EXPERIMENTAL MODEL OF SPINAL CORD INJURY (SCI) IN RATS: MANAGEMENT GUIDELINES

MODELO EXPERIMENTAL DE LESÃO MEDULAR (SCI) EM RATOS: DIRETRIZES DE MANEJO

MODELO EXPERIMENTAL DE LESIÓN DE MÉDULA ESPINAL (SCI) EN RATAS: GUÍAS DE MANEJO

Asdrubal Falavigna[1], Fernanda Cechetti[2], Guilherme Finger[3], Leonardo Gilmone Ruschel[3], Grasiela Marcon[3], Pedro Guarise da Silva[3]

ABSTRACT

Surgical experiments with laboratory animals are necessary for medical research. These studies aim to clarify the mechanism of disease, investigate the action and efficacy of new drugs or biological markers, as well as develop and enhance new therapies and apply new techniques. Regarding the models of spinal cord injury (SCI), there are several different methods that address the handling of the animals, especially concerning the use of analgesics, antibiotics and pre- and postoperative management. The lack of uniformity and standardization among the studies does not allow the understanding of the model of SCI or the proper handling of the paraplegic animals, hampering the adequate interpretation and comparison of results. The goal of this study is to establish a standard protocol on the handling of animals subjected to experimental models of SCI.

Keywords: Models, Animal; Spinal cord injuries; Paraplegia; Biomedical research.

RESUMO

Experimentações cirúrgicas em nível laboratorial com o uso de animais são necessárias para o desenvolvimento da pesquisa médica. Estes estudos têm o objetivo de identificar o mecanismo das doenças, pesquisar a ação e eficácia de novos medicamentos ou marcadores biológicos, além de desenvolver e aprimorar novas terapêuticas. Em relação aos modelos experimentais relacionados à lesão raquimedular, há diversas metodologias descritas sobres o manejo desses animais, especialmente em relação ao uso de analgésicos, antibióticos e manejo pré e pós operatórios. Essa variedade metodológica resulta na falta de uniformidade e padronização entre os estudos, prejudicando a interpretação adequada e a comparação entre os resultados. Diante deste cenário, este estudo tem objetivo de estabelecer um protocolo padrão sobre o manejo dos ratos submetidos a modelos experimentais de trauma raquimedular.

Descritores: Modelos animais; Traumatismos da medula espinal; Paraplegia; Pesquisa biomédica.

RESUMEN

La realización de experimentos quirúrgicos con animales de laboratorio son necesarios para la investigación médica. Estos estudios tienen por objeto aclarar el mecanismo de las enfermedades, investigar la acción de nuevos medicamentos y marcadores biológicos, así como desarrollar y mejorar nuevas terapias y aplicar nuevas técnicas. En cuanto a los modelos animales de lesión de la médula espinal (SCI), existen varios métodos diferentes que abordan el cuidado de estos animales, especialmente en relación con el uso de analgésicos, antibióticos y manejo pre y post operatorio. La falta de uniformidad y estandarización entre los estudios no permite la comprensión del modelo de SCI o el manejo adecuado del animal parapléjico, lo que dificulta la interpretación y comparación adecuada de los resultados. El objetivo de este estudio es establece un protocolo estándar de manejo de animales sometidos a modelos experimentales de SCI.

Descriptores: Modelos animales; Traumatismos de la médula espinal; Paraplejía; Investigación biomédica.

INTRODUCTION

Since the time of Aristotle and other philosophers, it has been sought to establish the bests methods to manage animals during experiments, with as little suffering as possible, while maintaining an adequate experimental disease model[1,2]. In Brazil, the importance of using animals to benefit science is well known but, in order to protect the animals, their mistreatment has already been defined by law. The monotony and slowness of the court system led universities, research institutions and other organizations to set up special ethics committees to perform an advance review of procedures involving animals. They have the power to stop studies before they begin[3,4].

Surgical experiments with laboratory animals are essential for medical research. The main goals of these research studies are to clarify the mechanism of diseases, perform research with new pharmaceuticals and biological markers, and to apply new techniques[3]. However, it is important that projects involving animals only begin after considering their value to science and weighing the potential effects on the welfare of these animals[5].

Most of the research performed nowadays is developed with small animals[3]. Rats are most used in experimental research because they are easily handled, a large number can be housed in a relatively small area and their physiology, anatomy, genetics and behavior allow meaningful results, which, if interpreted with care, can be extrapolated to humans[6].

The objective is to establish a management protocol of care for animals undergoing spinal cord injury (SCI) experimental models, due to the lack of standards for the daily management of animals undergoing experimental models of SCI.

1. Professor of Neurosurgery, Dean of the Medical School of the University of Caxias do Sul, Caxias do Sul, RS, Brazil.
2. Professor of Physioterapy, University of Caxias do Sul - Caxias do Sul, RS, Brazil. Doctorate in Neuroscience, UFRGS, RS, Brazil
3. Medical Student, University of Caxias do Sul, Caxias do Sul, RS, Brazil.

This project was developed in the University of Caxias do Sul, Caxias do Sul, RS, Brazil.
Correspondence: Rua General Arcy da Rocha Nóbrega, 401/602 - Caxias do Sul, RS, Brazil. CEP: 95040-290. asdrubalmd@gmail.com

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BASIC CARE

It is recommended that laboratory animal care should follow the guidelines of animal practices, such as the "Guiding Principles in the Care and Use of Animals for Scientific Purposes"[5]. All housing facilities should be appropriately staffed, designed, constructed, equipped and maintained to achieve a high standard of animal care. The animals should be housed in special cages to ensure comfort and well-being[5].

The number of animals per cage will depend on the time when the research is performed. During the preoperative period, it is recommended to avoid social isolation, however, the week after surgery, the animals should be placed in individual cages to avoid cannibalism[5,7-9]. The cages should be placed on a shelf with temperature control and allocated to a room with a dark-light cycle every 12 hours, respecting the animal biology[7,10,11]. The temperature and humidity should be maintained between 18-26 Celsius degrees and 30-70%, respectively[5].

The animals should receive appropriate and uncontaminated chow, respecting the nutritional requirements for each species, and sufficient to maintain the normal growth of immature animals[7,9]. The animals should be kept constantly hydrated with clean, fresh and uncontaminated water[5].

PREOPERATIVE MANAGEMENT

Many of the anesthetic complications can be minimized or avoided in the healthy animal with no subclinical diseases. All animals must come from a specialized, reliable rat breeding source used in scientific research. This source should be appropriate to allow normal growth and development of the animal with prevention of infectious diseases. It is very important to provide a period of acclimatization, usually between 1 or 2 weeks before the surgical procedure. During this period, a clinical examinations to evaluate possible diseases before the initiation of the experiment is performed and the constant feeding allows the rats to gain weight[12]. Surgery is not recommended in rats before the age of 14 weeks or at a weight of less than 300g.

ANTISEPSIS OF THE SURGICAL PROCEDURE

The purpose of antiseptic techniques is to reduce the infection rates and maintain the normal physiology, although it is known that rats used for laboratory experiments are less susceptible to infections than other animals. There is evidence that using antisepsis techniques during surgical manipulation reduces the rates of infection and postoperative complications, and this is indispensable in research involving animals in order to reduce the variables that may interfere in the results of the experiment[7].

During the procedure, the surgeon should at least be wearing clean lab clothes, mask and sterile gloves. The use of sterilized surgical materials is the best method to avoid infections related to the surgical procedure. However, this procedure is not performed in most laboratories. At least the surgeon should take precautions to prevent contamination from one animal to the other by avoiding the use of the same instruments for more than one procedure[12].

After shaving, the skin should be cleaned and antisepsis should be performed with antiseptic products, such as aqueous Povidone, to avoid infections in the surgical site[7,10,11].

ANTIBIOTIC

There are two different indications for antibiotics, prophylaxis and therapy.

Antibiotic Prophylaxy

Antibiotic prophylaxis focuses on the prevention of surgical site infection, that is usually caused by skin microorganisms. The recommended prophylaxis is the intra-abdominal injection of ceftriaxone 100 mg/kg after finishing the procedure[7-10]. In our practice, besides the use of ceftriaxone, we mixed with the water that is available to the rat throughout the day, 320mg of amoxacilin-clavulinate diluted in 500ml of water for a total of 10 days, 5 days before and 5 days after the surgical procedure.

Antibiotic Therapy

Antibiotic therapy is guided by the presence of signs of infection. The most common site of infection in SCI rats is the urinary system. The animals lose control of the urinary sphincter after the trauma retaining more urine inside the bladder, despite the maneuver performed 4 times a day to empty the bladder, which predisposes to bacterial proliferation. The signs of urinary infection are the presence of blood or pus in the urine, identified during the Credé maneuver[10,13]. Animals with signs of bladder infection should be treated with amoxaciline-Clavulinate, at a 2mg/100g dose, with intraperitoneal injections 2 times a day until 24 hours after the symptoms disappear[10,13].

ANESTHETIC PROCEDURE

The rats must be weighed in order to adjust the anesthetic dosage[14] (Table 1). Most injectable anesthetics are administered to rats by intraperitoneal or intramuscular routes in a single dose. Apparently, when not contraindicated, intraperitoneal administration allows giving larger volumes of anesthetic, and if skillfully performed appears to cause a minimum of pain and distress to the rat[12].

An efficient anesthesia includes complete analgesia, sedation and muscle blockade. Muscular blockade implies the need for orotracheal intubation and mechanical ventilation, which requires specific equipment for rats.

The selection of the anesthetics will vary according to the type and duration of the surgical procedure. Ketamine (90mg/kg) is a potent hypnotic, but provides insufficient analgesia even for the most superficial surgical procedures. Xylazine is an alfa-2-adrenergic agonist

Table 1. Anesthetic dose of ketamine and xylazine adjusted according to the rat`s weight.

weight (g)	ketamine 10% (ml)	xylazine 2% (ml)
250	0,22	0,1
260	0,23	0,1
270	0,24	0,1
280	0,25	0,1
290	0,26	0,15
300	0,27	0,15
310	0,27	0,15
320	0,28	0,15
330	0,29	0,15
340	0,30	0,15
350	0,31	0,18
360	0,32	0,18
370	0,33	0,18
380	0,34	0,20
390	0,35	0,20
400	0,36	0,20
410	0,36	0,20
420	0,37	0,20
430	0,38	0,20
440	0,39	0,20
450	0,40	0,20
460	0,41	0,20
470	0,42	0,20
480	0,43	0,25
490	0,44	0,25
500	0,45	0,25

Source: Capra R. Protocolo de anestesia no rato. In: Falavigna A, Schenkel PC, editors. Praticando fisiologia. Caxias do Sul: EDUCS; 2010. p. 13-6.

with sedative and analgesic properties, resulting in medium planes of surgical anesthesia. The use in combination of ketamine plus xylazine, provides muscle blockade, sedation and analgesia, and is the most widely used anesthetic combination. In addition the animal is able to maintain ventilatory control throughout the surgical procedure[8-12,15-19].

POSTOPERATIVE MANAGEMENT

The main goal of postoperative management is to ensure the comfort and well-being of the rat, and at the same time to ensure that interferences do not influence the results. Since the animals will require some degree of special attention during the postoperative period, it is preferable that they recover in a separate room[12]. Postoperative management can be divided into two different moments: immediate postoperative and late postoperative procedures.

Immediate postoperative management: includes antibiotic prophylaxis immediately after surgery, animal temperature control, analgesic and fluid therapy. Hypothermia prevention is extremely important. This can be achieved with incubator or heating pads and lamps, maintaining the animal temperature between 25 and 30 degrees Celsius[9,10,13,18]. The cage floor should be comfortable[12]. Immediately after surgery the rat receives a subcutaneous injection of tramadol every 12 hours, at a dose of 1.5 mg/kg for the first 48 hours after surgery[10,13,18]. Tramadol provides adequate and safe analgesia because is a centrally acting analgesic used for the treatment of moderate to severe pain with a low affinity for μ-opioid receptors, and it increases the analgesic action by blocking nociceptive impulses at the spinal cord, it binds to monoaminergic receptors by inhibiting the serotonin and norepinephrine reuptake[17,20,21]. Besides the tramadol, instilling 20 drops of acetaminophen into the rats' water for every 500 ml of water during 48 hours optimizes the analgesic. The acetaminophen acts at the central and peripheral pathways of the pain, including stimulation of nitric oxide by direct inhibition of n-methyl-D-aspartate (NMDA) receptors and inhibition of the cyclo-oxygenase 2 pathway[16]. Oral fluid therapy is stimulated in the postoperative period because of the body fluid loss during the surgery and the rat is unwilling to drink water in the first 12-24 hours after surgery. If this happens, 40-80ml/kg of saline solution is administered by subcutaneous or intraperitoneal route. The measurement of the pre and postoperative body weight, urine and fecal output should be recorded to evaluate the animals' hydration[12].

Late postoperative care: the late complications are due to the fact that the animals become paraplegic, and the most common are skin ulcers and bladder infection. The former are caused by the paraplegic posture, the animals drag their paws, their movements are clumsy, and they stay in the same position for a long time leading to skin ulcers[22]. For these reasons, the substrate of the cages should be soft, smooth and absorbent in order to prevent complications on the skin, joints and bones. The second is caused by the loss of the bladder reflexes and more urine is retained in the bladder, which predisposes to bacterial proliferation. For these reason, the bladder should be manually emptied using the Credé maneuver, four times a day. The Credé maneuver is performed applying pressure over the bladder at the lower abdomen. After 3 to 4 weeks of the spinal cord lesion, the bladder automatism returns and the frequency of the Credé maneuver is progressively reduced. If during the Credé maneuver any sign of urinary tract infection is detected, like the presence of blood or pus in the urine, antibiotic therapy is indicated[13]. The rat should be examined daily to identity and manage any possible complications.

SACRIFICE OF THE ANIMALS

The animal is sacrificed after administration of a larger intraperitoneal dose of ketamina and xylasina, 100mg/kg each, in order to ensure that the rat does not feel any pain. Once deep analgesia and sedation are obtained, the animal is sacrificed with a guillotine, which has to be lubricated with Vaseline and accordingly sharpened, so that the decapitation will be as fast and the least harmful possible. The animal carcass must be put into special white bags, and stored in a refrigerator at -20 Celsius degrees, and afterwards all bags must be incinerated by a specialized service.

FINAL CONSIDERATIONS

A standard protocol for the management of laboratory animals submitted to SCI is necessary not only to protect the rats and ensure that their welfare is being achieved, but also to avoid comparing results obtained from different methodologies, which hampers adequate interpretation and analysis. Besides, in order to obtain reliable results and be able to compare them with other projects, every step of the research should be optimized, including proper selection and daily handling of the animals, the antibiotic and anesthetic scheme, and finally appropriate surgical procedure.

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