TAT-W61 peptide reduces the inflammation and apoptosis by inhibiting the binding S100b to the V-domain of Rage during ischemic stroke

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Research

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Abstract

Ischemic stroke is a nervous system disease associated with high mortality and morbidity rates. Currently, there are no neuroprotective drugs for this disease. S100 calcium-binding protein b (S100b), which is abnormally expressed in the cerebrospinal fluid of patients, can bind to various other proteins and exacerbate the progression of stroke; receptor for advanced glycation end-products (Rage) is one of these proteins. To inhibit the occurrence of this interaction, a peptide (TAT-W61) based on the V-domain of S100b that interacts with Rage was produced. This study aimed to use TAT-W61 to reduce the levels of inflammation and apoptosis after ischemic stroke and to improve learning memory and motor dysfunction. In this study, S100b and Rage expression increased after ischemic stroke. Possible binding sites of S100b on Rage were mapped through molecular docking and polar–contact assays. In immunoprecipitation experiments, TAT-W61 inhibited the binding of S100b to Rage after ischemic stroke. TAT-W61 peptide, derived from Rage, could reduce the inflammatory and apoptotic response caused by ischemic stroke. It may also improve the infarct volume of the pathological injury and reduce the apoptotic rate. In particular, the TAT-W61 peptide could significantly improve learning, memory, and motor dysfunction in an ischemic stroke model. TAT-W61 peptide may help alleviate ischemic stroke injury.

Introduction

The incidence of stroke is increasing globally, with a corresponding increase in the associated mortality and morbidity rates[1], thereby creating a public health challenge recognized by the World Health Organization[2]. Changes at the cellular and molecular levels caused by the sudden interruption of blood flow and reperfusion are the leading causes of ischemic injury. When an ischemic stroke occurs, astrocytes are activated and release various inflammatory factors, including JAK, STAT3, Olig2, TGF-β, and Smad. S100b is an inflammatory factor and a Ca^{2+}-associated protein of the S100 family, which acts as a diagnostic biomarker for central nervous system injury[3, 4]. The S100 family plays a key role in the development of the central nervous system, as well as in diseases such as pneumonia[5], Alzheimer's disease[6], multiple sclerosis[7], spontaneous preterm birth and preeclampsia, and ischemic stroke[8, 9].

Astrocytes secrete S100b into the extracellular space, where it interacts with other proteins, leading to further damage. Evidence from the STRING database suggests that S100b interacts with several proteins, such as receptor of advanced glycation end-products (Rage), CACYBP, CAPZA1, CAPZA2, FGF2, GFAP, GUCA1A, MAPT and NF-κB, and TRP53. Among these, the interaction between Rage and S100b is amplified after ischemic stroke. Rage is often present on the surface of neurons and is abnormally overexpressed in some diseases, such as chronic obstructive pulmonary disease[10],atherosclerosis[11], diabetes[12], and ischemic stroke[13]. Rage is a multi-ligand receptor that can bind to high mobility group box 1, non-histone DNA-binding protein, Rage, and the S100/calgranulin family[14]. After ischemic stroke, the Rage expression levels in the ischemic penumbra and ischemic core are significantly elevated; however, specific inhibition of Rage activity can reduce brain damage[15, 16]. Rage belongs to the immunoglobulin (Ig) family and includes three domains: Ig-like V-type (residues 23–109), Ig-like C2-type 1 (residues 123–219), and Ig-like C2-type 2 (residues 233–315)[17–20]. The V-domain is the primary

receptor that participates in binding to ligands such as AGEs and proteins of the S100 family. The Rage–ligand binding activates downstream signaling pathways associated with the onset of neurodegeneration, chronic vascular inflammation, and cancer[20–22]. Activation of these signaling pathways can lead to inflammation, apoptosis, tumor growth, cell proliferation, and migration. This evidence suggests that blocking the binding of the Rage to the S100 family members and inhibiting intracellular signaling may slow down the progression of disease.

S100b was involved in disease progression by interacting with the Rage V-domain. The S100b and V-domain peptide were co-crystallized in a previous study [19]. The binding affinities assessment of S100b for the intact Rage V-domain was performed in fluorescence titration experiments, showing that S100b binds to a synthetic peptide made of 15 amino acids in the physical layer that corresponds to the Rage residues 54–68. These 15-amino acid-residues were named W61. The affinity of W61 toward S100b is much stronger than that of the Rage wild-type V-domain. However, the biological function of W61 *in vivo* remains unclear. The pathological mechanisms associated with W61 are also unclear. Studies are required to elucidate the mechanisms involved in the differential regulation of the Rage-mediated signaling by S100b. Based on W61, we designed a peptide named TAT-W61. In the present study, we aimed to use TAT-W61 to reduce the levels of inflammation and apoptosis after ischemic stroke and to improve learning memory and motor dysfunction.

Methods

MCAO

Adult male *C57BL/6J* mice (weight, 25–28 g) were anesthetized with 1% pentobarbital sodium (50 mg/kg, i.p.) and subjected to MCAO using nylon monofilament（Jia Ling ,China）. The nylon monofilament was slowly inserted into the lumen of the internal carotid artery through the common carotid artery and advanced from the bifurcation until it blocked the origin of the right middle cerebral artery[23, 24]. In the sham group, the nylon monofilament was not advanced, although it was introduced into the common carotid artery. The nylon monofilament was left in the artery for 60 min and then withdrawn for blood reperfusion. All animals were randomly divided into four groups: sham group, ischemia-reperfusion group, ischemia-reperfusion+TAT-W61 group, and ischemia-reperfusion+TAT-Scr group. The peptide was injected into the cortical M1 and hippocampal CA1 regions. The MNSS score was used to assess neurological deficits.

Co-immunoprecipitation

The interaction of endogenous S100b with the Rage protein was detected by Co-IP. Anti-S100b (Santa, USA) or anti-Rage (Santa, USA) antibody and protein A/G (Proteintech, USA) magnetic bead (30 μL; added ferrite beads to each sample) were treated with 1000 μg of total protein and incubated overnight at 4°C in a rotary mixer. Subsequently, these samples were placed on a magnetic rack for several minutes until the ferrite beads were fully absorbed. Each sample was mixed with 2× protein loading buffer and incubated in a metal bath at 100°C for 10 min; it was then analyzed by western blotting.

Analysis of brain infarct volume

The brain was removed at 24 h after MCAO, and the infarct volume was calculated according to a previously described method[25]. Briefly, the brain was sliced into 3-mm-thick coronal sections. The sections were stained with 1% 2,3,5-triphenyltetrazolium chloride (TTC) for 15 min and then fixed in 4% buffered formaldehyde solution. These brain sections were photographed, and the infarct part of each section was measured using ImageJ.

Western blot analysis

Proteins were extracted with the RIPA lysis buffer containing PMSF. Protein concentration was determined using the BCA kit (Beyotime Biotechnology , China). Post-preparation was performed using the RIPA lysis buffer and 5× protein loading buffer. Electrophoresis and electrotransfer were performed sequentially. After electrotransfer, protein samples were loaded onto SDS-PAGE gels (Beyotime Biotechnology , China) and transferred to PVDF (Pall , USA) membranes. After blocking with 5% skimmed milk, the samples were incubated with primary antibodies against S100b (Abcam , UK), Rage (Abcam , UK) , IL-1β (Cell Signaling Technology , USA), TNF-α (Cell Signaling Technology , USA) , IL-6 (Santa , USA), Bcl2 (Cell Signaling Technology , USA) , Bax (Cell Signaling Technology , USA) , cleaved caspase-3 (1:1000 dilutions; Abclonal), caspase-3 (Cell Signaling Technology , USA) , and NF-κB (Cell Signaling Technology , USA) at 4°C overnight. Subsequently, the membranes were incubated with mouse or rabbit secondary antibodies for 2 h at room temperature and analyzed with ImageJ.

HE staining

At 24 h after surgery, the mice were euthanized with pentobarbital sodium. The mice were treated with 0.9% saline followed by 4% paraformaldehyde. Mice brains were fixed with 10% paraformaldehyde. Paraffin-embedded brains were cut into 5-μm sections. The slices were stained with HE Kit (Beyotime Biotechnology , China) and observed and photographed under an ordinary microscope.

Behavioral tests

To assess mice memory and spatial learning ability, we performed the Morris water maze experiment 7 days after MCAO. Before a formal experiment, the mice were subjected to water maze training for 4 consecutive days, including 60 s of being submerged to ascertain mice ability to find an underwater platform. The mice were randomly assigned a starting position. Mice that failed to find the platform within the specified time were guided to the platform, where they remained for 20 s. The fourth day was the testing day, and mice performance was recorded.

In the open field test, the mice were placed in a box sized 45 cm × 45 cm × 45 cm. The box was divided into central and surrounding areas, and the mice were placed at the center of the site. Video tracking software was used to record the action route of the mice in the specified time, and ImageJ was used for statistical analysis.

Contextual fear conditioning test was performed based on a previously reported method. On the first day of training, the mice were allowed to freely explore the chamber without any stimulation for 5 min. The mice were placed in the box and observed for 6 min. After 3 min, the mice were subjected to three rounds of auditory and electric stimuli of 10 s each (80 dB, 5 kHZ), and an additional electric stimulus for 2 s (0.7 mA) at the eighth second of stimulation. After the end of each mouse, the excreta in the box were wiped with alcohol. Subsequently, the mice were placed back in the box for 8 min without being subject to any stimuli. Finally, the sound fear experiment was conducted; the mice were put into the white box for 5 min. After 3 min, they were exposed to three successive auditory stimuli of 10 s each. The freezing time of each mouse was recorded and used as an index to evaluate the memory of situational fear in mice.

The rotarod test was performed to measure coordination and balance. Before the test, the mice were placed on a stick for 4 min, then the rotation speed was increased from 5 to 40 r/min within 5 min. The time on the rod was recorded until the mouse fell off the rotarod. Each mouse underwent three trials, and the mean of the observed values was recorded[15, 26-28].

Flow cytometry analysis for cell apoptosis

Experiments were performed using Annexin-V-kFluor647/PI (Keygen BioTECH , China), and cells were collected after treatment and gently washed and blown twice with PBS. The collected cells were resuspended in 20 μl binding buffer, 5 μl Annexin-V FITC, and incubated for 5–10 min at 22-25℃. The samples were evaluated on a computer, and the results are presented.

Results

S100b and Rage expression increase after ischemic stroke

Single-cell sequencing results showed that S100b is mainly distributed in the astrocytes, while Rage is primarily distributed in microglia and neurons (**Fig. S1**). We used western blotting to detect S100b expression levels after stroke. S100b expression levels were higher at 24 h in the ischemia-reperfusion group than in the control group **(Fig. 1a)**. The Human Protein Atlas was used to verify the location of S100b[20], revealing that S100b expression is higher in the brain cortex and hippocampus than elsewhere **(Fig. 1b , c)**. A chord diagram of correlations among the 10 proteins most relevant to S100b in the STRING database revealed that Rage expression correlates strongly with S100b expression **(Fig. 1d)**. The Human Protein Atlas revealed that Rage is expressed in the cortex and hippocampus **(Fig. 1e)**. Western blot analysis revealed that Rage protein expression levels increased after middle cerebral artery occlusion **(**MCAO) compared with those in the sham group **(Fig. 1f)**. Rage expression increased in the lung **(Fig. S2)**. Overall, these results indicate that both S100b and RGAE are involved in brain disease progression.

Binding assay of S100b/Rage V-domain

To further explore the binding characteristics of the S100b/Rage V-domain, we performed molecular docking according to the S100b and Rage V-domain protein structures. The results showed that the Rage V-domain contains multiple possible binding sites for S100b **(Fig. 2a)**. Close contact assays of each docking confirmation showed that K37, R77, S74, and F85 in the Rage V-domain were critical for binding. Meanwhile, F87, E89, E2, H42, and F88 in S100b are essential for interaction **(Fig. 2b , c)**. Since F85 in the Rage V-domain and F87 and F88 in S100b are residues with a phenol-ring side chain, the interaction between S100b and Rage V-domain may be achieved by the π−π stacking together with polar contacts, originating from the arginine, serine, glutamic acid, and histidine residues in S100b or Rage V-domain.

Functional annotation of S100b and Rage by GO and KEGG analyses

The results of the GO analysis revealed variations in biological processes, including positive regulation of cytokine production and positive regulation of intracellular signal transduction **(Fig. 3a)**. Cell components of DEGs were markedly enriched in the nucleus, forming an apoptosis-inducing signaling complex **(Fig. 3b)**. Molecular function components were dramatically enriched in cytokine activity and cysteine-type endopeptidase activity involved in the apoptotic process **(Fig. 3c)**. The KEGG pathway revealed that all S100b and Rage were primarily enriched in the tumor necrosis factor (TNF) signaling, apoptosis, and necroptosis pathways **(Fig. 3d)**. These results suggest that S100b and Rage participate in the inflammatory and apoptotic response.

TAT-W61 can reduce the inflammatory and apoptotic response after stroke

To test the therapeutic effects of TAT-W61, we used this peptide to block the binding of S100b to Rage *in vivo*. Since high doses of the peptide may have a toxic effect on the blood–brain barrier, we selected a suitable amount (1 mg/kg) in the following experiments. Western blot results showed no difference between the sham and TAT-W61 treatment groups. Hematoxylin-eosin (HE) staining results showed no toxic effects of TAT-W61 **(Fig. S3)**. These results suggest that this dose of TAT-W61 is unlikely to be harmful to the brain. The mice were divided into the sham group, I/R group, I/R+TAT-W61 group, and I/R+TAT-Scr group. Co-immunoprecipitation **(**Co-IP) revealed that TAT-W61 could decrease their interactions.**(Fig. 4b)**. To further evaluate the inhibitory effect of TAT-W61, the expression of NF-κB was examined by western blotting; the injection of TAT-W61 reduced the expression level of NF-κB in the nucleus **(Fig. 4c , d)**. Furthermore, the expression levels of interleukin (IL)-1β, IL-6, and TNF-α were significantly reduced after the injection of TAT-W61. The expression levels of cleaved caspase-3 and Bax also reduced. In contrast, the expression levels of caspase-3 were similar between the intervention and sham groups. Treatment with TAT-W61 increased the expression of Bcl2, suggesting that TAT-W61 reduced the inflammatory **(Fig. 4e–h)** and apoptotic **(Fig. 4i–m)** effects caused by MCAO. These results suggest that TAT-W61 can effectively protect the brain from the consequences of ischemic stroke.

TAT-W61 can improve the pathology of ischemic stroke

To explore the effect of TAT-W61 peptide in the ischemic stroke mice model **(Fig. 5a)**, we stained mouse brain samples from four different groups with 2,3,5-triphenyltetrazolium chloride (TTC) solution. As shown in (**Fig. 5b)**, no infarct volume was found in the sham group. In the I/R group, the infarct volume accounted for approximately 30% of the brain tissue. The injection of TAT-W61 peptide significantly decreased the infarct area. HE staining was performed to detect the extent of pathological injury in these groups; brain tissues from mice after MCAO showed cell morphological changes with the nucleus reduced and increased pathological marker levels. After being treated with TAT-W61, the injury was significantly reduced **(Fig. 5c)**. Flow cytometry was applied to examine apoptotic cells. Early apoptosis rate was higher in the OGD/R group than in the sham group **(Fig. 5d)**. The TAT-W61 groups had a lower apoptosis rate than the model group. These data suggest that TAT-W61 could reduce the injury from MCAO and OGD/R.

TAT-W61 reduces behavior dysfunction after ischemic stroke

Behavioral tests were performed 7 days after MCAO **(Fig. 6a).** The neurological deficit score was higher in the I/R group than in the TAT-W61 injection group **(Fig. 6b)**. To examine the effects of TAT-W61 on learning memory and motor dysfunction in post-stroke mice, motor function was assessed using the rotarod test; findings in the experimental groups were compared with those in the sham group. MCAO mice exhibited apparent motor behavioral deficits; after treatment with TAT-W61, the retention time improved **(Fig. 6c)**. In the fear test and contextual fear conditioning test, the MCAO mice showed a shorter freezing time than the sham mice; the freezing time recovered after treatment with TAT-W61 **(Fig. 6d , e)**. The open field test was applied to evaluate spontaneous mobility, revealing that the mobility of the MCAO mice was reduced. In the I/R group, total distance, crossing time, and time in the center significantly decreased; these parameters were recovered after treatment with TAT-W61 **(Fig. 6f–i)**. Using the Morris water maze, we explored the effect of TAT-W61 on stroke-induced memory impairment in the mice. The MCAO mice, when treated with TAT-W61 peptide, were quicker than the control mice at finding the hidden platform. Memory assessment was performed on the fifth day when the platform was removed, and each mouse was allowed to swim freely for 60 s; during this trial, the number of platform crossings, percent of the distance, and time required were recorded. The index was lower in the TAT-W61 treatment group than in the I/R group **(Fig. 6 j–n)**. These results suggest that TAT-W61 improves learning, memory, and motor dysfunction after ischemic stroke.

TAT-W61 inhibits S100b binding to the V-domain of Rage and reduces inflammation and apoptosis in ischemic stroke

Ischemic stroke promotes the binding of S100b to the V-domain of Rage, resulting in the transfer of NF-κB from the cytoplasm to the nucleus, activating downstream inflammatory and apoptotic signaling pathways, and producing inflammatory and apoptotic factors. The small peptide TAT-W61 inhibits the binding of S100b to the extracellular segment V structural domain of Rage and reduces the progression of inflammatory and apoptotic responses, thereby mitigating further deterioration of ischemic brain injury **(Fig. 7)**.

Discussion

Our study demonstrated that TAT-W61 could block the binding of S100b to Rage V-domain and reduce the release of inflammatory and apoptotic proteins after ischemic stroke. Previous studies have reported that TAT-W61 can support much higher affinities for S100b than Rage V-domains, and that calcium is required for S100b to bind either to the Rage V-domain or W61 in isothermal titration experiments. We speculate that this phenomenon is related to calcium overload after ischemic stroke. If the opening of calcium channels after stroke is mitigated, the injury caused by the binding of S100b to Rage may be reduced.

Despite medical advances, there are no effective neuroprotective drugs for ischemic stroke. In recent years, the use of ligand–receptor binding to design drugs has been increasingly recognized by the drug research community[29]. At present, it is used in the treatment of thrombolysis and thrombectomy. However, due to the limited treatment time window, it is not available for all patients, highlighting the need for alternative treatments[30, 31]. Inflammation and neuronal apoptosis are complications of ischemic stroke[32]. Inflammatory mechanisms are essential in early ischemic glial cell injury and neuronal death secondary to ischemia[33]. In most cases, inflammation is caused by the release of cytotoxic microglia, necrotic cells, reactive oxygen species, and pro-inflammatory factors such as IL-1β and TNF-α[34]. Pro-inflammatory cytokines are an important trigger of neuronal cell injury and apoptosis[35]. Meanwhile, apoptosis plays an important role in cerebral ischemia-reperfusion. The mechanisms of apoptosis in stroke include death receptor attachment, DNA damage, protease activation, and ion imbalance. After a period of occlusion, restoring cerebral circulation can help re-establish tissue oxygenation, leading to reperfusion injury. Local inflammation and increased levels of mitochondria-derived reactive oxygen species exacerbate cellular damage[36]. Excess reactive oxygen species disrupt the integrity of mitochondrial membranes, interrupting the electron transport chain, triggering cytosolic component leakage from the cytoplasmic lysate, and ultimately leading to apoptosis[37]. In addition, reactive oxygen species play a role in post-stroke thrombosis[38]. There is a need for a new treatment to reduce the levels of inflammation and apoptosis in the ischemic penumbra.

In this study, based on the binding sites of W61, we designed a peptide called TAT-W61 which can reduce the extent of injury 24 h after ischemic stroke, including infarct volume, pathological injury severity, and inflammatory factor release and neuronal apoptosis rates, helping improve cognitive and motor dysfunction caused by ischemic stroke. These findings suggest that TAT-W61 significantly reduces the binding of S100b to the Rage V-domain and subsequent inflammatory and apoptotic responses, thereby reducing the extent of brain damage. TAT-W61 peptide may help improve the condition of stroke patients. Our future research aims to translate the present findings into clinical trials.

Abbreviations

MCAO: middle cerebral artery occlusion; I/R:Ischemia/Reperfusion; S100b:S100 calcium binding protein b; Rage:Receptor of advanced glycation end products; TTC:2,3,5-Triphenyltetrazolium Chloride;

OGD/R:Oxygen glucose deprivation/ Reperfusion; MWM:Morris Water Maze;

Declarations

Acknowledgements

Not applicable.

Author's contributions

YJS and NW initiated and designed the project; JL performed the main experiments, analyzed experimental results and drafted the article ; HL and NW performed bioinformatics analysis and structure analysis. XYM and DL took part in western blot.YLC,QF and ZZ performed Behavioral experiments. XRW took part in immunohistochemistry. All authors read and approved the fnal manuscript.

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Availability of data and materials

The data and materials in the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The project "TAT-W61 peptide reduces the inflammation and apoptosis by inhibiting the binding S100b to the V-domain of Rage during ischemic stroke" was examined and verified by Laboratory Animal Ethics Committee of Xuzhou Medical University in accordance with Guide to Laboratory Animal Ethics Examination of Xuzhou Medical University (201907A008). And relative animal experiments are permitted.

Consent for Publication

Not applicable.

Competing interests

The authors declare no conflict of interest that could prejudice the impartiality of the present research.

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Supplementary Information

Supplementary Information is not available with this version.

Figures

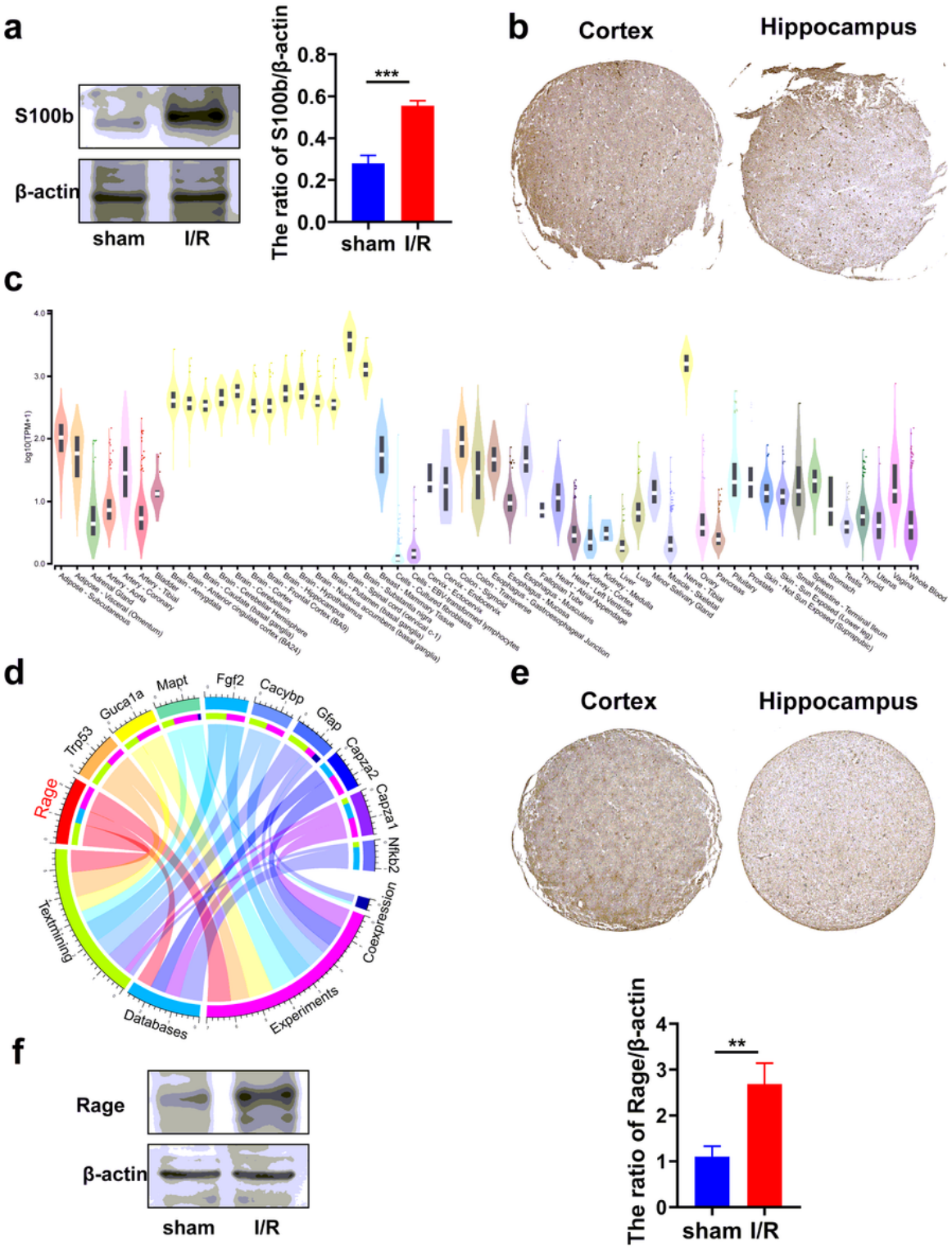


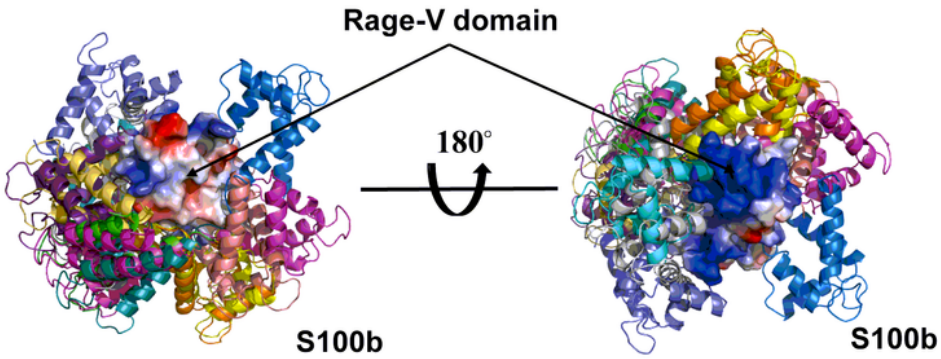
Figure 1

S100b and Rage expression levels increased after ischemic stroke

a Protein expression level of S100b was evaluated by western blot. **b** Expression of S100b in the Human Protein Atlas. **c** Expression of S100b in GTEx normal tissues. **d** Chord diagram reflecting the correlation of 10 most relevant proteins to S100b in the STRING database. **e** Expression of Rage in the Human Protein

Atlas. **f** Protein expression level of Rage was evaluated by western blot. n=3. ***p* < 0.01, and ****p* < 0.001. Data are represented as mean ± SD.

a



b

Predicted Binding Conformation	Contact Residue Pairs (Residue in Rage-V domain – Residue in S100b			
Conformation 1	K37-L44	R77-F87	S74-E89	R29-E2
Conformation 2	A40-E86	G40-A83		
Conformation 3	R77-E86	S74-E89		
Conformation 4	S65-H42	F85-F88	K39-E2	
Conformation 5	R77-E39	V78-H42		
Conformation 6	S111-S41	A41-K48		
Conformation 7	R116-S41	K37-E89		
Conformation 8	R77-H42	F85-F88		
Conformation 9	N81-E89			
Conformation 10	K37-E45	D73-E89	R77-E86	P33-H42
Conformation 11	R77-F87	L34-H42		
Conformation 12	Q24-E46	I26-S41	F85-F88	
Conformation 13	Y118-E45			
Conformation 14	R77-E86	K39-E45		
Conformation 15	S74-E89			

c

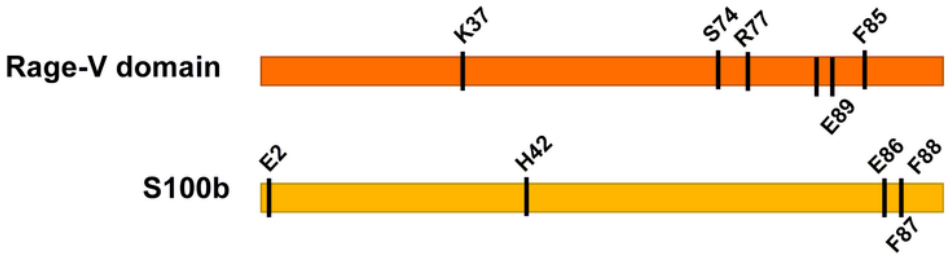


Figure 2

Binding model of S100b to Rage V-domain

a Docking model of S100b (PDB:4XYN) to Rage V-domain (PDB:2L7U). Rage V-domain was presented in the surface style and colored by charge (red for negative-charged surface, blue for positive-charge surface, and white for natural surface). S100b docking conformations were presented in cartoon style. **b** Close contact assay of the docking results. In each polar–contact pair, the first residue is from the Rage V-domain and the second residue is from S100b. Residues that are critical in the S100b/Rage V-domain are present in red. **c** Critical residues in S100b/Rage V-domain presentation as in **b** .

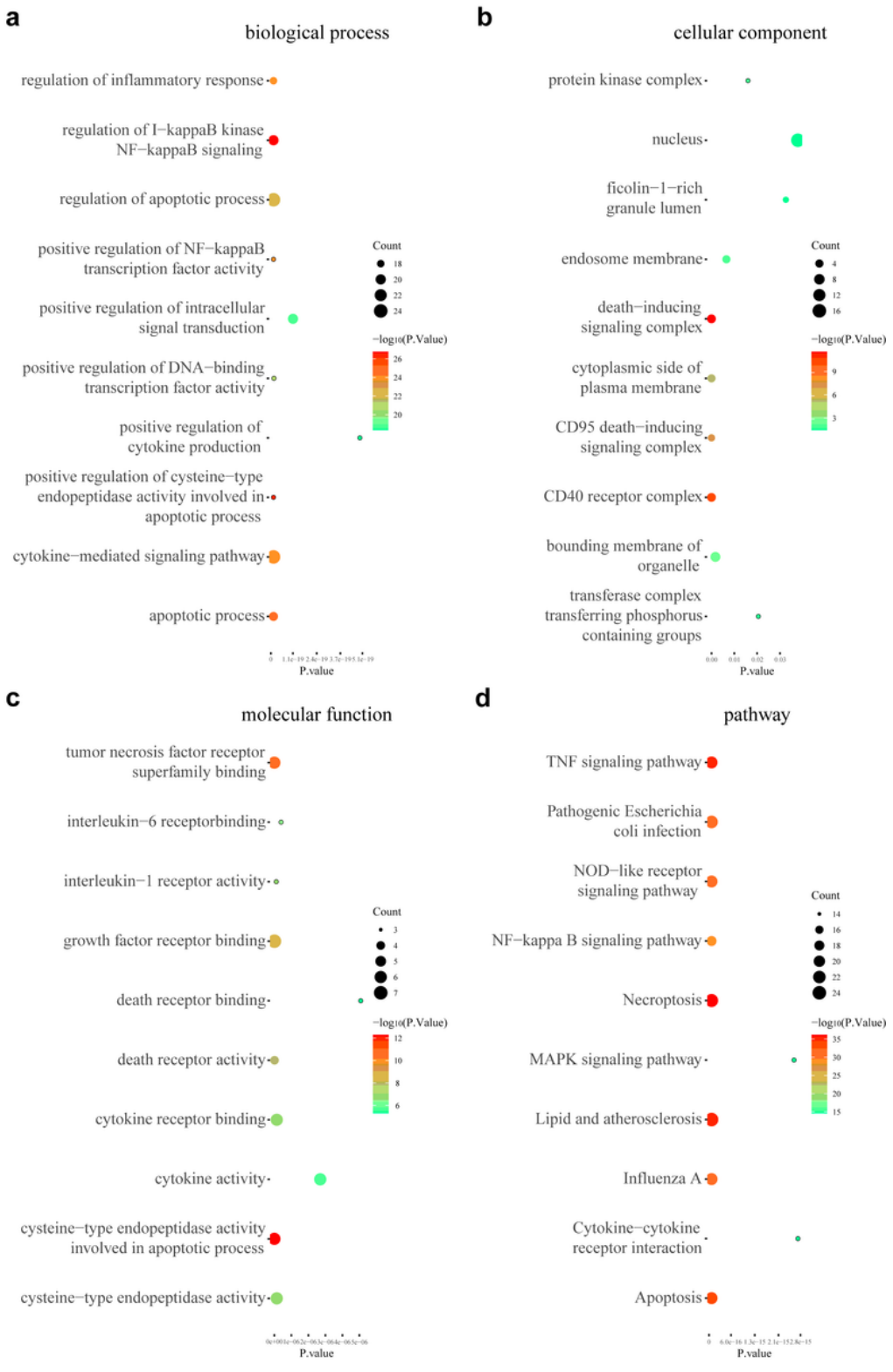


Figure 3

Functional annotation of S100b and Rage by GO and KEGG analyses

a-c Detailed information related to changes in the biological processes, cellular components, and molecular functions of S100b and Rage in atherosclerosis and control tissues through the GO enrichment analyses. **d** The KEGG pathway analysis of S100b and Rage. Representative images are shown.

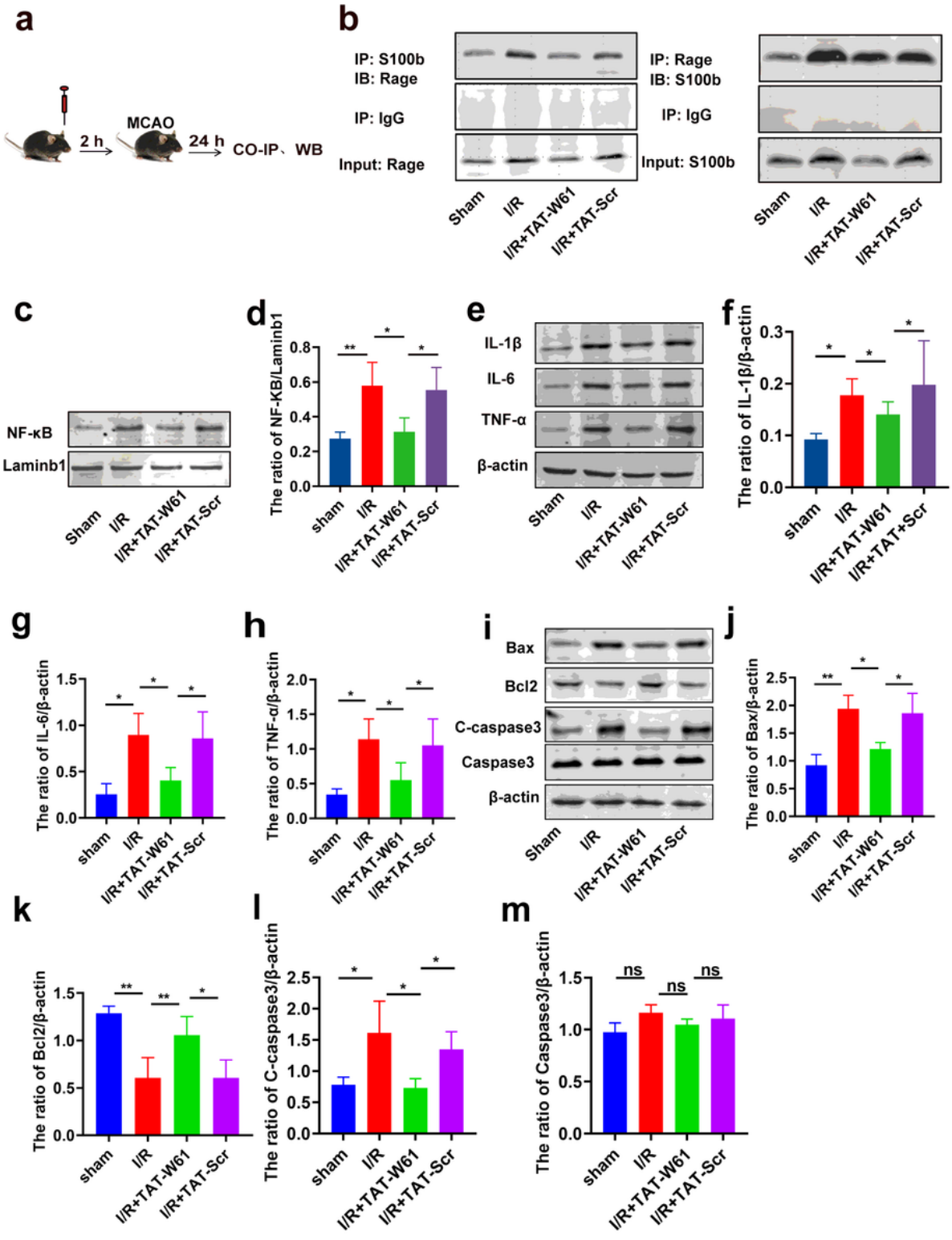


Figure 4

TAT-W61 reduces the inflammatory and apoptotic response after stroke

a Diagram capturing the experiment. **b** CO-IP assessment of the binding of S100b and Rage. **c–d** Protein expression level of NF-κB was evaluated by western blot. **e–h** Protein expression levels of IL-1β, IL-6, and TNF-α were determined by western blot. **i–m** Protein expression levels of Bax, Bcl2, cleaved caspase-3, and caspase-3 were determined by western blot. n = 3. ***p < 0.05, ****p < 0.01; ns, no significance. Data are represented as mean ± SD.

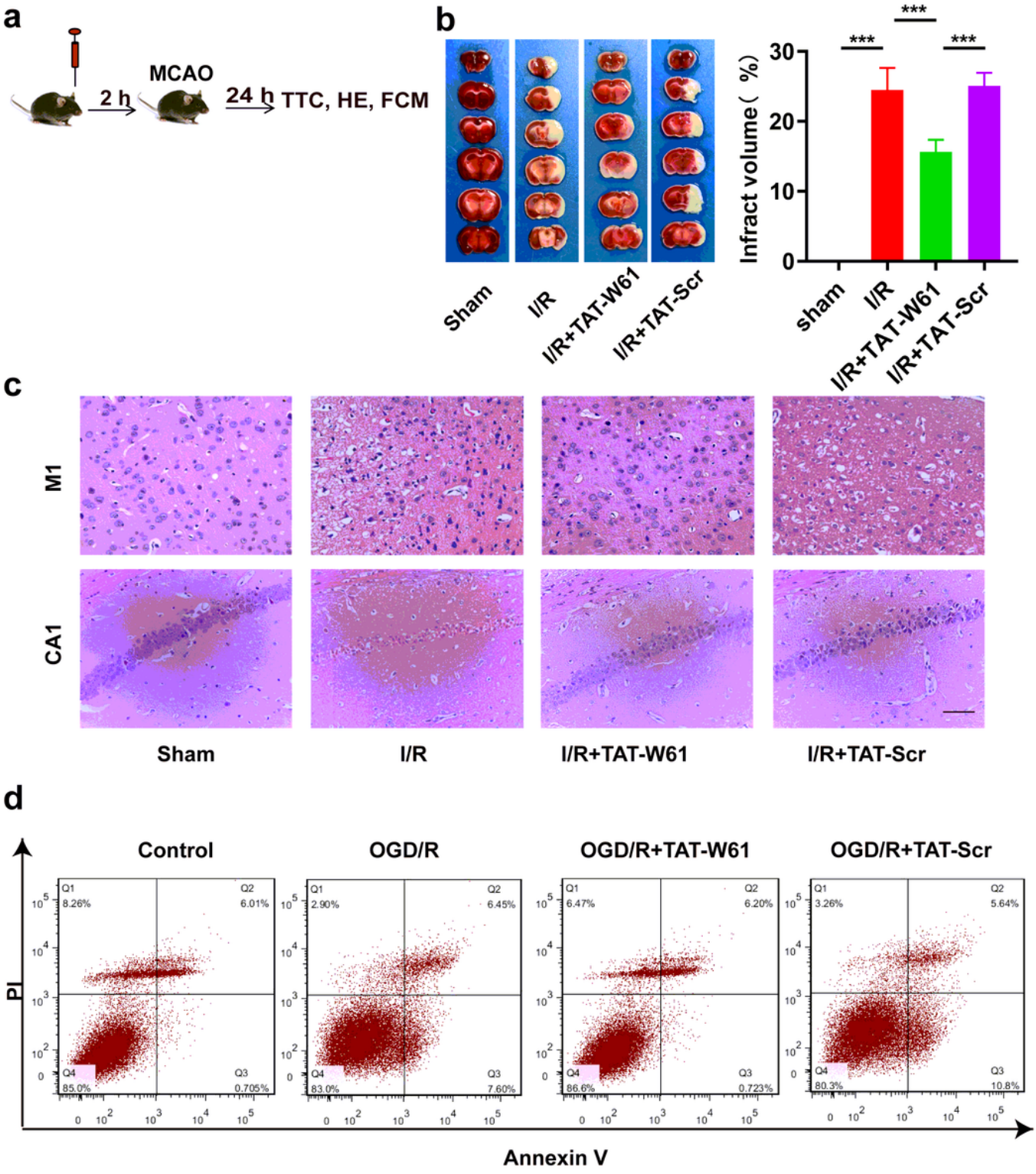


Figure 5

TAT-W61 inhibits the damage induced by stroke

a Diagram capturing the experiment. **b** TTC staining. The red part of the brain section represents the non-ischemic area, and the pale part represents the ischemic part. **c** HE staining detected pathological

changes in the cortex and hippocampus. Scale bar: 50 μm. **d** Flow cytometry image; n=3. ***$p < 0.001$. Data are represented as mean ± SD.

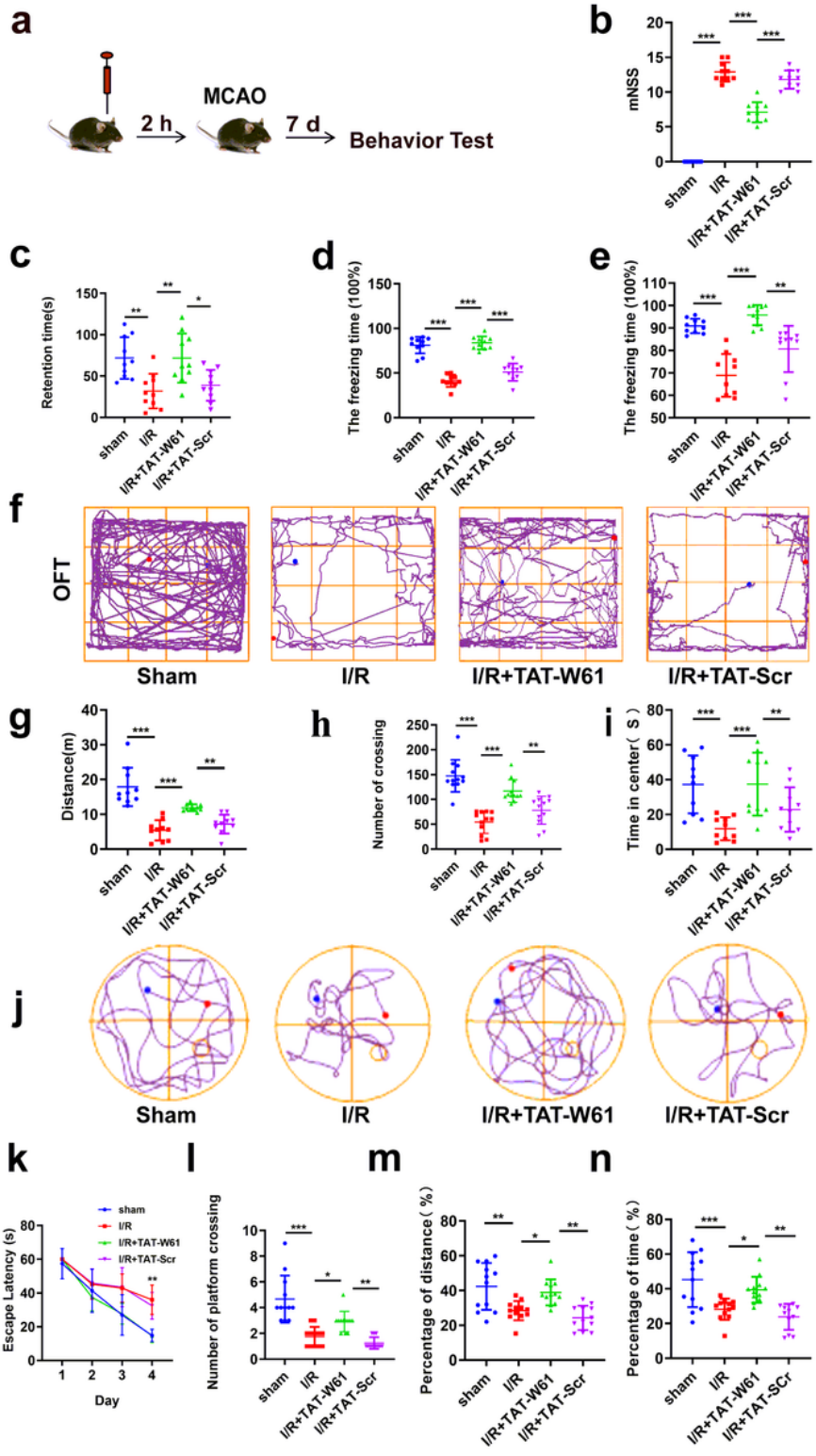


Figure 6

Function of TAT-W61 on stroke-induced behavior dysfunction

a Diagram capturing the behavioral experiment. **b** Neurological deficit scores for mice. **c** Rotarod test was performed to analyze the motor function of MCAO mice. **d-e** Percentage of freezing time in the context mode and in the cued mode of mice. **f-i** The trajectories of the mice in the open field test experiment, total distance moved in the field, time spent in the central area, and number of crossings. **j-n** The trajectory diagram of the water maze experiment, time to find the platform in the positioning navigation test, number of crossing the platform, and proportion of time and distance in the quadrant where the platform is located. n = 10. ***p* < 0.05, ****p* < 0.01, and *****p* < 0.001. Data are represented as mean ± SD.

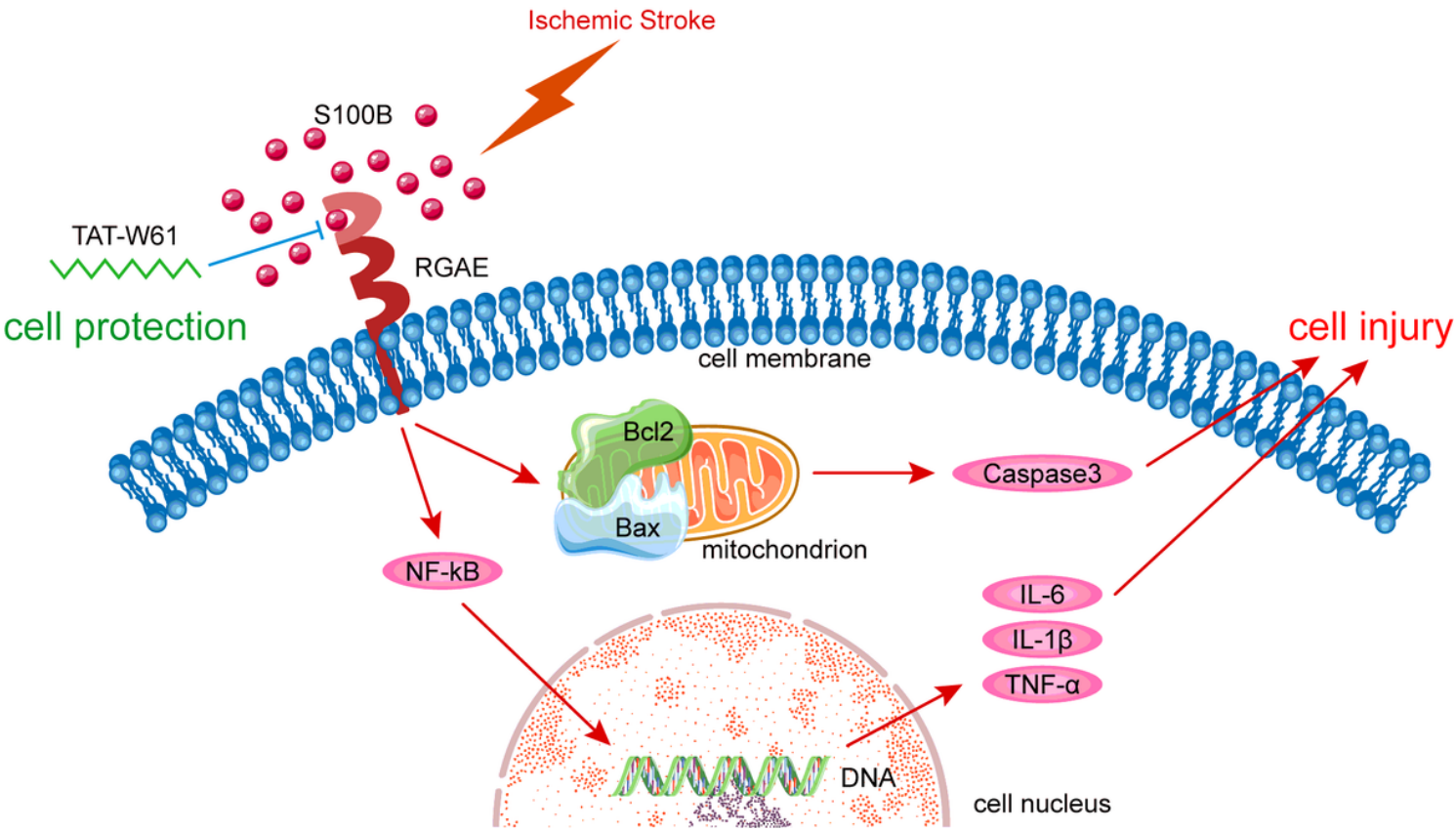


Figure 7

TAT-W61 inhibits S100b binding to the V-domain of Rage and reduces inflammation and apoptosis in ischemic stroke

Ischemia-reperfusion promotes the binding of S100b and the V-domain of Rage, resulting in the transfer of NF-κB from the cytoplasm to the nucleus and activating astrocytes and neurons, thus releasing inflammatory and apoptotic factors. TAT-W61 can inhibit the binding of S100b and the V-domain of Rage, reduce the transfer of NF-κB, inhibit inflammatory and apoptotic signaling pathways, and thus reduce damage after cerebral ischemia-reperfusion.