hunan Province (grant number 19C1423); and the Foundation of China Center for Evidence Based Traditional Chinese Medicine (grant number 2019XZZX-NB004).

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

L.X., Y.X., G.M., R.F., and T.Y. performed the experiments, analyzed the data and wrote the article. D.W. and J.G. designed the study and revised the article. S.C., S.Z., and D.L. interpreted the results and revised the article. J.J. and J.H. analyzed the data. All authors read and approved the final article.

Ethical approval for the use of experimental animals was obtained from the Laboratory Animal Welfare and Ethics Committee, Hunan Normal University (Changsha, Hunan, China).

Conflicts of interest

There are no conflicts of interest.

References

1 GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2019; **18**:439–458.
2 Kuo CL, Hu GC. Post-stroke spasticity: a review of epidemiology, pathophysiology, and treatments. *Int J Gerontol* 2018; **12**:280–284.

3 Schinwelski MJ, Sitek EJ, Wąż P, Sławek JW. Prevalence and predictors of post-stroke spasticity and its impact on daily living and quality of life. *Neurol Neurochir Pol* 2019; **53**:449–457.
4 Lee KB, Hong BY, Kim JS, Sul B, Yoon SC, Ji EK, *et al*. Which brain lesions produce spasticity? An observational study on 45 stroke patients. *PLoS One* 2019; **14**:e0210038.
5 Raluy-Callado M, Cox A, MacLachlan S, Bakheit AM, Moore AP, Dinet J, Gabriel S. A retrospective study to assess resource utilization and costs in patients with post-stroke spasticity in the United Kingdom. *Curr Med Res Opin* 2018; **34**:1317–1324.
6 Li S, Chen YT, Francisco GE, Zhou P, Rymer WZ. A unifying pathophysiological account for post-stroke spasticity and disordered motor control. *Front Neurol* 2019; **10**:468.
7 Ha SY, Han JH, Ko YJ, Sung YH. Ankle exercise with functional electrical stimulation affects spasticity and balance in stroke patients. *J Exerc Rehabil* 2020; **16**:496–502.
8 Raol YH, Joksimovic SM, Sampath D, Matter BA, Lam PM, Kompella UB, *et al*. The role of KCC2 in hyperexcitability of the neonatal brain. *Neurosci Lett* 2020; **738**:135324.
9 Chen B, Li Y, Yu B, Zhang Z, Brommer B, Williams PR, *et al*. Reactivation of dormant relay pathways in injured spinal cord by KCC2 manipulations. *Cell* 2018; **174**:1599.
10 Olde Engberink AHO, Meijer JH, Michel S. Chloride cotransporter KCC2 is essential for GABAergic inhibition in the SCN. *Neuropharmacology* 2018; **138**:80–86.
11 Nakamura K, Moorhouse AJ, Cheung DL, Eto K, Takeda I, Rozenbroek PW, Nabekura J. Overexpression of neuronal K+-Cl- co-transporter enhances dendritic spine plasticity and motor learning. *J Physiol Sci* 2019; **69**:453–463.
12 Lee-Hotta S, Uchiyama Y, Kametaka S. Role of the BDNF-TrkB pathway in KCC2 regulation and rehabilitation following neuronal injury: a mini review. *Neurochem Int* 2019; **128**:32–38.
13 Xie L, Wu D, Fang R, Zhang F, Li H, Zhou S. Effect of Baishao Luoshi Decoction on Spasticity after Ischemic Stroke. *Journal of Hunan University of Chinese Medicine* 2018; **38**:77–81.
14 Zhu X, Hu H, Li Z, Lin R, Mao J, Chen L. Gua Lou Gui Zhi decoction attenuates post-stroke spasticity via the modulation of GABAB receptors. *Mol Med Rep* 2015; **12**:5957–5962.
15 Bohannon RW, Smith MB. Interrater reliability of a Modified Ashworth Scale of muscle spasticity. *Phys Ther* 1987; **67**:206–207.
16 Chen CL, Chen CY, Chen HC, Wu CY, Lin KC, Hsieh YW, Shen IH. Responsiveness and minimal clinically important difference of Modified Ashworth Scale in patients with stroke. *Eur J Phys Rehabil Med* 2019; **55**:754–760.
17 Payne JA. Functional characterization of the neuronal-specific K-Cl cotransporter: implications for K+ (0) regulation. *Am J Physiol* 1997; **273**:C1516–C1525.
18 Goutierre M, Al Awabdh S, Donneger F, François E, Gomez-Dominguez D, Irinopoulou T, *et al*. KCC2 regulates neuronal excitability and hippocampal activity via interaction with task-3 channels. *Cell Rep* 2019; **28**:91–103.e7.
19 Chamma I, Chevy Q, Poncer JC, Lévi S. Role of the neuronal K-Cl co-transporter KCC2 in inhibitory and excitatory neurotransmission. *Front Cell Neurosci* 2012; **6**:5.
20 Côme E, Heubl M, Schwartz EJ, Poncer JC, Lévi S. Reciprocal regulation of KCC2 trafficking and synaptic activity. *Front Cell Neurosci* 2019; **13**:48.
21 Côme E, Marques X, Poncer JC, Lévi S. KCC2 membrane diffusion tunes neuronal chloride homeostasis. *Neuropharmacology* 2020; **169**:107571.
22 Awad PN, Amegandjin CA, Szczurkowska J, Carriço JN, Fernandes do Nascimento AS, Baho E, *et al*. KCC2 regulates dendritic spine formation in a brain-region specific and BDNF dependent manner. *Cereb Cortex* 2018; **28**:4049–4062.
23 Chudotvorova I, Ivanov A, Rama S, Hübner CA, Pellegrino C, Ben-Ari Y, Medina I. Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. *J Physiol* 2005; **566**:671–679.
24 Otsu Y, Donneger F, Schwartz EJ, Poncer JC. Cation-chloride cotransporters and the polarity of GABA signalling in mouse hippocampal parvalbumin interneurons. *J Physiol* 2020; **598**:1865–1880.
25 Beverungen H, Klaszky SC, Klaszky M, Côté MP. Rehabilitation decreases spasticity by restoring chloride homeostasis through the brain-derived neurotrophic factor-KCC2 pathway after spinal cord injury. *J Neurotrauma* 2020; **37**:846–859.
26 Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, *et al*. Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* 2010; **16**:302–307.
27 Liabeuf S, Stuhl-Gourmand L, Gackière F, Mancuso R, Sanchez Brualla I, Marino P, *et al*. Prochlorperazine increases KCC2 function and reduces spasticity after spinal cord injury. *J Neurotrauma* 2017; **34**:3397–3406.
28 Khirug S, Soni S, Saez Garcia M, Tessier M, Zhou L, Kulesskaya N, *et al*. Protective role of low ethanol administration following ischemic stroke via recovery of KCC2 and p75NTR expression. *Mol Neurobiol* 2021; **58**:1145–1161.
29 Ben-Ari Y, Khalilov I, Kahle KT, Cherubini E. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *Neuroscientist* 2012; **18**:467–486.
30 Plantier V, Sanchez-Brualla I, Dingu N, Brocard C, Liabeuf S, Gackière F, Brocard F. Calpain fosters the hyperexcitability of motoneurons after spinal cord injury and leads to spasticity. *Elife* 2019; **8**:e51404.
31 Aguado F, Carmona MA, Pozas E, Aguiló A, Martínez-Guijarro FJ, Alcantara S, *et al*. BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2. *Development* 2003; **130**:1267–1280.
32 Carmona MA, Pozas E, Martínez A, Espinosa-Parrilla JF, Soriano E, Aguado F. Age-dependent spontaneous hyperexcitability and impairment of GABAergic function in the hippocampus of mice lacking trkB. *Cereb Cortex* 2006; **16**:47–63.