Nitric oxide in focal cerebral ischemia, an experimental study

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Chapter 2

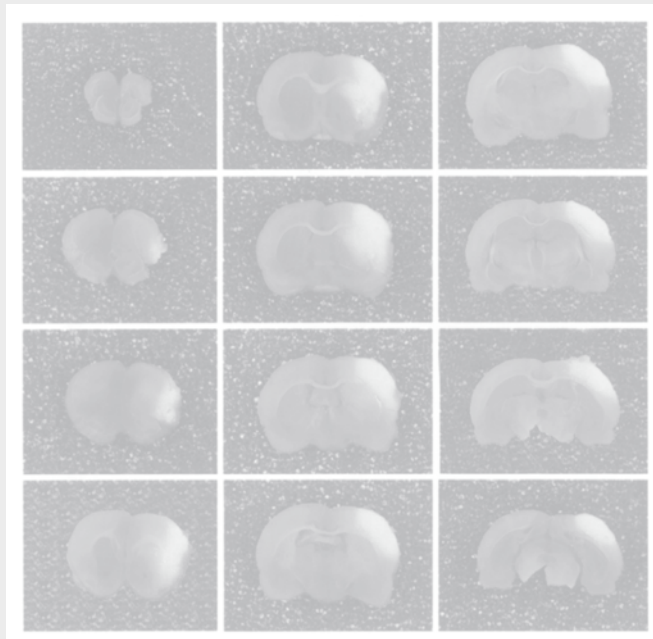## Reproducibility of Cerebral Cortical Infarction in the Wistar Rat After Middle Cerebral Artery Occlusion

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ABSTRACT

Although middle cerebral artery (MCA) occlusion in the rat is often used to study focal cerebral ischemia, the model of ischemia affects the size and reproducibility of infarction. The purpose of this experiment was to methodically examine different preparations to determine the optimum focal cerebral ischemia model to produce a reproducible severe ischemic injury. Eighty-two Wistar rats underwent 1 hour, 3 hour, or permanent MCA occlusion combined with no, unilateral, or bilateral common carotid artery (CCA) occlusion. Three days after ischemia, the animals were prepared for tetrazolium chloride assessment of infarction size. One-hour MCA occlusion produced a coefficient of variation (CV) of 200% with an infarction volume of 20.3 ± 10.5 mm^3. Adding unilateral or bilateral CCA occlusion resulted in a CV of 134% and 101%, respectively. Three-hour MCA occlusion combined with bilateral CCA occlusion decreased the CV to 58% with a cortical infarction volume of 82.6 ± 12.1 mm^3, $P < 0.05$, compared with 1-hour MCA occlusion with or without CCA occlusion. Permanent MCA occlusion combined with 3 hours of bilateral CCA occlusion resulted in a CV of 47% with a cortical infarction volume of 89.6 ± 16.0 mm^3. These results indicate that 3-hour MCA occlusion combined with bilateral CCA occlusion consistently provides a large infarction volume after temporary focal cerebral ischemia.

INTRODUCTION

Numerous experimental animal models have been developed to study the pathophysiology and to test therapeutic interventions in focal cerebral ischemia. Rodent models have gained considerable interest for a variety of reasons including their low cost and wide availability, and new possibilities with genetically altered animals. Because of Tamura et al's[1] description of a middle cerebral artery occlusion (MCAO) technique, MCAO in rats has been widely used as a model of focal cerebral ischemia. Unfortunately, there is significant variability in the reproducibility and size of cortical infarction using the MCAO model in Wistar rats[2,3]. This makes it problematic to investigate the pathophysiology and to compare the efficacy of therapeutic interventions. This variability or inconsistency can be attributed to the model (location and duration of occlusion), strain/vendor[4,5], age/weight[6,7], anesthesia[8], brain and body temperature[9], systemic parameters (PaO_2, $PaCO_2$, and pH); blood pressure[10,11] and serum glucose levels (Table 1) [12-14]. The purpose of this experiment was to rigorously examine various models of temporary focal cerebral ischemia in the Wistar rat to improve consistency and reproducibility of cortical infarction.

MATERIAL AND METHODS

After review and approval by the Institutional Animal Care and Use Committee, 82 adult male Wistar rats weighing between 350 and 450 g were anesthetized with halothane at 1.5% during the surgical exposure and at 1.0% during the occlusion period. The animals were spontaneously breathing with a mixture of air and oxygen through a face mask. Atropine was administered preoperatively at 0.1 mg/kg subcutaneously to reduce respiratory secretions. Core body temperature was monitored with a rectal probe and was maintained at 37 ± 0.5°C by using an infrared heating lamp which warmed both the head and body simultaneously. Head and brain temperature, therefore, was maintained at 37 ± 0.5°C throughout the entire experiment from the beginning of the surgical preparation. A polyethylene catheter (PE-50; IntraMedic AB, Balsta, Sweden) was inserted into the right femoral artery to monitor arterial blood pressure and to sample blood for measurements of PaO_2, $PaCO_2$, pH, and serum glucose. All animals had free access to water and food before and after surgery. Body weight of the rats was recorded preoperatively and before they were killed to examine weight loss.

Temporary Left MCAO

Temporary MCAO was obtained through a modification of the technique originally described by Tamura et al[1]. Briefly, a 2-cm skin incision was made between the left outer

Table 1. Review of temporary clip and ligature MCA occlusions in rats (mean ± SE)

	Morikawa et al.[39]	Buchan et al.[17]	Margaill et al.[34]	Selman et al.[40]	David et al.[41]	Hiramatsu et al.[3]	Xue et al.[35]	Drummond et al.[42]	Coert et al.
Strain (all male)	Sprague-Dawley	Wistar/SHR	Sprague-Dawley	Wistar/SHR	Wistar	Sprague-Dawley	Wistar	SHR	Wistar
Vendor	Charles River	n.a.	n.a.	n.a.	n.a.	Hilltop	n.a.	n.a.	Harlan SD
Weight (g)	200-480	W: 180-230/ S: 230-280	300-350	250-350	350-450	270-320	200-250	350-400	270-470
Anesthesia	Halothane (1.5%)	Halothane (1-2%)	Chloralhydrate (sb)	Halothane (2%)	Halothane (0.5-1.0%)	Halothane (1.5%)	Halothane (1-2%)	Halothane (0.9%)	Halothane (1-1.5%); sb
Temperature (°C)	36.5-37.5	37 ± 0.5	37-38	37 ± 0.3	37.4 ± 0.2	35.5	37	37	36.5-37.5
Glucose (mmol/L)	9.9	W: 6.2/S: 7.5	n.a.	n.a. (fasted)	6.8	n.a. (nonfasted)	n.a. (fasted)	6.9	9.6
MABP (mm Hg)	100	W: 126/S: 189	87	W: 65/S: 106	97	100	84	130	92
MCAO method	Perm/temp coag/Zen clip (olf)	Perm/temp: coag/Sundt #1 (distal inferior vein) + bilat CCAO	Temp: Zen clip (proximal of lenticulo-striate) + bilat CCAO	Temp: ligature (anterior of lenticulo-striate)	Temp: Zen clip (proximal occlusion)	Temp: Sundt #1 (between olf Branch-lenticulo-striate) + bilat CCAO	Perm/temp: coag/Sundt #1 (rhina fissure) + bilat CCAO	Temp: 2 ligatures (proximal to lenticulostriate + distal to inferior cerebral vein)	Perm/temp: coag./Sundt #2 (olf) + bilat CCAO
Occlusion time	Perm/2 hr	Perm/1, 2, 3 hr	45/60/90 min	60 min	60 min	30 min/3 hr	Perm/3 hr	180 min	Perm/3 hr
Reperfusion time	72 hr	24 hr	24 hr	72 hr	72 hr	72 hr	6 hrs/21 hr	120 min	72 hr
Staining	H & E	H & E	Cresyl Violet	H & E	H & E	TTC immersion	H & E	TTC perfusion	TTC perfusion
Mean cortical infarction volume (mm³)	Cortex: Perm: 67.6 ± 9.1 Temp: 22.5 ± 9.1	Perm: W: 214 ± 28 S: 209 ± 12 Temp (W): 1 hr: 21 ± 5 2 hr: 150 ± 43 3 hr: 211 ± 14	Perm: 45 min: 135 ± 28 60 min: 212 ± 13 90 min: 217 ± 13	W: 49.4 ± 17.3 S: 126.7 ± 18.7	50 ± 12.1	1 hr: 71 ± 32.2 2 hr: 132 ± 44.1 3 hr: 177 ± 6.3	Perm: 142 ± 18 Temp: 211 ± 14	99 ± 4.6	Cortex: Perm: 89.6 ± 16.0 Temp: 82.6 ± 12.1

(Abbreviations: MCA, middle cerebral artery; SE, standard error; n.a., not available; sb, spontaneous breathing; MABP, mean arterial blood pressure; MCAO, middle cerebral artery occlusion; perm, permanent; temp, temporary; coag, coagulation; olf, olfactory tract; bilat, bilateral; CCAO, common carotid artery occlusion; H & E, hematoxylin and eosin; TTC, 2,3,5-triphenyl tetrazolium.)

canthus and the tragus. After deflecting the temporal muscle anteriorly, the middle part of the left zygomatic arch was removed without damaging the facial nerve. Muscles were retracted both downward and anteriorly, after which the mandibular nerve was identified and followed back to the foramen ovale. A 4 mm craniectomy was made just anterior and superior to the foramen ovale with the use of a high speed air drill. After opening the dura with a sharp needle, the left MCA was dissected free of the arachnoid. A Sundt #2 AVM microclip (Codman and Shurtleff, Inc, Raynham, MA) was applied to the MCA crossing the olfactory tract.

Bilateral Common Carotid Artery Occlusion (CCAO)

A ventral midline incision was made and both CCAs were exposed and isolated from the surrounding tissue. The contralateral CCA was permanently ligated using a 3.0 silk suture, the ipsilateral CCAO with a miniature Mayfield aneurysm clip was temporary (1 hour or 3 hours).

Permanent Left MCAO

Surgical exposure was previously described for the temporary occlusion of the MCA. After freeing the MCA from the arachnoid, a bipolar forceps was used to carefully coagulate the MCA from the olfactory tract to the inferior cerebral vein. To prevent recanalization, the MCA was transected using microscissors.

Experimental Study Groups

The animals were divided into 7 groups. The first 3 groups underwent 1-hour MCAO with either 1 day (n = 13), 3 days (n =15), or 7 days (n = 6) of reperfusion to examine for possible effects of reperfusion time on infarction volume. Groups 4 through 7 had 3 days of reperfusion after the ischemic experiment before histological preparation. Group 4 (n = 10) underwent 1-hour MCAO with ipsilateral CCA occlusion. Group 5 (n = 16) had 1-hour MCAO and bilateral CCAO (MCAO + 2 CCAO). In group 6 (n = 16), the duration of occlusion was 3 hours for both MCAO and bilateral CCAO. In group 7 (n = 6), animals underwent permanent MCAO with 3-hour-bilateral CCAO. Ipsilateral CCAO was always temporary, whereas the contralateral CCAO (only in the bilateral CCAO groups) was permanent.

Histopathology

After the designated reperfusion period, the animals were reanesthetized, weighed, and perfused intracardially with a warm (37˚C) 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution. The brains were quickly removed and then immersed in 37˚C, 2% TTC solution for 15 minutes to enhance staining and then placed in a 10% buffered formaldehyde solution for 5 days. Twelve serial coronal sections were cut at 1-mm intervals, beginning at the frontal pole, by using a rodent brain matrix (ASI Instruments Inc, Warren, MI), and photographed (Fig 1). Areas of unstained tissue (infarcted) were easily disseminated
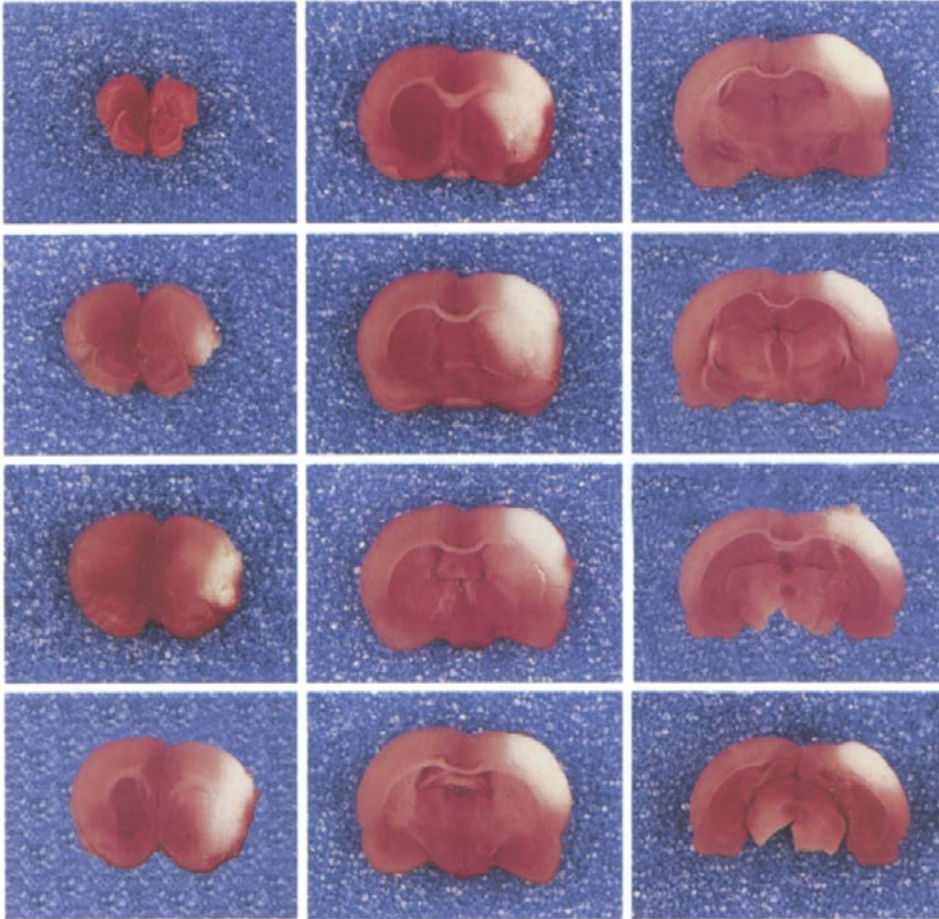
Figure 1. TTC histology of adjacent serial coronal brain sections that were cut at I-mm intervals after 3 hours MCA and bilateral CCA occlusion, followed by a 3-day reperfusion period. For colour image see front cover.

from areas of viable tissue, which stained red or pink. The infarcted area of each tissue section was traced by a computer-assisted image analyzer (JAVA; Jandel Scientific Software, SPSS, Inc, Chicago, IL). This analyzer was previously calibrated to express the measurement in square millimeters. Total cortical infarction volume was calculated by integrating the infarcted area in each slide (area of infarction in square millimeters times thickness of the slice, 1 mm). The percentage of animals with infarction was determined by dividing the number of infarcted animals by the total number of animals within each study group. Edema ratio was determined by dividing the left hemispheric area by the right hemispheric area.

Statistical Analysis

Analysis of variance (ANOVA) with Tukey's posthoc test for multiple comparisons was used for statistical analysis of infarction volume, edema ratio, and percentage of weight loss. The coefficient of variation (CV) was computed to provide an index of experimental variation for each group. Two-sample F test was used for statistical analysis of rate of infarction and CV. Statistical analysis of systemic parameters were performed using the unpaired t-test. Data were expressed as mean and standard error. P values less than 0.05 were considered to be significant.

RESULTS

Systemic Parameters

The physiologic parameters, which were mean arterial blood pressure (MABP), PaO_2, $PaCO_2$, pH, serum glucose, and core body temperature, are shown in Table 2. There were no significant differences between the groups studied.

Table 2. Systemic parameters (mean ± SE)

Groups	Sacrifice time (n)	Weight (g)	pH	$PaCO_2$ (mm/Hg)	PaO_2 (mm/Hg)	Glucose (mmol/L)	MABP (mm/Hg)
1 hr MCAO	1 day (13)	395 ± 14.6	7.46 ± 0.03	42.8 ± 2.0	202.9 ± 18.6	n.a.	87.4 ± 3.7
1 hr MCAO	3 days (15)	376 ± 7.1	7.41 ± 0.02	41.4 ± 1.8	242.7 ± 12.3	11.0 ± 0.6	84.7 ± 2.3
1 hr MCAO	7 days (6)	403 ± 17.8	7.40 ± 0.03	41.5 ± 4.0	195.3 ± 19.4	n.a.	93.3 ± 13.4
1 hr MCAO + 1 CCAO	3 days (10)	337 ± 16.9	7.40 ± 0.02	42.6 ± 2.1	221.5 ± 17.8	8.2 ± 0.5	91.3 ± 3.6
1 hr MCAO + 2 CCAO	3 days (16)	341 ± 8.0	7.46 ± 0.02	42.1 ± 2.0	179.3 ± 7.2	9.7 ± 0.4	95.3 ± 4.3
3 hr MCAO + 2 CCAO	3 days (16)	349 ± 15.3	7.42 ± 0.02	45.0 ± 1.8	236.9 ± 19.9	10.4 ± 0.7	94.9 ± 2.0
Permanent + 2 CCAO	3 days (6)	340 ± 7.5	7.36 ± 0.01	42.4 ± 4.6	156.3 ± 24.0	9.4 ± 0.2	95.6 ± 1.6

(Abbreviations: SE, standard error; MABP mean arterial blood pressure; MCAO, middle cerebral artery occlusion; n.a., not available; CCAO, common carotid artery occlusion). NOTE: Core body and head temperature were maintained at 37 ± 0.5ºC. These systemic parameters were measured at the end of the ischemic period.

Table 3. Measured parameters (mean ± SE)

Groups	Sacrifice time (n)	% with infarction	Infarction volume	% weight loss	Edema ratio	CV (%)
1 hr MCAO	1 day (13)	53	18.9 ± 9.2	5.2 ± 1.0	1.04	175
1 hr MCAO	3 days (15)	40	20.3 ± 10.5	13.0 ± 1.7‡	1.00	200
1 hr MCAO	7 days (6)	43	16.3 ± 10.0	24.2 ± 1.7§	1.01	150
1 hr MCAO + 1 CCAO	3 days (10)	60	21.3 ± 9.0	14.0 ± 2.8	1.02	134
1 hr MCAO + 2 CCAO	3 days (16)	75	51.9 ± 13.1*	14.6 ± 0.8	1.01	101
3 hr MCAO + 2 CCAO	3 days (16)	88	82.6 ± 12.1*†	17.1 ± 1.3§	1.00	58§
Permanent + 2 CCAO	3 days (6)	100	89.6 ± 16.0*†	21.8 ± 1.5§	0.99	47§

(Abbreviations: SE, standard error; CV, coefficient of variation; MCAO, middle cerebral artery occlusion; CCAO, common carotid artery occlusion.)

*Significantly different from 1 hr MCAO 1-, 3-, and 7-day sacrifice groups and 1 hr MCAO + 1 CCAO group, P < 0.05.

† Significantly different from 1 hr MCAO + 2 CCAO group, P < 0.05.

‡ Significantly different from 1 hr MCAO 1-day sacrifice group, P < 0.05.

§ Significantly different from 1 hr MCAO 3-day sacrifice group, P < 0.05.

Experimental Groups

Effect of Reperfusion on Infarction Rate and Volume (groups 1, 2, and 3)

The infarction rates and infarction volumes for the 3 1-hour MCAO without CCAO groups that were killed 1, 3, or 7 days after ischemia are described in Table 3. There were no significant differences in infarction rate, CV, or infarction volume among these groups.

72-Hour Reperfusion (groups 4, 5, 6, and 7)

The 1-hour MCAO group without CCAO resulted in infarction in 7 of 15 animals with a CV of 200%. Adding ipsilateral and bilateral CCAO resulted in infarction in 6 of 10 animals with a CV of 134% and in 12 of 16 animals with a CV of 101%. The group with 3-hour MCAO and 2 CCAOs increased the infarction rate to 88% (14/16) with a significant decrease in the CV to 58%, compared with the 1-hour MCAO group. Permanent MCAO combined with 3-hour bilateral CCAO resulted in cortical infarction in all animals (6/6) with a CV of 47%, significantly different from the 1-hour MCAO group ($P < 0.05$) (Table 3).

The cortical infarction volume in the 1-hour MCAO measured 20.3 ± 10.5 mm^3. Ipsilateral CCAO added to the 1-hour MCAO did not significantly alter the average infarction volume (21.3 ± 9.0 mm^3), whereas bilateral CCAO did significantly ($P < 0.05$) increase infarction volume to 51.9 ± 13.1 mm^3 (Table 3). When the occlusion time for MCA and bilateral CCA was extended to 3 hours, there was a significant ($P < 0.05$) increase in the volume of infarction to 82.6 ± 12.1 mm^3, when compared with the group with 1-hour MCAO and 2 CCAOs. In the group with permanent MCAO and 3-hour bilateral CCAO, the infarction volume was 89.6 ± 16.0 mm^3. The difference in infarction volume between the group with 3-hour MCAO and bilateral CCAO and the group with 1-hour MCAO and CCAO was significant ($P < 0.05$). There was no significant difference between the 3-hour MCAO with bilateral CCAO group and the group with permanent MCAO and 2 CCAOs.

Edema

Infarction volumes of both hemispheres were measured and compared to identify possible edema. Edema in the ischemic hemisphere would increase the overall measured infarction volume. Because edema has been described to peak at 24 hours after occlusion[15], as the different reperfusion intervals (1, 3, and 7 days) were considered separately (Table 3). Overall, the average ischemic hemispheric volumes were 580.3 ± 6.1 mm^3 and 574.4 ± 6.6 mm^3 for the nonischemic (right) hemisphere and ischemic (left) hemisphere, respectively. There were no differences among any of these groups.

Weight Loss

Average weight loss in the total group of 82 animals measured was 52.1 g (19.1% of the preoperative body weight). Weight loss correlated well with the time between surgery and death when comparing the 3 1-hour MCAO (1, 3, and 7 day) groups that were killed (Table 3). Increasing the severity of the ischemic insult from 1-hour MCAO with 2 CCAOs, to 3-hour MCAO with 2 CCAOs, to permanent MCAO with 2 CCAOs significantly increased weight loss in these groups (P < 0.05) (Table 3).

DISCUSSION

Volume and reproducibility of cortical infarction in rat MCAO models depend on many factors including exact location of occlusion[16], duration of occlusion[17], anesthetic[8], brain and body temperature[9], blood pressure[18], glucose[12], and histological techniques. Rat strain and vendor have also been identified in several studies to effect infarction volume and reproducibility in rat MCAO models[17,19]. Different start-up stocks and breeding strategies make outbred strains like Sprague Dawley and Wistar more susceptible to vendor-dependent genetic divergence[5]. The incidence of abnormalities in the rat's circle of Willis could explain the reported higher variability of infarction volume in these strains when compared with F344 rats[19]. Anatomical studies have shown that the presence of more proximal MCA side branches may be responsible for the differences in variability in the neuropathological outcome of focal cerebral ischemia between F344 and Wistar rats[4]. Age-related differences in ischemic susceptibility in rats have been proposed by Sutherland et al.[7], who found significantly larger infarctions in older Wistar rats (26 to 28 months, 595 g) as compared with younger Wistar rats (2 to 3 months, 353 g). However, Duverger and MacKenzie[19], in a study with F344 rats, did not find significant differences in the 3 different age groups.

The staining technique with TTC in this study is an accurate, rapid, and inexpensive way to delineate cerebral infarction[20]. Tissue changes caused by ischemia have been reported to parallel irreversible changes seen in hematoxylin and eosin (H&E) staining at 1 day and 3 days[15,16]. The accuracy of TTC at 7 days is unknown because inflammatory response may result in unreliable TTC staining[21]. This is the rationale of choosing the 3-day time for comparing the models in this experiment. Isayama et al.[20], compared the TTC staining technique with that of H&E staining in a permanent MCAO model. The TTC staining technique tended to show larger infarctions than H&E staining, for which 2 possible explanations were given: compromised accessibility of TTC to the tissue in permanent MCAO and tissue shrinkage in conventional histologic techniques[20]. Random assessment by H&E staining did not show any difference when compared with TTC staining.

Brain edema in rats can cause overestimation of infarction volume by up to 22%[2]. Some investigators have found that maximal infarction volume was reached at 24 hours after occlusion[22,23]. Other investigators have suggested that the duration of occlusion determines the incidence and time course of cerebral edema[24]. Indirect measurements have been proposed to minimize this error[25]. Lin et al.[15], reported smaller infarction volumes at 7 days of reperfusion when compared with 1 day and 3 days, even with indirect measurement[23]. Anesthetics, such as halothane, in higher doses can reduce ischemic brain edema by counteracting superimposed vasogenic edema during reflow by reducing hyperemia through a decrease in MABP and cerebral perfusion pressure[26]. Although reperfusion has been reported to aggravate edema formation in other species[23], Kaplan et al.[27] found no difference in edema volume between the 3 and 4 hours of temporary MCAO and permanent occlusion groups. Specific gravity measurements in Shigeno's study showed edema in permanent occlusion groups and not in any of the reperfusion groups[28]. A close correlation was found between the size of infarction and edema volume[27]. This could indicate that the origin of the edema was more likely cytotoxic than vasogenic. Left and right hemispheric volumes were not significantly different in any group in our study.

One-hour MCAO in our experience did not consistently result in infarction. Hiramatsu et al.[3], showed that none of the 1-hour and 2-hour MCA-occluded rats had infarctions. Focal permanent occlusion in Bederson's[16] study resulted in infarction in 67% (site of occlusion proximal to the olfactory tract), 13% (MCA origin from internal carotid artery), or 0% (distal of the inferior cerebral vein). Coyle, in his study on the collateral circulation in rats, concluded that the location of MCAO determined the collateral field[29]. Reduction of collateral flow after MCAO will increase the severity of cerebral ischemia and MCA territory infarction[30]. Divergent genetic coding for vascular collateral flow could explain variability in infarctions after MCAO in rats[31]. In our study, adding ipsilateral CCAO to reduce collateral flow resulted in a 60% infarction rate, whereas, in the bilateral CCAO group, 75% of rats had infarctions. Relative blood flow in the cortex was reduced from 62% with distal MCAO without CCAO, to 48% with ipsilateral CCAO, and to 18% with bilateral CCAO in laser Doppler flow studies[32]. Addition of the bilateral CCAO with 3 hours of MCAO resulted in an increase in the infarction rate from 25% to 100% in Hiramatsu's study[3]. Extending occlusion time from 1 hour to 3 hours in his study resulted in a more reproducible infarction volume; this suggests that the severity of ischemia may affect variability of outcome. When occlusion time is prolonged, infarction volume enlarges progressively until it approximates permanent occlusion[27,33,34]. In our study, the duration of occlusion was extended from 1 hour to 3 hours to improve reproducibility. In the group with 3-hour MCA and 2 CCAO, the CV was 58%. To confirm that 3-hour MCAO and 2 CCAOs could equal permanent occlusion, a group of rats was permanently occluded with 3 hours of 2 CCAOs. This resulted in a CV of 47% with an average infarction volume of 89.6 ± 16.0 mm^3, whereas 3 hours of MCAO and 2 CCAOs resulted in an average infarction volume of 82.6 ± 12.1 mm^3. The difference between these groups

was not significant. By contrast, Hiramatsu's study[3] showed that 3 hours of MCAO with 2 CCAOs in Sprague Dawley rats resulted in an infarction volume of 177.4 ± 6.3 mm^3 with a CV of 11%. Herz et al.[4], using permanent MCAO in Wistar rats, showed an infarction volume of 44.2 ± 11.3 mm^3, and the CV was 26%. Xue et al.[35] in Wistar rats, showed an infarction volume of 211 ± 14 mm^3, with a CV of 17% as a result of 3 hours of MCAO. Xue, using permanent MCAO, however, showed an infarction volume of 142 ± 18 mm^3 with a CV of 31%. These results depict a wide variation in infarction volume and reproducibility among investigators and also within an investigation (permanent v temporary occlusion). In a review of the literature, it is not possible to ascertain the reasons for the discrepancies in the same species (e.g. the Wistar rat), because of the extreme variability in experimental protocols. Brain temperature during and after ischemia has been reported to affect distribution and extent of ischemic injury[8,9,36]. Anesthetics, such as halothane, isoflurane, and thiopental, are cerebroprotective during ischemic injury[37]. Hypothermia induced by anesthesia could be partly responsible for this effect. The effect of halothane on MABP should also be taken into consideration. Oliff et al.[5] reported that raising the halothane concentration from 2% to 2.5% reduced MABP from 102 mmHg to 74 mmHg, which resulted in an 8-fold increase in cortical infarct volumes from 14.2 mm^3 (2% halothane) to 113.6 mm^3 (2.5% halothane). Lowering MABP increased the severity of the ischemic event[18] by decreasing cerebral perfusion pressure, which reduced collateral flow. Zhu and Auer[11] reported mortality rates of 50% for rats undergoing 2-hour MCAO at a MABP of 40 mmHg, which resulted in an average infarction volume of 174 mm^3. In our study, core body and head temperature was controlled at 37 ± 0.5°C with an average MABP of 91.4 ± 1.0 mmHg under 1% halothane anesthesia. Data from the study by Oliff et al.[5], including average MABPs for different rat strains and average infarction volumes with MCAO and CCAO, showed an obvious decrease in infarction volume with increasing MABP. Phenylephrine-induced hypertension during MCAO attenuated size of infarction zones of severely decreased local cerebral blood flow (LCBF) (0 to 15 mL/100 g/min)[10]. Increased cerebral perfusion pressure-dependent collateral CBF and inverse steal caused by phenylephrine-induced vasoconstriction in normal brain regions were identified as possible mechanisms for these changes in CBF[10].

Temporomandibular joint dysfunction has been described as interfering with the animal's ability to eat[38]. Weight loss did appear after surgery in our study. Neurological deficit could also account for the reduced body weight when the animal was killed. Although we did not perform an extensive neurological evaluation in our study, no focal neurological signs were observed except for some limping, which can be attributed to femoral artery occlusion after removal of the femoral arterial catheter. In general, agility appeared to be reduced in most animals. Duverger and MacKenzie [19] described hyperactive and aggressive behaviour postoperatively that we did not observe. In comparing our 1-, 3-, and 7-day reperfusion groups, we observed an almost linear weight loss over time.

Weight loss has also been described in the intraluminal cerebral ischemia model in which temporomandibular dysfunction does not occur. A correlation was found in this study between duration of MCAO, MABP, and weight loss[11]. Yamamoto et al., in their study, found a 12.5% weight loss after 1-hour MCAO and 7 days of reperfusion[38].

In conclusion, 1-hour proximal MCAO in Wistar rats did not consistently result in infarction. Because edema has been reported to maximize at approximately 24 hours[22,23], and post-operative weight loss in our study averaged 24.2% of total body weight at 7 days, a 3-day reperfusion period was chosen. Reducing collateral flow through unilateral or bilateral CCAO augmented infarction rate to 60% and 75% and reduced variability to 134% and 101%, respectively. Because increased severity of ischemia can reduce variability[3], occlusion time was extended to 3 hours, resulting in an infarction rate of 88% and a CV of 58%, with an infarction volume of 82.6 ± 12.1 mm^3, which was significantly greater when compared with the 1-hour MCAO groups with or without CCAO. Reproducibility is a major concern in rat models of temporary focal cerebral ischemia. This study shows that 3-hour MCAO combined with bilateral CCAO produces a reliable infarction after temporary focal ischemia.

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