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Collagenase-Induced Rat Intra-Striatal Hemorrhage Mimicking Severe Human Intra-Striatal Hemorrhage

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Abstract

Basal ganglia hemorrhage accounts for approximately 50% of all hemorrhagic strokes. A good rat model that produces severe intrastriatal hemorrhage (ISH) mimicking human severe ISH is lacking. The present study compared the intra-striatal injection of 0.2 U with that of 0.6 U of collagenase in inducing severe ISH in rats. Three-Tesla (3T) magnetic resonance imaging (MRI) was used to evaluate brain injuries in terms of hematoma size (volume), midline shift (MLS), and brain edema. This evaluation was further substantiated by determination of behavior and neurologic functions and mortality over 56 h. The 0.2 U collagenase caused hematoma volume increases for 10.3 to 30.1 mm^3, while the 0.6 U caused 36.4 to 114.8 mm^3, at post-ISH 1 h to 56 h. The 0.6 U collagenase significantly increased MLS to 1.5-3.0 times greater than the 0.2 U did at all post-intracerebral hemorrhage (ICH) time points. The MLS increased dependently with hematoma expansion with high correlation coefficients, yet no mortality occurred. These two dosages, nevertheless, caused the same pattern and severity in relative apparent diffusion coefficient (rADC) changes for three regions of interest (ROIs). Both ISH models induced consistent behavior deficits. The larger dosage produced severe brain injuries as well as neurological deficits, more closely mimicking severe human ISH. Hematoma volume and MLS can be the most useful parameters for evaluating the ISH severity in the present experimental model. The larger dosage, therefore, would be useful for investigating the pathophysiology of the severer ISH in the striatum. This may be applied for evaluating potential therapeutic strategies and outcomes in the future.

Key Words: collagenase, hematoma, intracerebral hemorrhage, intra-striatal, midline shift

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Introduction

Accounting for approximately 10-15% of all type of stroke, intracerebral hemorrhage (ICH) is the most devastating type of stroke in humans (19, 20). Intrastriatal hemorrhage (ISH) accounts for approximately 50% of all ICH strokes and is associated with high rate of morbidity and mortality (10, 20). Currently, there is no effective treatment for ISH that increases the survival and improves the quality of life. For the development of new treatments, a good rat model that produces brain injuries comparable to clinically severer ISH in humans is needed.

The collagenase-induced ICH model enables successful and reproducible evaluation of ICH-derived edema formation and histological changes that mimic spontaneous ICH (21, 23). However, this model appears to have limitations and reflects only certain clinical features of ICH (10, 21, 23). Bacterial collagenase enzymatically disrupts the basal lamina of brain capillaries and causes active bleeding that generally progresses over several hours (16). Therefore, the collagenase-induced ICH is widely used, particularly having been focusing on the striatum of the preclinical animal studies (14, 15, 23).

Intrastriatal infusion of 0.2 U collagenase is the most common model for experimental ICH in rat (11, 15, 23). Infusions of 0.01–0.5 U bacterial collagenase into the striatum to induce bleeding in Sprague–Dawley rats have produced dose-dependent brain damages as manifested by increments of hematoma volume and brain edema (23). However, all rats injected with 1 U collagenase died of massive edema and brain herniation within 24 h (23). Thus, we used a rat model induced with 0.6 U collagenase and assumed that it would produce more severe ISH with low mortality in the present investigation (27).

Enlargement of a hematoma after ICH ictus contributes to brain midline shift (MLS), brain edema, and neurological deterioration (26). MLS is a sign that has been correlated with increased mortality and poor clinical outcome of ICH (4, 9, 25). In clinical examinations, computed tomography (CT) is the most commonly used technique to measure MLS with reasonable accuracy in patients (13, 17). For imaging of ICH animal models, most investigations focus on monitoring hematoma volume and brain edema, rarely monitoring MLS (18, 23). In clinical imaging, CT is preferred over magnetic resonance imaging (MRI) for visualization, but when specialized pulse sequences are used, the accuracy of MRI exceeds that of CT (22). Thus, we used MRI to measure hematoma volume, brain edema, and MLS.

A good animal model that can mimic the clinical features of human ICH should meet the standards for high reproducibility and clinically relevance (27). The collagenase-induced ICH model enables successful and reproducible evaluation of ICH-derived edema formation and histological changes that mimic spontaneous ICH (21, 23). We assumed that 0.6 U collagenase would produce more severe ISH with low mortality and would probably produce brain injuries, including hematoma, brain edema, and MLS, comparable to clinically spontaneous ISH.

Materials and Methods

Animal Ethics

The animals were cared under the guidelines of the Tzu Chi University, Taiwan in accordance with guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

ISH

Twelve male adult Sprague-Dawley rats weighing from 280 to 350 g were arbitrarily assigned to two groups, six rats were injected for 0.2 U collagenase and the other six rats for 0.6 U collagenase. Rats anesthetized with pentobarbital (50 mg/kg, IP; Sigma-Aldrich, St. Louis, MO, USA) were placed in a stereotactic frame. A 30-gauge needle was introduced through a burr hole into the caudate nucleus (3 mm lateral to midline, 0.2 mm posterior to bregma, depth 6 mm below the surface of the skull) (15, 23). A microinfusion pump was used to infuse 1 μl and 3 μl saline containing 0.2 U or 0.6 U of bacterial collagenase (Type VII-S, Sigma-Aldrich) (flow rate 0.2 μl/min; total volume 1 μl for 0.2 U and 3 μl for 0.6 U). Ten min after the completion of infusion, the needle was removed, the burr hole was sealed with bone wax, the wound was sutured, and the animal was placed in a warm box with free access to food and water. During the surgery, the body temperature was automatically maintained at 37.5°C by a rectal temperature sensor and a heating pad (CMA 150, CMA Microdialysis AB, Stockholm, Sweden).

MRI

The imaging, using a 3-Tesla (3T) MRI (Signa HDx, GE healthcare Milwaukee, WI, USA) with 4.3 cm diameter surface coil, was performed on the 12 rats. They were placed into an induction chamber and anesthetized by 5% isoflurane for induction and 2.5 to 3.0% isoflurane/O_2 mixture *via* a nose cone throughout the sequential MRI examination. Two groups of rats underwent sequential MRI examinations after collagenase injection. A baseline MRI before ICH injection was performed on one day before surgery. Time points at 1, 3,

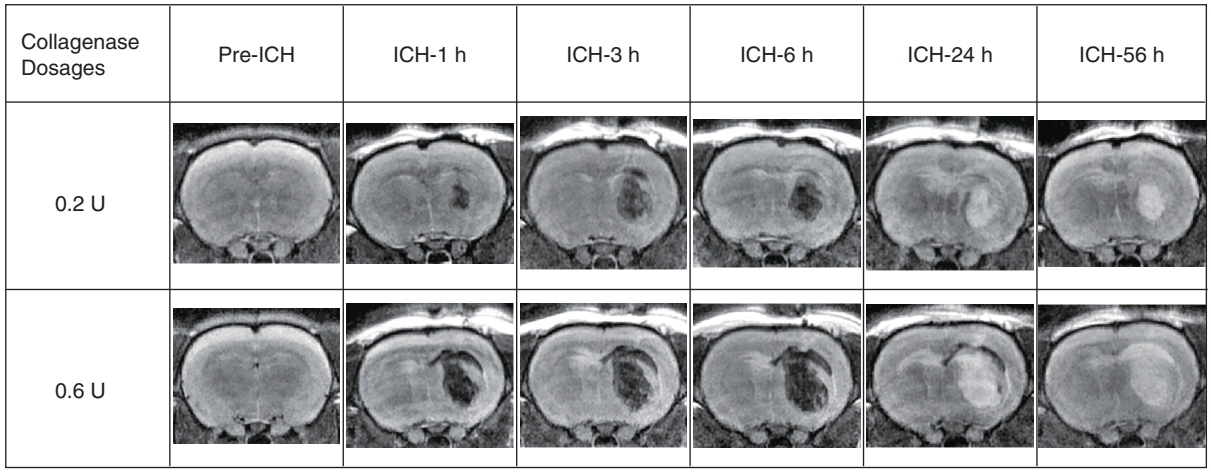

Fig. 1. Time series of T2WI show changes in hematoma size and signal intensity before (pre-ICH), and at 1 h (ICH-1 h), 3 h (ICH-3 h), 6 h (ICH-6 h), 24 h (ICH-24 h), and 56 h (ICH-56 h) after injections of 0.2 U (upper) and 0.6 U (lower) collagenase in the rat striatum.

6, 24 and 56 h post-ICH were acquired. MRI protocols consisted of a T2-weighted imaging 3 dimension fast recovery fast spin echo (FRFSE) sequence with the following relevant parameters: repetition time (TR)/echo time (TE): 2000/85 ms, matrix size = 256 × 224, field of view (FOV) = 4 cm, slice thickness = 1 mm; diffuse weighted imaging (DWI) parameters set as: TR/TE: 10,000/min, matrix size = 128 × 128, FOV = 6 cm, number of excitation (NEX) = 4, slice thickness = 3 mm, gap = 0.3 mm, b value = 0 and 1000 s/mm^2.

Hematoma Size and MLS

The hematoma size was determined from the 3D T2W FRFSE images. In brief, lesioned area was outlined manually in each slice and the hematoma volume (in mm^3) was determined by integration of the lesioned area in each slice using ITK-SNAP software (www.itksnap.org). Determination of the MLS was modified by Llompart Pou *et al*. (13). The measurements were taken from 1-mm T2WI FRFSE. The bilateral distances from the external edge of the hemorrhagic brain side and its contralateral side to the center of the third ventricle were measured as distances B and A, respectively. The following mathematical formula was applied: MLS = (B – A)/2.

Changes in striatal water content were expressed as changes in apparent diffusion coefficient (ADC) and relative ADC (rADC). The ADC map was derived directly from the DWI calculated by the scanner software (GE). As the absolute ADC values (mm^2/sec) differ in each experimental setup, rADC values were calculated. The rADC is a ratio of the ADC value of the ISH (ipsilateral) side to the healthy (contralateral) side of a corresponding mirror region of interest (ROI),

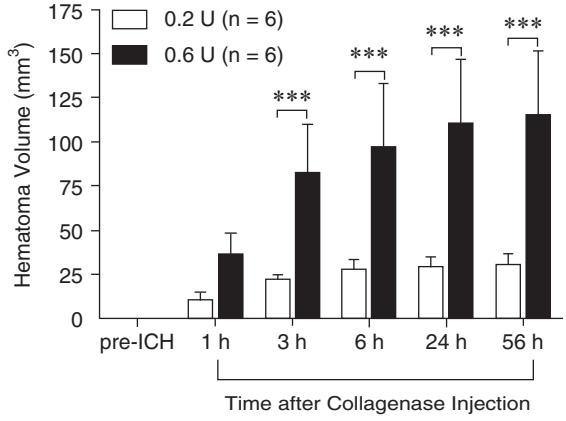

Fig. 2. Hematoma volumes before and at 1, 3, 6, 24 and 56 h after infusion of 0.2 and 0.6 U collagenase. Bars indicate means ± SD. ***$P < 0.001$ indicates significant differences between 0.6 U and 0.2 U ICH groups by two-way ANOVA and Bonferroni multiple comparison.

calculated as rADC = $ADC_{ipsilateral}/ADC_{contralateral}$. Three areas were manually outlined to define ROI in the ipsilateral lesion side first and then mirrored another three ROIs on the contralateral side. Measurements were made in six ROIs.

Neurological functions of rat were evaluated by a modified Neurological Severity Score (mNSS) (5) method at pre surgery and 24 and 56 h after induction of ICH. The evaluation was performed by an investigator blinded to the experimental treatment scheme. The mNSS is a composite test of motor, sensory, balance, reflexes absent, and abnormal movement functions. Neurological function is graded on a scale of 0 (normal) - 18 (maximal deficit). All the rats were weighted before functional be-

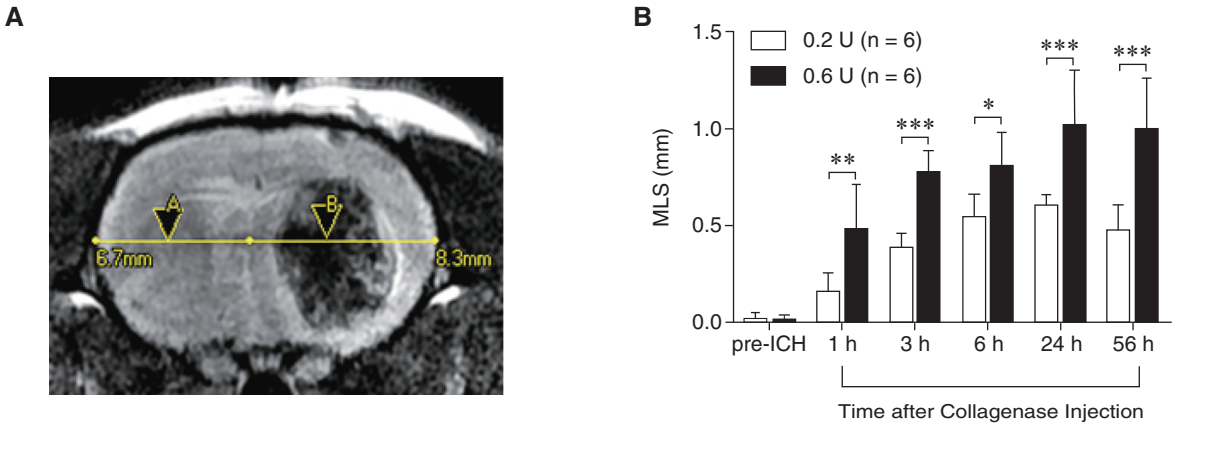

Fig. 3. MLS before and at 1, 3, 6, 24 and 56 h after infusion of 0.2 and 0.6 U collagenase. (A) An image shows MLS caused by a large hematoma. The MLS is calculated as: (B – A)/2. The ipsilateral and contralateral MLS distances are indicated by B and A, respectively. (B) Statistic analysis. (C) MLS change on pre ICH and post ICH between 2 groups. Data are expressed as means ± SD, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ are considered significant differences by two-way ANOVA.

havioral testing and on 1 and 3 days after ICH. All behavioral tests were evaluated and analyzed by an investigator blinded to the groups.

Data were statistically analyzed using statistical software package (SPSS, version 21; SPSS Inc., Chicago, Ill, USA) and were presented as mean ± standard deviation (SD). The statistical comparisons among multiple time points by two-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. In all instances, n refers to the number of animals in a particular group. The mNSS between two groups compared by nonparametric Mann-Whitney U test. Correlation between hematoma volume and MLS used correlation coefficient to analyze. Statistical significance was considered at $P < 0.05$.

Results

Hematoma Size/Volume and MLS

The hematoma size (Fig. 1) and volume (Fig. 2) increased time dependently in each group during 56 h of the observation period. At all post-ICH time points, the 0.6 U collagenase significantly induced 3.0-3.6 time greater hemorrhage volume than the 0.2 U did. The 0.2 U collagenase caused hematoma volume increases for 10.3 ± 4.7, 22.2 ± 2.2, 26.8 ± 6.8, 28.9 ± 5.6 and 30.1 ± 6.8 mm^3, while the 0.6 U caused 36.4 ± 12.3, 81.5 ± 28.6, 96.1 ± 37.8, 109.7 ± 38.4 and 114.8 ± 38.1 mm^3, respectively, at post ICH-1 h, 3 h, 6 h, 24 h, and 56 h (Fig. 2). The maximum volume increase in 0.6 U group was 114.8 mm^3, while that in 0.2 U group was about 30.09 mm^3.

The 0.2 U collagenase caused MLS 0.2 ± 0.1, 0.4 ± 0.1, 0.5 ± 0.1, 0.6 ± 0.1 and 0.5 ± 0.1 mm, while the 0.6 U caused 0.5 ± 0.2, 0.8 ± 0.1, 0.8 ± 0.2, 1.0 ± 0.3 and 1.0 ± 0.3 mm, respectively, at post ICH-1 h, 3 h, 6 h, 24 h, and 56 h (Fig. 3). The 0.6 U collagenase significantly increased MLS about 1.5-3.0 times greater than the 0.2 U did at all post-ICH time points. The maximum MLS in 0.6 U group was 1.01 mm, while that in 0.2 U group was 0.60 mm.

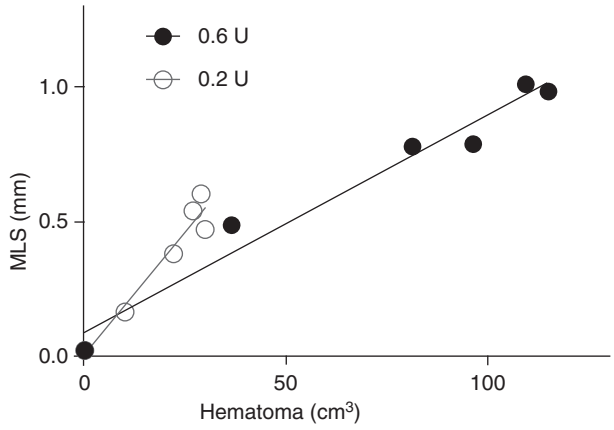

Fig. 4. Correlation between hematoma volume *vs.* MLS. Hematoma volume with MLS has high positive correlation.

Correlation of MLS with Hematoma Expansion

MLS increased dependently with hematoma expansion with high correlation coefficients, r = 0.818 ($P < 0.01$) for 0.2 U group and r = 0.879 ($P < 0.01$) for 0.6 U group (Fig. 4).

Striatal Water Content

In the pre-ICH striatum, ADC and rADC (ROI 1) were 0.70 × 10^{-3} (mm^2/sec) and 0.98 respectively. Fig. 5 shows that rADC changes in three ROIs of the hematoma side between two groups were not significantly different. At the post ICH-3 h and 6 h, the rADC in ROI 1 of both groups, however, decreased to the lowest value, indicating cytotoxic edema, and then

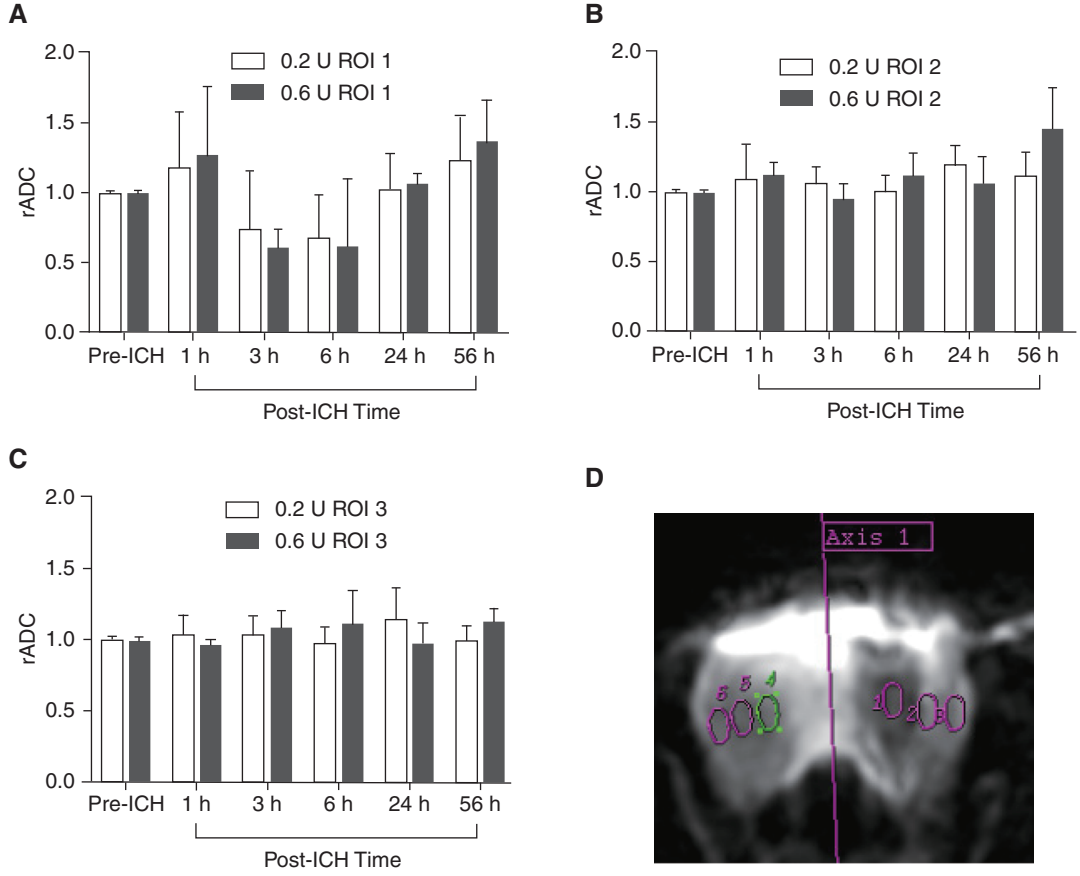

Fig. 5. Time courses of relative ADC (rADC) changes for ROIs 1, 2, and 3 in 0.2 U and 0.6 U groups. The rADC of a ROI is a ratio of ADC value of the ROI on ipsilateral side (ROIs 1, 2, and 3) to that of a corresponding mirror ROI on contralateral side (ROIs 4, 5, and 6). (A) rADC for hematoma core; (B) rADC for periphery rim; (C) rADC for outer rim. (D) A schematic drawing illustrates ROIs 1, 2, and 3 on the ipsilateral (ISH) side with their mirror ROIs 4, 5, and 6 on the contralateral (healthy) side. Values are expressed as means ± S.D. In comparison of the 0.2 U with 0.6 U groups, $P > 0.05$ is considered as non-significant by two-way ANOVA.

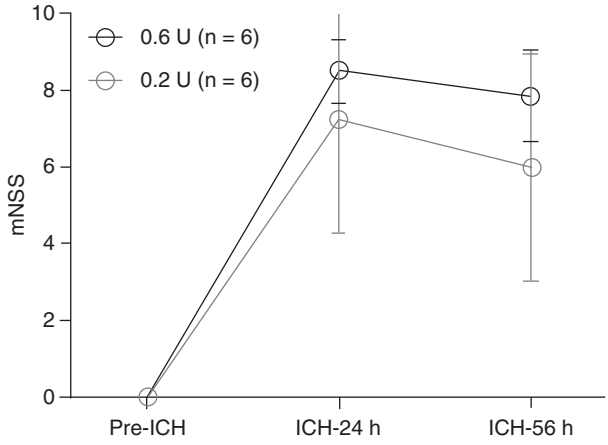

Fig. 6. The mNSS determined the neurological deficits of the rats on day 0 (pre), at post-ICH 24 and 56 h. Data are expressed as mean ± SD. $P > 0.05$ is considered as insignificantly different between 0.2 U and 0.6 U groups by Mann-Whitney U test.

returned to normal at ICH-24 h. The rADC values in ROIs 2 and 3 did not change over time. Two groups had the same pattern in rADC changes for these three ROIs (Fig. 5).

Changes in Neurologic Function and Survived Rate

Neurological impairment (deficit score) of the 0.6 U group appeared to be not significantly severer than that of 0.2 U group at post ICH 1 and 3 days ($P > 0.05$) (Fig. 6). All rats subjected to experimental ICH surgery survived until day of sacrifice (mortality = 0%). Survival rates for both groups were 100%.

Discussion

Compared with 0.2 U collagenase, the use of 0.6 U collagenase resulted in significantly larger hematoma volume and MLS (Figs. 2, 3). Increasing MLS was highly correlated with expansion of hematoma in both 0.2 U and 0.6 U groups (Fig. 4). The model generated reproducible hematoma volumes, MLS, brain edema, (Fig. 5) and consistent neurological deficits (Fig. 6). It must be emphasized that neither of the dosages was associated with mortality (both groups showed 100% survival rate). These results differed from those observed clinically and are limitations of the current ICH animal model.

Collagenase Dosage and ISH

The most commonly used collagenase dose to induce ICH in previous animal experiments was 0.2 U (12, 15). However, the resulting damage has been too mild to effectively simulate a clinically severe ICH. A suitable dosage of collagenase to induce more severe brain injuries (hematoma volume, MLS, and brain edema) with a high survival rate is important in ICH experiments. Our findings showed that hematoma volume and MLS were greater for a dosage of 0.6 U than that of 0.2 U (Fig. 3). This result is similar to that of a report by Rosenberg (1990) in which the lesion extent depended on the amount of enzyme injection (23). Rosenberg *et al*. demonstrated most of the rats injected with 0.5 U collagenase survived, whereas the rats injected with 1.0 U died of massive edema and brain herniation within 24 h. Our study using 0.6 U of collagenase to induce ISH in rats showed that all rats survived for at least 56 h. Therefore, we think that 0.6 U is a suitable dosage for inducing severe brain injuries that mimic clinically severe ISH.

Effect of Hematoma Volume and MLS on ICH Outcome

Large hematoma volume has been associated with poor outcomes and high mortality rates in patients with ICH (3). Patients with ICH with larger MLS (>10 mm) have been shown to have higher mortality rates than those with smaller MLS (<9 mm; 53% *vs*. 25%) (1). It has been reported that a MLS >12 mm at any time during the clinical course was predictive of 100% fatal outcome within 6 months (9). In the present study, we demonstrated high correlations between hematoma volume and MLS in both the groups (Fig. 4), indicating that these two parameters may be the most useful in evaluating the ISH severity, even though we have not yet demonstrated the degree of MLS that may cause mortality.

Aging and ICH

Increasing age is a risk factor for ICH in humans (8, 20, 22), and aging has been shown to exacerbate ICH-induced brain damage and neurological deficits (6, 7). However, young rats have been primarily used as experimental ICH models. Young rats may not mimic the clinical features observed in older people because young rats may have better recovery ability; therefore, use of young rat model data may result in poor treatment outcomes. To more closely mimic clinical features, investigators may either use older rats in which ICH itself exacerbates brain injuries or younger rats in which ICH injuries are aggravated by higher doses of collagenase. Hence, we used a higher dose of collagenase (0.6 U) to induce ICH in young rats to mimic the clinical features observed in older patients with ICH.

Brain Water Content and Diffusion

Brain edema is an important clinical complication of ICH. Both 0.2 and 0.6 U-induced ISH models show the same pattern in rADC, showing decreases at 1 h post-ICH and recovery at 24 h in the hematoma core (Fig. 5). In addition, the extent of cerebral edema was not significantly different between the two groups. Therefore, this parameter was not a good indicator of brain damage after ISH induced by 0.2 U and 0.6 U of collagenase.

MRI is a noninvasive method that can repeatedly detect changes in hematoma volume, brain edema, and MLS at different time points after ISH in the same rats (2, 24). In addition, the method has an advantage of reducing experimental animal numbers.

There were two limitations in our study. First, the number of rats was small, so significant differences in changes in edema formation and neurological deficits may not have been detected. Second, ICH produced in young rats may not ideally mimic ICH produced in older rats because older people are more vulnerable to ICH, and aging exacerbates ICH-induced brain damage and neurological deficits (6, 7). Therefore, increasing experimental rat number is needed in the future experiment. Therefore, future studies using larger numbers of rats are needed.

In conclusion, we established and validated ISH in rats by inducing 0.6 U of collagenase. This ISH model produced reproducibly larger hematoma volumes and MLS that closely mimicked clinically severe ISH in humans. Hematoma volume and MLS were found to be the most useful parameters for evaluating ISH severity in our experimental model. The present ISH rat model can be used to investigate the pathophysiology of ISH and evaluate new therapeutic strategies for ISH or ICH.

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Conflict of Interests

The authors declare that there are no conflicts of interests.

References

1. Becker, D.P., Miller, J.D., Ward, J.D., Greenberg, R.P., Young, H.F. and Sakalas, R. The outcome from severe head injury with early diagnosis and intensive management. *J. Neurosurg*. 47: 491-502, 1977.
2. Belayev, L., Obenaus, A., Zhao, W., Saul, I., Busto, R., Wu, C., Vigdorchik, A., Lin, B. and Ginsberg, M.D. Experimental intracerebral hematoma in the rat: Characterization by sequential magnetic resonance imaging, behavior, and histopathology. Effect of albumin therapy. *Brain Res*. 1157: 146-155, 2007.
3. Broderick, J.P., Brott, T.G., Duldner, J.E., Tomsick, T. and Huster, G. Volume of intracerebral hemorrhage. A powerful and easy-to-use predictor of 30-day mortality. *Stroke* 24: 987-993, 1993.
4. Celikbilek, A., Goksel, B.K., Zararsiz, G. and Benli, S. Spontaneous intra-cerebral hemorrhage: A retrospective study of risk factors and outcome in a Turkish population. *J. Neurosci. Rural Pract*. 4: 271-277, 2013.
5. Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M. and Chopp, M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 32: 1005-1011, 2001.
6. Gong, Y., Hua, Y., Keep, R.F., Hoff, J.T. and Xi, G. Intracerebral hemorrhage: effects of aging on brain edema and neurological deficits. *Stroke* 35: 2571-2575, 2004.
7. Gong, Y., Xi, G.H., Keep, R.F., Hoff, J.T. and Hua, Y. Aging enhances intracerebral hemorrhage-induced brain injury in rats. *Acta Neurochir. Suppl*. 95: 425-427, 2005.
8. Ikram, M.A., Wieberdink, R.G. and Koudstaal, P.J. International epidemiology of intracerebral hemorrhage. *Curr. Atheroscler. Rep*. 14: 300-306, 2012.
9. Kiphuth, I.C., Huttner, H.B., Breuer, L., Schwab, S. and Kohrmann, M. Sonographic monitoring of midline shift predicts outcome after intracerebral hemorrhage. *Cerebrovasc. Dis*. 34: 297-304, 2012.
10. Krafft, P.R., Rolland, W.B., Duris, K., Lekic, T., Campbell, A., Tang, J. and Zhang, J.H. Modeling intracerebral hemorrhage in mice: injection of autologous blood or bacterial collagenase. *J. Vis. Exp*. 67: e4289, 2012.
11. Lekic, T., Rolland, W., Hartman, R., Kamper, J., Suzuki, H., Tang, J. and Zhang, J.H. Characterization of the brain injury, neurobehavioral profiles, and histopathology in a rat model of cerebellar hemorrhage. *Exp. Neurol*. 227: 96-103, 2011.
12. Liew, H.K., Cheng, H.Y., Huang, L.C., Li, K.W., Peng, H.F., Yang, H.I., Lin, P.B., Kuo, J.S. and Pang, C.Y. Acute alcohol intoxication aggravates brain injury caused by intracerebral hemorrhage in rats. *J. Stroke Cerebrovasc. Dis*. 25: 15-25, 2016.
13. Llompart Pou, J.A., Abadal Centellas, J.M., Palmer Sans, M., Perez Barcena, J., Casares Vivas, M., Homar Ramirez, J. and Ibanez Juve, J. Monitoring midline shift by transcranial color-coded sonography in traumatic brain injury. A comparison with cranial computerized tomography. *Intensive Care Med*. 30: 1672-1675, 2004.
14. MacLellan, C.L., Auriat, A.M., McGie, S.C., Yan, R.H., Huynh, H.D., De Butte, M.F. and Colbourne, F. Gauging recovery after hemorrhagic stroke in rats: implications for cytoprotection studies. *J. Cereb. Blood Flow Metab*. 26: 1031-1042, 2006.
15. MacLellan, C.L., Silasi, G., Poon, C.C., Edmundson, C.L., Buist, R., Peeling, J. and Colbourne, F. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J. Cereb. Blood Flow Metab*. 28: 516-525, 2008.
16. Manaenko, A., Chen, H., Zhang, J.H. and Tang, J. Comparison of different preclinical models of intracerebral hemorrhage. *Acta Neurochir Suppl*. 111: 9-14, 2011.
17. Motuel, J., Biette, I., Srairi, M., Mrozek, S., Kurrek, M.M., Chaynes, P., Cognard, C., Fourcade, O. and Geeraerts, T. Assessment of brain midline shift using sonography in neurosurgical ICU patients. *Crit. Care* 18: 676, 2014.
18. Orakcioglu, B., Becker, K., Sakowitz, O.W., Herweh, C., Kohrmann, M., Huttner, H.B., Steiner, T., Unterberg, A. and Schellinger, P.D. MRI of the perihemorrhagic zone in a rat ICH model: effect of hematoma evacuation. *Neurocrit. Care* 8: 448-455, 2008.

19. Qureshi, A.I., Mendelow, A.D. and Hanley, D.F. Intracerebral haemorrhage. *Lancet* 373: 1632-1644, 2009.
20. Qureshi, A.I., Tuhrim, S., Broderick, J.P., Batjer, H.H., Hondo, H. and Hanley, D.F. Spontaneous intracerebral hemorrhage. *N. Engl. J. Med*. 344: 1450-1460, 2001.
21. Rosenberg, G.A., Estrada, E., Kelley, R.O. and Kornfeld, M. Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neurosci. Lett*. 160: 117-119, 1993.
22. Rosenberg, G.A., Grossetete, M. and Mun-Bryce, S. Chapter 15 Experimental models in intracerebral hemorrhage. In: *Handbook of Clinical Neurology*, edited by Marc, F., Stroke: Elsevier, pp. 307-324, 2008.
23. Rosenberg, G.A., Mun-Bryce, S., Wesley, M. and Kornfeld, M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke* 21: 801-807, 1990.
24. Silva-Candal, A.D., Vieites-Prado, A., Gutierrez-Fernandez, M., Rey, R.I., Argibay, B., Mirelman, D., Sobrino, T., Rodriguez-Frutos, B., Castillo, J. and Campos, F. Blood glutamate grabbing does not reduce the hematoma in an intracerebral hemorrhage model but it is a safe excitotoxic treatment modality. *J. Cereb. Blood Flow Metab*. 35: 1206-1212, 2015.
25. Suthar, N.N., Patel, K.L., Saparia, C. and Parikh, A.P. Study of clinical and radiological profile and outcome in patients of intracranial hemorrhage. *Ann. Afr. Med*. 15: 69-77, 2016.
26. Xi, G., Keep, R.F. and Hoff, J.T. Mechanisms of brain injury after intracerebral haemorrhage. *Lancet Neurol*. 5: 53-63, 2006.
27. Zhu, W., Gao, Y., Chang, C.F., Wan, J.R., Zhu, S.S. and Wang, J. Mouse models of intracerebral hemorrhage in ventricle, cortex, and hippocampus by injections of autologous blood or collagenase. *PLoS One* 9: e97423, 2014.