**Tirilazad**

**Table A: Tirilazad: Location, institution, ethics, animals, numbers**

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| **Study ID** | **Location** | **Institution** | **Ethics statement** | **Animal** | **Number animals** |
| 139 | UK | Manchester University | No | Rats | Not reported\* |
| 140 | USA; Germany | Columbia University; Philipps Universitiit | No | Rats | 58 |
| 141 | Canada; USA | Ottawa Civic Hospital; Robarts Research Institute; Ohio State University | No | Rats; and spontaneously hypertensive rats | 97 |
| 142 | USA | University of Vermont | No | Rabbits | 24 |
| 143 | USA | The Johns Hopkins Medical Institutions | No | Cats | 25 |
| 144 | Sweden | Uppsala University Hospital | The investigations were carried out under a project licence approved by the local ethics committee for Animal Research. | Rats | 30 |
| 145 | South Korea; USA | Catholic University Medical College; The Upjohn Company | No | Rats | 36 |
| 146 | Japan | Hamamatsu University School of Medicine | No | Rats | 30 |
| 147 | USA | University of Arizona; University of Oaklahoma | No | Rabbits | 35 |
| 148 | USA | University of Vermont; Albany Medical Centre | Animal care use committee mentioned, but only in relation to method of euthanasia | Rabbits | 24 |
| 149 | Germany | Ludwig-Maximilians-Universität | Animals were cared for before and at all stages of the experiment in compliance with The Principles of laboratory animal care (NIH publication no. 86-23, revised 1985) as well as with the current version of the German Law on the Protection of Animals. | Rats | 44 |
| 150 | Switzerland | Cantonal Hospital Basel; Cantonal Hospital Aarau | All experiments described in this paper were approved by the Animal Welfare Committee of the Canton Basel and were incompliance with the Swiss Guidelines for the Care and Use of Animals. | Spontaneously hypertensive rats | 20\* |
| 151 | Turkey | Erciyes University Medical School | No | Rabbits | 28 |
| 152 | Germany | Ludwig-Maximilians-Universität | Rats were cared for prior to and at all stages of the experiment in compliance with applicable institutional guidelines and regulations of the Government of Bavaria. | Rats | 55 |
| 153 | Germany | Ludwig-Maximilians-Universität | Rats were cared for prior to and at all stages of the experiment in compliance with applicable institutional guidelines and regulations of the Government of Bavaria. | Rats | 42 |
| 154 | Germany | Ludwig-Maximilians-Universität | No | Rats | 43 |
| 155 | Germany | Ludwig-Maximilians-Universität | Animals were cared for before and at all stages during the experiment in compliance with applicable institutional guidelines and regulations of the government of Bavaria. AND: 'In light of the increased mortality and missing improvement in neurological recovery, group 5 was terminated after 6 animals because of restrictions formulated in the permission to conduct animal experiments.' | Rats | 53 |
| 156 | Germany | Ludwig-Maximilians-Universität | Rats were cared for prior to and at all stages of the experiment in compliance with applicable institutional guidelines and regulations of the Government of Bavaria. | Rats | 120 |
| **Total 18 studies** | **Germany 7; USA 7; UK 1; Canada 1; Sweden 1; South Korea 1; Japan 1; Switzerland 1; Turkey 1** | **Universities 14; hospital 1; hospital and university collaboration 2; university and pharmaceutical company collaboration 1** | **Not reported 10**  **Ethical statement 8**  **(approved by committee 1; according to guidelines/ principles/ legal requirements 5; approved by committee and according to guidelines 1; animal care use committee mentioned in relation to method of euthanasia 1)** | **Rats 13 studies; rabbits 4 studies; cats 1 study** | **764\***  **Average no. animals used per study 42** |

\* Actual total will be higher as the number in Study 139 was not reported and an unspecified number were excluded from Study 150

**Table B: Tirilazad: Animal model, anaesthesia, how and when killed, what animals experienced**

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| --- | --- | --- | --- | --- | --- |
| **Study ID** | **Animal model** | **Anaesthesia for experimental procedures** (excluding anaesthesia prior to death) | **How killed** | **When killed (endpoint)** | **What animals experienced** |
| 139 | Rat model of Permanent Middle Cerebral Artery Occlusion (MCAO) | Sodium pentobarbitone anaesthesia for MRI 24h post occlusion. No details of anaesthesia for surgery to induce occlusion (but this is a poster). | Not reported (poster) | Not reported (poster) | Operation to occlude MCA. *(Almost certainly under anaesthesia but this not reported - this is a poster.)* Some rats had iv test agent 10 mins and 3h after operation. Other rats had either test agent or vehicle i.p 16h and 24h after operation. 24 hrs after operation all rats had anaesthesia and then MRI. |
| 140 | Rat model of MCAO | The rats were anesthetized with halothane in a mixture of nitrous oxide/ oxygen | The rats were transcardially  perfusion-fixed with 4% paraformaldehyde freshly dissolved in  phosphate-buffered saline | After 2 days | Rats were anaesthetised and surgery was conducted to remove part of the skull and operate to occlude the left MCA using coagulation. After the wounds were sutured and anaesthesia discontinued, rats were kept warm until they had regained consciousness and motility. They were given one of two different test agents (at one of two different doses), or vehicle (all i.p.) 30 mins before the MCAO and 2, 6, and 24 after. |
| 141 | Rat models of both transient MCAO and permanent MCAO | Animals were initially anesthetized with halothane mixed with nitrogen and oxygen and then maintained on the same (with less halothane). Animals were re-anaesthetised for second procedure and with halothane prior to killing. | Decapitation. | 24 hours after the onset of CCA/MCA occlusion | Prior to the experiment rats had a procedure to drill away a layer of skull and place a laser Doppler flowmetry probe onto the surface of the dura*.* No details on anaesthesia for this procedure. For the experiment (we don’t know how long after) rats were anaesthetised and surgery was conducted to occlude the MCA either transiently or permanently, through a ventral midline cervical incision. Wounds were closed, anaesthesia was discontinued and animals were returned to their cages and kept warm using a rectal thermistor until they were fully awake. Two hours later, those that were subjected to transient ischaemia were reanaesthetised and had operation to restore blood flow. The wounds were reclosed and rats were returned to their cages. Some rats received test agents i.p. prior to surgery and 4 and 10 hours afterwards. Some rats received test agents i.p prior to surgery and 6 and 12 h later. Some received test agents i.v. 10 mins and 3 h after surgery, then i.p. 6 and 12h after surgery. |
| 142 | Rabbit model of thrombo-embolic stroke | Rabbits were anesthetized with acepromazine and ketamine. Anaesthetic depth and the need for supplemental anaesthesia was followed by assessing the blink reflex, response to paw pinch, and variations in mean arterial pressure. | An i.v. dose of sodium pentobarbital. | After the 4-hour interval after embolization | Prior to the experiment an autologous clot was formed by incubating blood from the auricular artery in PE-90 tubing at 37°C for 4 hours. We don’t know how much later the main experiment was conducted. For this, animals were anaesthetised and surgery was conducted to remove part of the skull bilaterally, to insert electrodes into the brain, to place an intracranial pressure monitor and to inject an embolus into the artery. The animals were ‘supported’ for 4 hours after the embolic event during which time physiological measurements were recorded at five separate intervals. |
| 143 | Cat model of transient focal cerebral ischaemia | Cats were anesthetized with halothane in oxygen during spontaneous ventilation. Anaesthesia was maintained with halothane in oxygen-enriched air. Halothane concentration was increased for signs of cardiovascular stimulation during surgery but was not changed throughout the experimental protocol. | The animal was killed with KC1. (Potassium chloride) | After 3 hours | Cats were anaesthetised and an operation conducted to produce focal ischaemia by occluding the MCA with a clip and by tightening the CCAs with ligatures. Just after tightening the ligatures and by continuous intravenous infusion, some cats had, vehicle, others had the test agent followed by vehicle at 70 minutes of ischemia, others had vehicle followed by the test agent at 70 minutes of ischaemia. After 90 minutes of ischemia the carotid ligatures were loosened, and the clip on the MCA removed. Reperfusion lasted 180 minutes. Measurements were made at 15, 30, 60, 90, 120, 150, and 180 minutes of reperfusion.  This would appear to be ‘non-recovery’ as cats appear to have been anaesthetised throughout and killed at the end of the operation. |
| 144 | Rat model of Permanent MCAO | To induce anaesthesia we used a closed box with a mixture of halothane in oxygen. The anaesthesia was continued with halothane in a mixture of N20 and 30% O2. | Intraperitoneal injection of a mixture containing chloral hydrate and  pentobarbitaI followed by intracardiac perfusion with phosphate buffer and buffered formaldehyde. | After 3 days | Rats were anaesthetised and surgery was conducted to occlude the MCA. Test agents or vehicle were injected into the tail vein over 2 mins at 10 min and 3 h after occlusion. Rats were allowed to recover and the tail catheter was left in place until 3h. Rats were observed for three days and were graded daily for neurological status using methods that included being pushed laterally to assess resistance. |
| 145 | Rat model of Permanent MCAO | The animals were anesthetized with a nitrous oxide mixture containing halothane. Anaesthesia was stopped just after MCA occlusion. Rats were re-anaesthetised prior to being killed. | All the rats were re-anesthetized and killed with an i.v. injection of KC1. | 24 h after MCA occlusion | Rats were anaesthetised and surgery taking 20-30 mins was conducted to occlude the left MCA using a micro-bipolar coagulator. (A subtemporal approach was taken, without removal of zygomatic arch or temporal muscle.) Anaesthesia was stopped just after MCA occlusion and animals were kept warm. At 2, 6, 12 and 24 hours after discontinuing anaesthesia, each rat's level of consciousness and motor activity were graded using methods that include attempting to arouse the rat using tactile and painful stimulation. At 24h neurological status was graded using methods that included being pushed laterally to assess resistance. Test agents were administered i.v. at 15 min, 2 h and 6 h post occlusion, then (at higher doses) i.p. 12 h post occlusion.  In a preliminary study, some conscious normal rats had their temporal muscle and rectal temperatures continuously monitored for 5h. |
| 146 | Rat model of MCAO | Pentobarbital anaesthesia for surgery and then for MRI scanning and then for killing. | The head skin was removed and the head was frozen by dipping it into liquid nitrogen under pentobarbital anaesthesia. The brains were then quickly removed. | After magnetic resonance imaging, which was 24h after occlusion | Rats were anaesthetised and surgery was conducted to remove a portion of the skull and to expose the MCA. A portion of the MCA was thrombotically occluded using irradiation with green light. Rose bengal and test agents were administered IV to the animals. The incisions were closed after the operation. Spontaneous reperfusion occurred about 3 h hours after the occlusion. Twenty-four hours after occlusion the extent of ischaemic damage was measured with MRI.  In a preliminary experiment, physiological parameters were measured just before and 15 min after MCAO. |
| 147 | Rabbit model of thrombo-embolic stroke | Short term anaesthesia for creation of thrombus. For surgery two days later rabbits were anaesthetised with a tranquillizing mixture of acepromazine and xylazine. They were intubated and remained on oxygen supplemented by halothane during surgery. Halothane was discontinued after embolisation, but at this point a second dose of tranquillising mixture was given and then again at approx 60min intervals. | Not reported | 4 h after administration of treatment | Rabbits were anaesthetised for procedure to ear for creation of autologous clots. They were returned to their cages and given routine care for two days, after which they were anaesthetised, the clot was harvested from the ear and surgery was conducted to cannulate the internal jugular and inject the clot into the internal carotid artery. Surgery was completed and the skin incision closed. Test agents or vehicle were then administered through the cannulated jugular. |
| 148 | Rabbit model of thrombo-embolic stroke | We are told that animals were anaesthetised for surgery but given no details. | Exsanguination and bilateral thoracotomy, 'accepted by the AVMA  and the UVM Animal Care and Use Committee as effective and painless.' | After eight hours | Rabbits had blood taken to create autologous clot. No info on details. For main experiment rabbits were anaesthetised and surgery conducted to remove a section of the skull and to place an intracranial pressure monitor, an epidural brain temperature probe, electrodes to measure rCBF and to inject an autologous clot into the anterior brain circulation. U74006F I.V. and L644,711 I.V. or their vehicle control were given 3.5 hours following autologous clot embolization. Rabbits received test agents or vehicle I.V. 3.5 hours after embolization. All received t-PA I.V. beginning 4 hours after stroke, 20% as an IV bolus with the remainder infused over a 2 hours. |
| 149 | Rat model of transient focal cerebral ischaemia | For operative procedures animals received atropine subcutaneously and anaesthesia was induced with halothane. The rats were orally intubated and mechanically ventilated with halothane in a mixture of N2O and O2. Rats were also anaesthetised before being killed. | Rats were re-anesthetized and perfused transcardially by 2% paraformaldehyde | Seven days after ischemia | Rats were anaesthetised and surgery was conducted to place a laser Doppler probe over each brain hemisphere. Al rats had the right MCA occluded for 90 mins by the introduction of a nylon monofilament. Rats received either vehicle or test agents Test agents were administered iv over 15 mins, starting 15 min before ischemia and at reperfusion.  Neurological deficits were evaluated daily using methods that included being pushed laterally to assess resistance. Rectal temperature was measured 2 h after reperfusion and then daily. |
| 150 | Rat model of Permanent MCAO | We are told that 'anaesthesia was induced and maintained with halothane' - this seems to be only for the MCAO, not for the micro-dialysis probe implantation? For killing animals were anaesthetised with pentobarbital. | Animals were anaesthetised with pentobarbital and a  transcardial perfusion-fixation was performed using heparinised  phosphate-buffered saline and formaldehyde. | On day 3 | Surgery was conducted to implant a micro-dialysis probe in the brain. (Anaesthesia not reported for this procedure. We don’t know when this procedure was conducted, in relation to the main experiment.) For the main experiment, under anaesthesia surgery was conducted to remove part of the skull and to occlude the MCAO with bipolar coagulation, or a sham operation was performed. One hour after MCAO the rat was allowed to regain consciousness in a heated cage. Postoperatively, rats were treated with buprenorphine (an analgesic) and ampillicin. Vehicle or test agents were injected i.v. 15 min, 2h, 6 h and i.p. 12 h and 24 h after surgery. The micro-dialysis probe was left in place.  On days 2 and 3 perfusion was restarted but we’re not told how. Presumably it required a further operation but we’re not told. It wasn’t spontaneous.  Occlusion was monitored by laser Doppler measurement but no details given on placement of this, which requires brain surgery. It may have been done at the same time as the MCAO. |
| 151 | Rabbit model of permanent MCAO | All rabbits were sedated with ketamine and atropine i.m. and then anaesthestized with thiopentone i.v. Paralysis and analgesia were maintained with pancuronium bromide i.v. and fentanyl. | The surviving animals were killed with a lethal intracardiac KCl injection | At the end of the third day. | Rabbits were anaesthetised and surgery was conducted to perform either a sham operation or to occlude the MCA using bipolar cautery. This involved removing the eye and part of the skull just above the eye. The wound was closed. After MCAO the rabbits were given vehicle or test agents IV either over 2 minutes, 15 min and 3 h after MCA occlusion, or twice at an interval of 15 min every 3h. Neurological status was evaluated as normal, abnormal, or dead at 24, 48, and 72 h after occlusion. Abnormal findings included generalized weakness, markedly reduced motor activity (decreased feeding, grooming), or obtundation (less than fully alert). |
| 152 | Rat model of permanent focal cerebral ischaemia | Anaesthesia was conducted as previously described (ref given). In Part I animals received 6 h of halothane-anaesthesia. In Part II anaesthesia was terminated 180 min after ischemia onset. | Not reported | Some after 6 h ischemia. Some after 7 days. | Rats had anaesthesia. For placement of the LDF probe animals had surgery to remove part of the skull and to position an LDF probe above the surface of each brain hemisphere. Animals were subjected to permanent MCAO by insertion of a silicone-coated nylon monofilament via the external carotid artery.  Prior to surgery some rats had ice packs applied until their temperature dropped. Rewarming started after 2h of hypothermia. (Other similar experiments indicate that rats were anaesthetised during ice pack application)  Animals were either normothermic and had vehicle infused over 30 mins, or were hypothermic and had two test agents serially infused, each over 15 mins, commencing 30 min prior to ischemia. Drugs were administered at 90 min intervals.  Anaesthesia was for either 6 hours after which animals were killed (NON-RECOVERY GROUP) or was terminated 180 min after ischemia onset and animals observed for 7 days. In the latter group neurological function was evaluated daily using a method that included being pushed laterally to assess resistance. |
| 153 | Rat model of transient MCAO | For the operative procedures, the animals received atropine SC and anaesthesia was induced with halothane. The animals were orally intubated and mechanically ventilated with halothane in a mixture of N2O and O2 to maintain normal arterial blood gases. Rats were also anaesthetised prior to killing but we're not told how. | Each rat was anesthetized and perfused transcardially with isotonic heparinized saline. | Seven days after transient cerebral ischemia | Rats were anaesthetised. Hypothermia was induced in some rats with ice packs until a temperature of 33°C was reached and maintained. Before induction of ischemia, an interval of 20 minutes was allowed for physiological stabilization.  Rats had surgery to remove part of the skull, place LDF probes over each brain hemisphere and occlude the MCA for 90 minutes by insertion of a silicone-coated nylon monofilament. After 90 mins reperfusion was achieved by withdrawing the filament.  Rats were either normothermic and had vehicle or two test agents, or they were hypothermic and had vehicle or two test agents. Vehicle and test agents were administered iv over 15 minutes.  Each animal received 2 doses of vehicle or drugs, with the first dose administered before ischemia and the second at reperfusion.  Postoperatively, each animal’s neurological function was evaluated daily using a method that included being pushed laterally to assess resistance and being pulled by the tail to assess circling behaviour. |
| 154 | Rat model of transient focal ischaemia | For the operative procedures, the animals received atropine SC and anaesthesia was induced with halothane. The animals were orally intubated and mechanically ventilated with halothane in a mixture of N2O and O2 to maintain normal arterial blood gases. | Each rat was anesthetized and perfused transcardially with isotonic heparinized saline, followed by paraformaldehyde | Seven days after transient cerebral ischemia | Rats were anaesthetised and had surgery to remove part of the skull, place LDF probes over each brain hemisphere and occlude the right MCA for 90 minutes by insertion of a silicone-coated nylon monofilament.  Rats had either vehicle or test agents administered iv over 15 mins, at 15 mins before onset of ischemia, during ischemia, 15 min before reperfusion, and 45 min after reperfusion.  Rectal temperature was measured daily.  Neurological function was evaluated daily using a method that included being pushed laterally to assess resistance and being pulled by the tail to assess circling behaviour. |
| 155 | Rat model of transient focal ischaemia | After induction of anaesthesia with halothane, animals were intubated and ventilated with halothane in a mixture of N2O and 30% O2  Animals in the control group received 4.5 to 7.5 hours of halothane-anaesthesia proportionally to the duration of anaesthesia of the various treatment groups. | ‘the brains were subjected to perfusion  fixation …' | Seven days after ischemia | Rats were anaesthetised. Hypothermia was induced in some rats with ice packs until a temperature of 33°C was reached and maintained. Before induction of ischemia, an interval of 20 minutes was allowed for physiological stabilization.  Rats had surgery to remove part of the skull, place LDF probes over each brain hemisphere and occlude the MCA for 90 minutes by insertion of a silicone-coated nylon monofilament. After 90 mins reperfusion was achieved by withdrawing the filament.  Normothermic animals had vehicle. Hypothermic animals had two test agents beginning either at ischemia onset, or after 1 hour, 3 hours or 5 hours. Hypothermia was maintained for 2 hours.  Neurological function was evaluated daily using a method that included being pushed laterally to assess resistance and being pulled by the tail to assess circling behaviour. |
| 156 | Rat model of transient focal ischaemia | Animals were orally intubated and mechanically ventilated with halothane in N2O and O2 AND: In the groups subjected to methohexital, anaesthesia was introduced by halothane and followed by methohexital before ischemia. 30 mins after onset of reperfusion, methohexital was discontinued; halothane was given again to allow extubation. | Animals were anesthetized and perfused transcardially by isotonic heparinized saline, followed by 2% paraformaldehyde. | Seven days after ischemia | Under anaesthesia rats had surgery to remove part of the skull, place LDF probes over each brain hemisphere and occlude the MCA for 90 minutes by insertion of a silicone-coated nylon monofilament.  In one experiment rats received either a constant IV infusion of saline, or test agents administered from 30 minutes before ischemia to 60 minutes after reperfusion, or iv as 2 short 15 min infusions 30 minutes before ischemia and 15 minutes before reperfusion, or a combination of test agents.  In another experiment animals received either saline, methohexital in a dose causing burst suppression [an EEG pattern], or methohexital and a combination of test agents. In the groups having methohexital, anaesthesia was given prior to methohexital and discontinued thirty minutes after onset of reperfusion. Halothane was given again to allow extubation.  In another experiment animals were either normothermic and received vehicle infusion, or were normothermic and had methohexital and test agents, or were hypothermic and had test agents administered iv over 15 minutes, the first dose before ischemia and the second at reperfusion. Hypothermia was induced by ice packs and an interval of 20 mins was allowed prior to ischemia, to allow for stabilization.  Postoperatively, the neurological function of all animals was evaluated daily using a method that included being pushed laterally to assess resistance and being pulled by the tail to assess circling behaviour. |
| **Total 18** | **Permanent MCAO 5; transient MCAO 3; both transient and permanent MCAO 1; thromboembolic stroke 3; transient focal cerebral ischaemia 5; permanent focal cerebral ischaemia 1** | **Anaesthesia reported in all 18 studies, but usually unclear how long this continued for post-operatively but while experiment still in progress** | **Not reported 3; anaesthesia followed by transcardial perfusion 6; transcardial perfusion (no anaesthesia reported) 1; KC1 3; pentobarbital 1; decapitation 1; pentobarbital followed by immersion of head in liquid nitrogen 1; exsanguination and bilateral thoracotomy 1; ‘brains subjected to perfusion fixation’ 1** | **Not reported 1**  **Reported 17 (after 3 hours 1; after 4 hours 2; after 6 hours 1; after 8 hours 1; after 1 day 3; after 2 days 1; after 3 days 3; after 7 days 6)**  ***nb: some studies had more than 1 endpoint*** |  |

**Table C: Tirilazad: Unexpected deaths and events, paralytics, painkillers, welfare**

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| **Study ID** | **Unexpected deaths** | **Unexpected events** | **Paralytics** | **Painkillers** | **Welfare** |
| 139 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 140 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 141 | Reported that no unexpected deaths | Not reported | Not reported | Not reported | Before experiments animals were subjected to fasting overnight but allowed free access to water. |
| 142 | Not reported | 6 rabbits did not meet ischemic rCBF criteria | Not reported | Not reported | Not reported |
| 143 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 144 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 145 | Reported that no unexpected deaths | Not reported | Not reported | Not reported | Not reported |
| 146 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 147 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 148 | Not reported | 5 animals either did not meet rCBF criteria or clot placement could not be verified | Not reported | Not reported | Not reported |
| 149 | Not reported | 4 animals excluded due to iatrogenic subarachnoid haemorrhage after insertion of filament. | Not reported | Not reported | Animals were denied food overnight before surgery, but had free access to water. |
| 150 | Not reported | Not reported | Not reported | Rats had analgesia post operatively | Rats had free access to food and water.  Rats had analgesia and penicillin post-operatively |
| 151 | Five animals died: one on the first day and the other four on the second day post-operatively. | Not reported | Paralysis maintained with pancuronium bromide | Analgesia maintained with fentanyl | Not reported |
| 152 | 13 animals died before being killed. The authors speculate that the deaths were possibly due to perforation of the internal carotid artery (ICA) by the intraluminal filament *after* the monitoring period, due to the animal flexing and extending its neck. Some animals had the filament in place for 7 days, longer than usual. | 1 animal excluded due to inadequate MCAO.  5 animals were excluded and replaced due to vessel perforation (during insertion of the monofilament) and subarachnoid haemorrhage, confirmed by autopsy. | Not reported | Not reported | Rats were fasted overnight before surgery with free access to water. |
| 153 | Not reported | Two animals were excluded from the study and replaced because they had SAH after insertion of the filament, confirmed by autopsy. | Not reported | Not reported | Rats were fasted overnight before surgery with free access to water. |
| 154 | Not reported | 3 animals were excluded and replaced because the placement of the filament caused a SAH, confirmed by autopsy. | Not reported | Not reported | Rats were denied food overnight before surgery but had free access to water. |
| 155 | 1 control animal died within 2 days and 2 animals in group 5 died within 1 day post ischemia. Due to increased mortality / lack of neurological recovery, group 5 terminated after 6 animals due to restrictions in permission to conduct experiments. | Five animals were excluded and replaced because of vessel perforation after insertion of filament, with subsequent subarachnoid haemorrhage. | Not reported | Not reported | Not reported |
| 156 | Not reported | 10 animals excluded and replaced because of vessel perforation and SAH on filament insertion. | Not reported | Not reported | Not reported |
| **Total 18 studies** | **Not reported 13; reported that no unexpected deaths 2; deaths reported 3, some due to vessel perforation by filament insertion.**  **(In total 21 deaths reported)** | **Not reported 10; did not meet rCBF criteria/ inadequate MCAO/ clot placement not verified 3; vessel perforation and SAH due to filament insertion 6**  **(In total 29 animals ‘excluded’ due to vessel perforation)** | **Pancuronium 1** | **Not reported 16; painkillers reported 2** | **Not reported 12**  **Reported 6 (overnight fasting pre-operatively but free access to water 5; free access to food and water 1; post-operative analgesia and penicillin 1)** |

**Table D: Tirilizad: Procedures**

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| **Study ID** | **Procedures** |
| 139 | The left MCA was occluded by electrocautery. The rats were injected i.v. with U74006F 10 mins and 3h after occlusion at a dose of 3mg/kg OR i.p. 16h and 24 h after occlusion at a dose of 10mg/kg OR vehicle. |
| 140 | The tail artery was cannulated. Temperature was closely held at 37 °C using a rectal thermoprobe. The animals underwent left subtemporal craniectomy, the dura mater was incised and focal cerebral ischemia was induced by irreversible occlusion of the left middle cerebral artery by microbipolar coagulation. The wounds were sutured, anaesthesia was discontinued and external heating was maintained until the rats had regained complete consciousness and motility. The 21-aminosteroids, U74006F (one of two different doses) and U75412 E (one of two different doses), were given i.p. 30 min prior and 2, 6, and 24 h after MCAO. Control groups were used. |
| 141 | Body temperature was maintained during surgery using a rectal thermistor. The tail artery was cannulated. The common carotid arteries (CCAs) were isolated through a ventral midline cervical incision, and surgical silk ligatures were placed around both CCAs in the Wistar rats and the right CCA in the spontaneously hypertensive rat. The right MCA was exposed through a burr hole drilled near the fusion of the zygomatic arch with the squamosal bone. An aneurysm clip was used to temporarily or permanently occlude the MCA. Wounds were closed with surgical clips, anaesthesia was discontinued and animals were returned to their cages. Temperature was maintained using the rectal thermistor until animals had regained homeostatic control and were fully awake (within minutes). 2 hours later rats subjected to transient ischemia were re-anesthetized, the clip was removed and restored blood flow in the MCA confirmed visually. In Wistar rats, the suture in the left CCA was also cut (to restore blood flow?). Wounds were reclosed, and rats were returned to their cages. Regional CBF was recorded using laser Doppler flowmetry. For this a burr hole had apparently previously been drilled and a thin bone layer removed. A laser Doppler flowmetry probe was advanced under microscopic guidance to the surface of the dura*.* CBF was measured before and just after CCA/ MCA occlusion, before and just after reperfusion in experiments 1 and 2 and at the time of decapitation.In Exp.1 rats received agents i.p. prior to surgery and 4 and 10 hours after perfusion (i.p). In Exp. 2 rats received agents i.p. prior to surgery and 4 and 10 hours after reperfusion (i.p). In Exp. 3 rats received agents i.p prior to permanent occlusion and 6 and 12 h later. In Exp. 4 rats received agents i.v. 10 mins and 3 h after permanent occlusion. Further doses were given i.p. 6 and 12 h after permanent occlusion. Arterial blood gases, hematocrit, and glucose were measured twice. |
| 142 | The femoral arteries were cannulated. The animal was tracheostomized, mechanically ventilated and bilateral craniectomies were performed. Electrodes were inserted 2 mm into the cortical mantle for monitoring rCBF; three electrodes were evenly spaced in a 15-mm long trough over the hemisphere to be embolized. A single electrode was similarly placed over the contralateral hemisphere. An epidural fiberoptic intracranial pressure (ICP) monitor was placed anterior to the coronal suture after craniectomy. The ICP monitor and rCBF electrodes were then hermetically sealed in place, essentially reconstructing the calvarium. Core temperature was maintained with a heating blanket. The region of the carotid bifurcation was then exposed, the external carotid artery ligated, and the common and internal carotid artery isolated. Autologous clot was formed by incubating blood from the auricular artery in PE-90 tubing at 37°C for 4 hours. (When?) An autologous clot embolus was injected under direct visualization and the artery was sutured. Regional CBF determinations were made within 10 minutes of embolization. The animals were ‘supported’ for a total of 4 hours from the time of the embolic event. Physiological measurements were recorded at base line, within 30 minutes of embolization, and at 1, 2, 3, and 4 hours after embolization. |
| 143 | Cats were orally intubated and mechanically ventilated. Catheters were placed in both femoral veins, in the descending aorta via a femoral artery and in the left atrium via a left thoracotomy. The cat was turned prone and its head positioned in a stereotaxic frame. A thermistor was placed in the right temporal epidural space. Epidural temperature was maintained using a heating pad and a heating lamp. The left MCA was exposed. To produce focal ischemia, the MCA was reversibly occluded using a microvascular clip, and both common carotid arteries were reversibly occluded by tightening previously placed ligatures. Regional cerebral blood flow (CBF) was measured with injected radiolabelled microspheres. In the control group, just after tightening the ligatures, vehicle was administered by continuous intravenous infusion. In the pre-treatment group, just after tightening the ligatures, tirilazad was administered by continuous intravenous infusion followed by intravenous infusion of vehicle at 70 minutes of ischemia. In the posttreatment group, just after tightening the ligatures, vehicle was administered by continuous intravenous infusion followed by tirilazad by continuous intravenous infusion at 70 mins. After 90 minutes of ischemia the carotid ligatures were loosened, and the clip on the MCA was removed. Reperfusion lasted 180 minutes. Measurements were made at 15, 30, 60, 90, 120, 150, and 180 minutes of reperfusion. Arterial blood gas and CBF measurements were made before ischemia, at 20 and 85 minutes of ischemia, and at 60,120, and 180 minutes of reperfusion. |
| 144 | The rats were intubated and artificially ventilated. The tail artery and a tail vein were cannulated. The body core temperature was maintained using a rectal thermometer. Exposure and permanent occlusion of the left MCA was accomplished with a technique they do not describe but provide a reference for. Tirilazad mesylate, U-74006F or vehicle was injected over a 2 minute period into the tail vein 10 min and 3 h after MCA occlusion. The rats were allowed to recover and were extubated. The tail vein catheter was sealed and left in place until the 3 hour administration had been given. Neurological status was tested on day 1, 2, and 3 using the following grading system: Grade 0, normal behaviour; Grade 1, forelimb flexion; Grade 2, forelimb flexion and decreased resistance to lateral push; Grade 3, same as grade 2, with circling behaviour. |
| 145 | A mask was put on the nose and self-respiration was maintained during the surgery, which took 20-30 min. The right femoral artery and vein were catheterized. Needle temperature sensors were inserted into the bilateral temporal muscles. Focal cerebral infarction was achieved as follows: all animals underwent left MCA occlusion via a subtemporal approach without removal of zygomatic arch or temporal muscle. Under a surgical microscope, the left MCA was coagulated with a micro-bipolar coagulator from the olfactory tract to the most proximal portion of the MCA through a cranial window, about 3 to 4 mm in diameter. The animals were maintained normothermic with a homeothermic blanket. Two, six, twelve and twenty-four hours after discontinuing anaesthesia, each rat's level of consciousness and motor activity were evaluated using a grading scale: (0: normal activity; 1: spontaneous activity; 2: not arousable by tactile stimulation; 3: not arousable by painful stimulation, no spontaneous activity). At 24 h post MCA occlusion, neurological status was examined using a grading scale of 0 to 3 (0: no observable deficit; 1: forelimb flexion; 2: decreased resistance to lateral push without circling; 3: same behaviour as grade 2, with circling). Agents were administered i.v. at 15 min, 2 h and 6 h post occlusion, then (at higher doses) i.p. 12 h post occlusion. ALSO, In a preliminary study, the temporal muscle and rectal temperatures were continuously monitored for 5 h in conscious normal rats to investigate the effect of U-74006F upon temporal muscle and body temperature during the early stage of drug treatment after cessation of anaesthesia. |
| 146 | Body temperature was maintained with a heating-pad. In the main experiment, a catheter was inserted into the femoral vein. The scalp and temporalis muscle were reflected, and a subtemporal craniotomy was performed, using a dental drill. A 4-mm-diameter window in the skull base was opened and a segment of the main trunk of the middle cerebral artery was exposed. A 3-mm-diameter circular area of the window was illuminated with green light and the entire illuminated segment became thrombotically occluded. The irradiation was directed by an optic fibre, the head of which was placed on the window in the skull base. After 10 mins of photo-illumination rose bengal was injected iv and then photo-illumination was continued for a further 10 min. U74006F or vehicle was administered iv in each of the animals 10 min after the rose bengal injection. The incisions were closed after the operation. Spontaneous reperfusion occurred about 3 h hours after the occlusion. Twenty-four hours after occlusion the extent of ischaemic damage was measured by MRI. |
| 147 | A thrombus was created in rabbits by puncturing the central auricular artery with a needle. The intima of the artery was then traumatised with another needle to produce an arterial thrombus. A ligature was placed around the artery distal to the puncture and tied to reduce blood flow by 50%. Rabbits were returned to their cages and given routine care for the next two days. Two days later the rabbits were anaesthetised. An arterial line was established. The thrombosed section of the artery was harvested and the thrombus extracted. (*Is this how all autologous thrombi are created?*) Surgery was conducted to cannulate the internal jugular and inject the thrombus into the internal carotid. Surgery was completed and the skin incision closed with staples. Monitoring continued for 2 h and then treatment was given (tpa, 21-amino steroid, combination of both, control - administered via cannulated jugular). |
| 148 | Briefly, following anaesthesia, bilateral femoral cutdowns were performed for the introduction of aortic and venous catheters. A tracheostomy was then performed and the animal mechanically ventilated throughout. A craniectomy was performed for placement of the following monitors: intracranial pressure monitor, an epidural brain temperature probe, and electrodes to measure rCBF. An autologous clot embolus was introduced into the right internal carotid artery and injected into the anterior circulation of the brain. The autologous clot had been previously prepared 2-3 hours prior by admixing 1 ml of whole blood with 50-60 mg of 20 tin granules in a thrombin-treated segment of polyethylene tubing. Following embolisation the artery was sutured. A submental vertex X-ray was taken for verification of clot placement. U74006F I.V. and L644,711 I.V. or their vehicle control were given 3.5 hours following autologous clot embolization. Both groups received t-PA I.V. beginning 4 hours after thromboembolic stroke; 20% bolus I.V. with remainder continuing over a 2-hour infusion period. |
| 149 | Temporalis muscle and rectal probes were used to monitor temperature. The tail artery and left femoral vein were cannulated. Local cerebral blood flow (LCBF) was bilaterally recorded by continuous laser Doppler flowmetry). For this, bilateral 1-mm burr holes were drilled. Each animal was placed supine and the head was firmly immobilized in a stereotaxic frame with non-rupture ear bars. A laser Doppler probe was positioned over each brain hemisphere. Local CBF was continuously measured from before the onset of ischemia until 1 h after reperfusion. All rats were subjected to 90 min of right MCA occlusion by introduction of a nylon monofilament via the external carotid artery. Rats received either: (1) vehicle, (2) DM, (3) tirilazad, or (4) DM+tirilazad. DM and Tirilazad were administered iv in succession over 15 min, starting 15 min before ischemia and at reperfusion. Neurological deficits were evaluated daily using a 6-scale grading score: (0) no spontaneous activity, (1) spontaneous circling, (2) circling if pulled by tail, (3) lowered resistance to lateral push without circling, (4) contralateral forelimb flexion, (5) no apparent deficit. Rectal temperature was measured 2 h after reperfusion and then daily. |
| 150 | Micro-dialysis: a cannula was implanted in the left frontoparietal cortex and secured on the skull. A micro-dialysis probe was inserted and perfused with Ringer solution, with half hourly collection. On day 1 the probe was perfused for 2–2.5 h, then MCAO or sham operation was performed. Samples were collected for a further 4.5 h. Micro-dialysis was discontinued overnight, but the probe was left in place. On days 2 and 3 perfusion was restarted. Thereafter, beginning exactly 25 and 49 h after the operation, samples were collected for 4.5 h. For the MCAO, rectal body temperature was maintained with a heating pad. The tail artery was cannulated. A minimal left subtemporal craniotomy was performed and the stem of the MCA was occluded with bipolar coagulation and cut to prevent spontaneous reperfusion. Occlusion was monitored by laser Doppler measurement. (no details on placement of this) For the sham operation, the same anaesthetic and surgical procedures were performed, except that the edge of the dura was coagulated instead of the MCA. One hour after MCAO the rat was allowed to regain consciousness in a heated cage. Postoperatively, rats were treated with buprenorphine (an analgesic) and ampillicin. Either vehicle alone or U74 solution was injected i.v. 15 min, 2h, 6 h and i.p. 12 h and 24 h after MCAO or sham operation. |
| 151 | Tracheostomy was performed and lungs were mechanically ventilated with air. Catheters were inserted into the femoral artery and vein. Occlusion of the MCA was performed with the head stabilized. Evacuation of the orbital content was performed (i.e. eye removed) to prevent wound infection and bleeding. Craniectomy was performed just above the optic foramen using a high-speed drill. The dura mater was incised and the MCA occluded using bipolar cautery. The middle of the closed artery was cut with micro-scissors to prevent recanalization. The craniectomy defect was covered with Gelfoam and the wound closed. After MCAO the rabbits were given either i.v. vehicle; U-74006F over 2 min in the femoral vein 15 min and 3 h after MCA occlusion; mannitol twice at an interval of 15 min every 3 h (how? iv?); U-74006F plus mannitol using a combination of the above procedures. Sham-operated animals were prepared in the same way. Physiological parameters were recorded before and at 15 min and 3 h after MCA occlusion. Body temperature was monitored by a rectal thermometer. The neurological status of each rabbit was evaluated 24, 48, and 72 h after occlusion using the following grading scale: normal, abnormal, or dead. Abnormal findings include generalized weakness, markedly reduced motor activity (decreased feeding, grooming), or obtundation (less than fully alert). |
| 152 | A temporalis muscle and rectal probe were used to monitor temperature. In the treatment group, whole-body hypothermia was induced by the application of ice packs over 30 min until 33C rectal and temporalis muscle temperature were reached. Rewarming was started after 120 min of 33C hypothermia. The tail artery and left femoral vein were cannulated. Continuous laser-Doppler flowmetry (LDF) was employed, I think in all animals. For placement of the LDF probe, a burr hole was drilled. The animals were placed in a supine position and the skull was immobilized in a stereotaxic frame. An LDF probe was positioned above the surface of each brain hemisphere. LCBF was continuously measured, starting prior to ischemia with termination prior to the end of the acute experimental phase. Animals were subjected to permanent MCAO by insertion of a silicone-coated nylon monofilament via the external carotid artery. Animals were either normothermic and had vehicle (saline infused over 30 min), or were hypothermic (33C maintained for 2h) and had MgCl2 + tirilazad serially infused, each drug over 15 min, commencing 30 min prior to ischemia. Drugs were administered at 90 min intervals. Anaesthesia was terminated 180 min after ischemia onset. Postoperatively, neurological function was evaluated daily using a 6-point scale: 5=no apparent deficit, 4=contralateral forelimb flexion, 3=lowered resistance to lateral push without circling, 2=circling if pulled by tail, 1=spontaneous circling, 0=no spontaneous activity. Each animal’s body weight was determined daily. Some animals were killed after 6 h and some after seven days. |
| 153 | The animals were orally intubated and mechanically ventilated. Temporalis muscle and rectal probes were used to monitor temperature. The tail artery and left femoral vein were cannulated. A continuous laser-Doppler flowmeter (LDF) was used. To allow placement of the LDF probe, a burr hole (1-mm diameter) was drilled. The animals were placed in a supine position, and the head was firmly immobilized in a stereotaxic frame. The LDF probe was positioned over each brain hemisphere. LCBF was continuously measured from before the onset of ischemia until 2 hours after reperfusion. All rats were subjected to 90 minutes of MCA occlusion by insertion of a silicone-coated nylon monofilament via the external carotid artery. Reperfusion was achieved by withdrawing the filament into the external carotid artery after 90 minutes. Rats were either vehicle-treated, normothermic; tirilazad and MgCl2 normothermic; vehicle-treated, hypothermic; tirilazad and MgCl2 hypothermic. Vehicle or drugs were administered intravenously over 15 minutes. Each animal received 2 doses of vehicle or drugs, with the first dose being administered before ischemia and the second dose at reperfusion. In the hypothermic groups, whole-body hypothermia was induced with the use of ice packs until a temperature of 33°C was reached and maintained. Before induction of ischemia, an interval of 20 minutes was allowed for physiological stabilization. Rewarming started 30 minutes after reperfusion. Postoperatively, each animal’s neurological function was evaluated daily on a 6-point grading scale: 5, no apparent deficit; 4, contralateral forelimb flexion; 3, lowered resistance to lateral push without circling; 2, circling if pulled by tail; 1, spontaneous circling; and 0, no spontaneous activity. In addition, each animal’s body weight was determined daily. |
| 154 | The animals were orally intubated and mechanically ventilated. Temporalis muscle and rectal probes were used to monitor temperature. The tail artery and left femoral vein were cannulated. Continuous laser Doppler flowmetry (LDF) was used. To allow placement of the LDF probe, a burr hole 1 mm diameter was drilled. The animals were placed supine and the head was firmly immobilized in a stereotaxic frame. The LDF probe was positioned over each brain hemi-sphere. LCBF was continuously measured from before the onset of ischemia until 1h after reperfusion. All rats were subjected to 90 min of right MCA occlusion by the introduction of a silicone-coated nylon monofilament via the external carotid artery. Rats had either: vehicle; U-101033E; U-74389G; U-101033EqU-74389G. Drugs were administered 15 mins before onset of ischemia, during ischemia, 15 min before reperfusion, and 45 min after reperfusion. Each dose was administered iv over 15 min. Postoperatively, each animal’s neurological function was evaluated daily, using a six-point grading scale: 5. no apparent deficit, 4. contralateral forelimb flexion, 3. lowered resistance to lateral push without circling, 2. circling if pulled by tail, 1. spontaneous circling, 0. No spontaneous activity. In addition, each animal’s body weight was determined daily and rectal temperature was measured daily. |
| 155 | Animals were intubated and ventilated. Temporalis muscle and rectal temperature were monitored. In the hypothermic groups, whole-body hypothermia was induced with ice packs until 33°C rectal and temporalis muscle temperatures were reached. Before induction of ischemia, a 20-minute interval was allowed for physiological stabilization. Rewarming (1°C every 10 minutes) was started 30 minutes after reperfusion. Femoral vein and tail artery were cannulated. Continuous laser Doppler flowmetry (LDF) was used to monitor local cerebral blood flow (LCBF). To allow placement of the LDF probe, a burr hole (1-mm diameter) was drilled. The animal was placed supine, and the head was firmly immobilized in a stereotaxic frame. The LDF probe was positioned over each brain hemisphere. Cortical CBF was continuously measured from before the onset of ischemia until 1 hour after reperfusion. All animals were subjected to 90 minutes of MCA occlusion by introduction of a silicone-coated nylon monofilament inserted  via the external carotid artery. Reperfusion was established by withdrawal of the filament back into the external carotid artery after 90 minutes. Animals were either: normothermic vehicle treated controls; MgCl2 + tirilazad + hypothermia beginning either at ischemia onset, or after 1 hour, 3 hours or 5 hours. Drugs were given in 1-hour intervals, and hypothermia was maintained for 2 hours. Animals in the control group received 4.5 to 7.5 hours of halothane-anesthesia proportionally to the duration of anesthesia of the various treatment groups. Post-operatively, the neurological function of all animals was evaluated daily with a 6-point scale: 5 no apparent deficit, 4 contralateral forelimb flexion, 3 lowered resistance to lateral push without circling, 2 circling if pulled by tail, 1 spontaneous circling, and 0 no spontaneous activity. |
| 156 | Animals were orally intubated and mechanically ventilated. Temporalis muscle and rectal probes were used to monitor temperature. The tail artery and left femoral vein were cannulated. Continuous laser Doppler flowmetry (LDF) was used. To allow placement of the LDF probe, a burr hole 1 mm diameter was drilled. The animals were placed supine and the head was firmly immobilized in a stereotaxic frame. The LDF probe was positioned over each brain hemi-sphere. LCBF was measured continuously, beginning before ischemia with termination 1 hour after start of reperfusion. All animals were subjected to 90 minutes of MCA occlusion by a silicone-coated nylon monofilament inserted via the external carotid artery. **Experiment 1A**: Animals received either (1) vehicle, (2) nimodipine, (3) mannitol, (4) dexamethasone, or (5) nimodipine mannitol dexamethasone (N M D). Control animals received saline I.V. as constant infusion. Nimodipine was administered from 30 minutes before induction of ischemia until 60 minutes after reperfusion. Mannitol and dexamethasone were applied iv as short infusion for 15 minutes, with the first dose given 30 minutes before induction of ischemia and the second at 15 minutes before reperfusion.  **Experiment 1B:** Animals received either (1) saline, (2) methohexital in a dose causing burst suppression [an EEG pattern], or (3) methohexital and N M D. In the groups subjected to methohexital, anesthesia was introduced by halothane and followed by methohexital before ischemia. Thirty minutes after onset of reperfusion, methohexital was discontinued; halothane was given again to allow extubation. In group 3, burst suppression was induced by methohexital together with administration of N M D as described above. **Experiment 2:** Animals received either (1) vehicle infusion in normothermia, (2) methohexital and N M D, or (3) MTH. In the normothermic control group, anaesthesia was maintained by halothane. Group 2 received methohexital and N M D as described above. In group 3, MgCl2 and tirilazad mesylate were administered iv over 15 minutes. The first dose was given before ischemia and the second at reperfusion. Whole-body hypothermia was induced by ice packs until a rectal and temporalis muscle temperature of 33°C was reached. Before ischemia, an interval of 20 minutes was allowed for stabilization. Rewarming to normothermia (1°C per 10 minutes) was started 30 minutes after onset of reperfusion. Postoperatively, the neurological function of all animals was evaluated daily using a 6-point scale: 5 points, no deficit; 4 points, contralateral forelimb flexion; 3 points, lowered resistance to lateral push; 2 points, circling if pulled by tail; 1 point, spontaneous circling; 0 points, no spontaneous activity. |

**Table E: Tirilizad: Model**

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| **Summary of model**  The core model is an operation under anaesthesia to occlude the middle cerebral artery (MCAO), or a sham operation, and then to test the effect of various agents on recovery after this operation. During the operation probes and monitors may also be placed in the brain or on the surface of the brain. In some studies hypothermia is also induced in animals. After the operation animals are given test agents or vehicle at various time points, usually intra-peritoneally or intravenously. Some of these test agents may be administered before or during the operation, most are administered post-operatively up to various time points (3h, 4h, 5h, 12 h or 24h).  **Pre-operative variations:** In two studies blood was taken from auricular artery (to produce an autologous clot) prior to the main experiment. In one study animals had an operation under anaesthesia to produce an autologous clot.  **Second operation:** In one studya second operation was performed under anaesthesia (to restore flow in the brain after transient ischaemia) after an unspecified recovery period.  **Post-operative assessments:** MRI scans were performed 24 h after operation in two studies. Some animals had daily neurological assessments using methods that included attempting to arouse the animal using tactile and painful stimulation, pushing the animal against resistance and pulling the animal’s tail.  **Broad categories of model**  Animals that had MCAO operation, test agents and killed up to 24 h.  Animals that had MCAO operation, test agents, daily assessments and killed up to 2- 3 days.  Animals that had MCAO operation, test agents, daily assessments and killed at 7 days. |