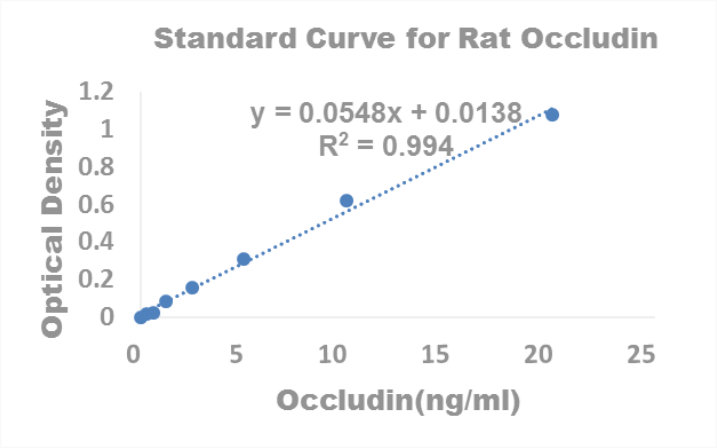
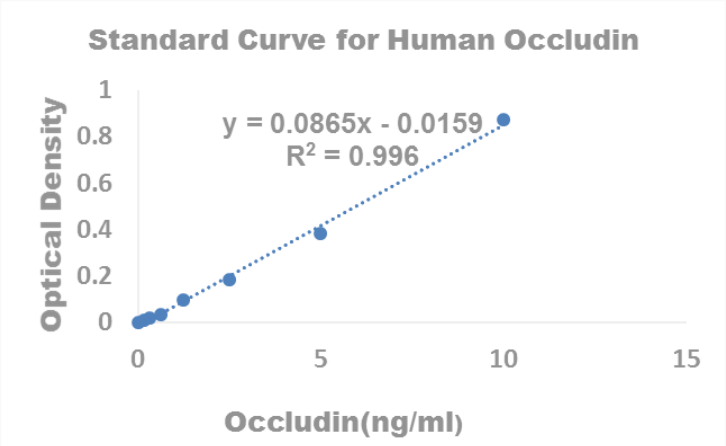
SUPPLEMENTAL MATERIAL

Detailed information on occludin antibodies for the ELISA assay

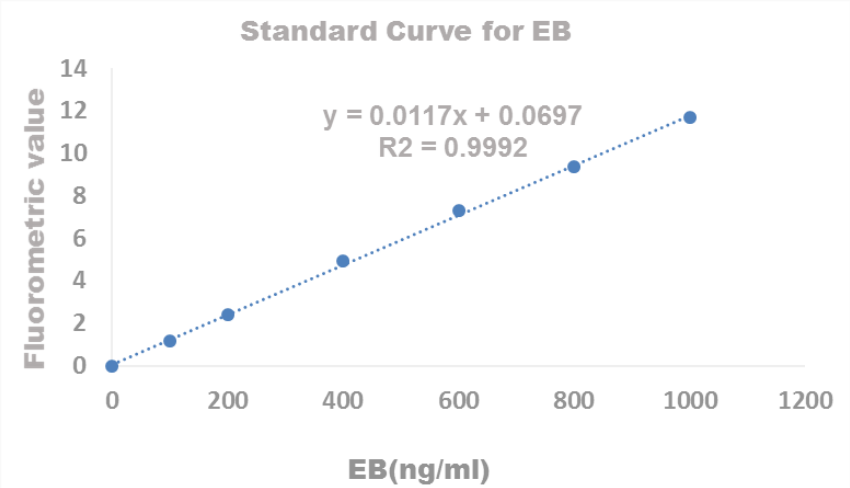
Both rat and human ELISA assay kits for occludin were purchased from USCN Life Science Inc., China. According to the information provided by the vendor, the specifics for the antibody for rats are: target sequences, Ala88~Arg269; sensitivity, 0.312ng/ml-20ng/ml; minimum, 0.112ng/ml; and specificity, 100%. The specifics for human kit are: target sequences, Ser370~Thr522; sensitivity, 0.156ng/ml-10ng/ml; minimum, 0.055ng/ml; and specificity, 100%. The standard calibration curves for rat and human occludin are shown below：





Evan’s blue assay to measure BBB damage

Evan’s blue dye binds to albumin in plasma and the complex enter into brain tissue through compromised BBB. Quantitative analysis of Evan’s blue dye in brain tissue is commonly used to measure the extent of BBB injury in animal models (Stroke, 2015, 46: 1344-1351; J Neurosci. 2013, 33: 19579-89). Evan’s blue dye (EB, 2% wt/vol in PBS, 3mL/kg) (Sigma, USA) was administered (I.V.) via tail vein at the onset of reperfusion. At the end of 2 h reperfusion, the rats were transcardially perfused with 250 mL cold PBS. The brain was then removed, and hemispheric tissues were homogenized in 1 ml 50% trichloroacetic acid. The contents of the dye were assessed by measuring the fluorescence intensity using Odyssey Infrared Imaging System (emission wavelength of 680nm). The total Evan’s blue content (ng/g) in each sample was calculated according to the external standard curve. The difference of dye contents in ischemic hemispheric tissue between Normoxia and NBO groups reflected the extent of BBB damage. The standard curve for Evan’s blue assay is shown below：



Supplemental Table I. **Baseline Data for 8 AIS patients and 8 healthy volunteers (Human Study I)**

Characteristic	AIS patients (n=8)	Healthy volunteers (n=8)
Age, y （means, range）	64.6(33-77)	50.6(40-69)
Female	3(37.5%)	4(50%)
Onset time	3.5(1-4.5)	N/A
Prior medical history		
Prior stroke	2(25.0%)	0(0.0%)
Myocardial infarction	0(0.0%)	0(0.0%)
Atrial fibrillation	2(25.0%)	0(0.0%)
Hypertension*	5(62.5%)	2(25.0%)
Diabetes	2(25.0%)	1(12.5%)
Malignancy	0(0.0%)	0(0.0%)

*P<0.05 AIS patients vs Healthy volunteers

Supplemental Table II. Baseline data for stroke patients in treatment and control groups (Human Study II)

Characteristic	NBO group (n=9)	Controls group (n=9)
Age, y （mean, range）	61.6(51-75)	58.0(47-70)
Female	3(33.3%)	2(28.6%)
Onset time	3.2(1-4.5)	3.0(1.2-4.5)
Admission NIHSS	12.0(5-20)	12.3(7-18)
Prior medical history		
Prior stroke	1(11.1%)	2(22.2%)
Myocardial infarction	1(11.1%)	0(0.0%)
Atrial fibrillation	1(11.1%)	0(0.0%)
Hypertension	6(66.7%)	7(77.8%)
Diabetes	5(55.6%)	3(33.3%)
Malignancy	0(0%)	0(0%)

Preclinical checklist information

Methodological and Reporting Aspects	Description of Procedures
Experimental groups and study timeline	√ The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study. √ An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated. √ An overall study timeline is provided.
Inclusion and exclusion criteria	√ A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article.
Randomization	√ Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided. √ Type and methods of randomization have been described. √ Methods used for allocation concealment have been reported.
Blinding	√ Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible. √ Blinding procedures have been described with regard to masking of group assignment during outcome assessment.
Sample size and power calculations	√ Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided.
Data reporting and statistical methods	√ Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups.

	√ Baseline data on assessed outcome(s) for all experimental groups have been reported. √ Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms. √ Statistical methods used have been reported. √ Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures.
Experimental details, ethics, and funding statements	√ Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described. √ Different sex animals have been used. If not, the reason/justification is provided. √ Statements on approval by ethics boards and ethical conduct of studies have been provided. √ Statements on funding and conflicts of interests have been provided.

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