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Anomaly in aortic arch alters pathological outcome of transient global ischemia in Rhesus macaques

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Abstract

We investigated a non-human primate (NHP) transient global ischemia (TGI) model which was induced by clipping the arteries originating from the aortic arch. Previously we demonstrated that our TGI model in adult Rhesus macaques (*Macaca mulatta*) results in marked neuronal cell loss in the hippocampal region, specifically the cornu Ammonis (CA1) region. However, we observed varying degrees of hippocampal cell loss among animals. Here, we report for the first time an anomaly of the aortic arch in some Rhesus macaques that appears as a key surgical factor in ensuring the success of the TGI model in this particular NHP. Eleven adult Rhesus macaques underwent the TGI surgery, which involved 10-15-minute clipping of both innominate and subclavian arteries. Animals were allowed to survive between 1 day and 28 days after TGI. Because of our experience and knowledge that Japanese macaques exhibited only innominate and subclavian arteries arising from the aortic arch, macroscopic visualization of these two arteries alone in the Rhesus macaques initially assured us that clipping both arteries was sufficient to produce TGI. During the course of one TGI operation, however, we detected 3 arterial branches arising from the aortic arch, which prompted us to subsequently search for 3 branches in succeeding TGI surgeries. In addition, we performed post-mortem examination of the heart to confirm the number of arterial branches in the aortic arch. Finally, in order to reveal the pathological effect of the aortic arch anomaly, we compared the hippocampal cell loss between animals found to have 3 arterial branches but had all or only two branches clipped during TGI operation. Post-mortem examination revealed eight NHPs had the typical two arterial aortic branches, but three NHPs displayed an extra arterial aortic branch, indicating that about 30% of Rhesus macaques had 3 arterial branches arising from the aorta. Histological analyses using Nissl staining showed that in NHPs with the aortic arch anomaly clipping only two of three arterial branches led to a partial cell loss and minimal alteration in number of cell layers in the hippocampal region

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when compared with clipping all three branches, with the hippocampal cell death in the latter resembling the pathological outcome achieved by clipping the two arterial branches in NHPs displaying the typical two-artery aortic arch. The finding that 3 of 11 NHPs exhibited an extra arterial aortic branch recognizes this aortic arch anomaly in Rhesus macaques that warrants a critical surgical maneuver in order to successfully produce consistent TGI-induced hippocampal cell loss.

Keywords

non-human primate; cerebral ischemia; aortic arch; anatomy; hippocampal neuronal loss

1. Introduction

Ischemic brain injury is a major cause of death and disability around the world. An effective therapy remains elusive, except for thrombolytic treatment via urokinase and tissue plasminogen activator which unfortunately only benefits the acute stage of the disease. The development of novel treatments for cerebral ischemia is warranted. Experimental therapies have been tested in animal models of ischemic brain injury, but mostly employing rodents as subjects (Bliss et al., 2006). In our desire to facilitate the translation of experimental treatments for cerebral ischemia from the laboratory to the clinic, we sought to study these therapeutic modalities in ischemic non-human primate (NHP) models.

The anatomy and behavioral repertoire of the monkey are considered to be more advanced than rodents because of its closer proximity to human within the phylogenetic tree (Frykholm et al., 2005). The adult mammalian hippocampus is a resident to neural progenitor cells, and experimental brain injuries, such as ischemia, in rodents have been shown to promote endogenous neurogenesis in the dentate gyrus, DG) and CA1 region (Gage et al. 1998; Nakatoma et al., 2002). Neurogenesis has been implicated as a robust endogenous repair mechanism, and equally a potential target for cell therapy (Guzman et al., 2008; Hara et al., 2008; Hess and Borlongan, 2008; Kondziolka and Wechsler, 2008; Kondziolka et al., 2000; Kondziolka et al., 2005), as well as neuroprotective and neurorestorative drugs, thereby soliciting investigations into this cell survival pathway as a strategy for treating ischemic injury.

However, little is known about neurogenesis and brain ischemia in primates. Recent studies have used Japanese macaques (*Macaca fuscata*) to demonstrate neurogenesis in primates following cerebral ischemia (Tonchev et al., 2005; Tonchev and Yamashima, 2006; Tonchev et al., 2006; Tonchev et al., 2003; Yamashima, 2000; Yamashima et al., 2004; Yukie et al., 2006), but only a few reports have utilized the Rhesus macaques (*Macaca mulatta*), which is a more common NHP strain in western world, especially in the US (Nemoto et al., 2005). We recently developed a transient global ischemia (TGI) model in Rhesus macaques by clipping the arteries originating from the aortic arch (Hara et al., 2007). Although we observed ischemic cells in the hippocampus of all NHPs that underwent our TGI surgical procedure, the extent of cell loss varied among animals. Accordingly, we conducted a post-mortem study on these NHPs to confirm whether we successfully clipped the aortic branches. Here, we report for the first time an anomaly in the aortic structure of Rhesus macaques – the existence of a third branch from the aortic arch in about 30% of this NHP strain. The recognition of this anomaly should prompt the surgeon to isolate and clip this extra branch during the TGI operation in order to produce consistent hippocampal cell loss, which is a prerequisite when using this NHP model for evaluation of experimental treatments for ischemic injury.

2. Results

2.1. Experiment 1: Perioperative condition

Based on our experience with Japanese macaques, during the TGI surgery in Rhesus macaques we focused on isolating and clipping the two branches originating from the aortic arch, which were easily visualized in all NHPs. In the case of NHP4, to our surprise we detected three aortic branches during operation; accordingly all three branches were clipped for this particular animal. However, at that time we were not aware of this anomaly of the aortic branch, thus no additional effort was made to search for such an extra aortic branch in other operated NHPs. Moreover, as an index of successful ischemia, we relied on the dilation of pupils which was confirmed in all NHPs.

2.2. Experiment 2: Aortic arch anomaly

Upon examination of the heart, we detected the anomaly in the aortic anatomy. Out of the eight ischemic NHPS, 6 displayed the typical two aortic branches (NHP1, NHP2, NHP5-NHP8) corresponding to the innominate artery and the left subclavian artery (Fig. 1). However, two NHPs (NHP3, NHP4) exhibited three branches (Fig. 2), namely the innominate artery, the left subclavian artery, and the left common carotid artery. These three branches were visualized in NHP4 during operation and were clipped, but for NHP3 this anomaly was only recognized after the surgery thus we were only able to clip the innominate artery and the left common carotid artery. Despite an unclipped left subclavian artery, the pupils of NHP3 were dilated during the 10-15-min ligation. In addition, such aortic anomaly was found in one of three non-operated NHPs. Taken together, three animals from 11 NHPs examined here (27.3%) possessed an extra aortic branch (Fig. 3 and Table 1).

2.3. Experiment 3: Histological study

Histological examination of ischemic brains via Nissl staining revealed ischemic cell loss in the hippocampus, especially at day 30 after TGI, characterized by chromatolysis (Figs. 4 and 5). Of note, only partial cell loss and almost unaffected number of cell layers in the hippocampus were detected in NHP3 which had only two of three aortic branches clipped. In contrast, NHP4 which also had three aortic branches, but with all three branches clipped during operation, showed obvious hippocampus cell loss and cell layer reduction comparable to NHPs that had the typical two aortic branches (Figs. 4 and 5). Hence blood circulation supplied by the left subclavian artery in NHP3 likely prevented a complete TGI, thereby resulting in a limited damage to the hippocampus (Table 1).

3. Discussion

The present study shows an anomaly in the aortic arch with the left common carotid artery, in addition to the innominate artery and the left subclavian artery, originating from the aortic branch in about 30% of Rhesus macaques. This finding precludes that ligating this third aortic branch is necessary for successful TGI-induced hippocampal cell loss.

We recently reported that the TGI model in Rhesus macaques led to neuronal cell loss and delayed cell apoptosis in the hippocampus (Hara et al., 2008) resembling that seen in Japanese macaques (Tsukada et al., 2001; Yamashima, 2000; Yamashima et al., 2003; Yamashima et al., 2004; Yamashima et al., 1996). This ischemic injury model is useful for investigation of neurogenesis in CA1 region (Yamashima et al., 2004). The present TGI surgical procedure is less invasive than other TGI models, e.g. bilateral carotid and vertebral artery occlusion (Tabuchi et al., 1992; Tsukada et al., 2001; Yukie et al., 2006). In this procedure, the most severe complication is pneumothorax, which can be easily avoided by covering the visceropleura on the lung by a sterile rubber piece after removing the sternum. There is no any

other critical organ in the operative field. The surgical operation itself is straightforward. First, the innominate artery could be found out by its pulsation. Second, after exposing the innominate artery, the aortic arch could be visualized. In the distal part of the aortic arch, directly adjacent to the innominate artery, the second branch corresponding to the left subclavian artery should be isolated. Third, equipped with the knowledge of an anomaly in the aortic branch, one should advance along the distal aorta and attempt to isolate a third branch from the aortic arch. If such a third branch was detected, that branch should be identified as the left subclavian artery, while the second artery should be assigned as the left common carotid artery (Fig. 2). If a third branch was confirmed not to exist, the second branch should be designated as the left subclavian artery (Fig. 1). In order to confirm the number of aortic branches, exposing the distal innominate artery was helpful because the left carotid common artery should normally originate from the innominate artery. All branches (two or three) should be clipped to achieve complete ischemia, because the arteries perfusing the entire brain (i.e., bilateral common carotid arteries and vertebral arteries) can be occluded by ligating all branches from the aortic arch. Here, we show that the success of the TGI model depended on clipping all branches arising from the aortic arch. Three of 11 NHPs displayed three aortic branches, which have not been observed in Japanese macaques or other NHP strains. Indeed, a complete TGI model is accomplished in Japanese macaques by occluding only two branches (Tonchev et al., 2005; Tonchev and Yamashima, 2006; Tonchev et al., 2006; Tonchev et al., 2003; Tsukada et al., 2001; Yamashima, 2000; Yamashima et al., 2004; Yukie et al., 2006). With the simple additional step of isolating and clipping the third aortic branch in Rhesus macaques with such anomaly, we achieved comparable cell loss and cell layer depletion in hippocampal CA1 region. Accordingly, the TGI approach, which was initially developed in Japanese macaques, can be successfully extended to Rhesus macaques despite this aortic arch anomaly. Such subtle differences in blood circulatory systems between NHP strains may prove critical in animal disease modeling as shown in the present ischemic injury model.

The anatomy of the aortic arch in Rhesus monkeys has been described 70 years ago using sketches of the normal aortic arch and arterial branch anomalies found in Rhesus macaques (de Garis, 1938). In addition to the photographic confirmation made in the present study, we characterized for the first time the consequent histological effects of this aortic branch anomaly when this species is used for disease modeling. We acknowledge the small series in our study, but subsequent surgical operations using additional Rhesus macaques revealed 1 of 3 animals displaying such anomaly (data not shown).

Our TGI model in NHPs is a much simpler and less traumatic approach compared to other ischemic models whereby animals are exposed to complicated and invasive surgical procedures (Frykholm et al., 2005; Hudgins and Garcia, 1970; Nemoto et al., 2005; O'Brien and Waltz, 1973). However, our model is different from clinical situation of brain ischemic attack, and may actually closely resemble the cardiac arrest scenario. Notwithstanding, our minimally invasive TGI model appears as a suitable platform for examination of therapeutic regimens designed to reveal the participation of neurogenesis in the hippocampus.

In summary, we show a modified TGI procedure in Rhesus macaques that effectively circumvents the anomaly in the aortic branch. The third branch should be detected and clipped during surgery to achieve a complete TGI. Such refinements of surgical methods in laboratory models are necessary to advance their validity in clinical application (Agazzi et al., 2007; Froelich et al., 2007). We envision that this NHP model will serve as a valuable tool for evaluating experimental treatments in ischemic injury.

4. Experimental procedures

4.1. Animals

Eleven (about 8 years of age) Rhesus macaques (*Macaca mulatta*), weighing between 4.8 and 6.0 kg were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Bethesda, MD, USA). The animals were housed in an AAALAC-International accredited NHP facility. During pre-surgery period, animals were allowed access to an enclosed outdoor exercise facility on an individual basis. Both cages and exercise facilities contained perch bars and enrichment gadgets. The animals were maintained on an automatic watering system and standard laboratory non-human primate chow (Harlan Teklad 20% Protein NHP diet, 2050) supplemented with fruits and vegetables. Surgical procedures were conducted under aseptic conditions, and every effort was made to minimize animal discomfort.

4.2. Transient global ischemia

Eight NHPs underwent TGI surgery, while 3 NHPs served as non-operated controls. The TGI procedure involved clipping both the innominate and left subclavian arteries as described in detail previously (Tsukada et al., 2001; Yamashima et al., 1996). The ischemia surgery was carried out under general anesthesia. Animals were sedated with ketamine (10 mg/kg) IM and preanesthetized with Atropine (0.02 mg/kg) SC. The animals were intubated with an appropriately sized endotracheal tube, and anesthetized with 2% isoflurane by a respirator prior to the surgical procedure. Body temperature was monitored with a rectal probe and maintained within normal physiological limits (36.5–38.5 °C) using a warming blanket. Body temperature, pupil size, respiratory rate, SpO₂ were monitored throughout the surgical procedure. To achieve transient forebrain ischemia, both the innominate artery and the left subclavian artery were initially exposed in the mediastinum by removing the sternum, then they were clipped with two vascular clamps under the normotension of 80–100 mmHg (Fig. 1). We used the 10–15-min occlusion which worked consistently in Japanese macaques (Yamashima et al., 1996). We kept the animals alive up to 1 day (NHP1), 5 days (NHP2) or 30 days (NHP3–NHP8) after surgery. As a control, brains obtained from the three non-operated NHPs were processed similarly together with the ischemic brains for histological examination.

4.3. Histology

The animals were sacrificed for histological analyses using the sedative dose of ketamine (20 mg/kg), then perfused with 0.9% saline and 4% paraformaldehyde (PFA) transcardially, followed by brains excision and cryopreservation with 30% sucrose in PB (Sigma, St. Louis, MO, USA). Brains were sectioned at 25 µm thickness using a cryostat (Leica, Nussloch, Germany) then processed for Nissl staining with cresyl violet solution (Sigma, St. Louis, MO, USA). The brain section coordinates corresponded to 10–11 cm from the anterior pole of the brain, which approximated the same hippocampal region of the Japanese macaques shown to be highly vulnerable to TGI (Yamashima et al., 2003). Since we made all the brain blocks using the same coordinates, we can approximate equivalence in the specific hippocampal regions and the subsequent fields of view selected for cell count analyses across animals.

4.4. Aortic anatomical evaluation

Following perfusion and brain harvesting, the heart with intact ascending aorta and aortic arch was dissected and fixed in 4% PFA. The heart specimens were obtained from the eight ischemic NHPs, as well as from the three non-operated control NHPs. The number of branches arising from aortic arch and anatomy of main vessels from these branches were examined and digitally photographed.

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Fig. 1. Anatomy of the aortic arch in Rhesus macaques

Typical anatomy of the aortic branch is characterized by innominate artery and left subclavian artery originating from aortic arch. By clipping these two aortic branches, all arteries perfusing the entire brain (e.g., bilateral common carotid and bilateral vertebral arteries) should be occluded. Red lines show the clipping sites.

Fig. 2. Anomaly of the aortic arch in Rhesus macaques

Three branches (innominate, left common carotid, and left subclavian artery) are shown to originate from the aorta. This anatomy closely resembles that seen in human. Red lines show the clipping sites in the case of 3 aortic branches.

Fig. 3. Summary of anatomy of the aortic arch in Rhesus macaques
Out of 11 NHPs, three (Panels I, J and K) showed the “three-branch anomaly”.

Only 2 of 3 branches were clamped.

incomplete model

All 3 branches were clamped.

complete model

Fig. 4. Aortic branch representations

Recognition of the “three-branch anomaly” allows a surgical maneuver of clipping all 3 aortic branches to achieve a complete TGI model, resulting in consistent hippocampal cell loss than an incomplete 2-branch clipping TGI approach.

Fig. 5. Hippocampal cell loss in animals with aortic branch anomaly

Nissl staining showed less cell loss and minimal depletion of cell layers (4-5 cell layers maintained) in NHP4 (2 of 3 branches clipped; Panel A). In contrast, NHP3 (all 3 branches clipped; Panel B) displayed rampant cell loss, with only sparing of 1-2 layers, in the hippocampal CA1 region. Scale bar=30um.

Table 1

Summary of Experimental Procedures and Results

Subject Code	Surgery	# Clipped Aortic Branches	# Aortic Branches	# Day Survival Post-TGI	Hippocampal Cell Loss
NHP1	TGI	2	2	1 POD	-
NHP2	TGI	2	2	5 POD	+
NHP3	TGI	2	3	30 POD	+
NHP4	TGI	3	3	30 POD	++
NHP5	TGI	2	2	30 POD	++
NHP6	TGI	2	2	30 POD	++
NHP7	TGI	2	2	30 POD	++
NHP8	TGI	2	2	30 POD	++
NHP9	control	NA	2	NA	-
NHP10	control	NA	3	NA	-
NHP11	control	NA	2	NA	-