Expression and role of CaMKII and Cx43 in a rat model of post-stroke depression

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Abstract. Expression of Ca^{2+}/CaM-dependent protein kinase II (CaMKII) and connexin 43 (Cx43) in a rat model of post-stroke depression (PSD) was investigated. Rats were separated into control group (10 rats underwent a sham operation and were not ligated after incision), PSD group (13 PSD rats) and KN93 group (12 rats were treated with KN93, an inhibitor of CaMKII, on the basis of the PSD group). After PSD modeling, Longa scoring was performed, and an open field test as well as a step-through test were carried out to observe rat behavior. RT-qPCR and western blot analysis were used to detect the expression of CaMKII and CX43 in the hippocampus tissue. On the 14th day, the Longa scores in the PSD and KN93 groups were higher than that in the control group (P<0.05), while on the 18th day, Longa score was higher in the PSD group than that in the control and KN93 groups, and higher in the KN93 group than that in the control group (both P<0.05). In the PSD group, the Longa score on the 18th day was significantly higher than that on the 14th day, whereas in the KN93 group, the Longa score on the 18th day was significantly lower than that on the 14th day (both P<0.05). Compared with the PSD group on the 18th day, the passive avoidance defects in the KN93 group were improved, and the frequency of activity in the open field test was significantly increased. On the 18th day, the expression levels of the mRNA and protein of CaMKII were higher in the PSD group than in the control group, whereas those of Cx43 were lower in the PSD group than those in the control group (P<0.05). The mRNA and protein expression levels of CaMKII in the KN93 group were lower than those in the PSD group, but higher than those in the control group. In PSD rats, CaMKII expression is upregulated, but Cx43 expression is downregulated, and both CaMKII and Cx43 may participate in PSD. The inhibitor of CaMKII, KN93, can improve the depression in PSD rats.

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Introduction

Post-stroke depression (PSD), one of the most common complications of stroke, refers to different degrees of depression, for >2 weeks, in patients with stroke. A study has shown that at least 60% of patients with stroke suffer from PSD (1), the clinical manifestations of which are similar to those of primary depression, such as low mood, loss of interest, slow behavior, insomnia/hypersomnia and guilt without a source (2). PSD is not conducive to the recovery of brain function, weakens the treatment effect and reduces the quality of life, and increases the mortality rate (3), the pathogenesis of which is still investigated. Studies have shown that the incidence of PSD is higher in patients with damage to the left brain, is decreased in 5-HT and NE, or derepression of dexamethasone (DST), and in elderly and female patients with stroke (4,5). Therefore, it is particularly important to clarify the pathogenesis of PSD and develop targeted therapeutic drugs and protocols, for improving the prognosis of PSD patients.

As an important material basis for learning and memory function, Ca^{2+}/CaM-dependent protein kinase II (CaMKII) is mainly present in postsynaptic densities in the hippocampus (6) and involved in glutamatergic excitatory transmission (7). Glutamate is an excitatory neurotransmitter that causes Ca^{2+} influx. When intracellular Ca^{2+} concentration is increased, Ca^{2+}/CaM binds to CaMKII to activate CaMKII and induce long-term potentiation (7,8).

Connexin 43 (Cx43) is mainly present in astrocytes and vascular endothelial cells and is involved in gap junction, maintaining direct communication between blood-brain barrier (BBB) and cells (10). If BBB is damaged, inflammatory factors and harmful substances enter the brain, inducing neuritic degeneration and brain damage which are related to the occurrence and development of PSD (11). Studies have shown that Cx43 expression is decreased and gap junction is widened in the hippocampus tissue and cortical areas in patients with depression (12,13). As an indicator for judging whether BBB is damaged, Cx43 is closely related to depression (14). A study has shown that the pathophysiological process of depression is closely associated with the glutamatergic system (9).

CaMKII, an important component of glutamatergic excitatory transmission, is speculated to have a relationship with depression. However, there are no reports on whether CaMKII

is indeed involved in PSD, whether BBB damage is related to PSD, and whether CaMKII affects Cx43 expression with Cx43 considered as an important indicator for judging BBB damage. Therefore, in the present study, CaMKII and Cx43 expression levels in PSD rats and the effect of CaMKII inhibitor on Cx43 were explored in order to provide a new understanding of the etiology of PSD and a theoretical basis for the treatment and development of PSD.

Materials and methods

Experimental animals. Thirty-five SPF male SD rats, 12-15 months of age and weighing 300-350 g, were provided by Changzhou Cavens Experimental Animal Co., Ltd., with license SCXK (Su) 2011-0003. After 1 week of conventional adaptive feeding (rats were fed with basal feed and were free to drink water and move; 12-h light/dark cycle), rats were screened according to their behavior, based on the open field test and step-through test. Ten rats were included in the control group, 13 rats in the PSD group and 12 rats in the KN93 group (rats were treated with KN93, an inhibitor of CaMKII, on the basis of the PSD group), based on the principle that the three groups of behavioral scores should be similar. The ages of rats in the three groups were 12.63±2.26, 13.21±2.19 and 13.87±2.77 months, respectively, and the body weights were 322.32±27.54, 330.21±31.23 and 326.53±28.32 g, respectively, without significant differences (P>0.05). The study was approved by the Ethics Committee of Wenzhou Seventh People's Hospital (Wenzhou, China).

Compound modeling of PSD. Compound modeling of PSD (15) is to establish a depression model after a stroke model. A rat model of ischemic stroke was established using the suture embolic method in the PSD and KN93 groups. Chloral hydrate (10%) was intraperitoneally injected at a dose of 300 mg/kg for anesthesia. The muscle was incised and separated at the center of the neck after the preoperative preparation, and the bilateral common carotid artery was permanently ligated with a line no. 9. Rats in the control group underwent a sham operation and were not ligated after incision. The rats were intraperitoneally injected with 3 units of penicillin for 3 consecutive days after operation to prevent infection and peritonitis. Longa scoring (16) was performed at the 6th hour after operation, and rats with a Longa score of 2-3 points were considered as a successful modeling of stroke. Twelve rat models of stroke were successfully established in the PSD and KN93 groups, respectively. After the rats with ischemic stroke were orphaned for 2 weeks, 2 weeks of unpredictable stress was performed, including behavioral limitation, foot shock, tail squeezing, thermal stimulation and a reversal of day and night. Fifty micrograms of KN93 were dissolved into 10 μl of normal saline containing 10% DMSO to obtain KN93 injection, which was intrathecally injected into the rats in the KN93 group. Equal amount of normal saline containing 10% DMSO was intrathecally injected into the rats in the PSD group. After 4 days of KN93 injection, the open field test and step-through test were carried out to observe rat behavior.

Materials and reagents. TransScript II All-in-One First-Strand cDNA Synthesis SuperMix for PCR and TransScript II SYBR-Green Two-Step RT-qPCR SuperMix kit (AH321-01 and AQ301-01; both from Transgen Biotech Co., Ltd.); RT-qPCR primers [Sangon Biotech (Shanghai) Co., Ltd.]; Trizol kit (10296028; Thermo Fisher Scientific, Inc.); RIPA lysis buffer, BCA kit and ECL chromogenic reagent (P0013C, P0012S, and P0018FS; all from Beyotime Institute of Biotechnology); monoclonal rabbit anti-rat CaMKII, monoclonal rabbit anti-rat Cx43, and monoclonal rabbit anti-rat β-actin antibodies, as well as polyclonal horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (ab5683, ab79010, ab179467, and ab6728; all from Abcam); KN93 (CSN11255; CSNpharm).

Table I. Longa score criteria.

Symptom	Score
No neurologic deficit	0 points
Failure to fully extend left forepaw, a mild focal neurologic deficit	1 point
Circling to the left, a moderate focal neurologic deficit	2 points
Falling to the left, a severe focal deficit	3 points
Not spontaneous walking and a depressed level of consciousness	4 points

Longa score. Longa scoring was performed on the 7th, 14th and 18th day, with a total score of 4 points. The higher the score, the more severe the neurological deficit was (16). The specific scoring standards are shown in Table I.

Open field test. The open field test was performed on the 7th, 14th and 18th day. Rats were exposed to white noise at 95 dB for 1 h, and then placed in the open field from the same corner (80 cm x 80 cm x 40 cm, a total of 25 squares). After they were adapted for 1 min, their activities in the open field within 5 min were photographed and recorded, including the square number of horizontal movements (four paws into the grid was considered as one time) and the vertical standing condition (two paws in the air and then putting them down was considered as one time). The autonomous activity frequency was the sum of all conditions.

Step-through test. The step-through test was performed on the 7th, 14th and 18th day. Rats were firstly placed in a dark room. Escaping to the bright room after an electric shock, and returning to the dark room was considered as one time. The test was stopped when rats stayed in the bright room for 2 min or the number of electric shocks reached 20 times. The number of electric shocks and the durations were recorded.

RT-qPCR. On the 18th day, RT-qPCR was performed using the TransScript II SYBR-Green Two-Step RT-qPCR SuperMix kit to detect CaMKII and Cx43 expression levels. Rats in each group were decapitated after anesthesia with 300 mg/kg 10% chloral hydrate. The hippocampus tissue was taken on an ice tray, placed in normal saline, precooled at 4˚C, and stored at 0˚C with the remaining blood washed away. TRIzol was used to extract total RNA, and the concentration and purity were detected using an UV spectrophotometer (S117578; Shanghai

Table II. Primer sequences.

Gene	Upstream primers	Downstream primers
β-actin	5'-CACGGCATTGTAACCAACTG-3'	5'-TCTCAGCTGTGGTGGTGAGG-3'
CaMKII	5'-AAGATGTGCGACCCTGGAATG-3'	5'-TGTAGGCGATGCAGGCTGAC-3'
Cx43	5'-TTGTTTCTGTCACCAGTAAC-3'	5'-GATGAGGAAGGAAGAGAAGC-3'

CaMKII, Ca^{2+}/CaM-dependent protein kinase II; Cx43, connexin 43.

Kemin Biological Technology Co., Ltd.). A260/A280 between 1.9 and 2.0 was considered as qualified. Twenty microliters of reverse transcription reaction system were prepared with 1 μg of total RNA, 4 μl of TransScript II All-in-One First-Strand cDNA Synthesis SuperMix for PCR, and 1 μl of gDNA Remover (Transgen Biotech Co., Ltd.) and RNase-free Water (Takara Biotechnology Co., Ltd.). The mixture was incubated at 50˚C for 15 min, and at 85˚C for 5 sec. With β-actin as an internal reference, the amplification reaction was performed, and the final system was 20 μl: 1 μl of cDNA, each 0.4 μl of upstream and downstream primers (10 μM), 10 μl of 2X TransScript Tip SYBR-Green qPCR SuperMix (Transgen Biotech Co., Ltd.) and RNase-free Water used to complement 20 μl. The two-step amplification conditions were as follows: 94˚C for 30 sec, 94˚C for 5 sec and 60˚C for 30 sec, for 40 cycles. The results were analyzed using the $2^{-\Delta\Delta Cq}$ method (17). Primer sequences are shown in Table II.

Western blot analysis of CaMKII and Cx43 protein expression levels. On the 18th day, western blot analysis was performed to detect the expression of CaMKII and Cx43. RIPA was used to lyse and extract the total protein from the hippocampus tissue, and BCA was used to measure its concentration. A total of 10 μl of protein was loaded per lane. Total protein was separated with 10% SDS-PAGE electrophoresis, transferred to the NC membrane, and blocked at 22˚C for 1 h with 5% skim milk (M230-42G-5PK; Beijing Jiehui Bogao Biotechnology Co., Ltd.). Rabbit anti-rat CaMKII (1:300) and Cx43 (1:400) primary antibodies were blocked overnight at 4˚C, with β-actin (1:4,000) as an internal reference. After the membrane was washed 3 times with PBS, the HRP-labeled goat anti-rabbit secondary antibody was diluted at 1:5,000, and incubated at room temperature for 2 h. The ECL chromogenic agent was incubated at room temperature and developed for 1 min. Two parallel experiments were conducted simultaneously, and gel imaged using the ImageJ software (National Institutes of Health). The gray value of each protein band was analyzed to calculate the relative protein content of target protein (the gray value of target protein/the gray value of internal reference protein).

Statistical analysis. SPSS 20.0 (IBM Corp.) was used in this study. Measurement data were expressed as mean ± SD. t-test was used for comparisons between two groups. Analysis of variance was used for comparisons between three or more groups, and LSD test was the post hoc test. Paired t-test was used for comparisons between two time-points in the same group, and Chi-square test for comparisons between multiple time-points in the same group. P<0.05 was considered to indicate a statistically significant difference.

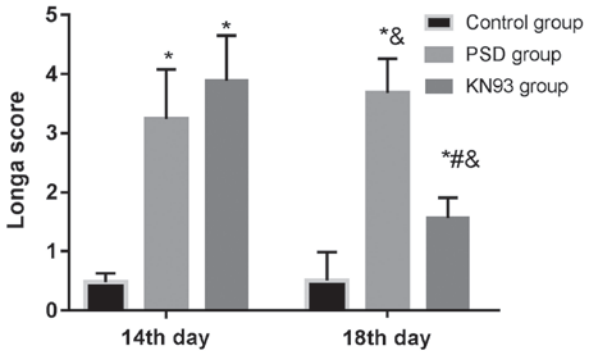

Figure 1. Comparison of Longa scores among groups. On the 14th day, the Longa score was not different between the PSD and KN93 groups (P>0.05), but the scores in the two groups were higher than that in the control group (both P<0.05). On the 18th day, the score in the PSD group was higher than that in the control and KN93 groups (P<0.05), and higher in the KN93 group than that in the control group (P<0.05). In the control group, the scores on the 14th and 18th day were not significantly different (P>0.05); in the PSD group, the score on the 18th day was significantly higher than that on the 14th day, whereas in the KN93 group, the score on the 18th day was significantly lower than that on the 14th day (both P<0.05). *P<0.05, compared with control group at the same time-point; #P<0.05, compared with PSD group at the same time-point; &P<0.05 compared with the 14th day in the same group. PSD, post-stroke depression.

Results

Comparison of Longa scores among groups. On the 14th and 18th day, the Longa scores in the control group were 0.48±0.15 and 0.51±0.48, respectively; in the PSD group were 3.24±0.84 and 3.68±0.58, respectively; and in the KN93 group were 3.89±0.76 and 1.57±0.34, respectively. On the 14th day, the Longa score was not different between the PSD and KN93 groups (P>0.05), but in both groups the Longa score was higher than that in the control group (both P<0.05). On the 18th day, the Longa score was higher in the PSD group than that in the control and KN93 groups (both P<0.05), and higher in the KN93 group than that in the control group (P<0.05). In the control group, the score was not significantly different between the 18th and 14th day (P>0.05). In the PSD group, the score on the 18th day was significantly higher than that on the 14th day, whereas in the KN93 group, the score on the 18th day was significantly lower than that on the 14th day (both P<0.05) (Fig. 1).

Open field test. On the 7th day, there were no differences in the results of the open field test between the control, PSD

Table III. Results of open field test.

Group	Autonomous activity frequency (times)			F value	P-value
	7th day	14th day	18th day		
Control group (n=10)	48±15	42±12	40±13	1.048	0.363
PSD group (n=12)	43±12	13±8[a,b]	11±9[a,b]	36.500	<0.001
KN93 group (n=12)	46±11	14±9[a,b]	39±11[b-d]	30.120	<0.001
F value	0.441	23.680	26.060		
P-value	0.648	<0.001	<0.001		

[a]P<0.05, compared with control group at the same time-point; [b]P<0.05, compared with the 7th day in the same group; [c]P<0.05, compared with PSD group at the same time-point; [d]P<0.05, compared with the 14th day in the same group. PSD, post-stroke depression.

Table IV. Results of step-through test.

Variables	Control group (n=10)	PSD group (n=12)	KN93 group (n=12)	F value	P-value
Electric shock number					
7th day	1.86±0.55	1.92±0.61	1.89±0.58	0.029	0.971
14th day	1.76±0.42	5.88±1.43[a,b]	6.35±1.11[a,b]	55.680	<0.001
18th day	1.98±0.61	6.98±1.56[a,b]	2.21±0.76[a-d]	78.530	<0.001
F value	0.428	52.560	103.800		
P-value	0.656	<0.001	<0.001		
Duration					
7th day	2.78±0.67	2.76±0.87	2.98±0.88	0.258	0.774
14th day	2.61±0.57	8.98±1.53[a,b]	9.23±1.21[a,b]	103.200	<0.001
18th day	2.87±0.87	9.16±1.44[a,b]	3.12±0.91[b-d]	117.800	<0.001
F value	0.342	92.450	149.500		
P-value	0.714	<0.001	<0.001		

[a]P<0.05, compared with control group at the same time-point; [b]P<0.05, compared with the 7th day in the same group; [c]P<0.05, compared with PSD group at the same time-point; [d]P<0.05, compared with the 14th day in the same group. PSD, post-stroke depression.

and KN93 groups (P>0.05). On the 14th day, there were no differences between the PSD and KN93 groups (P>0.05), but the results in the PSD and KN93 groups were lower than those in the control group (both P<0.05). On the 18th day, the results in the PSD group were lower than those in the control and KN93 groups (both P<0.05), but there was no difference between the KN93 and control groups (P>0.05). In the control group, the results were not significantly different on the 7th, 14th or 18th day (P>0.05). In the PSD group, the results were not different on the 14th and 18th day (P>0.05), whereas they were lower on the 14th and 18th day than those on the 7th day (both P<0.05). In the KN93 group, the results were lower on the 14th and 18th day than those on the 7th day (both P<0.05), but those on the 18th day were higher than that on the 14th day (P<0.05) (Table III).

Step-through test. On the 7th day, there was no difference in the number of electric shocks between the control, PSD and KN93 groups (P>0.05). On the 14th day, the number in the PSD and KN93 groups was higher than that in the control group (both P<0.05), but there was no difference between the PSD and KN93 groups (P>0.05). On the 18th day, the number in the PSD and KN93 groups was higher than that in the control group (both P<0.05), and was lower in the KN93 group than that in the PSD group (P<0.05). In the control group, the number was not significantly different on the 7th, 14th or 18th day (P>0.05). In the PSD group, the number on the 14th and 18th day was significantly higher than that on the 7th day (both P<0.05), but there was no difference between the number of shocks on the 14th and 18th day (P>0.05). In the KN93 group, the number on the 4th and 18th day was significantly higher than that on the 7th day (both P<0.05), and was significantly lower on the 18th day than that on the 14th day (P<0.05) (Table IV).

On the 7th day, there was no difference in the duration of electric shock between the control, PSD and KN93 groups (P>0.05). On the 14th day, the duration in the PSD and KN93 groups was higher than that in the control group (both P<0.05), but there was no difference between the PSD and KN93 groups (P>0.05). On the 18th day, the duration in the PSD group was higher than that in the control and KN93 groups (both P<0.05), but there was no difference between the control and KN93

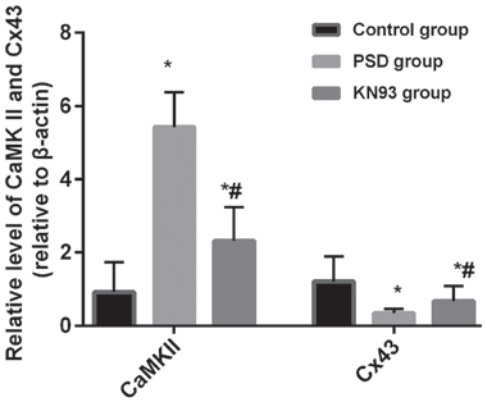

Figure 2. RT-qPCR detection of CaMKII and Cx43 expression. The relative expression of CaMKII mRNA in the PSD and KN93 groups was significantly higher than that in the control group (both P<0.05), and it was significantly lower in the KN93 group than that in the PSD group (P<0.05). The expression of Cx43 mRNA in the PSD and KN93 groups was significantly lower than that in the control group (both P<0.05), and it was significantly higher in the KN93 group than that in the PSD group (P<0.05). *P<0.05, compared with the respective control group; #P<0.05, compared with the respective PSD group. CaMKII, Ca^{2+}/CaM-dependent protein kinase II; Cx43, connexin 43; PSD, post-stroke depression.

groups (P>0.05). In the control group, the duration was not significantly different on the 7th, 14th or 18th day (P>0.05). In the PSD group, the duration on the 14th and 18th day was significantly higher than that on the 7th day (both P<0.05), but there was no difference on the 14th and 18th day (P>0.05). In the KN93 group, the duration on the 7th day was lower than that on the 14th and 18th day (both P<0.05), and was significantly lower on the 18th day than that on the 14th day (P<0.05) (Table IV).

RT-qPCR detection of CaMKII and Cx43 expression. The relative expression of CaMKII mRNA in the control, PSD and KN93 groups was 0.92±0.82, 5.42±0.96 and 2.32±0.92, respectively, and of Cx43 mRNA expression was 1.21±0.68, 0.34±0.12 and 0.67±0.42, respectively. The relative expression of CaMKII mRNA in the PSD and KN93 groups was significantly higher than that in the control group (both P<0.05), and was significantly lower in the KN93 group than that in the PSD group (P<0.05). The expression of Cx43 mRNA in the PSD and KN93 groups was significantly lower than that in the control group (both P<0.05), and was significantly higher in the KN93 group than that in the PSD group (P<0.05) (Fig. 2).

Western blot analysis of CaMKII and Cx43 expression. The relative protein expression of CaMKII in the control, PSD and KN93 groups was 0.89±0.34, 4.32±0.87 and 2.32±0.84, respectively, and of Cx43 was 1.11±0.87, 0.45±0.21 and 0.97±0.66, respectively. The relative protein expression levels of CaMKII in the PSD and KN93 groups was significantly higher than that in the control group (both P<0.05), and was significantly lower in the KN93 group than that in the PSD group (P<0.05). The relative protein expression of Cx43 in the PSD group was lower than that in the control and KN93 groups (both P<0.05), and there was no difference in the expression of Cx43 between the KN93 and PSD groups (P>0.05) (Fig. 3).

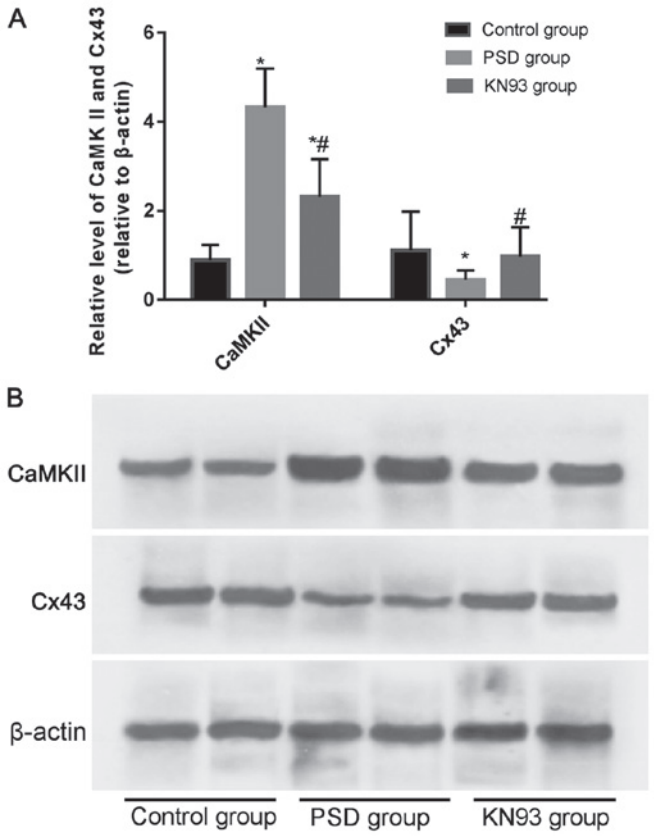

Figure 3. Western blot analysis of CaMKII and Cx43 expression. (A) The relative protein expression of CaMKII in the PSD and KN93 groups was significantly higher than that in the control group (both P<0.05), and it was significantly lower in the KN93 group than that in the PSD group (P<0.05). The expression of Cx43 in the PSD group was significantly lower than that in the control and KN93 groups (both P<0.05). (B) Western blots of CaMKII and Cx43 expression. *P<0.05, compared with the respective control group; #P<0.05, compared with the respective PSD group. CaMKII, Ca^{2+}/CaM-dependent protein kinase II; Cx43, connexin 43; PSD, post-stroke depression.

Discussion

With the aging of the population and the increase in social competition, the incidence, disability rate and mortality rate of PSD are increasing year by year, but the pathogenesis of it has not yet been elucidated, which may be related to brain injury site, neurotransmitter, endocrine, age and sex (18,19). CaMKII is an important component of the glutamatergic nervous system that plays an important role in the pathogenesis of PSD (20). There is also a study showing that the pathological process of depression is closely related to the integrity of BBB, and Cx43 is an important protein for maintaining BBB, and an important indicator for judging the integrity of BBB (21). Therefore, in the present study, the expression of CaMKII and Cx43 in the hippocampus tissue of PSD rats and their association with PSD were explored, in order to provide a new understanding for the etiology of PSD and a theoretical basis for the treatment and development of PSD.

On the 14th day, the Longa score was not different between the PSD and KN93 groups, but was higher in the two groups than that in the control group, indicating that the rat model of stroke was successfully established in the PSD and KN93 groups. On the 18th day, the score was higher in the PSD group than that in the control and KN93 groups, and higher in the KN93 group

than that in the control group. In the control group, the score was not significantly different between the 18th and 14th day; in the PSD group, the score was significantly higher on the 18th day than that on the 14th day; in the KN93 group, the score was significantly lower on the 18th day than that on the 14th day. The results suggest that after treated with KN93, the nerve damage of rats in the KN93 group was repaired, and the nerve function was recovered to a certain extent. However, stroke becomes more severe in the PSD group, and the increase in the Longa score indicates the aggravated neurological deficit.

Studies have shown that compared with healthy rats, PSD rats have less spontaneous and inquiry activities, less curiosity to new things and environment, lower reactivity to avoid injury, and more reaction time, because of fear of the new environment (22,23). On the 7th day, the results of the open field test and the step-through test were not different between the control, PSD and KN93 groups, suggesting that the grouping is reasonable, and ensures the comparability of subsequent test results. On the 14th day, compared with the control group, rats in the PSD and KN93 groups had different degrees of passive avoidance defects, significantly decreased reactivity to external stimuli, and significantly reduced number of activity in the open field test, indicating that different degrees of PSD occur in rats after PSD compound modeling, showing successful compound modeling. In a study on learning and memory levels of PSD rats, Wu *et al* (24) have found that compared with normal rats, PSD rats have weaker activity in the open field test, and prolonged reaction time in the step-through test. On the 18th day, compared with the PSD group, the passive avoidance defects of rats in the KN93 group were improved, and the number of activities was significantly increased in the open field test, indicating that the depression in rats was alleviated after treated with KN93. The results of RT-qPCR and western blot analysis showed that on the 18th day, compared with the control group, the PSD group had higher CaMKII expression but lower Cx43 expression, indicating that compared with healthy rats, CaMKII expression is upregulated but Cx43 expression is downregulated in PSD rats. Kozoriz *et al* (25) observed Cx43 expression in middle cerebral artery occlusion and the relationship with neuronal injury, and found that Cx43 protects the nerves in the model of stroke, and Cx43 expression is significantly increased in the short term after stroke, followed by a sustained and significant decrease. In this study, Cx43 expression was decreased on the 18th day in the PSD group, which is consistent with the findings of Kozoriz *et al* (25). CaMKII expression in the KN93 group was lower than that in the PSD group, but higher than that in the control group; Cx43 expression in the KN93 group was higher than that in the PSD group, but not different from that in the control group. The results suggest that KN93 can downregulate CaMKII expression, upregulate Cx43 expression, and alleviate the depression in PSD rats. Margrie *et al* (26) have found that KN93, an antagonist of CaMKII, can completely block the long-term depression in young chickens caused by low frequency stimulation, which is consistent with the results of the present study. However, the way in which CaMKII participates in depression was not previously explored in depth (26). The results of the present study show that CaMKII involved in PSD may be related to Cx43 protein expression, which provides a new idea for understanding the pathogenesis of depression and PSD. Therefore, it is speculated that KN93 is an inhibitor of CaMKII, so CaMKII expression is downregulated after KN93 treatment. In this study, KN93 was found to upregulate Cx43 expression, suggesting that CaMKII may negatively regulate Cx43. Cx43 is an important protein that maintains BBB (9). The increase in CaMKII expression in PSD rats inhibits Cx43 expression. The rat BBB is damaged, and harmful substances enter the brain, which cause damage to related nerves and participate in PSD. KN93 alleviates the progress of PSD by upregulating Cx43 expression, which needs more experiments for verifiction, and the specific regulatory pathway needs more research.

In this study, an ischemic stroke animal model was established using carotid artery ligation. This model has advantages, such as simple operation, low mortality, and long observation time after operation. The disadvantage is that it only causes incomplete cerebral ischemia, that is, chronic hypoperfusion. The pathological changes of cerebrovascular vessels in this model are close to the pathological basis of clinical stroke (15). Some of the stroke models in this study may be further developed into vascular dementia, but it is not excluded that these rats still have stroke. At the same time, long-term ligation of the bilateral common carotid arteries may lead to damage of the BBB, resulting in decreased expression of Cx43 and may be a pathological pathway for the reduction of clinical PSD Cx43 expression. Because carotid artery ligation simulates a stroke model, clinical stroke may also lead to BBB damage leading to decreased Cx43 expression, and decreased Cx43 expression induces depression in patients, which may be the possible pathological process of secondary depression in stroke patients. However, this is only our speculation based on other related research, and more sophisticated experiments are required. The severity of stroke does have an impact on the severity of depression. This is indeed not considered and is one of the limitations of this study. However, there is no difference in the open field test and step-through test on the 7th day in the three groups. On the 14th and 18th day, the results of the open field test and step-through test were decreased and were lower than those on the 7th day. The effects of stroke severity on the severity of depression were evenly distributed among the three groups. The data of this study were comparable, and more experimental methods will be applied in future studies.

Stroke consists of ischemic stroke and hemorrhagic stroke. No less than 60% of patients with stroke have ischemic stroke, and the incidence of ischemic stroke is much higher than that of hemorrhagic stroke (27,28). In this study, the rat model of stroke is ischemic stroke, without a rat model of hemorrhagic stroke, which saves cost and time of the test, but limits the universality of the results. Another limitation of this study is that MRI was not used to evaluate cerebral infarction, and the Longa score was the only means used, which may increase bias in outcomes due to subjective factors.

In this study, CaMKII was found to negatively regulate Cx43 expression and be involved in PSD. However, the specific signal transduction pathway has not been elucidated, and it needs to be further explored for harmful substances entering the brain and participating in the pathogenesis of PSD after BBB damage, due to the downregulation of Cx43 expression, as well as the specific biological role of the downstream target genes of Cx43.

In conclusion, CaMKII leads to PSD through regulating Cx43 protein expression and gap junction function, and the inhibitor of CaMKII, KN93, can improve depression.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ST and MJ performed PCR and western blot analysis. ST drafted the manuscript. TQ assisted with open field test. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Wenzhou Seventh People's Hospital (Wenzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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