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Variations in the Circle of Willis in the New Zealand White Rabbit

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Abstract

The New Zealand White rabbit serves an important role as an experimental model for vascular research, specifically in the area of stroke. We document vascular variations in the circle of Willis in New Zealand White rabbits (N=100) using sub-selective internal carotid digital subtraction angiograms. Important variations include hypoplasia in 36%, duplication of the middle cerebral artery in 29%, asymmetries of the posterior region in 19% and multiple variations in 28%. The complete classical symmetric COW without significant variation is present in less than 30% of animals. With recognition of the variations, the New Zealand White rabbit becomes an improved research model.

Introduction

Collateral blood supply to the brain is provided by a ring-like structure of arteries collectively referred to as the Circle of Willis (COW) (1). Human anatomical variations of the COW are well known and variations have been described for common laboratory animals used as ischemic stroke models including the dog and rhesus monkey (2–3).

The rabbit serves as an attractive model for the study of acute ischemic stroke (4–5), and results from rabbits have made a successful transition into human therapy (6). Despite the long term use of the rabbit as a model for embolic stroke, only limited description of anatomical variations within the COW is available (7). Digital subtraction angiography (DSA) has been utilized to provide detailed images of cerebral vessels (8) and to describe anatomical variations along the aortic arch (9) in New Zealand White rabbits. However, a detailed description of anatomical variations within the COW in New Zealand White rabbits does not exist.

In order to facilitate the development of novel therapies for acute ischemic stroke using the rabbit as a model, a better understanding of anatomical variations within the COW in the

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New Zealand White rabbit is warranted. The objective herein was to utilize sub-selective DSA to evaluate and describe vascular variations within the COW of New Zealand White rabbits.

Materials and Methods

Angiography was performed on New Zealand white rabbits (NZWR) ($n = 100$, mean age = 1.0 ± 0.02 yr, mean body weight = 5.2 ± 0.07 kg) of both genders under the approval of the Institutional Animal Care and Use Committee. These rabbits were part of an experimental stroke study and were euthanized at 24 hours. The procedures of Culp et al. (8) were utilized to obtain magnified angiographic images of the COW. In brief, rabbits received an intramuscular injection of ketamine (30 mg/kg, Ketaset; Fort Dodge, Fort Dodge, IA) and xylazine (3 mg/kg, AnaSed, Lloyd Laboratories, Shenandoah, IA) and subsequently were anesthetized with isoflurane (Novaplus, Hospira Inc., Lake Forrest, IL). Following sedation and anesthesia a femoral cut down was performed and sub-selective angiography of the internal carotid was performed with a 65-cm 3 French catheter (SlipCath, Cook Inc., Bloomington, IN). A 0.025 inch angled-tip guide wire (Glidewire, Terumo Medical Corp., Elkton, MD) was utilized to advance the 3F catheter under fluoroscopy to the common carotid artery and subsequently the internal carotid artery. Images in multiple projections were used to evaluate variations within the COW using a single plane C-arm digital subtraction angiography machine (OEC 9800, GE Healthcare; Salt Lake City, UT). Digital subtraction angiography at 8 frames per second was performed by injection of 3 to 5 mL of an iodinated contrast agent (Visipaque, GE Healthcare; Princeton, NJ) in the anteroposterior and lateral views.

The complete COW comprised of the classic symmetric pattern was recorded. In addition, all anatomical variations of the COW were documented. Vessels were labeled as human anatomical equivalents in accordance with the literature (6). The vessels examined included: internal and external ophthalmic arteries, anterior cerebral arteries, middle cerebral arteries, posterior communicating arteries, posterior cerebral arteries, superior cerebellar arteries, basilar artery, anterior inferior cerebellar arteries, posterior inferior cerebellar arteries, and vertebral arteries. Additionally, the anterior cerebral-middle cerebral artery junctions, middle cerebral-internal carotid artery junctions, posterior communicating-posterior cerebral artery junctions, and the posterior cerebral-basilar junctions were observed. The presence of a hypoplastic artery, defined as a very small or incompletely developed artery of less than 50% of the expected diameter was recorded. Variations of less severity were quite common and not documented. Additional variations, such as abnormal origination points, abnormal connections, or duplicate vessels, were recorded. The proportions of rabbits having these variations was calculated.

Results

Overall, 29% of rabbits had the complete classical symmetric COW without significant variation (Fig 1A and Fig 2A). Hypoplastic vessels occurred in 36% of rabbits. Hypoplasias of anterior cerebral and vertebral arteries were observed in 13% and 10% of rabbits, respectively with other sites widely distributed (Fig 1D and Fig 2C). Other main variations were duplicate middle cerebral arteries (Fig 1B and Fig 2B) and an asymmetric posterior cerebral-basilar artery variation (Fig 1C and Fig 2D). Unilateral dual middle cerebral arteries occurred in 27% of rabbits. Duplication occurred on the left side in 20% while occurring only on the right side in 7% of rabbits. Middle cerebral artery duplication was bilateral in 2%. In 19%, a posterior cerebral-basilar artery asymmetric variation was observed and occurred on the left side in 18 out of 19 rabbits (Fig 1C and Fig 2D). A vertebral artery was absent in 7% (Fig 1D). Two or more variations occurred in 28% of rabbits.

The superselective magnification angiographic technique is reliably performed and shows good detail in all studies. Some debatable spasm resulting from catheterization and subsequent embolization was documented in 20% of rabbits. Subselective internal carotid injections routinely provided filling of the entire COW with reflux well up the vertebrals and opposite internal carotid artery. Scattered findings outside the COW include occasional fenestration in the vertebral junctions and at the origin of the ICA, along with other variations, beyond the scope of this study. The lateral projections are most helpful for sub-selection of the ICA but COW elements are often overlapped in these views and the anteroposterior view is required for this information.

Discussion

Stroke is the leading cause of long-term disability in the United States and remains an important and unresolved disease, which affects 700,000 people and accounts for more than 150,000 deaths each year (10). In the field of ischemic stroke research, rabbits have proven to be good research models with the successful transition of thrombolytic therapy from rabbit data to human practice (6). However, as more detailed models became necessary, it was apparent that little data was available on the angiographic variations that occur in the rabbit COW. An understanding of the variations is an important step in producing standard size strokes and comparing treatments.

The general gross morphology and distribution of the COW in the NZWR were first presented in 1884 (11) and the first angiographic description of the COW was given in 1962 (7). However, few researchers have reported cerebrovascular variations in the NZWR. Other studies involve fewer animals and do not use current super-selective angiography. Our results differ from that of the data reported by other authors in that 71% had a variation in the COW. Jeppsson and Olin (7) found that only one in seventy-six rabbits had a posterior cerebral-basilar artery variation, where as we found that 19% of our NZWR had this variation. This configuration can impact embolic distribution and catheter access to specific vessels. We also found duplication of the middle cerebral artery in 29% of NZWR, but Kirchoff et al. (5) described only four out of twenty six NZWR having duplication of the middle cerebral artery. Additionally, Kapoor et al. (3) states that no NZWR out of twenty five had a variation in the COW. However, we found that only 29% had the complete classical symmetric COW in our study of 100 rabbits.

Angiographic access to the cerebral vessels can be complicated by the frequent internal carotid variation involving a complex origin from the common carotid in conjunction with the occipital artery (8). Additionally, a vertebral approach to access the COW can be complicated by absent or hypoplastic vertebral arteries when using either a catheter or flush of embolic agents, a technique used in many rabbit studies in the past (6).

The common appearance of two smaller vessels feeding the MCA distribution found in 29% here can be found in up to 2.9% of humans (12). This variation can be very important when using a MCA target for a stroke model. Occlusion of only one of these branches will decrease the stroke volume in that animal and skew results. Therefore these animals must be excluded or the occlusion must be designed to be small enough to work either in a duplicated MCA or single MCA. A large embolus may not fit into either element of a duplicated MCA and lodge elsewhere. A micro-catheter occlusion of one element will not effect the whole distribution and produce a decreased volume of infarct (13).

Limitations include the small size of these animals, which requires excellent angiographic technique and equipment for successful imaging. Catheters are not readily available and must be purchased specifically for this use. While generalization of rabbit findings to human

disease promises to be more applicable than smaller models (14), reliable translation to human disease is not assured.

Still, the NZWR has many good attributes. It is small enough to be cost effective yet large enough to provide angiographic access the cerebrovascular vessels and has advantages of scale compared to mice and rats (15). Additionally, the rabbit possesses a well-developed COW similar to that of humans (16). Eftekhari et al. 2006 (17) described that in the COW of 92 humans, variations occurred in 68.5%, which makes the occurrence of variation similar to the 71% of the NZWR.

In summary, variations in the NZWR COW are common, occurring in 71%. Many do not greatly impact the use of this animal model, but the duplications of the MCA and vertebral variations can affect stroke models. While the frequency of variation is high, the rabbit remains a valuable and improved model with better understanding of these factors.

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Figure 1.

Cerebral digital subtraction angiography of the NZWR circle of Willis. (A) The normal cerebrovascular pattern is described in Culp et al. 2007, used with permission. Labeled arteries include the anterior cerebral (AC), anterior inferior cerebellar (AICA), basilar (B), external ophthalmic (EO), internal carotid (IC), internal ophthalmic (IO), middle cerebral (MC), maxillary (MX), posterior cerebral (PC), posterior communicating (PCOM), posterior inferior cerebellar (PICA), superior cerebellar (SC), and the vertebral (V). (B) Duplication of a middle cerebral artery. (C) An asymmetric posterior cerebral-basilar arterial variation. (D) An absent vertebral artery.

Figure 2.
Common anatomical variations of the anterior, middle, and posterior segments of the NZWR COW. (A) Complete COW. (B) Duplication of a middle cerebral artery. (C) Hypoplastic anterior cerebral artery. (D) A posterior cerebral-basilar variation.