

Models of Graded Closed Craniocerebral Injury in Rats

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Abstract

To build closed traumatic brain injury models in varying degrees with high accuracy, repeatability as well as easy operation, We use lateral fluid percussion method to simulate the rats' three types of closed traumatic brain injury models in slight, moderate and serious degrees after 24 hours and evaluate the situations of neurological deficit of the rats in each group through NSS, then conduct quantitative measurement of brain water content as well as blood brain barrier at the same time, finding that the more serious the rat's traumatic brain injury degree is, the higher NSS scores shows, and the more serious damage brain water content as well as blood brain barrier possess, the differences of which have statistical significance ($P < 0.05$). So we have built a traumatic brain injury model in varying degrees with high stability and easy operation, which lays the foundation for the preliminary study of traumatic brain injury to a certain extent.

Keywords—Trauma brain injury, Model Brain water content, Blood brain barrier, Lateral fluid percussion,

I. INTRODUCTION

Traumatic brain injury (TBI) is becoming more and more common among young people in the world. In the United States, the morbidity of TBI stands first on the list of all traumatism, and takes up 9%-12% of the traumatism on all parts of the body[1]-[2]. In recent years, with the TBI taken seriously by the governments and individuals, the treatment and prognosis of TBI have been greatly improved. However, some potential pathophysiology problems of TBI are still sort of unclear[2]. This study firstly builds a gradable closed traumatic brain

injury model with high accuracy and repeatability, which is also easy to observe and able to reflect the features of human brain's injury. It provides the preliminary basis for the follow-up pathophysiology study of traumatic brain injury.

II. MATERIAL AND METHODS

A. Models of craniocerebral injury

The experiment animals are bought in Shanghai Experimental Animal Center and the laboratory animal center of Wenzhou Medical College. And all the experimental operations and feeding are carried out in terms of experimental animal feeding management regulations of Wenzhou Medical College. Take one hundred healthy male SD rats (250-300g), and randomly divide them into blank group with 20 rats, sham operation group with 20 rats and operation group with 60 rats. Furthermore, operation group is sub-divided into slight TBI group, moderate TBI group and Serious TBI group. Adopt lateral fluid percussion method method[3] in operation group. Inject 10% chloral hydrate (0.3ml/100g of weight) into abdominal cavities of the rats in operation group to carry out anesthesia. Fix its head in the supine position on the brain stereotactic instrument. Then after the disinfection of preoperative skin preparation, use dental drill to conduct one-side parietal lobe craniotomy under aseptic conditions (near midline by 3.5mm, behind bregma by 2.5mm, small bone window with a diameter of 5mm, epidural intact). Next, connect hydraulic striking apparatus (Fig 1). Choose different pressure to strike the rats through adjusting different striking force (2.10-3.10atm, 3.10-4.10atm, 4.10-5.00atm), so as to make slight, moderate and serious TBI. Finally, suture the skin of brain. As for

sham operation group, just conduct craniotomy. In addition, do no treatment to blank control group. After the operations, put all the rats into the cage and feed them, moreover, keep the room's temperature at 37 ± 0.5 °C.

B. Neurological Severity Score (NSS)

Conduct NSS analysis to the rats in each group 24 hours later after the experiment. By lifting the tail of the rat, observe whether the forelegs on both sides are bent, the height that its head rise, sensory testing, reflection and abnormal activities, as well as scores of multiple indexes in beam balance test (the grand total is 18 scores). Reward one score if it can't complete one item among them or any one of them is missing. So, 1-6 scores are thought to be slight neurological function impairment, 7-12 scores are moderate neurological function impairment, and 13-18 scores are serious neurological function impairment.

C. Contents of Brain Water

Take 15 rats in each group to conduct brain water content measurement. Dry-wet weight method is adopted in this experiment. After taking the brain out within 30 seconds, suck up the blood on the surface of the brain with a filter paper. Then separate about 100mg of the left parietal cortex by sharp dissection. After obtaining wet weight with electronic analytical balance, put it into 110°C constant temperature drying oven to dry for 24 hours until with constant weight (the difference between the two weight should be less than 0.2mg). Calculate the percentage of brain water content in each part after obtaining dry weight: brain water content (%) = $(\text{wet weight} - \text{dry weight}) / \text{wet weight} \times 100$.

D. Blood Brain Barrier (BBB)

Take 15 rats in each group to conduct quantitative measurement of blood brain barrier. Inject 2% Evans blue to blank control group and sham operation group respectively (3ml/kg for each rat), and inject the same amount of Evans blue (EB) to damage group one hour before injury. Put all the rats to death after 24 hours. And get the injured lateral cortex and weigh respectively. Add double

volume of dimethylformamide, incubate in 50°C water for 72 hours, carry out 1500g centrifuge for 10 minutes and get the upper clear liquid. By using spectrophotometer, detect the absorbance where Evans blue has maximum absorption spectrum of 635nm. In the end, query the content of Evans blue on the standard curve

E. Statistical Analysis

Measurement data is showed as mean \pm standard deviation ($x \pm s$). If the data shows as a normal distribution and homogeneity of variance, ANOVA will be used for comparison of means among several groups. And LSD-t test is used to make comparisons of the means between two groups. If the data shows as an abnormal distribution or heterogeneity of variance, Kruskal-Wallis H rank test will be use to compare among several samples, and Mann-Whitney U rank test for comparison between two groups. All the results are thought to be statistically significant if they meet the condition that $P < 0.05$.

III. RESULTS

No rats die in blank control group or sham operation group during the experiment, while six rats died in the operation group. Rats with brain injury of different degrees have varying degrees of bleeding in cortex, subarachnoid space and sub-cortical white matter white after 24h. The rats' parts of brain tissue in laceration focal also rupture in operation group. As for rats with moderate and serious TBI, they are more likely to have splinter hemorrhage, form obvious edema zone around lesion focal. It is clear to see that most of astrocyte are markedly swollen in the edema zone, some nucleus neurons are missing, the vessel are deformed under pressure with rupture and bleeding. However, normal brain cortex should be shown in pink. In addition, the NSS scores of all rats in operation group are significantly higher than those in blank control group and sham operation group 24 hours later. Moreover, with the increase of the TBI degree, the scores are higher and higher as well ($P < 0.05$), and symptoms of neurological deficit are more and more serious (Table 1). At last, the brain water

content and BBB of rats in operation group are more and more obvious(Table 2).

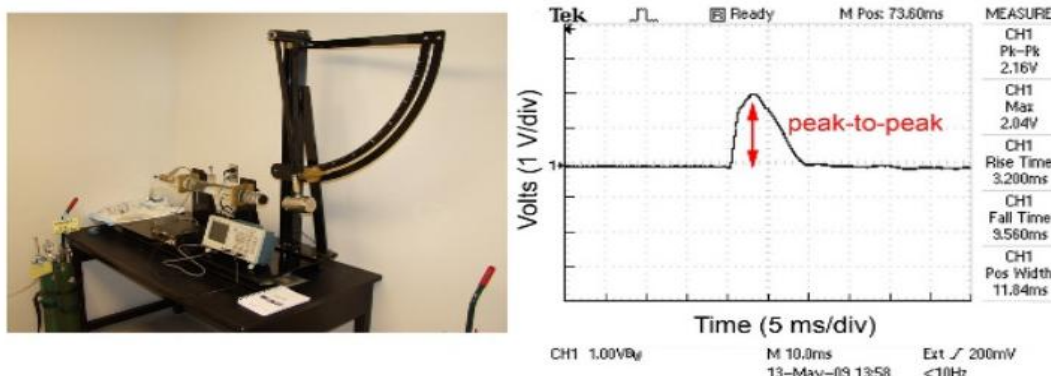


Fig 1 Hydraulic Blow Apparatus

Table 1 NSS Score Among Different Groups

Group	Surgery			Sham-operated	Control
	Mild-TBI	Moderate-TBI	Heavy-TBI		
NSS	3.30 ± 1.16	9.60 ± 1.43	14.40 ± 1.43	1.42 ± 0.47	1.20 ± 0.42

Table 2 Contents of Brain Water and Eb Among Each Group

Group	Surgery			Sham-operated	Control
	Mild-TBI	Moderate-TBI	Heavy-TBI		
Water Content (%)	32.480 ± 0.232	32.594 ± 0.197	32.768 ± 0.136	31.997 ± 0.167	31.858 ± 0.180
EB (ug/g)	84.06 ± 3.03	195.67 ± 4.08	243.22 ± 10.98	5.98 ± 0.15	4.38 ± 0.17

IV. CONCLUSIONS

Firstly, this study uses lateral fluid percussion method to clinically simulate traumatic brain injury animal models in varying degrees. At present, there are three main methods to simulate clinical closed TBI models, which are free falling, frostbiting and fluid percussion[4]. The basic requirements of TBI models built in this study include high repeatability, sensitivity and are similar to clinical TBI mechanism. The lateral fluid percussion method (LFP) that we use has already widely become animal model method to simulate TBI[5]-[6]. It mainly has the following advantages:

firstly, it can simulate neuropathology and the behavior results after the injury have access to TBI from slight to serious degrees, and the striking force can be graded quantifiably; secondly, the operation has high sensitivity, which won't cause TBI for a second time, and the injury mechanism is most similar to TBI in clinical practice. However, we still should note that during the process of experiment, we must guarantee the integrity of rats' dura after striking and improve the stability of the models. Futhermore, when conducting craniectomy, if there exist remaining skeletal in the rats' joint, or the bone wax is hard to take out, both of them will cause

impact on the experimental results. At last, we should adequately consider the rats' recovering time of reflex and the death rates, or we need to adjust the striking force in time. During the process of this experiment, the rats in operation group all recover reflex two hours after the operation, and a total of six rats die during the process of building model, of which two rats are in slight TBI group, one in moderate TBI group and three in serious TBI group, so the death rate is 6/100. While no rats die in blank control group during the experimental process, the striking forces are all controlled within the scope of operation (the striking forces in operation group are respectively 2.10-3.10atm for slight TBI group, 3.10-4.10atm for moderate TBI group, and 4.10-5.00atm for serious TBI group). Through analyzing NSS scores of animal models 24 hours after the operation, we will find that the scores of rats in operation group are all higher than those in blank control group and sham operation group, which the differences are statistically significant ($P < 0.05$). Moreover, they all conform to the standards of TBI models in varying degrees. In order to ensure the dependability of the experimental data, we also observe the pathological changes of rats' brain tissue after injuries. The rats' parts of brain tissue in laceration focal rupture in operation group. As for rats with moderate and serious TBI, they are more likely to have splinter hemorrhage, form obvious edema zone around lesion focal. It is clear to see that most of astrocytes are markedly swollen in the edema zone, some nucleus neurons are missing and the vessel are deformed under pressure with rupture and bleeding. The brain cortex in blank control group and sham operation group are normal. In this experiment, through observing brain water content and quantitatively measure BBB, we find that the increase of rats' brain water contents are obviously different among three TBI models, and they are in line with the degrees of BBB, which illustrates that the establishment of slight, moderate and serious TBI models are successful and stable. In conclusion, through LFP method in this experiment, we firstly build stable and reliable closed traumatic brain injury

models of rats in varying degrees, which provides important guarantees and foundations for the follow-up experiment.

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