Neurological Recovery Investigation - MCAO Mouse Model Evaluation System

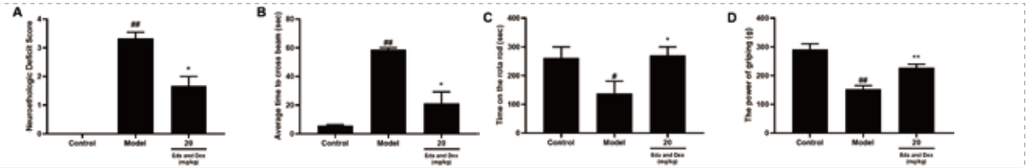
Animals: ICR mice, male

Group: Control Group, Model Group, Positive Drug Group (Sanbexin®)

Inspection indicators: Zea-longa score, Balance Beam Transit Time, Rotarod Dwell Time, Grip Strength

Methodology:

- The right middle artery of the mice was blocked by suture method, and the suture was removed after blocking for 1 h to achieve reperfusion.
- Neurological Recovery Investigation: Animals were intravenously injected with the positive drug Sanbexin® 20 mg/kg 0.5 h after infarction, and the treatment time window was investigated.



Pharmacodynamic Investigation of Neurological Function: A: Zea-longa score; B: Balance Beam Transit Time; C: Rotarod Dwell Time; D: Grip Strength

Rat Behavioural Examination - MCAO Rat Model Evaluation System

Animals: SD Rat, male

Group: Control Group, Model Group, Positive Drug Group 1 (Edaravone), Positive Drug Group 2 (Nimodipine)

Inspection indicators: Zea-longa score, Infarct Volume by TTC method

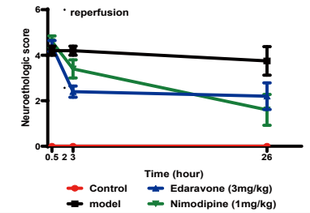
Methodology:

- The right middle artery of the mice was blocked by suture method, and the suture was removed after blocking for 2 h to achieve reperfusion.
- Pharmacodynamics Study: Animals were given the positive drugs by intravenous injection at 0.5 h after infarction, and the pharmacodynamic effects of the two positive drugs were investigated.

Zea-longa Score

Behavioral investigation of rats can grab the tail of the rat and let the rat be 10cm above the table. If the rat's front paw is in a straight state, the rat's behavior is normal; If the forelimb on the paralyzed side is retracted and bent under the abdomen after the tail is lifted, and rotated to the paralyzed side when walking, the MCAO rat model is successful. There is also the Longa score, the higher the score, the more severe the animal behavior disorder. Criteria for successful establishment of model rats: Rats exhibit symptoms such as hemiplegia, contralateral forelimb drooping and standing instability.

- No neurological deficits：0；
- Paralyzed front paw cannot fully extend：1；
- Circling to the paralyzed side while walking：2；
- Tipping to the paralyzed side while walking：3；
- Unable to walk automatically, there is a phenomenon of loss of consciousness：4.



Score of Animal Behaviour

Summary and Outlook

In recent years, with the continuous development of biomedical technology, more and more stroke drugs have gone from the target to the stage of preclinical testing. We believe the problem will be solved soon!

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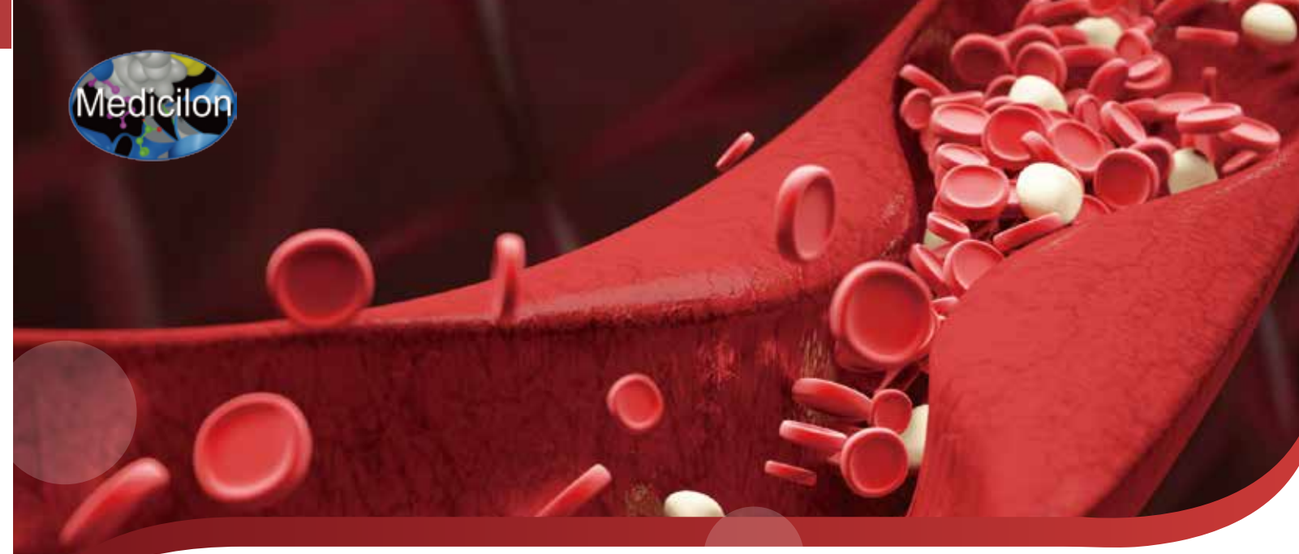
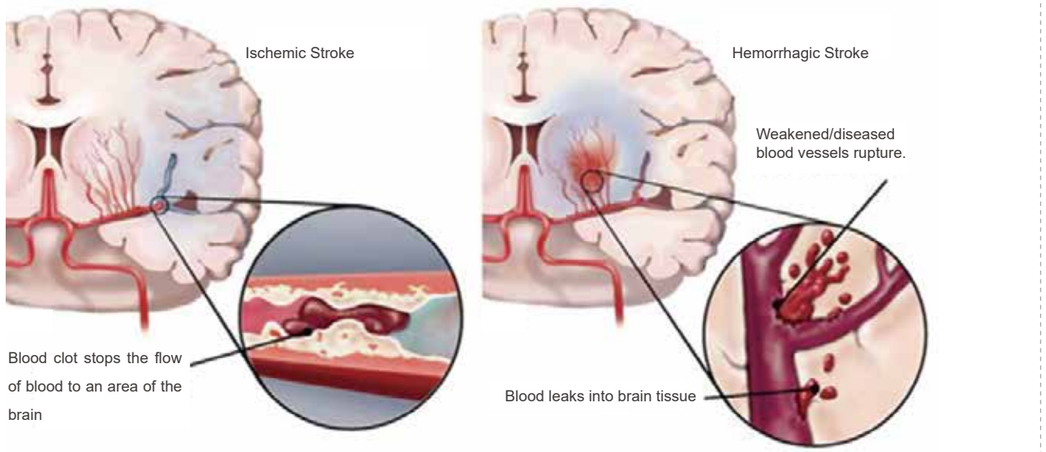


Medicilon Preclinical Efficacy Evaluation Service Platform for Cerebrovascular Disease

Stroke is the scientific name of cerebralvascular accident, which generally refers to ischemic or hemorrhagic diseases of the heart, brain and tissues of the whole province caused by hyperlipidemia, blood clotting, atherosclerosis, hypertension, etc. It is a sudden onset of cerebral blood circulation disorders. The clinical manifestations are mainly characterized by sudden dizziness, unconsciousness, or sudden deviated eyes, hemiplegia, and intellectual disability. Stroke mainly includes ischemic stroke (transient ischemic attack, atherosclerotic thrombotic cerebral infarction, lacunar cerebral infarction, cerebral embolism) and hemorrhagic stroke (cerebral hemorrhage, subarachnoid hemorrhage).



Stroke is known as "the number one killer of human health" because of its high prevalence, high morbidity, high mortality and high disability rate. In China, the annual treatment cost of cardiovascular and cerebrovascular diseases is 540.6 billion yuan. The annual treatment cost of cerebrovascular diseases accounts for 25.68% of cardiovascular and cerebrovascular diseases. Among them, ischemic stroke accounts for 18% and hemorrhagic stroke accounts for 7%. Although the annual treatment cost of stroke is so high, there is still a problem that there are few drugs for clinical specific treatment, and there is a huge gap in the demand for clinical drugs. Therefore, the market potential of stroke drugs is great and the development prospect is good. For the preclinical development of stroke drugs, the two mountains of pharmacokinetics and pharmacodynamics cannot be avoided, and the models used in both pharmacokinetics and pharmacodynamics are particularly important.

Pharmacodynamic Evaluation Model for Cerebral Stroke

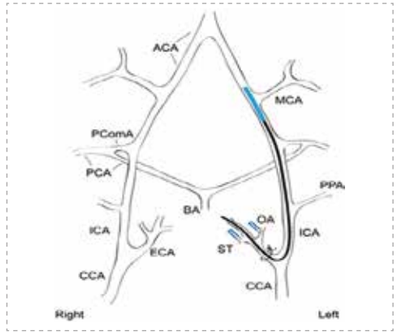
Rodent Models:

- Focal cerebral ischemia：middle cerebral artery occlusion/reperfusion (MCAO/R) model- suture-occluded method、Embolization、Photochemistry, etc.
- Total cerebral ischaemia (TCI)： Vessel Clamp Technique

***In vitro* model:**

- Oxygen-glucose deprivation (OGD)
- Stimuli such as hydrogen peroxide Oxidative Stress (OS)
- Others: Transwell to investigate BBB scale (Basso, Beattie & Bresnahan locomotor rating scale), *in vitro* brain slice, Neurovascular unit (NVU) and so on.

Because the pathogenesis of the MCAO is similar to that of human ischemic stroke, it is of great significance to create a simulated human cerebral ischemia model for the pathogenesis of cerebral ischemia and drug screening, and it is also the key recommendation of the pharmacodynamic evaluation system by the National Institutes of Health(NIH) and the Stroke Treatment Academic Industry Roundtable(STAIR). In the test, the classic suture-occluded method is usually used to establish the model: Exposed neck vessels are isolated, and nylon threads are inserted from the external carotid artery (ECA) or common carotid artery (CCA) to enter the internal carotid artery (ICA) blocks the blood supply to the origin of the Middle Cerebral Artery (MCA) and all its collaterals, resulting in focal ischemia in the MCA area. Model evaluation is a critical step in testing results. Medicilon strictly follows the STAIR preclinical drug development strategy for stroke, and has rich experience in MCAO model establishment and a mature MCAO model system evaluation system:



- Rat and mouse MCAO models with stable lesions, high success rate and high survival rate
- Reliable and effective evaluation of dose-effect/time-effect relationship
- Efficacy evaluation of transient cerebral ischemia and permanent cerebral ischemia
- Complete the investigation of various pharmacodynamic core indicators according to STAIR technical guidelines
- The model rodents could be customized with special diseases and complete activity evaluations according to customer requirements

Research Purposes	Model Type/ Test Method	Animal Species	Inspection Indicators
Dose-Response& Time-Response Relationship	MCAO	Mouse	• Infarct volume • Brain water content
Treatment Time Window			• Infarct volume • Brain water content • Brain volume
Behavioral Representation			• Grip strength • Rotor residence time • Zea-longa Score • Balance beam passing time
Overview		Rat	• Infarct volume • Behavioral Scores
Anticoagulant Efficacy	Capillary method	Mouse	• Clotting time

Investigation of Dose-Response and Time-Response Relationship - MCAO Mouse Model Evaluation System

Animals: ICR mice, male

Group: Control Group, Model Group, Positive Drug Group (Sanbexin@)

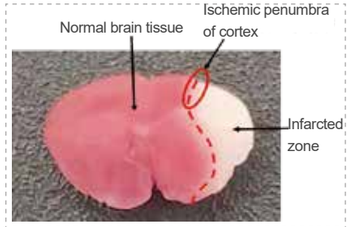
Inspection indicators: The infarct volume was measured by TTC method, and the brain water content was measured by drying method.

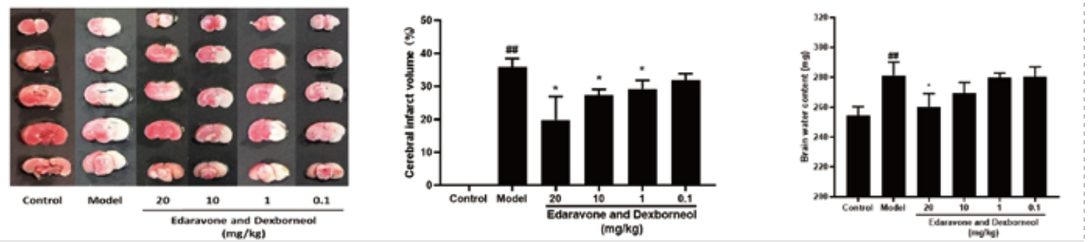
Methodology:

- The right middle artery of the mice was blocked by suture method, and the suture was removed after blocking for 1 h to achieve reperfusion.
- Dose-Response Investigation: Animals were given the positive drug Sanbexin® 20, 10, 1, 0.1 and 0.01 mg/kg intravenously at 0.5 h after infarction, respectively, and the dose-effect relationship was investigated.
- Time-Response Investigation: Animals were given the positive drug Sanbexin® 20 mg/kg intravenously at 0.5 h after infarction and 2 h, 6 h, 8 h and 10 h after reperfusion respectively, and the time-effect relationship was investigated.

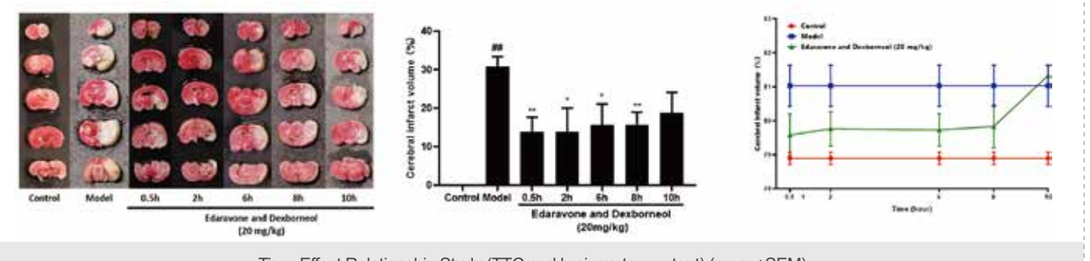
TTC Staining

TCC staining is a common method for staining and observing ischemic brain tissue. The specific operation is to quickly remove the rat brain, rinse it with cold saline, and quickly place it in a -20°C refrigerator for 10 minutes. After the brain tissue is slightly hardened, remove and cut off the olfactory bulb, pituitary, and lower brainstem, and coronally slice from anterior to posterior, evenly cut into 2 mm thick brain slices, equally cut into 5 slices, place them in 1% TTC solution, and incubate at 37°C for 30 minutes in the dark, turning every 5 minutes or so. TTC can react with the dehydrogenase system in normal tissue and be reduced to rose red, so the normal tissue of the group was stained rose red, and the infarcted tissue was white.





Dose-Effect Relationship Study (TTC and brain water content) (mean±SEM)



Time-Effect Relationship Study (TTC and brain water content) (mean±SEM)

Treatment Time Window Investigation - MCAO Mouse Model Evaluation System

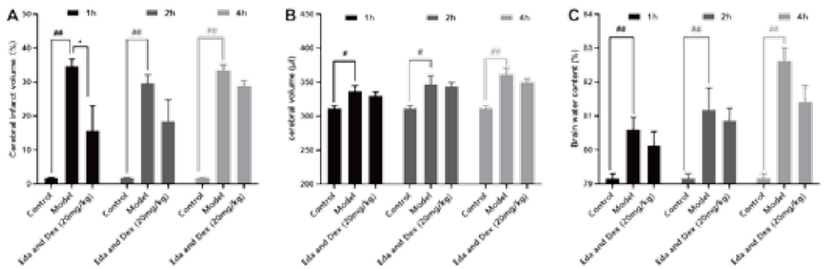
Animals: ICR mice, male

Group: Control Group, Model Group, Positive Drug Group (Sanbexin@)

Inspection indicators: Infarct volume was measured by TTC method, brain volume by drainage method, and brain water content by drying method.

Methodology:

- The right middle artery of the mice was blocked by suture method, and the suture was removed after 1 h, 2 h and 4 h of blocking to achieve reperfusion.
- Treatment Time Window Investigation: Animals were intravenously injected with the positive drug Sanbexin@ 20 mg/kg 0.5 h after infarction, and the treatment time window was investigated.



Treatment Time Window Investigation A: TTC; B: Brain Volume, C: Brain Water Content (mean±SEM)

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