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Research Article

Differential effects of common carotid artery occlusion models of ischaemic stroke on sensorimotor function and infarct sizes in rats.

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Abstract
One of the leading causes of death and disability worldwide is stroke. Experimental models used for studying brain infarction are important for the development of therapeutic interventions for all types of stroke but may differ in pathology post-injury. The aim of this study was to compare infarct sizes in different common carotid artery occlusion models of ischaemic stroke. Adult male rats were divided into five groups, each containing 12 rats: control, sham operated (SCCAO), temporary unilateral common carotid artery occlusion (TCCAO), permanent unilateral common carotid artery occlusion (PCCAO), and combined permanent and temporary common carotid artery occlusion (PTCCAO) groups. The common carotid artery was isolated, and occlusion was done by applying a silk suture either for 30minutes or permanently. Motor and sensory functions were tested 1 and 3days later, using the hanging wire and adhesive removal tests respectively. The rats were euthanized, brains removed, sectioned, and stained with H&E, Nissl and Triphenyltetrazolium chloride stains. Total brain infarct volumes and ischaemic neurons were compared across the groups. TCCAO and PCCAO caused slight ischemia and little infarct, however, PTCCAO caused substantial cerebral ischemia and infarctions in the cerebral cortex and striatum. In contrast to the other experimental groups, motor and sensory deficits were observed in the PTCCAO group when compared to controls. Only PTCCAO achieved significant infarct, but the type of infarct produced was inconsistent, possibly due to the existence of differences in the rat's cerebral circulation.

Key Words: Common carotid artery occlusion, Ischaemic stroke, Infarct size

INTRODUCTION

Stroke is caused by a change in blood flow within the brain and often leads to neurologic deficits. It involves injuries caused by haemodynamic perturbations and coagulation that are undetected in blood vessels, and has been adjudged as a devastating disease (Sommer, 2017). Currently, it is the leading cause of disability and third most common cause of death worldwide (Wayman et al., 2016). In Nigeria, studies have shown that the mortality rates for stroke are very high ranging from 21-45% in different regions (Njoku and Adeloju, 2004; Komolafe et al., 2007; Desalu et al., 2011). Stroke is classified as either ischaemic or haemorrhagic based on the etiology and characteristics of the lesion (Beal, 2010), with the ischaemic subtype accounting for the greater number of stroke cases, and vessel rupture associated with haemorrhage accounting for about 15% (Onwueke and Ezeala-Adikaibe, 2012; Perna and Temple, 2015).

A temporary or permanent reduction in cerebral blood circulation that is limited to the territory of a major cerebral artery or its branches leading to blockages and deficiency of oxygen in vital tissues results in ischaemic stroke (Fluri et al., 2015). It is heterogeneous in nature and occurs in several varieties but can be classed into a few clinical entities: global cerebral ischemia and focal cerebral ischaemia (Yan et al., 2015), or according to some authorities, in three forms i.e., global, focal and multifocal (Braeuninger and Kleinschnitz, 2009; Woodruff et al., 2011). Global ischemia results when there is pervasive reduction in cerebral blood flow in the brain. In focal cerebral ischemia, there is reduction in blood flow to specific region of the brain, although, there is usually some flow that reaches the affected area via collateral circulation. There is usually a gradient of blood flow from the inner core reaching out to the limits of the ischemic area. It is usually the result of occlusion of a major cerebral artery. In multifocal ischemia, there is a patchy pattern of reduced cerebral blood flow (Traystman, 2003).

Ischaemic stroke may also present like thrombotic stroke (large vessel and small vessel types), embolic stroke (with or without known cardiac and or arterial factor), systemic

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hypoperfusion or venous thrombosis. Regardless of the trigger, defective vascular supply to the brain is the sole event in majority of acute strokes (Mir et al., 2014). The telencephalic circulation is of utmost importance in the body because cessation of circulation for 5 minutes can result into neuronal death (Moss, 2001).

The arterial supply to rodents' encephalon is composed of two pairs of large arteries, the right and left internal carotid arteries and the right and left vertebral arteries which is similar to that of humans. The bilateral internal carotid artery (ICA) supplies the anterior cerebral circulation including the middle and anterior cerebral arteries (MCAs and ACAs) while the vertebral artery (VA) supplies the posterior circulation including the basilar artery (BA) and posterior cerebral arteries (PCAs) (Krishnaswamy et al., 2010). Both anterior and posterior circulations anastomose at the Circle of Willis via anterior and posterior communicating arteries. This circle gives rise to the anterior, middle, and posterior cerebral arteries, which through smaller arteries and arterioles supply corresponding areas of the cerebral cortex by running along the surface until they penetrate the brain tissue (Cipolla, 2009). In the event of occlusion of either the internal carotid arteries (anterior circulation) or the vertebral basilar system (posterior circulation), the collateral circulation in the circle of Willis provides blood to the affected area deprived of blood (Prince and Ahn, 2013).

Several studies have reported that the volume of an ischaemic lesion is strongly correlated with stroke severity and moderately correlated with impairment of motor function and activity limitations (Saver et al., 1999; Chen et al., 2000; Johnston et al., 2002). Knowledge about lesion volume might support predictions about future level of functioning and decisions about types of care in stroke (Yaghi et al., 2017). In addition to volume, infarct location has also been found to be fundamentally linked to neurological deficits, thus the combination of lesion volume and infarct location results in a stronger correlation with the severity of symptoms (Menezes et al, 2007; Laredo et al., 2018).

The advent of experimental stroke research was birthed with the use of higher species such as cats, pigs, sheep, dogs and non-human primates (Yan et al, 2015; Prinz and Endres, 2010; Macrae, 2011; Braeuninger et al., 2012; Sozmen et al., 2012), however, changes have occurred within the last 3-4 decades. Mice and rats are now increasingly being selected as in vivo stroke models, due to the lower costs of acquisition and maintenance, possession of similar cranial circulatory anatomy to humans, simpler monitoring methods and tissue processing as well as ethical issues (Sommer, 2017; Woodruff et al., 2011). Ischaemic stroke models can be separated into focal and global ischaemia models. Focal ischaemic stroke caused by an acute cerebral vessel occlusion can be reproduced by different techniques such as mechanical occlusion of the middle cerebral artery (MCA), unilateral occlusion of the common carotid artery or by thrombotic occlusion either via injection of blood clots or thrombin into the MCA or by photo-thrombosis after intravenous injection of Rose Bengal (Bacigaluppi et al., 2017). The cerebral arteries have been occluded through the common carotid arteries in rodents to induce hemispheric ischaemia. According to the location of arterial occlusion, global or focal ischaemic models are presented. Temporary occlusion of both common carotid arteries is deemed the most convenient model to induce transient global cerebral ischaemia (León-Moreno et al., 2020). Bilateral CCA occlusion has been performed to induce global cerebral ischaemia by reducing the blood flow to the brain through the arterial circle of Willis, while unilateral CCA occlusion induces focal ischemia (Kuraoka et al., 2009).

While validated models of bilateral CCA and unilateral CCA exist in rodents, comparison of the different common carotid artery occlusion models has not been well documented. We therefore compared the effect of different common carotid artery occlusion models of ischaemic stroke on sensorimotor function and infarct sizes in rats using permanent and temporary occlusion.

MATERIALS AND METHODS

Adult male Wistar rats weighing about 200g were acquired from the Central Animal House of the Faculty of Basic Medical Sciences, University of Ibadan, Ibadan. All the animals were housed in plastic cages at room temperature with food and water *ad libitum*. The animal experiments complied with the ARRIVE guidelines and were conducted in accordance with the NIH Guide for the Use and Care of Laboratory Animals, and the European Communities Council Directives (86/609/EEC), minimizing the number of animals used and avoiding their suffering.

To evaluate the effect of common carotid artery occlusion (CCAO) models of ischaemic stroke on infarct sizes, male rats were randomly assigned to five (5) groups. Group 1: Temporary unilateral CCA occlusion (TCCAO), Group 2: Permanent unilateral CCA occlusion (PCCAO), Group 3: Permanent and temporary CCA occlusion (PTCCAO), Group 4: Sham operated CCA animals (SCCAO) and Group 5: Control group. For temporary unilateral common carotid artery occlusion, a duration of 30 minutes was chosen to induce the infarct.

Induction of stroke: All animals were anesthetized with 60 mg/kg Ketamine hydrochloride and 10 mg/kg Xylazine hydrochloride given by intraperitoneal injection. After anesthesia, the animals were immobilized in the supine position on the surgical table using adhesive tape. The incision region was shaved and disinfected with ethanol and the midline neck skin incised. Through the incision, the common carotid artery was isolated from the vagal nerve and connective tissue by blunt dissection (Guzel *et al*., 2014; Lee *et al*., 2014). Temporary right common carotid artery occlusion was performed with a silk suture loop for 30 minutes, after which the suture was removed to restore blood flow. A permanent left common carotid artery occlusion was also performed using a silk suture. A combined permanent and temporary CCA occlusion was performed using a silk suture applied for 30minutes on the right and removed, a silk suture was then applied on the left permanently after the occlusion of the right (Cao et al., 2018). Sham-operated rats underwent the same procedure without CCAO. Thereafter, the neck incision was sutured. To eliminate bias regarding the effect of the procedure or anesthesia, control animals did not undergo the procedure. Postsurgical survival was checked and two rats each euthanized at 24hours, 3 days and 7 days (Kuraoka *et al*., 2009).

Neurobehavioral Tests: The functional effect of stroke was assessed using the hanging wire and adhesive removal tests 24 hours after the animals regained consciousness. Motor

performance was assessed using hanging wire test while the adhesive removal test was used to assess sensorimotor function in all the groups. Pre-training had earlier been done for the rats before induction of ischemia.

Hanging wire: Rats were placed to grip a wire stretched between two posts 60 cm high above soft padded surface. The time until each rat dropped off the wire (Latency to fall) was recorded. A time of zero was assigned if the rat dropped off immediately while 120seconds was the maximum period. Three trials were performed for each rat at each time point (Priyanga *et al.*, 2017).

Adhesive Removal Test

A small adhesive tape was applied to the forelimbs and the rat was then put into a cage. Time taken to contact and remove adhesive tape was recorded with 120 seconds being the time limit. Contact occurred when the mouth was first used to try to remove the patch (Bouet *et al*. 2010).

Tissue Collection: The rats were anesthetized with an intraperitoneal injection of 60 mg/kg Ketamine hydrochloride and 10mg/kg xylazine. Subsequently, they underwent transcardial perfusion with normal saline, followed by 10% neutral buffered formalin (NBF) after which the brain tissues were removed and post-fixed in the same solution for 48 hours. Five-millimetre coronal sections were obtained and stained with Hematoxylin and Eosin as well as cresyl violet to identify ischemic neurons. A subset of the rats (2 per group) was sacrificed under ketamine/xylazine anesthesia and their brains stained with Triphenyltetrazolium chloride (TTC) staining. The rats were decapitated, their brains were removed and sectioned coronally into five slices at an average thickness of 2mm each (Kuraoka *et al*., 2009).

Triphenyltetrazolium chloride (TTC) staining and infarct measurement: After brain removal, slices were immediately immersed in 2% TTC solution in a plastic dish for 25-30 minutes at room temperature. Then TTC solution around the brain tissue was gently aspirated and 10% NBF was added into the dish (to fix the brain tissue). This was done twice in order to ensure all the TTC solution was removed. The fixed brain tissues were arranged in a rostro-caudal sequence and photographed for analysis of infarct size. Areas of ischemic damage were evaluated using the Motic Image plus software. Total infarct volumes were calculated as the sum of the products of the infarcted area and thickness of the slice. Edema correction of the infarct volume was estimated using the following formula: volume correction (infarct volume × contralateral volume/ ipsilateral volume) (Dang *et al.*, 2011).

Statistical Analysis: Data collected for quantitative analysis were presented as means ± standard error of means (SEM) and compared among the groups using analysis of variance (ANOVA) and Bonferroni for post-hoc evaluations, with the GraphPad prism 7.0 statistical software (San Diego, California, USA). In addition, paired t-test was used for comparisons within groups and $p<0.05$ was set as the level of significance.

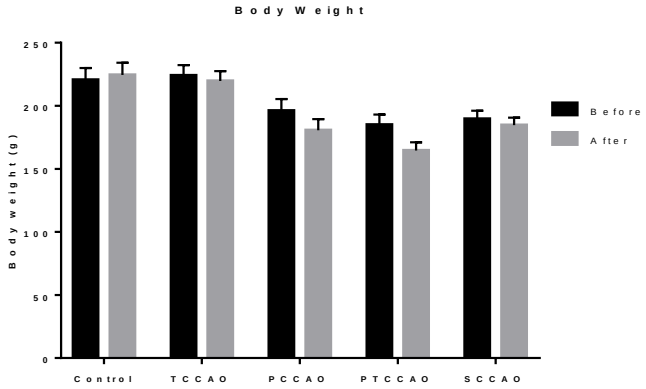
RESULTS

Gross Observation: Common carotid artery ligation caused unilateral ptosis (drooping eyelids) on the ipsilateral side in 22.7% of group 1 (TCCAO) rats and all (100%) of group 2 (PCCAO) rats, while bilateral ptosis was observed in all of group 3 (PTCCAO) animals. However, groups 4 (Sham operated) and 5 (Control) rats had no ptosis.

Body Weight: All experimental and sham-operated animals (groups 1-4) had a reduction in body weight after the common carotid artery occlusion procedure compared to weight taken before the surgical intervention (Figure 1A).

Brain Weight: Brain weight was significantly higher in rats that had PTCCAO and reduced in rats that were subjected to TCCAO and PCCAO in comparison to the controls but there was no significant difference between sham and control animals (Figure 1B).

A.



B.

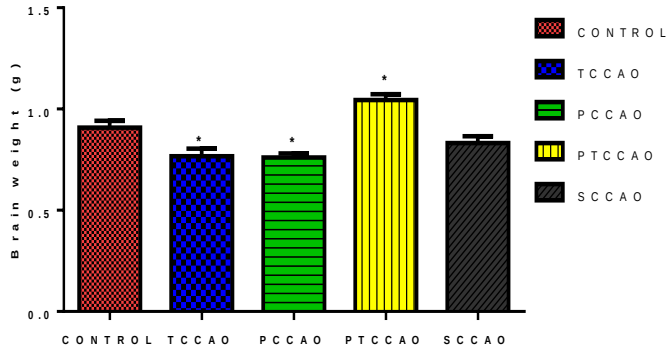


Figure 1:
(A) Body weight difference before and after induction of stroke in all the groups (B) Brain weight of rats after common carotid artery occlusion. Temporary unilateral CCA occlusion (TCCAO), Permanent unilateral CCA occlusion (PCCAO), Permanent and temporary CCA occlusion (PTCCAO), Sham operated CCA animals (SCCAO)

Mortality Rate: Mortality rate 24hours after the procedure was recorded. In the PTCCAO group, we observed a 58.33% mortality while all the rats in the TCCAO and PCCAO groups survived the procedure after 24hours.

Tests of Neurobehavioural functions

Hanging wire test

In the hanging wire test, there was no significant difference in the latency to fall between rats in TCCAO and PCCAO, sham-operated and controls but the rats in PTCCAO group had a significantly shorter latency to fall than the controls (Figure 2).

Permanent unilateral CCA occlusion (PCCAO), Permanent and temporary CCA occlusion (PTCCAO), Sham operated CCA animals (SCCAO)

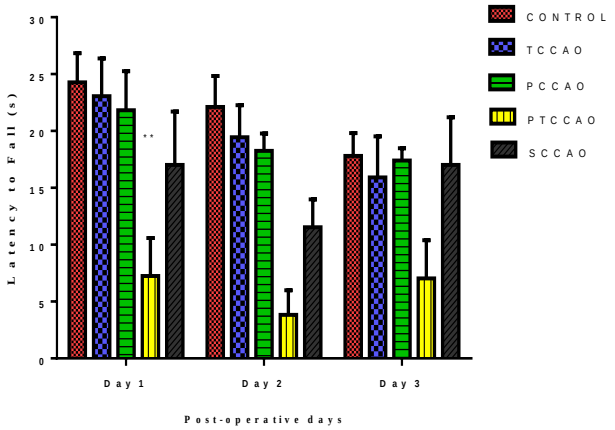


Figure 2:
Hanging wire test showing deficits in forelimb strength after CCA occlusion. Temporary unilateral CCA occlusion (TCCAO),

Adhesive removal test

Following the occlusion of the common carotid artery, the PTCCAO rats exhibited pronounced deficit in their ability to make contact in both the left and right forelimbs, compared to the control group. There was no significant difference in the time to contact in the TCCAO, PCCAO and SCCAO groups compared to the control group. We observed that the PTCCAO rats also took a longer time to remove the adhesive pads on days 1 and 3 post-occlusion (Figure 3).

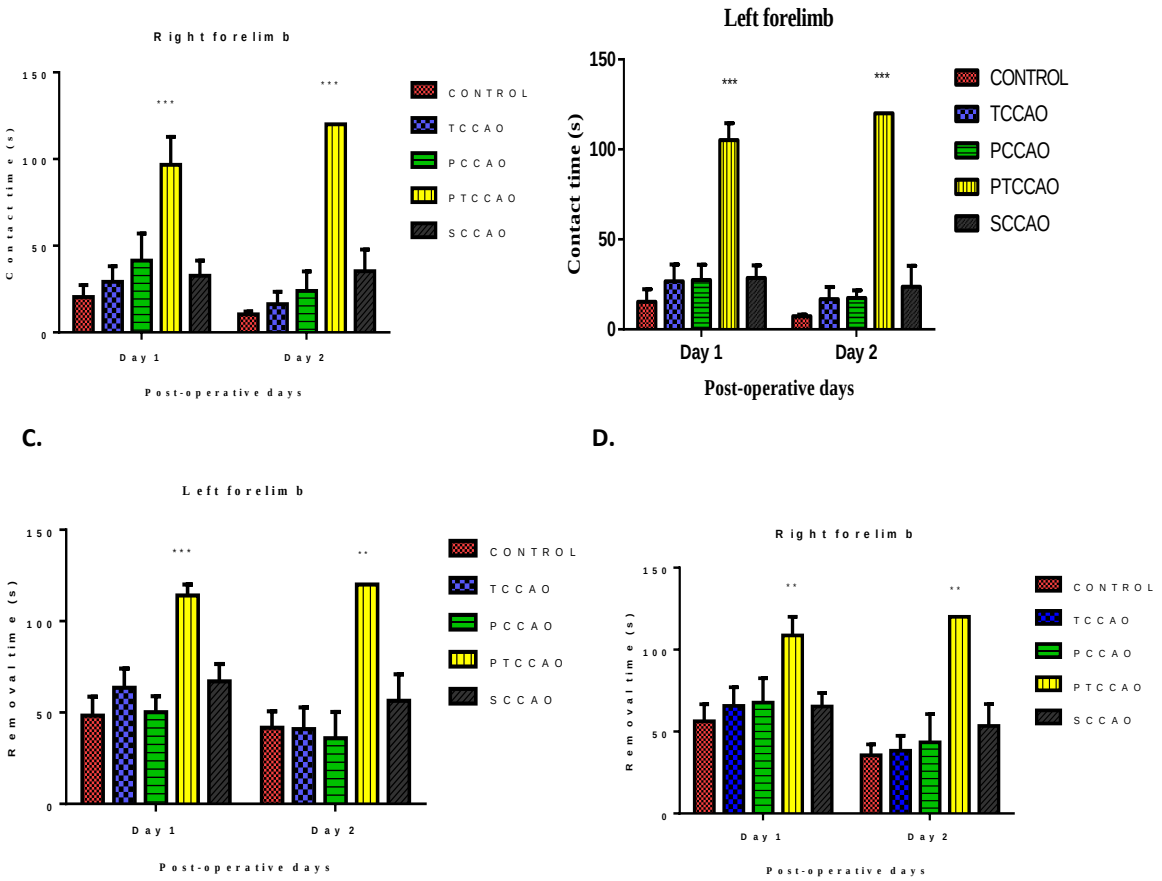


Figure 3:
Bar charts showing time-to-contact (A and B) and time-to-remove (C and D) the adhesive tape on the left and right paws of the rats on the 1st and 3rd day of observation. Temporary unilateral CCA occlusion (TCCAO), Permanent unilateral CCA occlusion (PCCAO), Permanent and temporaryCCA occlusion (PTCCAO), Sham operated CCA animals (SCCAO)

Differential effects of common artery occlusion on brain infarct size

In rats subjected to PTCCAO, the brains revealed infarcts in the cortex, caudate and putamen at day 1 and day 3. No result was presented for day 7 because none of the PTCCAO rats survived until that time. No clear demarcation of injured areas was found in brains of rat subjected to unilateral TCCAO and PCCAO 1, 3- and 7-days post occlusion, while the sham-operated and control brains had no unstained areas (Figure 4).

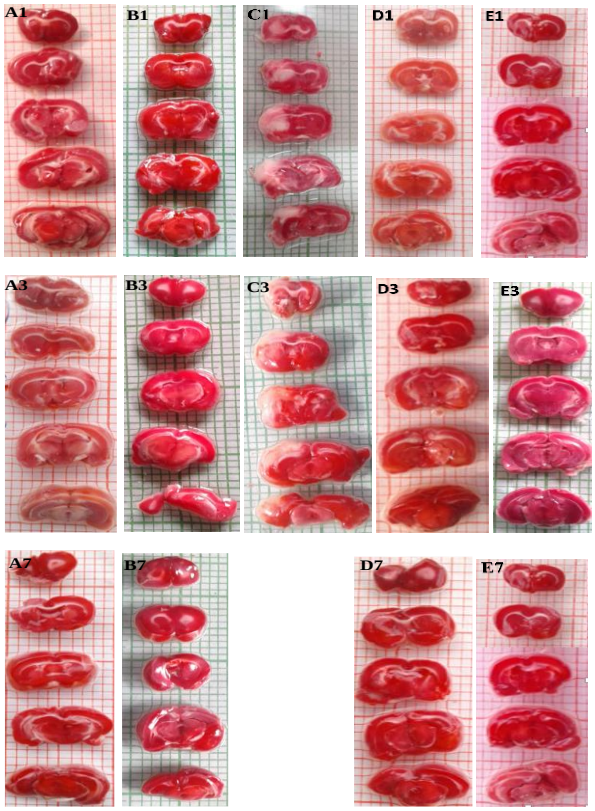
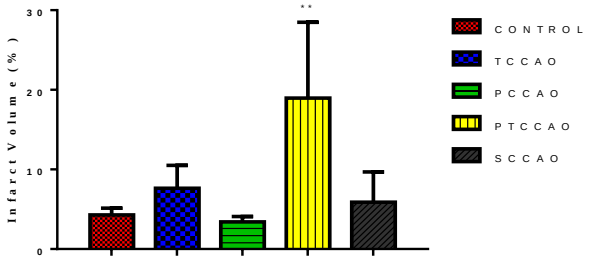


Figure 4:
A representation of TTC stained brain sections showing areas of healthy tissue (red) and ischemic injury (white) for (A1) TCCAO, (B1) PCCAO (C) PTCCAO (D1) SCCAO and (E1) Control at Day 1; (A3-E3) at Day 3 and (A7-E7) at Day 7

Subsequent quantification of infarct volume revealed a significantly higher infarct volume in the PTCCAO model when compared to the sham and control groups ($p<0.01$). The volume of infarct in the sham and TCCAO group however appeared slightly higher than the control group, but not to significant levels (Figure 5).

A.



B.

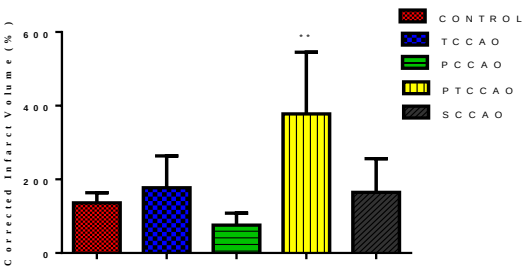


Figure 5:
(A) Infarct volume and (B) corrected infarct volume (%) calculated from TTC-stained coronal sections following CCAO or a simulated operation for different groups of rats. Temporary unilateral CCA occlusion (TCCAO), Permanent unilateral CCA occlusion (PCCAO), Permanent and temporary CCA occlusion (PTCCAO), Sham operated CCA animals (SCCAO)

Morphological changes

Microscopic examination of the sections revealed infarct lesions in the cerebral cortex characterized by shrunken neurons with perineuronal vacuolation in TCCAO and PCCAO brain samples at days 1, 3 and 7. The PTCCAO resulted in lesions in the cortices and striatum and was also characterized by shrunken neurons with perineuronal vacuolation and spongy neuropil. The brain sections of the Sham-operated and control rats had no features of vacuolation or shrunken neurons as they showed clear cytoplasm and the morphology of the neurons appear intact (Figure 6).

Ischemic cells with aberrant morphology were visible in the cortices of the TCCAO brains (at day 1, day 3 and day 7), PCCAO (at day 1, day 3 and day 7) and PTCCAO (at day 1 and day 3). They were triangular in shape and exhibited dark staining due to condensation of cytoplasm and karyoplasm (Figure 7).

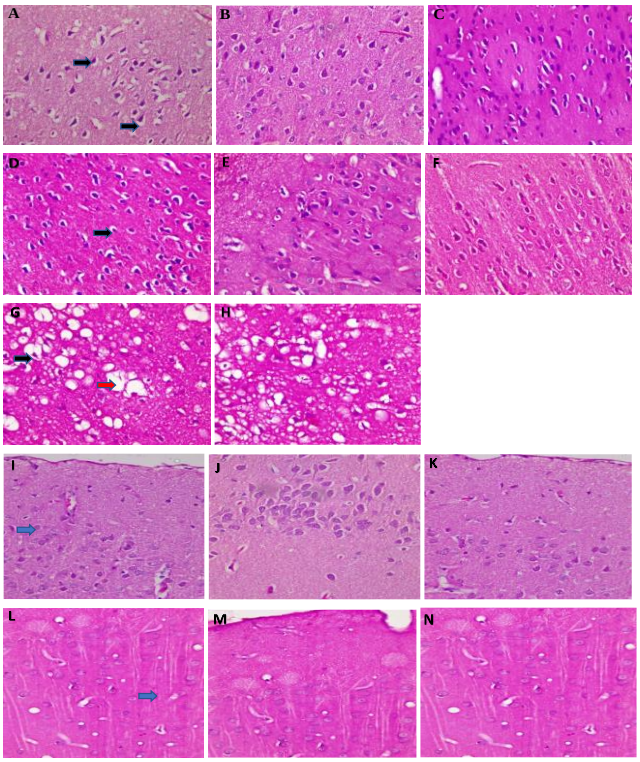


Figure 6:
Photomicrographs of the cerebral cortices of experimental and control rats; (A-C) TCCAO, (D-F) PCCAO, (G-H) PTCCAO, (I-K) SCCAO and (L-N) Controls. Vacuolations and pyknotic neurons were observed in the cortices of TCCAO, PCCAO and PTCCAO brain samples when compared to sham and control. Black arrows show pyknotic neurons, red arrow shows area of vacuolation and blue arrows show normal neurons. (Magnification: X40) H&E staining

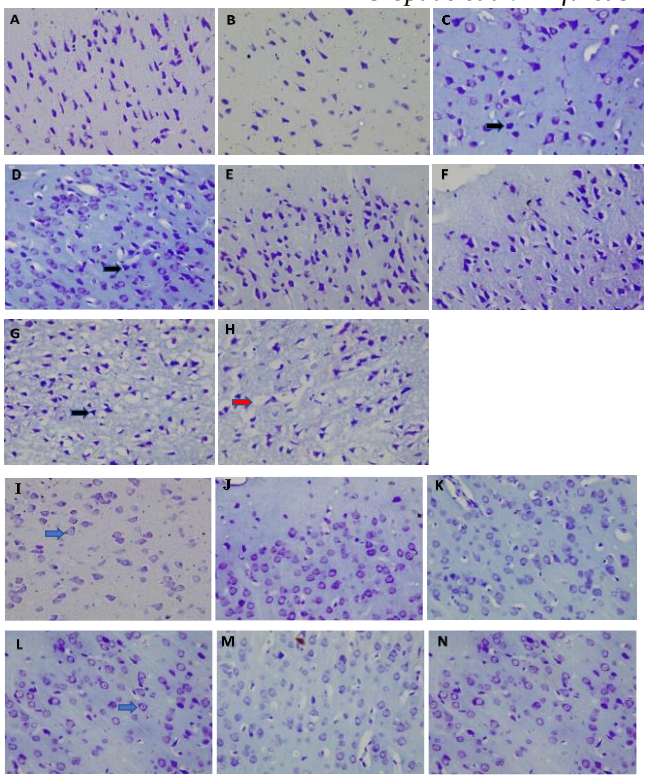


Figure 7:
Photomicrographs of the cerebral cortices of experimental and control rats; (A-C) TCCAO, (D-F) PCCAO, (G-H) PTCCAO, (I-K) SCCAO and (L-N) Controls. Pyknotic neurons were observed in TCCAO and PCCAO brain samples, andpyknotic neurons with spongy neuropil were observed in the PTCCAO brain samples. Black arrows show pyknotic neurons, red arrow shows area of vacuolation and blue arrows show normal neurons (Magnification: X40)Cresyl violet (Nissl) staining

DISCUSSION

Our study showed that unilateral common carotid artery occlusion alone, either transient or permanent, caused slight ischemia but a combination of permanent and temporary common carotid artery occlusion caused marked cerebral ischemia in the cerebral cortices and striatum. Unilateral carotid artery ligation alone has been found not to cause substantial changes in ipsilateral blood flow or energy state, but it markedly reduces the circulatory reserve in the ipsilateral hemisphere (Bronner et al., 1998). Occlusion of one of the common carotid arteries has been shown to cause only slight cerebral ischaemia as a result of the enlargement of the ipsilateral posterior communicating artery (Sun and Kuan, 2015; Chio et al., 2017). Smaller anastomotic vessels between the distributions of the anterior cerebral and middle cerebral arteries have been found to be enlarged following permanent unilateral occlusion of the middle cerebral artery in the rat (Bronner et al., 1998; Ma et al., 2016). However, bilateral common carotid artery occlusion caused widespread ischemia in the cerebral cortex, the corpus callosum, the caudate putamen, the anterior commissure, the hippocampal fimbria and the hippocampus (Vicente et al., 2009; Hattori et al., 2014).
Unilateral ptosis was noted in rats with temporary unilateral and permanent unilateral common carotid artery occlusion (TCCAO and PCCAO), while in rats with permanent and transient common carotid artery occlusion (PTCCAO), bilateral ptosis was observed when compared to the sham and control rats as also reported by Bronner et al. (1998) and Cao et. al. (2018). Bronner and his colleagues performed unilateral carotid artery ligation in male Wistar rats and observed ptosis in 23% of the animals, which they presumed was the result of damage to the sympathetic nerve trunk during isolation of the carotid artery, while Cao, with his colleagues, concluded that ptosis was an indication of a successful operation after bilateral common carotid artery occlusion in adult rats.

In this study, sham operation, transient unilateral, permanent unilateral, and permanent and temporary common carotid artery ligation were followed by decrease in body weight. Other studies have reported weight loss following bilateral common carotid artery occlusion but there is no report on weight loss after unilateral common carotid artery occlusion (Farkas et al. 2007; Hirabayashi et al., 2004). Farkas et al. (2007) attributed weight loss to hypertrophy of certain muscles in the ventral cervical region (e.g., the sternohyoid and the sternomastoid muscles) during the isolation of the carotid arteries leading to discomfort during movement of the head, mastication and swallowing. Reduced blood flow to the hypothalamus, thus compromising its function as a major center of autonomic control, might also be responsible. In contrast, Wayman et al. (2016) opined that weight loss is a primary indication of dehydration and stress rather than loss of body weight due to reduced feeding. Further studies are required to address the underlying cause of weight loss in these models.

We observed increased brain weight in the PTCCAO model and reduced brain weight in TCCAO and PCCAO rats, compared to the sham and controls. There have been reports in literature of brain swelling in the ipsilateral hemisphere due to edema after the middle cerebral artery occlusion (MCAO), since ischemia caused by the MCAO elevated brain water content in the lesioned hemisphere (Gerriets et al., 2004; Pasban et al., 2017). We speculate that this might be responsible for the increased brain weight we observed in the PTCCAO rats.

Mortality rate in the PTCCAO group was high and most deaths occurred within 24 hours after the procedure, but none of the rats in the TCCAO, PCCAO or sham-operated groups died after the procedure. Wang et al. (2020) reported a 25% mortality in rats that underwent bilateral CCA occlusion and Hattori et al. (2014) reported 58.8% mortality following bilateral implantation of ameroid constrictors to the CCAs, but most studies do not report any mortality following the unilateral common carotid artery occlusion (Bona et al., 1997; Yoshizaki et al., 2008). The standard procedure in which there is concomitant ligation of the arteries permanently has been reported to be associated with high mortality (Farkas et al., 2005) whereas a decrease in mortality was recorded in a modified protocol where either CCA were occluded within an interval, for example, an interval of one week, or when an analgesia was administered, (Institoris et al., 2007; Cechetti et al., 2010). However, occluding both common carotid arteries concomitantly but not permanently also resulted in a high mortality rate in this study. Raval et al. (2009) attributed the high mortality rate to post-operative complications such as respiratory difficulties during the post-ischemic period, long duration of surgery period, surgical damage and excessive dose of anaesthesia.

The high mortality rate observed in the PTCCAO rats in this study might also be due to reduced feeding as well as post-operative complications.

Neurobehavioral assessment was done 24 hours after the rats recovered in order to detect sensory and motor deficits respectively. In the hanging wire test, the PTCCAO rats exhibited inability to hold the wire and the latency to fall was reduced significantly when compared to the controls; this was not the case in the other groups. Our findings are consistent with previous investigations. Nagakannan et al. (2012) evaluated muscle strength using the hanging wire test after transient bilateral common carotid artery occlusion for 30 minutes in male Wistar rats and reported decreased grip strength in the ischaemic rats. Bona et al. (1997) performed unilateral common carotid artery occlusion in 7-day old rats and then exposed them to hypoxia and assessed muscle strength at day 42 using a horizontal rope, he observed that the latency to fall was shorter in the hypoxia ischemic animals compared to the controls. The decreased latency to fall was an indicator of motor deficits and/or loss of motor function in the rats. In the adhesive removal test, rats in PTCCAO group took a longer time to contact and remove the adhesive tapes when compared to the controls in both the left and right forelimbs, but no significant difference was observed in the other groups compared to the controls. Studies of other models of ischaemic stroke, such as the middle cerebral artery occlusion model report that animals with stroke took a longer time to remove the adhesive tape, indicating a deficit in sensorimotor function resulting from damage to the somatosensory cortex (Bouet et al., 2009; Bouet et al., 2010; Rewell et al., 2017; Trueman et al., 2017). We believe this must have led to the decreased ability of the PTCCAO rats to sense and then remove the adhesive tape on their whiskers.

Triphenyl tetrazolium chloride (TTC) evaluation of infarct volume remains the most regularly reported outcome in experimental stroke studies because it is a convenient procedure for detection of brain infarcts. TTC staining was performed to estimate infarct size after reperfusion. It reliably delineated the infarct region allowing one to differentiate between the pale injured areas and the surrounding red non-injured area. TTC staining in this study showed little infarcted areas in the cortices of the temporary unilateral and permanent unilateral common carotid artery occlusion rat brains. In contrast, Renolleau et al. (1998) reported no ischaemic lesion and infarct following temporary carotid artery occlusion for 60 or 90 minutes in 7days old rats. Our results however revealed distinct infarcted areas in the cortices and striatum of the PTCCAO rats, in consonance with that of Vicente et al. (2009) and Hattori et al. (2014). Although the infarct size is an essential and accurate measurement of stroke outcome, another significant outcome parameter of cerebral ischaemia is the impairment of function measurements using neurobehavioral tests, as earlier reported. Histological examination of our unilateral occlusion models revealed pyknotic neurons with perineuronal vacuolation in the cerebral cortex while the PTCCAO brains had shrunken neurons with more pronounced perineuronal vacuolation, pale and spongy neuropil in the cerebral cortex and striatum. These pyknotic neurons were almost certainly the products of ischaemic injury. These observations are in accordance with that of Kuroaka et al. (2009) who studied transient and permanent unilateral CCAO in mice. However, Shmonin et al. (2012) who compared 5 different rat models of middle cerebral artery occlusion, found no ischaemic lesions at all in their experimental models.

CONCLUSION

The transient unilateral CCAO or permanent unilateral CCAO model caused only slight ischemia and resulted in little or no infarct, but severe cerebral ischemia and a large infarct was observed following PTCCAO. However, the infarct produced in the PTCCAO model was not consistent and reproducible due to discrepancies in the rat's cerebral circulation and also due to previous observations that the more rostral the artery which is occluded is, the more inconsistent the outcomes. It is therefore necessary for more research to be done on discrepancies in the rat's circulation in order to fully describe the influences exerted by collateral circulation.

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