Experimental Animal Models of Neurogenic Bladder Dysfunction

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Neurogenic bladder is related to various types of neurogenic disease and injury, including cerebrovascular accident, brain tumor, spinal cord injury, and Parkinson’s disease. The results of urodynamic study show different types of detrusor and sphincter function. According to these urodynamic results, the physician decides on a treatment plan, such as anticholinergics or alpha-blockers. In the development of a new medication, it is necessary to test the medication’s efficacy and toxicity by using a laboratory animal. The proper laboratory animal should have several characteristics. These are biological similarity to humans, a short generation period, and an environment that is easy to control. We describe the development of laboratory animals for the study of neurogenic bladder by decerebration, stroke, and spinal cord injury.

Key Worlds: Laboratory animal; Neurogenic bladder; Procedure

Introduction

Laboratory animals, including mice, rats, hamsters, guinea pigs, rabbits, pigs, dogs, cats, and primates, are used for animal testing for study, examination, and education in life science and medicine [1,2]. Through such laboratory animals, we can study the metabolism and stability of new drugs and gain valuable data relevant to the cause and treatment of disease. Rodents, such as mice and rats, make up 90% of laboratory animals. They are similar to humans biologically and have a shortened generation period and easy-to-control environmental factors, which can influence experimental study [3]. Thus, to study neurologic bladder, we chose the mouse and rat.

Neurologic bladder is caused by stroke, brain tumor, Parkinson’s disease, spinal cord injury, herniation of inter-vertebral discs, diabetes mellitus, and pelvic surgery. Such diseases affect the coordination of muscle, such as the inner and outer sphincter, which causes voiding dysfunction [4]. Voiding dysfunction is sorted to storage dysfunction and elimination dysfunction, each of which has a particular treatment. Voiding dysfunction patients with neurologic bladder are treated by drugs, surgery, or conservative management, which restores bladder function [5,6].

Medical therapies for neurologic bladder include anti-cholinergics, tri-cyclic antidepressants, and calcium channel blockers, and multiple agents and drugs are currently under development. The effects and side effects of developed drugs are confirmed through animal experiments before clinical trials. To study neurologic bladder caused by central nervous system damage, laboratory animals are developed for applied decerebration, cerebral infarction, and spinal cord injury. In this article, we describe the methods of decerebration, cerebral infarction, and spinal cord injury.
Materials and Methods

A literature search of the PubMed database for all articles related to laboratory animals was performed. Key words used in our search included "laboratory animal," "decerebration," "cerebral infarction," and "spinal cord injury."

1. Development of a decerebrated animal model

Sherrington attempted surgical decerebration by ligation of both carotid arteries of dog, cat, and monkey for neurological study [7]. Mechanical decerebration results in massive bleeding, shock, and damage to the midbrain and brainstem, however. To address these cons, Pollock developed a less invasive method in the cat [8]. Because the mouse is the preferred laboratory animal, a method for decerebration of the mouse was developed [9]. Decerebration in mice is more difficult than in other laboratory animals, however, and even a little bleeding may lead to death.

In Yoshiyama’s method, the internal and external carotid arteries are ligated after halothane anesthesia [10]. The forebrain is then removed at the mid-collicular level and the posterior circulating artery is ligated. Cotton was filled up the space of the removed forebrain and then covered it with agar [11]. An 18-gauge needle is placed to the occipital ridge of the skull with its end point placed to the L6 level of the spine. After all preparation is complete, the decerebration status of the mouse is checked through 3 tests (head-up tilt, carotid occlusion, and sodium cyanide response) [11]. Because almost all decerebrated mice die within a few hours, researchers must immediately progress to the next study when the prior experiment is done. Mechanical decerebration has many problems, including a low success rate, the long time needed, and death caused by damage to the brainstem.

New methods that block cerebral blood flow are being developed to address such weaknesses of mechanical decerebration [12,13]. Decerebration by embolus blocks the circle of Willis and cause-sinterception of cerebral blood flow. Also, the embolus blocks the posterior communication artery originating from the basilar artery (Figure 1). Thrombi, latex, and polyvinyl acetate have been used as an embolus. Fouad et al reported using polyvinylsiloxane [12]. After haloxane anesthesia, steroids or mannitol is injected to reduce brainstem edema. The carotid artery is then dissected, and a little polyvinylsiloxane is injected. After the mouse awakens from the anesthesia, rigidity is checked. When the rigidity of the mouse is confirmed, the experiment is complete. Fouad et al reported that polyvinylsiloxane is a good embolus material. Because it is mucinous, it does not mix with the blood and is not transmitted into the capillary beds [12].

2. Development of a laboratory animal with cerebral infarction

As a laboratory animal model of cerebral infarction, the mouse is most similar to humans pathophysiology. The cerebral infarction mouse model caused by thrombus is appropriate for studying the effect of thrombus treatment [14]. Several methods have been used to produce a thrombus in the cerebral vessel in this animal model these include photochemical middle cerebral artery (MCA) occlusion, surgical occlusion, occlusion by electrocautery, blockage by thrombus, and occlusion using nylon [15-19]. Cerebral infarction by thrombus proceeds in two ways. The first is that many pieces of the thrombus result in a broad blockage that includes the frontal lobe, parietal lobe, and occipital lobe. The other way is that one small piece or nylon piece produces MCA occlusion only. To generate voiding dysfunction, most investigators choose MCA occlusion. Using thrombin and a mixture of thrombi, both carotid arteries are blocked and blood perfusion is checked by measuring cerebral blood flow over 2 hours [20].

Krueger reported a method for making a cerebral infarction mouse [18]. After halothane anesthesia, a PE-50 polyethylene tube is inserted into the femoral artery. Phosphate-buffered saline (PBS), 1 ml of a thrombin/PBS mixture, and 5 ml of an albumin/PBS mixture are then prepared and frozen. Thrombin (0.15 ml) and blood (0.6 ml) from the femoral artery are mixed and then kept at room temperature for 40 min. In a Petri dish, the needle shaped thrombus and remnant
thrombus are removed and washed with normal saline. Under a microscope, the white colored thrombus including fibrin is chosen and cut to 1.5 mm in size and put in the albumin/PBS mixture. The prepared thrombi are then ready for input (Figure 2). An incision is made in the mouse neck and the muscles near the neck are dissected. When the common carotid artery is exposed, the external carotid artery is dissected and the tube is inserted in the bifurcated portion of the common carotid artery. The inserted tube is fixed to the vessel, the common carotid artery is dissected, and an occluding device is prepared that prevents retrograde of the thrombus. The thrombi are put in the tube, the occluding device is blocked, the thrombus is slowly inserted into the MCA through the tube, and the occluding device is opened. However, it is difficult to place the tube. The tube, which is inserted into the external carotid artery, is the same diameter as the external carotid artery. If the angle of the internal carotid artery is larger than that of the external carotid artery, penetration of the thrombus is difficult. Because it is difficult to control the size and preparation of embolus materials using a thrombus, other studies have recently used nylon.

Kuge used 4-0 nylon to develop a cerebral infarction model to block the MCA [19]. A tube is inserted into the femoral artery to check blood pressure and blood sugar. The mouse is prepared as mentioned above, and then the right pterygopalatine branch is tied up and a 3-mm sized 4-0 nylon is placed 17 mm superior to the right common carotid artery bifurcation point. After complete infarction, the tube inserted in the femoral artery is removed. After 20 hours, nylon is inserted in the complete cerebral infarction mouse to
carry out the experiment. To check the region and degree of infarction, the size of infarction and cerebral blood flow are estimated after 24 hours [21,22]. The composition of thrombi in infarcted brain is confirmed by H-E staining and Ladewig staining [18]. The brain is cut into 1-2 mm sections by use of a tissue chopper and then stained with 2,3,5-phenyltetrazolium chloride (TTC) and its size measured [23-25]. The measured size is calculated by Swanson’s algorithm [26].

3. Development of spinal cord damaged laboratory animals

Since the development of Allen’s weight-drop method to make spinal cord damaged laboratory animals, this method has been recognized as a general method for making such laboratory animals [27-29]. The method has been applied to several animals, from monkeys, sheep, and dogs to mice [30-33]. Recently, interest has grown in the mouse as a spinal cord damaged laboratory animal. The method developed using the mouse is widely used [34,35]. Mice are relatively cheap, are easy to control, and it is simple to make gene transformations in the mouse.

The mechanism of spinal cord injury is dislocation or fracture of the spine. The spine and tissue are damaged by fast impact. The weight-drop method is similar to the general mechanism of spine injury [36]. However, the weight-drop method doesn’t provide detailed clinical results. Sometimes, the damaged spinal cord partially recovers therefore, it is not a perfect animal model. Bregman developed an animal model using a method of complete excision of the spinal cord [37]. After haloxane anesthesia, a laminectomy is performed at the T8-T9 level in an 8-week-old mouse. Then the spinal cord is cut completely at the same level with iridectomy scissors. The muscle and skin are sutured. Antibiotics, such as ampicillin, are administered for 3 days. After 3 weeks, further experiments can be carried out.

The classical injury methods do not provide the precision associated with the injury, especially in small animal such as mice. To control the impact precisely, several methods are now being developed. Seki reported using a pneumatic impact device to induce spinal cord damage [28]. After anesthesia is administered to 5-8-week-old mice, the skin and muscle are dissected at the T9-T13 level. The T10 level muscle is removed, and a laminectomy is performed. The pneumatic impact device is placed on the opened spinal cord. The pneumatic impact device is connected to a compressor, and the degree of shock and pressure can be calculated in real time. In the study by Seki, the mice were sorted into 3 experimental groups for which the impact velocity of the device was 1 m/s, 2 m/s, and 3 m/s, respectively. The tissue of the damaged region of the spinal cord was examined under a microscope. The damaged region of the spinal cord in each group was 0.86±0.06/100 mm³, 2.4±0.28/100 mm³, and 11.0±1.0/100 mm³, respectively. In Korea, Yeo reported similar results using mouse [29].

After the spinal cord is damaged, immediate forced voiding of the laboratory animals during the spinal shock period is performed. In most cases, twice daily voiding is sustained for 1 week. After this period, we perform a cystometrogram in the spinal cord damaged mouse [38,39]. A permanent catheter is needed for monitoring of continuous urodynamic tests. Thomas reported using a 7 Fr dual-lumen portacatheter or 2 mm polyethylene catheter as a permanent catheter. The catheter was inserted by the inner bladder through the subcutaneous and abdominal muscles [40]. The catheter is placed on the superior portion of the bladder to measure bladder inner pressure. Thus, it helps to smooth the release of urine and minimizes bladder stimulation [41,42]. Antibiotics must be administered to prevent infection. After 2 hours of catheterization, cystometrogram is performed.

Generally, a cystometrogram is recorded 2 hours after a test drug is administrated. The items to be inspected are the interval pressure to induced micturition, maximal voiding pressure, and inter-contraction intervals [10]. These data are used to estimate drug effect and status.

Conclusions

The development of laboratory animals is required to study neurologic bladder and to estab-
lish the effect of new drugs. Several methods have been used to make animal models, such as decerebration, cerebral infarction, and spinal cord injury. The ultimate purpose of the development of a laboratory animal is to make the desired lesion in an accurate region. The methods have to be easy and cheap. Thus, the development of a laboratory animal is based on an understanding of such methods.

References

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