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ORIGINAL ARTICLE

Evaluation of an ischemic model in ischemia prone and general Mongolian gerbils by neurological symptom, injury, and sex difference

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Abstract

Background: In the previous study, we established an ischemia-prone gerbil population (IG), which was selectively bred to increase the incidence of unilateral carotid arterial occlusion (UCO)-induced ischemia in Mongolian gerbils. However, if the characteristics of ischemia model in IG are the same as those in general gerbils (GG), and if the neurological symptoms are associated with the neurological insults in IG is still unclear.

Methods: In the present study, we evaluated the UCO model in IG by analyzing neurological symptoms, neurological injury in the hippocampal CA1 region and compared with GG.

Results: The data showed that the ratios of neurological symptom scores ≥ 2 in the IG and GG groups were 65.0% vs 30.0%, respectively, and were significantly different ($P < .01$).The neuronal damage following a UCO ischemic insult in the IG group was more severe compared to the GG group. There was a high correlation between the neurological insults' scale and the neurological symptom score in the IG and GG groups ($r = .979$ and .943 in the IG and GG groups, respectively). In animals with mild neurological symptom scores (2 and 3), the neuronal insults were significantly different between female and male gerbils in both IG and GG.

Conclusion: Our findings suggest that IG population would likely be more advantageous to establish an ischemic model.

KEYWORDS

anterior communicating artery, Circle of Willis, gerbil, ischemia-prone, neurological injury, posterior communicating artery, sex difference

1 | INTRODUCTION

The absence of the posterior communicating artery (PCoA) is found in most gerbils, and the anterior anastomosis of the anterior communicating artery (ACoA) is completely absent in approximately 30%-40% of the gerbil population.[1] Consequently, ipsilateral ischemic models induced by ligation of the common carotid artery (CCA) in the gerbil neck are widely used in ischemia research.[2-4] And the contralateral hemisphere could be used as an internal control. Compared to other models such as the MCAo (middle cerebral artery

Xiao-yan Du and Chang-long Li contributed equally to this work.

occlusion model of rats)[5,6] and models in rabbits in which an intraluminal filament is inserted into the MCA or a craniectomy performed using a dental drill to expose the MCA,[7,8] the gerbil ischemic model is more convenient, because of the simpler time-saving operations involved. Operations in both the ischemia and ischemia/reperfusion models are easier to perform with less equipment. However, only 30%-40% gerbils show ischemic symptoms following unilateral carotid arterial occlusion (UCO).[9,10] The remaining animals, with their ACoAs and PCoAs either complete on both sides or on one side, cannot be successfully operated to provide UCO ischemia models. Scientists have observed increased behavioral and histological variability in UCO experiments and this has been attributed to differences in the cerebrovascular anatomy of the Mongolian gerbils provided by the largest animal supply company.[11] Therefore, it seemed important to improve the proportion of successful UCOs in the gerbil ischemic model. From 2007 to 2010, we preliminarily established an ischemia-prone population by selective breeding. In the present study, we evaluated the neurological symptoms and insults in UCO model by comparing the ischemia-prone population and general population to determine whether the neurological symptoms are associated with the neurological injury in these populations.

2 | METHODS

2.1 | Ethics statement

All of the experiments and procedures on animals were approved by the Animal Welfare Committee of Capital Medical University, Beijing, China (Permit Number: AEEI-2017-032).

2.2 | Handling of animals

We obtained 132 Mongolian gerbils (aged 2½-3 months) from the ischemia-prone gerbil population newly established in Capital Medical University (referred to as the IG group) and from the general population at the Zhejiang Center of Laboratory Animals (referred to as the GG group). All animals were housed with 4 or 5 gerbils per cage under a room temperature of 20-24°C; relative humidity, 50%-70%; 12:12 light/dark cycle, with free access to a commercial rodent diet and water.

2.3 | Incomplete global cerebral ischemic model

Animals were divided into 3 groups: the experimental groups from IG (n = 60, 30 females and 30 males), and GG (n = 60, 32 females and 28 males) and the sham-operated group (SO) (n = 20, 5 females and 5 males from both IG and GG). The female gerbils were not synchronized and were allowed to cycle naturally at the time of operation. Incomplete global cerebral ischemia was achieved by the UCO method. Briefly, the animals were anesthetized with isoflurane (induced with 3%, maintained with 2%, in 30% O_2/70% N_2O). The right CCA was exposed by a ventral midline cervical incision. The CCA was tightly wrapped with a 4/0 silk thread, blood flow blockage was confirmed, and each animal was placed in an individual cage after closing the incision with glue. The cage temperature was maintained at approximately 36.5°C using a heated blanket until the animals were awake. To evaluate the effects of surgical stress on the brain, the sham-operated group underwent the same procedures and received the same treatment as the experimental group except for the ligation of the right CCA.

Gerbils were observed after at least 2 hours of permanent ligation of the right CCA, and rated for behavior from 0 to 5, with 0 being normal and 5 indicating death, according to our previous scale, by the individuals who were blinded to the treatment group. The SO group underwent the same procedures as the experimental group except for the ligation to evaluate the effects of surgical stress on the brain. After scoring their behavior, the circle of Willis (CoW) of each animal was observed as previously described.[12]

2.4 | Histology

The brains were then removed after evaluation of CoW and stained with hematoxylin and eosin by a process similar to Laidley et al.[11] Neurons exhibiting a distinct nucleus and lack of shrinkage or eosinophilia were counted in three random pictures on a scale of 0-6, where 0 = 0%-20% neurons remaining, 1 = 21%-40% neurons remaining, 2 = 41%-60% neurons remaining, 3 = 61%-80% neurons remaining, 4 = 81%-99% neurons remaining, and 5 = normal (no pyknotic or eosinophilic cells visible). The individual(s) performing the surgery and histological/functional outcomes were blinded to the treatment group.

2.5 | Statistical analysis

All data discussed are based on the 120 post-UCO gerbils, using Pearson's chi-squared test and the correlation between the neurological insult scale and the neurological symptom score using bivariate correlation analysis (software SPSS 13.0). A P value $< .05$ was considered statistically significant.

3 | RESULTS

3.1 | Neurological evaluation

The 20 gerbils in the SO group initially decreased their movements but returned to normal activity, accessing food and water after surgery. They showed no neurological symptoms and were scored 0 on the Longa scale. These results confirmed that the operating technique had no influence on neurological symptoms. The 120 post-UCO gerbils (60 gerbils each for the IG and GG groups) showed a variety of focal cerebral ischemia symptoms and neurological scores (Table 1). The ratios of scores ≥2 in the IG and GG were 65.0% (39/60) and 30.0% (18/60), respectively, and showed a significant difference (P = .00, <.01). The results (Table 1) also indicated that, in the IG group, the percentages of female gerbils with scores ≥2 were

TABLE 1 Neurological evaluation scores according to Longa's standard: a total of 120 experimental gerbils from the ischemia-prone group (IG) and general group (GG) were evaluated after 2 h of unilateral carotid occlusion

	Longa's standard neurological scores											
	0		1		2		3		4		5	
Group	IG	GG	IG	GG	IG	GG	IG	GG	IG	GG	IG	GG
Total number	10	28	11	14	22	6	8	5	7	7	2	0
Percentages (%)	16.7	46.7	18.3	23.3	36.7	10.0	13.3	8.3	11.7	11.7	3.3	0
Female number	5	12	5	9	13	2	4	4	2	5	1	0
Percentages (%)	16.7	37.5	16.7	28.1	43.3	6.3	13.3	12.5	6.7	15.6	3.3	0
Male number	5	16	6	5	9	4	4	1	5	2	1	0
Percentages (%)	16.7	57.1	20.0	17.9	30.0	14.3	13.3	3.6	16.7	7.1	3.3	0

33.3% (20/60) of the whole IG group and 66.7% (20/30) of the total female gerbils in this group. Similarly, the percentages of male gerbils with scores ≥2 were 31.66% (19/60) of the whole IG group and 63.3% (19/30) of the total male gerbils in this group. There were no significant sex differences in outcomes between females and males for all gerbils in IG (P = .845, >.05) or within sex groups (P = .787, >.05).

In the same way, we also analyzed the symptom score in the GG group (Table 1). The percentages of female gerbils with scores ≥2 were 18.33% (11/60) of the entire GG group, and 34.4% (11/32) of the total female gerbils in the GG group. Similarly, the percentages of male gerbils with scores ≥2 were 11.7% (7/60) of the GG group and 25.0% (7/28) of the total male gerbils in this group. These data also showed no significant difference in successful models between females and males in all gerbils of the GG group (P = .306, >.05) or within sex groups (P = .429, >.05).

3.2 | Histological variation related to neurological symptom score

The hippocampus is an important part of the brain that contributes to the formation of episodic and declarative memories.[13] It is well known that the hippocampus (which is divided into three regions: CA1, CA2 and CA3) is the most vulnerable region to ischemic insults in brain and that the CA1 is the most susceptible region to transient cerebral ischemia.[14] Here we investigated the neurons in the CA1 hippocampus to elucidate the relationship between neurological lesions and the neurological symptoms. To assess the ischemic lesions, we described the total number of CA1 neurons in the rostral hippocampus (Figure 1) in both right and left hemispheres. In the CA1 of sham-operatied animals, the neurons aligned regularly and the bounding was distinct (Figure 1A). The cytoplasm stained uniformly, and the nuclei of neurons were round and

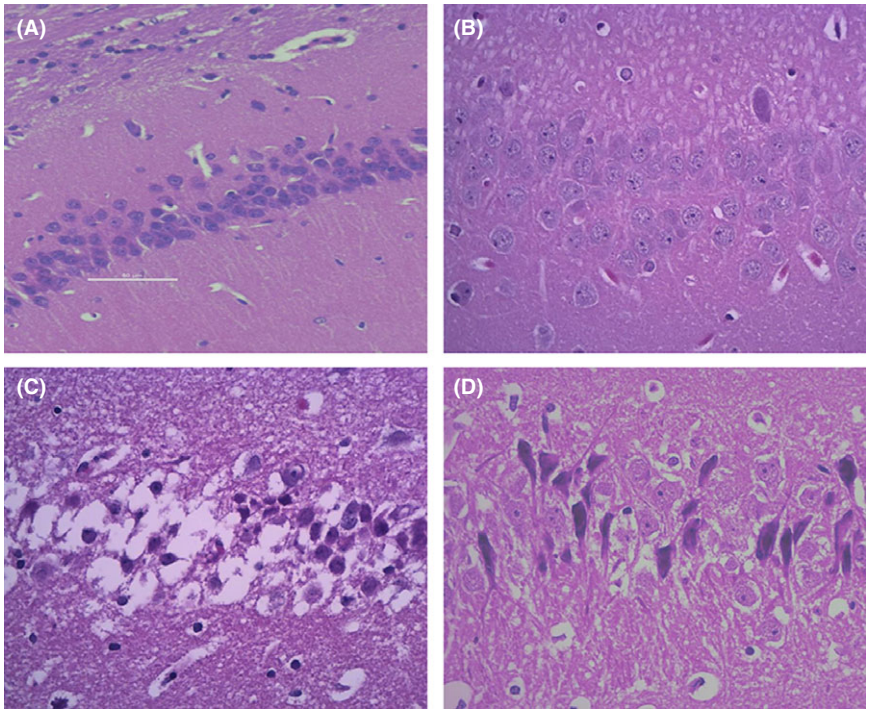

FIGURE 1 Neurons in the CA1 of the right hippocampus in a sham operation gerbil (A), and UCO gerbil with neurological scores of 0 (B), 2 (C), and 4 (D), respectively. The rating scale of neurological insults was 5, 4, 2, and 0 successively. The samples of Figure 1A, 1B, 1C, and 1D are from 4 animals that belonged to an IG (male), GG (female), IG (female), and IG (male) animal, respectively (scale bar: 60 μm)

stained light blue. The gerbils with a neurological symptom score of 0 (Figure 1B) were similar to those observed in sham-operated gerbils. The neurological insult scale of these gerbils was 4. Figure 1C shows the results of the gerbils with a neurological symptom score of 2. In the CA1 of the hippocampus, the neurons aligned in a disorderly fashion and the normal structure of the hippocampus was lost. The ischemic insults resulted in pyknotic and eosinophilic neurons, and the neurological insult scale was 2. Figure 1D shows the CA1 of the hippocampus in the gerbils with a neurological symptom score of 4. The neurons were necrotic, pyknotic and exhibited a profound loss. The neurological insult scale was 0.

The rating scale of neurological insults (0-5, with 0 being most severely injured and 5 being normal) of left hemispheres, right hemispheres and whole brains in the IG and GG groups were calculated. In both IG and GG groups, the UCO models with neurological symptoms showed a profound loss of CA1 neurons. Moreover, compared to the GG group, the cell loss in left, right, and whole brains in the IG group was more severe (the average values of the neurological insult scale were higher for each neurological symptom score). The neurological insult scale was highly correlated with the neurological symptom scores in the IG and GG groups (*P* values, .001 and .016, respectively, <.05). The lineage correlation coefficients were 0.979 and 0.943 in the IG and GG groups, respectively (Figure 2). We analyzed the average neurological symptom scores of the right hippocampus in the IG and GG groups, including the female and male animals separately (Table 2). The data showed that in the animals with high (4 and 5) and low (0 and 1) neurological symptom scores, the pathological insult rating scale values were not different between females and males. However, in the animals with mild neurological symptom scores (2 and 3), the neuronal damage was significantly different between female and male gerbils, with the value in female gerbils being significantly higher than that in male gerbils.

4 | DISCUSSION

Ischemia is the most important type of stroke in the world, and the development of a relevant animal model is important for extensive investigation of ischemic pathophysiology and molecular mechanisms and for the evaluation of potential therapies. Hence, many potential animal models have been developed, including mice, rats, and

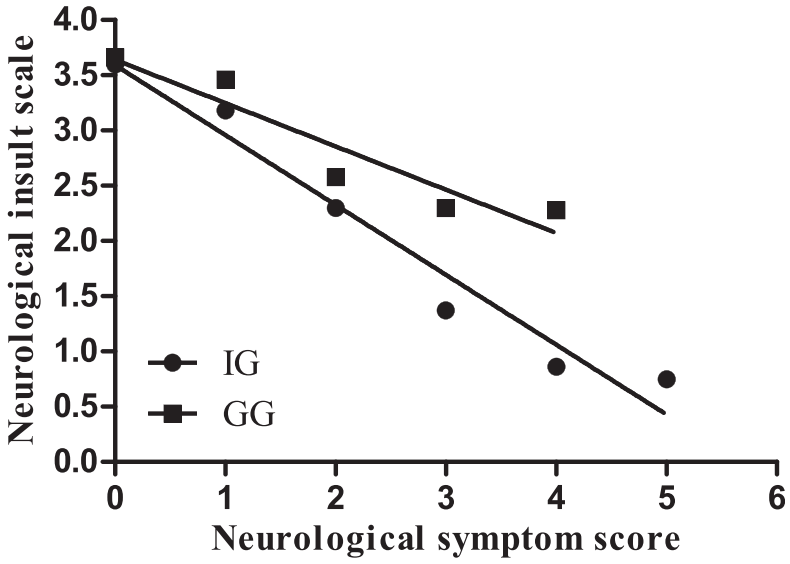

FIGURE 2 The relationship between the neurological symptom score and the neurological insult scale (average at each neurological symptom score) in the IG and GG groups. The lineage correlation coefficients were 0.979 and 0.943 in the IG and GG groups, respectively

TABLE 2 The average pathological insults score of right hippocampus in IG and GG group, including in female and male animals separately

		Longa's standard neurological scores					
	Group	0	1	2[a]	3[a]	4	5
Ave. NSS	IG GG	3.6 (36/10) 3.68 (103/28)	2.9 (32/11) 3.43(48/14)	0.955 (21/22) 1.33 (8/6)	0.75 (6/8) 1.4 (7/5)	0.14 (1/7) 0.71 (5/7)	0 (0/2) \
Ave. NSS in Female	IG GG	3.6 (18/5) 3.58 (60/16)	3 (15/5) 3.22 (29/9)	1.31 (17/13) 2.5 (5/2)	1.25 (5/4) 1.75 (7/4)	0 (0/2) 0.8 (4/5)	0 (0/1) \
Ave. NSS in Male	IG GG	3.6 (18/5) 3.75 (43/12)	2 (18/6) 3.8 (19/5)	0.44 (4/9) 0.75 (3/4)	0.25 (1/4) 0 (0/1)	0.2 (1/5) 0.5 (1/2)	0 (0/1) \

NSS, neurological insults score of right hippocampus.

[a]Significant differences between female and male gerbils. The numbers in parentheses indicate total pathological insults score and total number of gerbils in referred neurological scores group.

rabbits.[15] Despite these existing animal models of ischemia, researchers are still attempting to develop new animal models, such as injecting sodium laurate into the left internal carotid artery of rats to develop a new model of stroke, or in mice using transgenic techniques.[15,16] However, to the best of our knowledge, the UCO model of gerbils is a more convenient ischemic model involving a simple operation. Following the simple operation of UCO or bilateral carotid arterial occlusion (BCO) using silk thread or clips, an ischemic model can be established with less invasion and on a short time scale (within 5 minutes for our skillful technician). Due to the shortcomings of the general gerbil group among which only 30%-40% animals showed neurological deficits following UCO, we developed an ischemia-prone group of gerbils from 2008 to 2010. In the present study, the data indicate that, after comparison of neurological symptom scores, the incidence of UCO-induced ischemia models in the IG group is almost 2-fold greater than that in the general group. That is, to establish an ischemia model, the required number of gerbils from the IG group would be half of that from the general group.

These data confirm that a reliable ischemia-prone group of gerbils has been successfully established. In general, hormones have an important role in ischemia and exert some effects on brain ischemia. Female hormones, such as estradiol, exert a protective effect on brain ischemia or stroke.[17,18] Stages of the estrous cycle are important to ischemic outcomes in other rodents in that proestrus females showed significantly smaller infarcts than metestrus females in stroke-prone spontaneously hypertensive rats.[19,20] Thus, in most reports, male animals were chosen to study ischemia and stroke.[6,15] In our present research, both female and male gerbils were chosen and the data demonstrated that the numbers of animals with neurological symptom scores $\geq$2 were not significantly different between female and male gerbils in both the IG and GG groups. This result is not consistent with a previous report, in which the incidence of stroke-prone male gerbils was 42.9% vs 26.7% of females.[21] Because female gerbils in our study were not synchronized and were allowed to cycle naturally, the influence of estrous cycle stages in gerbil ischemia remains to be understood. We further analyzed the lesion for ischemic size by describing the residual number of neurons in the CA1 hippocampus and found that, in both IG and GG groups, the number of the dead neurons in the CA1 hippocampus was significantly higher in males than in females for gerbils with mild neurological symptom scores (2 and 3). However, the sex differences in the animals with high (4 and 5) and low (0 and 1) neurological symptom scores were not obvious. As mentioned before, because we have not synchronized the female animals, the difference in the residual number of neurons in the CA1in gerbils may result only from sex difference, not from the estrous cycle stage.

5 | CONCLUSIONS

Compared to the GG group, the IG group exhibited a higher incidence of ischemic models and more severe neuronal damage. In animals with mild neurological symptom scores, the neuronal damage was significantly different between female and male gerbils in both groups. These results suggest that selective breeding has no influence on sex-related IG symptoms. The IG population is therefore preferable to GG for establishing ischemic models.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All listed authors meet the requirements for authorship. XYD and CLL took part in the UCO model work, performed the pathological experiment, and wrote the original draft of the manuscript. MG and YW undertook the UCO model work. HGG and FWD analyzed the date. XYS and ZWC designed this study and provided comments. ZWC acts as guarantor. All authors read and approved the final manuscript.

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