LETTER TO THE EDITOR

The Outcomes of Stroke Induced by Middle Cerebral Artery Occlusion in Different Strains of Mice

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Stroke is the second most common cause of death and ischemic stroke is the largest subtype [1–4]. To investigate the mechanism of cerebral ischemic injury as well as to develop effective therapies to this disease, the use of ischemic stroke animal models is very important. Rodents, especially mice, are widely used for this purpose [5]. Clinically, the occlusion of middle cerebral artery is most of the cases in ischemic stroke [6]. Therefore, middle cerebral artery occlusion (MCAO) is a representative model for ischemic stroke. It is well known that there are many factors exert important effects on the outcome in the MCAO stroke model, including the age and sex of the animal used [6]. Here, we examined the outcomes of MCAO in the different strains of mice.

Four different strains of mice (25 ± 2 g in each group) were used in this study. Male ICR mice ($n = 33$), KM mice ($n = 25$), BALB/c mice ($n = 31$), and C57BL/6 mice ($n = 29$) were purchased from Sino-British SIPPR/BK Lab Animals (Shanghai, China). All animals were used in accordance with the guidelines of Second Military Medical University for Animal Care. The animals were anesthetized with chloral hydrate (300 mg/kg) and remained anesthetized during the following operation. Then, the mice were subjected to MCAO as previously described [7] without reperfusion. After 24 h, neurological deficits were examined using a 5-point scale [7]. Mice were then sacrificed. Brain slices were

prepared and stained with 1% 2, 3, 5-triphenyltetrazolium chloride (TTC, Sinopharm Chemical Reagent Co. Ltd. Shanghai, China) [8]. The infarction size was measured using an image analyzer (Image J 1.41; NIH, Bethesda, MD, USA). Data are expressed as mean $\pm$ SD and analyzed with one-way analysis of variance (ANOVA) followed by Dunnett's t-test for pairwise comparison.

During the following 24 h, 6, 6, 25, and 4 mice died in ICR, KM, BALB/c, and C57BL/6, respectively. The mortality is 18%, 24%, 81%, and 14%, respectively (Table 1). Then, all these brains of dead mice were removed, and signs of hemorrhage, edema or infarction were examined. The data showed that no animals died because of anesthetic or surgical accident. Edema or infarction but

Table 1 The infarction rate and mortality of operation in different strains of mice with MCAO

	ICR	KM	BALB/c	C57BL/6
Number of mice with MCAO	33	25	31	29
The death of the animals	6	6	25	4
Mortality, %	18	24	81	14
Mice without infarction	7	0	0	18
Failure rate, %	21	0	0	62

Figure 1 The disparity in the infarct size and neuralgic function in 4 mice strains with MCAO. The left panel: representative coronal brain sections from 4 strains of mice (ICR, KM, BALB/c, and C57BL/6). Sections were stained with 2-3-5-triphenyltetrazolium chloride (TTC). Data are expressed as mean $\pm$ SD and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. * $P < 0.05$, ** $P < 0.01$ versus ICR; # $P < 0.05$, ## $P < 0.01$ versus KM; $P < 0.05$, $P < 0.01$ versus BALB/c.

not hemorrhage existed in the brain, which suggested ischemic injury might be the main death reason. There are also 7 mice in ICR and 18 in C57BL/6 that failed to be induced cerebral infarction. The infarct size is $54.4 \pm 14.8\%$, $38.8 \pm 13.9\%$, $54.4 \pm 14.8\%$, $12.9 \pm 1.2\%$ in ICR, KM, BALB/c, and C57BL/6, respectively (shown in Figure 1). The infarct size is the smallest in C57BL/6 mice compared with the other 3 strains ($P < 0.01$). The infarct size in KM mice is smaller than ICR or BALB/c mice ($P < 0.05$). The neurological deficit score is significantly higher in BALB/c mice than all the other 3 strain mice, and the neurological function has no difference in ICR, KM, and C57BL/6 mice ($P > 0.05$).

Our results show that individual susceptibility to cerebral ischemic injury and neurological functions induced by MCAO vary greatly among these different strains of mice. BALB/c mice are

susceptible and sick to the ischemic injury induced by MCAO, while C57BL/6 mice are insensitive to MCAO. We recommend the ICR and KM strains but not BALB/c or C57BL/6 mice for the application of ischemic cerebral injury study in mice with MCAO.

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Conflict of Interest

The authors declare no conflict of interest.

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