pISSN 1976-7447, eISSN 2287-7363
J Biomed Res 2014;15(3):107-111
http://dx.doi.org/10.12729/jbr.2014.15.3.107
Received 18 Aug. 2014, Revised 17 Sep. 2014, Accepted 22 Sep. 2014

Original Article

Canine model of ischemic stroke with autologous thrombus in three dogs; Magnetic resonance imaging features and histopathological findings

Joon-Hyeok Jeon[1], Hae-Won Jung[1], Hee-Chun Lee[1], Byeong-Teck Kang[2], Jung-Hyang Sur[3], Dong-In Jung[1]*

[1]*Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea*
[2]*Laboratory of Veterinary Dermatology and Neurology, College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Korea*
[3]*Department of Pathobiology, Small Animal Tumor Diagnostic Center, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea*

Ischemic stroke is the most common type of stroke in humans. The purpose of this study was to evaluate the diagnostic value of magnetic resonance imaging (MRI) in a canine model of stroke. Ischemic stroke was induced by using prepared autologous thrombus. The dogs were placed in lateral recumbency on the operation table and the cervical area of each dog was sterilized by using alcohol. After making a cervical incision, the common carotid artery and internal carotid artery (a branch of the common carotid artery that supplies an anterior part of the brain) were exposed. A 200 μL injection of the autologous thrombus prepared 24 hr prior to surgery was delivered with a 20 gauge venous catheter through an internal carotid artery. After successful delivery of the autologous thrombus, the venous catheter was removed, and the cervical incision was sutured. Neurologic signs including generalized seizures, tetraparesis, and altered mental status, were observed in all 3 dogs after induction of ischemic stroke and the signs manifested immediately after awakening from anesthesia. T1- and T2-weighted images and fluid-attenuated inversion recovery (FLAIR) images of the brain were acquired 1 day before and 1 day after surgery. On the day following ischemic stroke induction, MRI revealed multifocal lesions in the cerebral cortex and subcortex such as T1 hypointensity, T2 hyperintensity, FLAIR hyperintensity, and diffusion-weighted hyperintensity in all 3 dogs. Upon postmortem examination, ischemic lesions were found to be consistent with the MRI findings and they were unstained with 2% triphenyltetrazolium chloride. Histologic features of the earliest neuronal changes such as cytoplasmic eosinophilia with pyknotic nuclei were identified. Neuropil spongiosis and perivascular cuffing were also prominently observed at the infarcted area. The present study demonstrated the features of MRI and histopathologic findings in canine ischemic stroke models.

Key words: dog, autologous thrombus, ischemic stroke, magnetic resonance imaging, histopathology

Introduction

Ischemic stroke is a leading cause of neurologic disability and human mortality worldwide. Transient or permanent occlusion of affected cerebral arteries can lead to a substantial reduction of blood flow in the territory of the affected arteries and cause cerebral ischemic stroke [1]. In addition, the subsequent proinflammatory cascade can lead to permanent neurologic deficit or severe disability. Although there are various animal models of ischemic stroke, most of them are small animal models (e.g., mice, rats, and gerbils). Compared with those animals, larger animals, including dogs, have brain and vasculature anatomy more similar to those of humans (e.g., gyrencephalic brains and substantial white matter among other similarities). Accordingly, larger animals may be more appropriate as ischemic stroke models [1, 2].

Magnetic resonance imaging (MRI) is considered an essential diagnostic device for evaluation of patients with symptoms suggestive of ischemic stroke. Especially in cases of acute stroke, MRI is not only superior to computed tomography for detection of ischemic lesions, but for differentiation of acute one from chronic hemorrhage [3]. Another advantage of MRI is its ability to differentiate stroke from other conditions that present similar focal and sudden-onset neurologic signs [3]. These advantages

***Corresponding author:** Dong-In Jung, Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea
Tel: +82-55-772-2361, E-mail: jungdi@gnu.ac.kr

make MRI an ideal diagnostic tool for ischemic stroke in human medicine [3]. Postmortem examination and histopathologic study should also be performed for definite diagnosis. The purpose of this study was to describe the neurologic signs, MRI features, and histopathologic findings of experimental acute ischemic stroke in dogs.

Materials and Methods

Animals

The study population included 3 healthy mongrel dogs (2 males and 1 female, about 3~5 years old, weighing 8.1, 8.5, and 9.5 kg, respectively). All 3 dogs were physically normal, with no history of neurologic disorders. Results of physical and neurologic examinations prior to the surgical procedure were all normal. Complete blood count and serum chemistry profile results were used as screening tests for metabolic diseases; they were found to be normal. The surgical procedure and experimental protocols, including euthanasia, were approved by the Institutional Animal Care and Use Committee of Gyeongsang National University.

Autologous thrombus preparation

Twenty-four hours prior to surgery, 5 mL of venous blood was obtained from the jugular vein and drawn into a plain tube. After 24 hr of coagulation at room temperature, the supernatant was decanted. A micropipette was used to quantify 200 µL of the autologous thrombus. A prepared 30 mL syringe was then filled with a mixture of quantified autologous thrombus and 20 mL of physiologic saline.

Animal preparation, monitoring, and surgical procedure

All dogs were made to fast for >12 hr before induction of general anesthesia. Atropine was administered as a premedication 10 min before induction, and anesthesia induction was performed using orally intubated propofol. Isofluran at 2~3% of the inspired volume was used to maintain general anesthesia during surgical procedures. The heart rate, body temperature, and blood oxygen saturation level were monitored and maintained within normal ranges during surgery. Ischemic stroke was induced by using the prepared autologous thrombus. The dogs were placed in lateral recumbency on the operation table, and the cervical area of each dog was sterilized by using alcohol. After making a cervical incision, the common carotid artery and internal carotid artery (a branch of common carotid artery that supplies the anterior part of the brain) were exposed. A 200-µL injection of the autologous thrombus was delivered with a 20-gauge venous catheter through the internal carotid artery. After successful delivery of the autologous thrombus, the venous catheter was removed and the cervical incision was sutured.

Magnetic resonance imaging analysis

MRI was performed 1 day before and 1 day after induction of ischemic stroke with a 0.4T magnet MRI system (APERTO 0.4, Hitachi Medical Co., Tokyo, Japan). Under general anesthesia, transverse T1-weighted imaging, T2-weighted imaging, FLAIR imaging, postcontrast T1-weighted imaging, and diffusion-weighted imaging (DWI) were performed (Table 1).

Staining with 2,3,5-triphenyltetrazolium chloride and histopathologic examination

A day after induction of ischemic stroke, MRI results were obtained, and all dogs were euthanized. The brains were carefully removed and dissected into 2 mm coronal slices. The fresh brain slices were immersed in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich, St. Louis, MO) in normal saline at 37°C for 30 min, as previously reported [4, 5]. Brain slices were also immersed in 10% paraformaldehyde in phosphate buffer for at least 72 hr for fixation. After immersion fixation,

Table 1. Magnetic resonance imaging protocol in the present study

Parameters	0.4 T			
Sequence	T1	T2	FLAIR	DWI
TR (ms)	391	5698	8720	6000
TE (ms)	13.0	115	100	126.2
Flip angle (degrees)	90	90	90	90
Field of view (mm)	180	180	180	220
Acquisition matrix	256 × 256	256 × 256	256 × 256	256 × 256
No. of slices	22	22	22	10
Thickness (mm)	3.0	3.0	3.0	5.5
No. of averages	4	4	2	2

the slices were dehydrated and embedded in paraffin. Transverse sections (5 μm) were cut. At the end of staining with hematoxylin and eosin, light microscopy was used to detect any histopathologic alterations caused by ischemic stroke.

Results

Postsurgical management and observed neurologic signs

Physiologic parameters before, during, and after surgery were well maintained until euthanasia. To prevent infection and pain after surgery, antibiotics and analgesics were administered until euthanasia. Neurologic signs, including generalized seizures, tetraparesis, and altered mental status, were observed in all 3 dogs after induction of ischemic stroke. These neurologic signs manifested immediately after awakening from anesthesia.

Magnetic resonance imaging findings

MRI findings before ischemic stroke induction were all normal; however, 1 day after ischemic stroke induction, MRI revealed multifocal lesions in the cerebral cortex and subcortex, with T1 hypointensity, T2 hyperintensity, FLAIR hyperintensity, and DWI hyperintensity in all 3 dogs (Fig. 1). Affected lesions were well differentiated from the adjacent normal brain parenchyma. Mild deviations of the falx cerebri caused by acute cerebral edema formation and mass effects were also identified (Fig. 1).

Staining with 2,3,5-triphenyltetrazolium chloride and histopathologic examination

No remarkable changes were identified in gross postmortem examination of the 3 dogs; however, the coronal brain slices showed edematous lesions, which obscured border lines between the gray and white matter (Fig. 1F). In addition, mild deviations of the falx cerebri due to acute cerebral edema formation were identified (Fig. 1F). These affected lesions were not stained with TTC, in contrast to the red-stained normal brain parenchyma (Fig. 1E). Histologic features of the earliest neuronal changes, such as cytoplasmic eosinophilia with pyknotic nuclei, were identified. Neuropil spongiosis and perivascular cuffing were also prominently observed at the infarcted

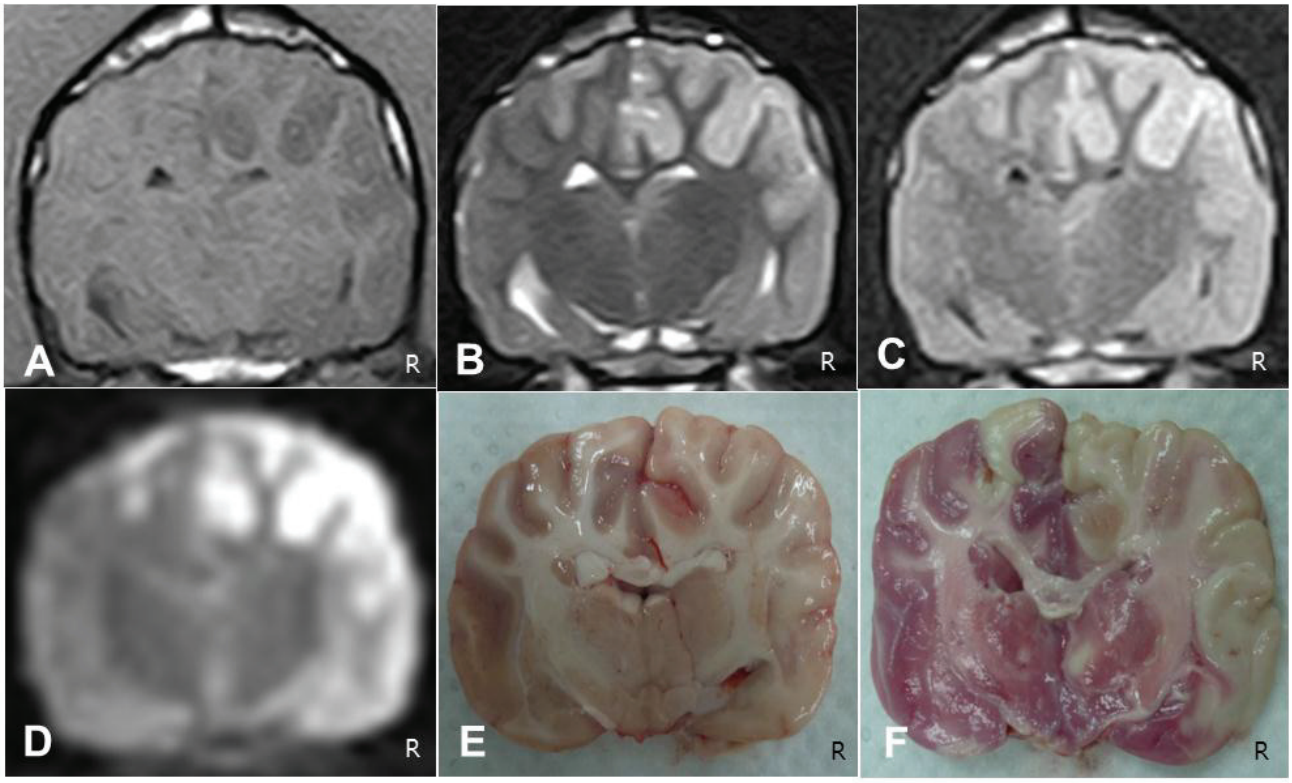

Fig. 1. Magnetic resonance imaging (MIR) scans and 2,3,5-triphenyltetrazolium chloride (TTC) staining of the brain from a dog with ischemic stroke. Image A, B, C, and D were obtained 1 day after induction of ischemic stroke. Images E and F were obtained after postmortem examination. T1 hypointensity (A), T2 hyperintensity (B), fluid-attenuated inversion recovery hyperintensity (C), diffusion-weighted imaging hyperintensity (D) were observed mainly in the lesions in the cerebral cortex and subcortex. Affected lesions were well differentiated from the adjacent normal brain parenchyma. Mild deviations of the falx cerebri caused by cerebral edema and mass effects were also identified on gross findings (E). Coronal section of the brain after of TTC staining showed unstained lesions on the right cerebral cortex (F).

area (Fig. 2).

Discussion

Conventional MRI sequences, including T1- weighted, T2-weighted, and FLAIR images, are only marginally useful for diagnosing ischemia within hours of onset because they are sensitive to vasogenic edema, a process that develops gradually over time. Identification of vasogenic edema with conventional MRI sequences may be difficult within the first 24 hr of stroke [4, 6, 7, 8]. In addition, during the first 24 hr after ischemic stroke, conventional MRI gives false-negative results 20% to 30% of the times [9, 10, 11]. This false-negative probability increases to 30% to 50% during the first 3 to 6 hr [12, 13]. In contrast, DWI sequences can detect ischemic lesions within minutes of onset. Signal changes on DWI sequences reflect the relative restriction of water diffusion due to influx of water from the extracellular to the intracellular space [4, 8]. Therefore, even at very early time points, DWI is highly sensitive and specific for acute ischemic lesion identification. In this study, however, ischemic lesions were also identified easily on conventional MRI sequences 1 day after ischemic stroke induction. Results of conventional MRI taken 1 day after induction of ischemic stroke were almost identical to the results of DWI. Based on these findings, conventional MRI sequences could also be a useful diagnostic tool for diagnosing acute to subacute ischemic stroke. Additionally, specific neurologic signs after ischemic stroke are associated with the location and size of the ischemic lesions [14, 15]. Observed neurologic signs in this study included generalized seizures, tetraparesis, and altered mental status. Of these, generalized seizures are considered one of the most common signs of forebrain disease [14, 16]. Observed lesions in this study were mainly located on the cerebral cortex and adjacent subcortex area of the forebrain. Those lesions were also identified on TTC staining and histopathologic examination. Histopathologic findings in the canine model revealed typical features of acute ischemic stroke such as hypoxic neuronal changes, neuropil spongiosis, and perivascular cuffing. In this study, we used clots derived from unmodified autologous blood to induce ischemic stroke. There were two main forms of clots; one was derived from unmodified blood [17], and one was derived from blood mixed with thrombin [18]. Although both types seemed to provide similar levels of occlusion, thrombin-induced clots appeared more resistant to the effects of tissue plasminogen activator as a thrombolytic therapy [19]. Because thrombolytic therapy within 3 to 6 hr of the onset of ischemic stroke is effective in restoring blood flow and improving stroke prognosis in humans [20], unmodified autologous blood clots are more suitable for studying thrombolytic therapy in acute ischemic stroke [2, 19].

In the present study, we described the induction of ischemic stroke in dogs with autologous thrombus. Characteristic features of ischemic stroke on MRI, histopathological analysis, and TTC staining were also investigated. On MRI, T1 hypointensity, T2 hyperintensity, FLAIR hyperintensity, and DWI hyperintensity lesions were consistent with MRI features of previously reported ischemic strokes in humans. Observed neurologic signs in this study were also consistent with reported effects of ischemic lesions. Based on the results of this study, further studies with highly sophisticated pathophysiologic processes and advanced imaging modalities would be helpful for the diagnosis and treatment of ischemic stroke in dogs.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of

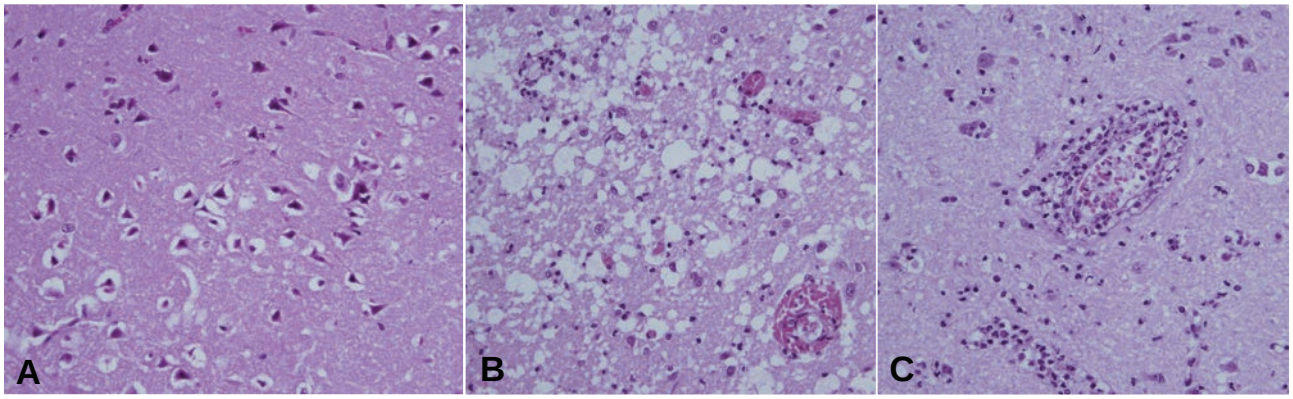

Fig. 2. Microscopic findings of infarcted cerebral parenchyma. Hypoxic neurons with pyknotic nuclei were observed extensively (A). Extensive neuropil spongiosis with increased oligodendrocytes (B) and perivascular cuffing with increased oligodendrocytes were also observed (C).

Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0008358).

References

1. Mergenthaler P, Meisel A. Do stroke models model stroke? Dis Model Mech 2012;5:718-725.
2. Howells DW, Porritt MJ, Rewell SS. Different strokes for different folks: the rich diversity of animal models of focal cerebral ischemia. J Cereb Blood Flow Metab 2010;30:1412-1431.
3. Arsava EM. The role of MRI as a prognostic tool in ischemic stroke. J Neurochem 2012;123:22-28.
4. Kang BT, Jang DP, Gu SH, Lee JH, Jung DI, Lim CY, Kim HJ, Kim YB, Kim HJ, Woo EJ, Cho ZH, Park HM. MRI features in a canine model of ischemic stroke: correlation between lesion volume and neurobehavioral status during the subacute stage. Comp Med 2009;59:459-464.
5. Kang BT, Lee JH, Jung DI, Park C, Gu SH, Jeon HW, Jang DP, Lim CY, Quan FS, Kim YB, Cho ZH, Woo EJ, Park HM. Canine model of ischemic stroke with permanent middle cerebral artery occlusion: clinical and histopathological findings. J Vet Sci 2007;8:369-376.
6. Lee DH, Kang DW, Ahn JS, Choi CG, Kim SJ, Suh DC. Imaging of the ischemic penumbra in acute stroke. Korean J Radiol 2005;6:64-74.
7. Rossmeisl JH Jr, Rohleder JJ, Pickett JP, Duncan R, Herring IP. Presumed and confirmed striatocapsular brain infarctions in six dogs. Vet Ophthalmol 2007;10:23-36.
8. van Everdingen KJ, van der Grond J, Kappelle LJ, Ramos LM, Mali WP. Diffusion-weighted magnetic resonance imaging in acute stroke. Stroke 1998;29:1783-1790.
9. Bryan RN, Levy LM, Whitlow WD, Killian JM, Preziosi TJ, Rosario JA. Diagnosis of acute cerebral infarction: comparison of CT and MR imaging. AJNR Am J Neuroradiol 1991;12:611-620.
10. Kertesz A, Black SE, Nicholson L, Carr T. The sensitivity and specificity of MRI in stroke. Neurology 1987;37:1580-1585.
11. Yuh WT, Crain MR, Loes DJ, Greene GM, Ryals TJ, Sato Y. MR imaging of cerebral ischemia: findings in the first 24 hours. AJNR Am J Neuroradiol 1991;12:621-629.
12. Mohr JP, Biller J, Hilal SK, Yuh WT, Tatemichi TK, Hedges S, Tali E, Nguyen H, Mun I, Adams HP Jr. Magnetic resonance versus computed tomographic imaging in acute stroke. Stroke 1995;26:807-812.
13. Shimosegawa E, Inugami A, Okudera T, Hatazawa J, Ogawa T, Fujita H, Toyoshima H, Uemura K. Embolic cerebral infarction: MR findings in the first 3 hours after onset. AJR Am J Roentgenol 1993;160:1077-1082.
14. Garosi L, McConnell JF, Platt SR, Barone G, Baron JC, de Lahunta A, Schatzberg SJ. Clinical and topographic magnetic resonance characteristics of suspected brain infarction in 40 dogs. J Vet Intern Med 2006;20:311-321.
15. Marks MP. Cerebral ischemia and infarction. In: Scott WA (ed.). Magnetic resonance imaging of the brain and spine, 3rd ed. Philadelphia: Lipincott Williams & Wilkins; 2002. p. 919-980.
16. De Lahunta A. The Neurologic Examination. In: De Lahunta A, Glass E (eds.). Veterinary neuroanatomy and clinical neurology. 3rd ed. Philadelphia: Saunders; 2009. p. 487-501.
17. Dinapoli VA, Rosen CL, Nagamine T, Crocco T. Selective MCA occlusion: a precise embolic stroke model. J Neurosci Methods 2006;154:233-238.
18. Wang CX, Yang T, Shuaib A. An improved version of embolic model of brain ischemic injury in the rat. J Neurosci Methods 2001;109:147-151.
19. Niessen F, Hilger T, Hoehn M, Hossmann KA. Differences in clot preparation determine outcome of recombinant tissue plasminogen activator treatment in experimental thromboembolic stroke. Stroke 2003;34:2019-2024.
20. Shaibani A, Khawar S, Shin W, Cashen TA, Schirf B, Rohany M, Kakodkar S, Carroll TJ. First results in an MR imaging compatible canine model of acute stroke. AJNR Am J Neuroradiol 2006;27:1788-1793.