

## EXPERIMENTAL MODEL OF REVERSIBLE FOCAL ISCHAEMIA IN THE RAT

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### Abstract

The final outcome of brain ischaemia depends on its severity and duration and also on conditions provided for the reperfusion phase. Studies on reversible brain ischaemia can give meaningful results only if experimental animals survive for a long time and the reperfusion phase is long enough to permit the full development of ischaemia. The model of reversible focal brain ischaemia in the rat described here was used in the Czech Republic for the first time. It is based on insertion of a monofilament fibre through the internal carotid artery into the middle cerebral artery. This approach produced reproducible ischaemia that was manifested by the development of hemiparesis and confirmed by histological findings, including the presence of apoptotic cells in brain tissue.

### Key words

Focal brain ischaemia, Suture model

### Abbreviations used

ACC, arteria carotis communis; ACI, arteria carotis interna; ACE, arteria carotis externa; ACM, arteria cerebra media

### INTRODUCTION

In order to restore perfusion in an ischaemic brain area, new methods, such as systemic or selective thrombolysis, angioplasty or stenting, have been developed and used in clinical practice. By means of any of them, ischaemia can be reversed and oxygen and glucose supply restored.

It is known that the final infarction size is determined not only by the degree and duration of ischaemia, but also by the conditions of the reperfusion phase. The development of an ischaemic lesion may still continue after the blood flow has been restored (1).

Experimental models that require an extensive surgical preparation phase are often devastating (craniectomy, eyeball removal) (2) and the animal has to be

killed right after the experiment. The reperfusion phase is then short (maximum of several hours) and the final ischaemia is different than it would be if the period of reperfusion was sufficiently long. Therefore many authors currently use a "suture" model of focal brain ischaemia in the rat to study reversible brain ischaemia and various neuroprotective methods (3,4,5,6). An advantage of this model is that the results of these experiments can readily be utilised in clinical practice, particularly if they are obtained by a research team working at a department specialised in the treatment of vascular brain disorders and in research on neuroprotective methods (2,7,8).

However, adoption of this model is not easy. Apart from the necessary equipment, such as microscopes, instruments for microsurgery and anaesthetic facilities, surgical skills and enough training and experience are required. According to our knowledge, this model has not so far been used in the Czech Republic.

## MATERIALS AND METHODS

### SUTURE PREPARATION

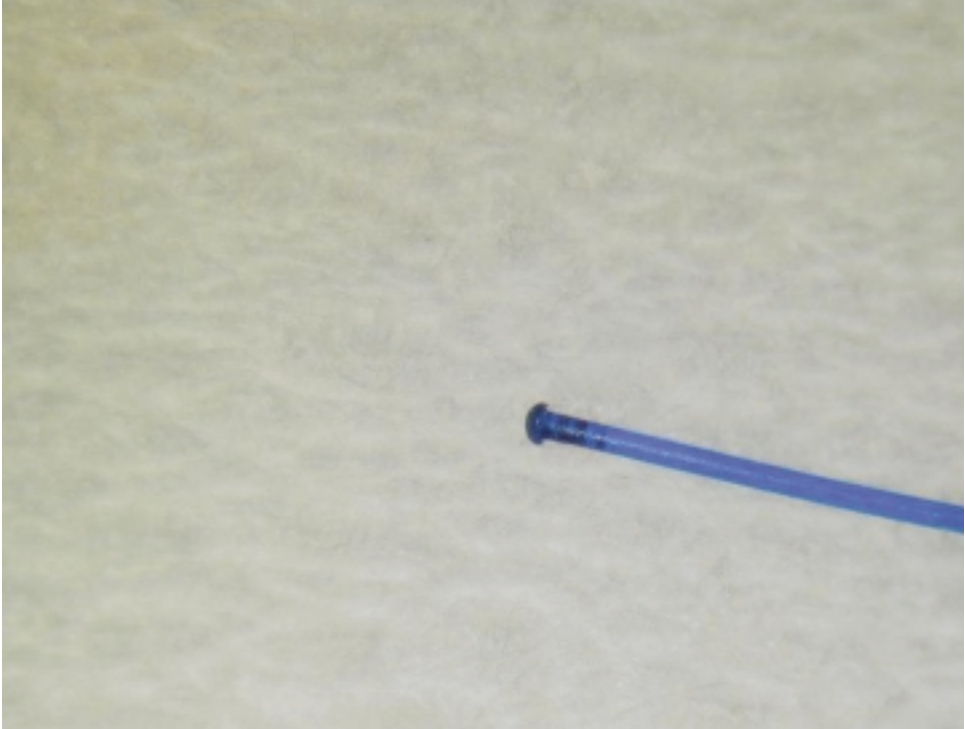
A 4-0 monofilament fibre was cut into parts 4 cm long. One end of each segment was carefully sealed over a flame to reduce its sharpness without increasing its diameter (*Fig. 1*). The fibre segments were then immersed in poly-L-lysine and allowed to stand for 1 h at 60 °C. After subsequent drying, a 1-cm scale (three marks) was marked on each fibre. The segments were stored in a clean Petri dish until use.

### SURGICAL TECHNIQUE

The protocol and methods used in this study were in agreement with the current Czech law on laboratory animal handling. They were approved by the Ethical Committee of the Bohunice Teaching Hospital, Masaryk University, Brno.

A Wistar rat (250–400g) with no access to food for 12 hours was anaesthetised with a mixture of Narkamon and Rometar (0.5ml/100g body weight) and the operative field was shaved. First a skin incision was made from the petrous bone to the scapula. Then the neck muscles were dissected to gain access to the common carotid artery (ACC). Dissection of the carotid bifurcation and glomus caroticum was performed in the vicinity of the vagus nerve (*Fig. 2*). The occipital artery branching off the external carotid artery (ACE) was ligated close behind the carotid bifurcation. The internal carotid artery (ACI) was carefully dissected as distal to its entrance to the skull as possible. Proximal to this entrance, ACI gives off a branch, described in the literature as the pterygopalatine artery, that runs more dorsal and lateral to ACE (it is important to avoid inserting the intraluminal fibre into this branch). Subsequently, ACE was ligated as distal as possible but still proximal to its branching, i.e., 3 to 5 mm from the bifurcation according to the size of the animal (*Fig. 3*). The proximal ligation of ACE was not cut off but was used to pull the blind end of ACE. Then temporary clips were applied to ACC and ACI and, pulling slightly the blind end of ACE, a cut through ACE was performed (*Fig. 4*). A prepared segment of the 4-0 monofilament fibre was carefully inserted into the lumen of ACE, right to the clip on ACI, then the clip was removed and the fibre continued to be gently inserted until it was wedged intracranially, which was usually 18 to 25 mm from the bifurcation according to the size of the animal (*Fig. 5*). Eventually, the stump of ACE was clipped together with the fibre inside and the clip on ACC was released. It was necessary to keep the animal under adequate anaesthesia.

When the ischaemic phase was terminated, the ACE stump was ligated below the clip, then the clip was removed and the fibre was gently pulled out through the ligature. (In some cases it was



*Fig.1*

Wider smooth end of a 4-0 monofilament suture (fibre) adjusted in order not to harm the vessel wall from inside.

necessary to cut off the sealed end of the fibre and leave some 1mm of it inside the stump of ACE). The wound on the neck was sutured in one layer and disinfected with iodine. The animal was placed in a clean cage.

When the animal woke up from anaesthesia, neurological symptoms resulting from the damage to the motor centre related to the duration of ischaemia were observed. The animal limped, with both extremities of one side affected, went round in a circle, etc. After 2 to 3 days of observation during the reperfusion phase, the rats were anaesthetised by ether and killed. The brain was removed from the skull, fixed and processed for histological and immunohistochemical examination.

## RESULTS

A total of 30 animals were operated on using the standardised methodology with the objective to achieve ischaemic phases of varying duration (range 15 to 180 min). All animals survived the reperfusion phase.

In brain sections, aggregates of apoptotic cells were detected and their numbers correlated well with the duration of ischaemia. We detected a rapid onset of



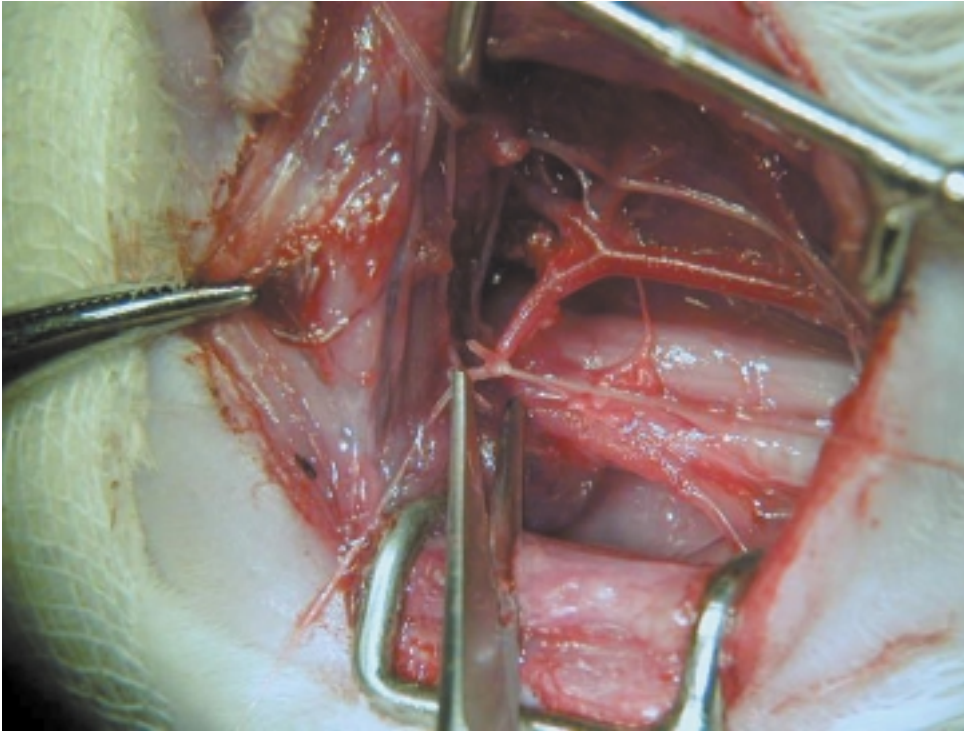
*Fig.2*

Branching of the common carotid artery, the glomus caroticum and the vagus nerve

nucleolar segregation in cortical and subcortical neurons damaged by ischaemia (15min) reperfusion injury, which coincided with a nuclear/nucleolar accumulation of MADD, a stress activated neuron specific death domain. Active caspase 3 and TUNEL positivity were found after prolonged ischaemia (30,60 and 120 min.). The results show that even short periods of temporary focal ischaemia may be harmful.

Histological and immunohistochemical examination also confirmed brain infarction; however, this was positive only in ischaemia lasting 60 to 120 min.

The exact results of the extent of ischaemic changes were not the subject of this paper. Our aim was to describe the technique of this experimental method, a novel model of brain reversible ischaemia in the Czech Republic.



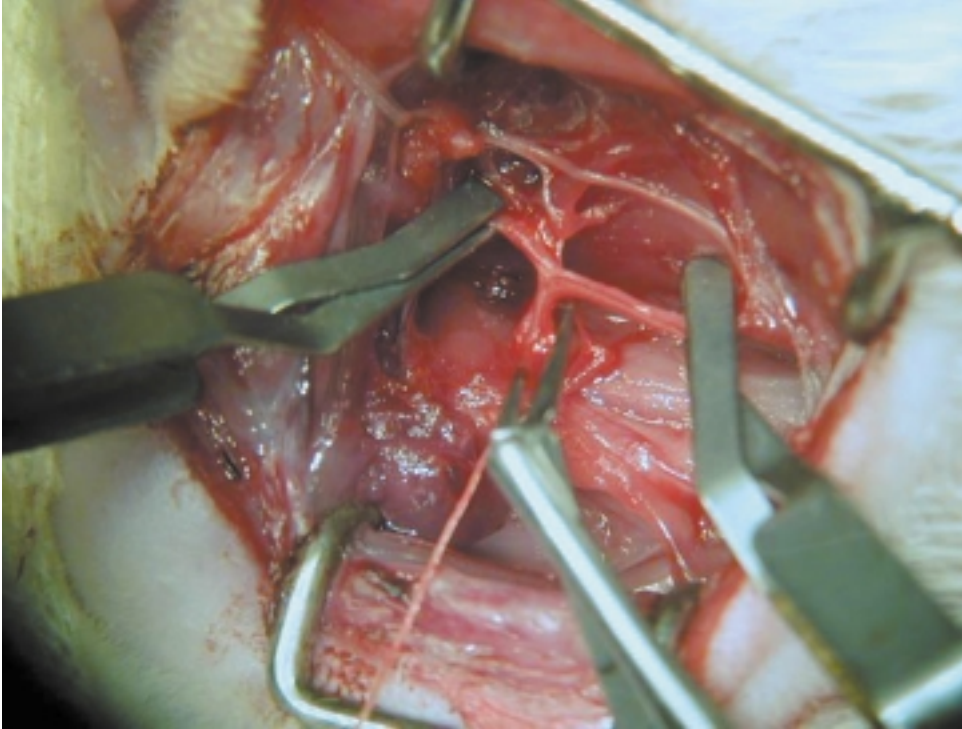
*Fig.3*

Ligation of the external carotid artery was made as far from the carotid bifurcation as possible.

#### DISCUSSION

We performed a total of 60 operations. At the beginning, we encountered some methodological difficulties, particularly with the preparation of such fibres that could be successfully introduced into the lumen of ACE. In the first 30 animals, we were not able to insert the fibre far enough into the cranium, because the fibre was either too thick or not stiff enough, and therefore the outcomes of ischaemia were inconsistent. Several animals died prematurely because of vessel wall perforation with a fibre that was too rigid or sharp.

It is known that not all species of rats are useful for this kind of experiment. The most consistent ischaemia with minimal mortality is achieved in Wistar rats, but Sprague-Dawley and Fischer-344 rats provide less consistent results (9). This is why we used Wistar rats for our purposes.

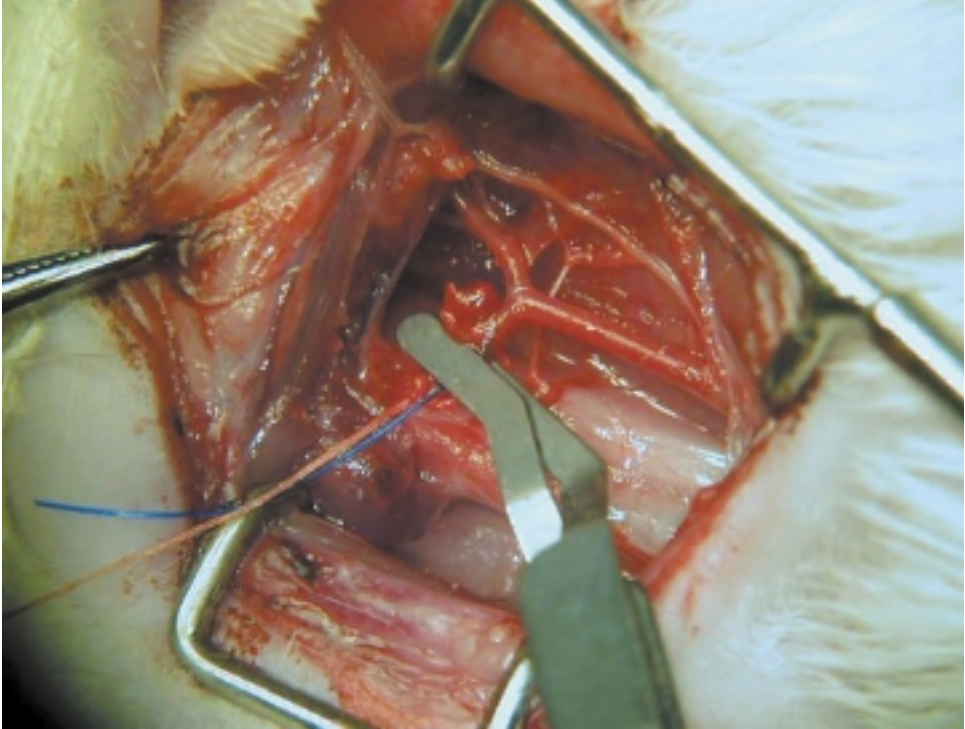


*Fig.4*

Incision in the stump of the external carotid artery. Transposition of the stump in order to facilitate insertion of a fibre into the artery lumen and further into the internal carotid artery.

In humans, carotid arteries are responsible for two thirds of cerebral perfusion whereas vertebral arteries only for one third. In rats, it is exactly the opposite. Therefore, a ligation applied alone to ACI in the neck region is not sufficient to cause ischaemia in the rat brain. The suture model offers an advantage in that the fibre, if correctly introduced, is wedged either into the intracranial bifurcation of the carotid artery or directly into the middle cerebral artery (ACM). This results in focal ischaemia in the region of ACM and the influence of collateral blood flow is minimised. This model of focal ischaemia takes into account a closure of ACM right at its beginning. This means that ischaemia influences not only the cortex and subcortex but also the basal ganglia which are supplied by lenticulostriate arteries of the proximal part of ACM. Therefore, the suture technique is not





*Fig.5*

The introduction of the fibre to be wedged intracranially, at a length of 18–25 mm.

suitable for experiments designed to study effects of the distal occlusion of ACM when the basal ganglia are excluded (6).

Evidence on the onset and development of ischaemia after fibre insertion can be obtained by several means. During the ischaemic phase, EEG can be recorded, evoked potentials can be monitored, blood flow can be measured (e.g., by laser Doppler flowmetry) or brain tissue oxymetry (ptiO<sub>2</sub>) can be carried out. After the animal wakes up from anaesthesia, the presence of hemiparesis and cognitive abilities of the animal are investigated. The definite proof of ischaemia is provided by histochemical and histological examination (e.g., the presence of apoptosis).

In this paper we describe the model of reversible focal brain ischaemia in the rat that has routinely been used world-wide but, in the Czech Republic, was adopted

for the first time. This model is universal and applicable to the study of all aspects of brain ischaemia, including the involvement of various neuroprotective methods. Its advantage is in that the reperfusion phase can be extended for as long as required. It can be expected that this model will gain further importance in clinical practice with the increasing use of thrombolytic therapy and other recanalisation techniques.

#### A c k n o w l e d g e m e n t

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#### MODEL REVERSIBILNÍ FOKÁLNÍ MOZKOVÉ ISCHEMIE U POTKANA

##### S o u h r n

Konečný rozsah mozkové ischemie je určen nejen hloubkou a délkou ischemie, ale také podmínkami reperfuční fáze. Experimentální zvířecí modely, které jsou založeny na extenzivní chirurgické přípravě, jsou často mutilující a zvíře musí být utraceno hned po skončení experimentu. Reperfuční fáze je proto velmi krátká (maximálně několik hodin) a vývoj ischemie nemusí být dokončen. Z toho důvodu jsou výhodné takové modely, kde zvíře může přežít experiment po dlouhou dobu. Pro tento účel jsme jako první v České republice vyvinuli model reverzibilní fokální mozkové ischemie u potkana, který je založen na aplikaci vyztužené nitě do arteria carotis interna a dále do arteria cerebri media. Jsme nyní schopni dosahovat reprodukovatelnou ischemii, která může být objektivně potvrzena přítomností hemiparézy, histologicky nebo pomocí detekce apoptózy v mozkové tkáni.

##### REFERENCES

1. *Neumann-Haefelin T, Kastrup A, de Crespigny A, Yenari MA, Ringer T, Sun GH, Mosley ME.* Serial MRI after transient focal cerebral ischemia in rats: dynamics of tissue injury, blood-brain barrier damage, and edema formation. *Stroke* 2000 31: 1965–1972.
2. *Smrčka M, Ogilvy CS, Crow RJ, Maynard KI, Kawamata T, Ames A III.* Induced hypertension improves regional blood flow and protects against infarction during focal ischemia: time course of changes in blood flow measured by Laser Doppler imaging. *Neurosurgery* 1998 42: 617–625.
3. *Liu Y, Belayev L, Zhao W, Busto R, Ginsberg MD.* MRŽ 2/579, a novel non-competitive N-methyl-D-aspartate antagonist, reduces infarct volume and brain swelling and improves neurological deficit after focal cerebral ischemia in rats. *Brain Res* 2000 862: 111–119.
4. *Paczynski RP, Venkatesan R, Diringer MN, He YY, Hsu CY, Lin W.* Effects of fluid management on edema volume and midline shift in a rat model of ischemic stroke. *Stroke* 2000 31: 1702–1708.
5. *Quartermain D, Li Y, Jonas S.* Enoxaparin, a low molecular weight heparin decreases infarct size and improves sensorimotor function in a rat model of focal cerebral ischemia. *Neurosci Lett* 2000 288: 155–158.
6. *Zea Longa E, Weinstein PR, Carlson S, Cummins RW.* Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989 20: 84–91.
7. *Gál R, Čundrle I, Zimová I.* Řízená hypotermie u pacientů s těžkým poraněním mozku [Controlled hypothermia in patients with severe brain trauma]. *Anest Neodkl Péče* 2000 11: 174–175.
8. *Gál R, Čundrle I, Zimová I.* Biochemické hodnoty během mírné hypotermie u pacientů s těžkým poraněním mozku [Biochemical values during mild hypothermia in patients with severe brain trauma]. *Anest Neodkl Péče* 2002 13 (in press).
9. *Aspey BS, Taylor FL, Terruli M, Harrison MJ.* Temporary middle cerebral artery occlusion in the rat: consistent protocol for a model of stroke and reperfusion. *Neuropathol Appl Neurobiol* 2000 26:232–242.