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Protocol

New canine spinal cord injury model free from laminectomy

Seijun Fukuda[a,*], Tatsuo Nakamura[a], Yoshihiro Kishigami[a,b], Katsuaki Endo[a], Takashi Azuma[c], Takamitsu Fujikawa[a], Sadami Tsutsumi[c], Yasuhiko Shimizu[a]

[a]*Department of Bioartificial Organs, Institute for Frontier Medical Sciences, Kyoto University, East Building 309, 53 Kawahara-cho Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*

[b]*Kishigami Veterinary Hospital, Japan*

[c]*Department of Medical Simulation Engineering, Institute for Frontier Medical Sciences, Kyoto University, Japan*

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Abstract

The present report details the successful development of a model for spinal cord injury (SCI). This model is simple, reproducible, and requires no laminectomy. Development of the model was carried out using fourteen dogs. A balloon catheter was inserted into the extradural space via the intervertebral foramen of each dog, then the balloon was inflated at the L1 level by injection of saline. Six dogs underwent compression with a balloon volume of 1.5 ml, three dogs with a volume of 1.0 ml, and the remaining five dogs were used as uninjured controls.

We applied the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale to the dogs. Compression of the spinal cord for 10 min at 1.5 ml produced severe paraplegia (BBB remained zero or one for 6 months following surgery), while compression for the same time interval at 1.0 ml produced moderate paraplegia. Electrophysiological tests showed no hindlimb movement upon stimulation cranial to the site of injury in the 1.5-ml group. The volume of abnormal-intensity lesions in the 1.0-ml group calculated using MR imaging showed no marked changes in either high- or low-intensity lesions after 3 months, whereas in the 1.5-ml group, the low-intensity lesions alone showed a marked increase. Pathological examination of the damaged spinal cord showed the formation of cavities surrounded by scar tissue containing high levels of collagen. These findings closely resembled those of clinical cases. It was concluded that 10 min of balloon compression with a volume of 1.5 ml caused irreversible paraplegia in dogs.

Theme: Motor systems and sensorimotor integration

Topic: Spinal cord and brainstem

Keywords: Spinal cord injury (SCI); Intervertebral foramen; Magnetic resonance (MR) imaging; Magnetic stimulation

1. Introduction

Although many studies have aimed to develop new therapeutic strategies for spinal cord injury (SCI), they have yet to produce an effective clinical treatment. Before such a treatment can emerge, however, more relevant animal models must be developed. In particular, the following conditions are necessary for an animal model to provide a uniform basis upon which new treatments can be evaluated.

1. Models that do not induce changes to the surrounding tissues, unlike those involving laminectomy.
2. Models that create injury of constant severity.
3. Models that are applicable to laboratory animals of sufficient size to be representative of human cases.

Over the past 50 years, a number of SCI models have been developed. Khan and Griebel compared three of them–weight dropping, clip compression and balloon compression–and

* Corresponding author. Fax: +81 75 751 4844.
E-mail address: sfukuda@frontier.kyoto-u.ac.jp (S. Fukuda).

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concluded that, from a technical and practical standpoint, the balloon compression model was the most easily applicable of the three, producing a closed injury similar to injuries seen in clinical cases [12]. The procedure used in this experiment involved the insertion of a balloon catheter on the dorsal side of the spinal cord, which was subsequently inflated to produce a SCI. Prior to insertion of the catheter, however, their method required a laminectomy to be performed—a requirement common to all conventional contusion models developed to date. Duncan et al. used a modified aneurysm clip applied epidurally after laminectomy [6]. Fukuoka et al. implanted fragments of *Laminaria*, a natural seaweed that absorbs water and swells, after thinning the lamina [9]. Ohta et al. placed a 20-g or 35-g weight on the exposed cervical cord [16]. Though these studies were based on detailed analyses using 2.0-T MR imaging, they all required laminectomy.

Laminectomy involves removal of a portion of a vertebra, and is performed in contusion models to expose the spinal cord; this subsequently allows an injury to be inflicted. However, laminectomy has a number of associated deleterious effects. Exposure of the spinal cord can be accompanied by serious complications such as spinal dislocation. Furthermore, laminectomy involves removal of not only a portion of the vertebral column itself, but also the muscles attached to (or crossing) the resected bone and ligaments. Since the condition of the tissues surrounding the spinal cord plays an important role in the regeneration process, this effect is decidedly undesirable for studies of spinal cord regeneration.

The balloon-induced closed injury method was pioneered by Tarlov, who determined the time limits for recovery after both acute and gradual compression in dogs, using two types of balloon compression device [22–24]. These studies led to the development of a volume-dependent SCI model in rats, produced using a subdural inflatable balloon [15]. More recently, the balloon volumes needed to produce the minimum compression required to cause irreversible paraplegia were investigated in rats [17,25]. In 2002, we reported a study detailing the optimization of the inflation volumes needed to produce this condition in larger animals (i.e., dogs) [8]. Subsequently, a recent study reported by Purdy et al. showed that the instruments used for humans (e.g., a 1.5-T MR imaging unit) were suitable for use with these animals and that interventional therapies could be easily developed using them [19,20].

The present study details the development of a new canine model for SCI, which involves the use of a balloon catheter without the need for laminectomy.

2. Materials and methods

2.1. Animal selection and experimental design

Fourteen adult female dogs, weighing 8.5 to 19.0 kg, were used in this study. Animals were assigned to experimental groups to evaluate the effects of catheter inflation at two volumes (1.0 ml and 1.5 ml). Six dogs were assigned to receive spinal injury induced by compression with a final balloon volume of 1.5 ml, three for compression with a final volume of 1.0 ml, and the remaining five to act as non-treated controls. All the animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University (1989).

The dogs were anesthetized by intramuscular administration of ketamine hydrochloride at a dose of 12.5 mg/kg and xylazine hydrochloride at 5 mg/kg together with atropine sulfate at 0.25 mg per dog. Anesthesia was maintained by inhalation of 70% N_2O and 1.0% halothane through an endotracheal tube. The concentration of halothane was increased to 3.0% during creation of the spinal injury.

2.2. Surgical operation

A schematic drawing of the operative procedure is illustrated in Fig. 1A. Dogs were laid in ventral recumbency on the operating bed with their limbs immobilized and pulled caudally. A left paramedian approach was selected to spare the ligaments and the muscles between the spinous processes. By opening the superficial external fascia of the trunk and cutting the erector muscles of the spine apart from the transverse processes of L4 with a flat chisel, we identified the spinal nerve, which protruded from the intervertebral foramen.

A spinal branch of the segmental artery and an intervertebral vein run along the spinal nerve root, and there was often severe bleeding from these blood vessels during handling. In such cases, hemostasis was achieved by compression with curved mosquito forceps inserted into the intervertebral foramen for 3 min.

The distance between the L1 and L4 spinous processes of the vertebrae was measured. A silicone balloon catheter (size 6 Fr; Mitsuya Medical, Osaka, Japan) was then inserted into the vertebral canal via the intervertebral foramen between L3 and L4. The balloon catheter was inserted in the cranial direction, to a distance corresponding to that previously measured between L1 and L4, thereby positioning it for inflation at the center of L1 (Fig. 1A). The balloon is shown in Fig. 1B.

The balloon was then inflated to the desired final volume (1.0 ml or 1.5 ml) for 10 min in the spinal extradural space by injection of saline with an inflation device (Bard MAX 30; C.R. Bard, Convington, GA). Compression of the spinal cord by the balloon consequently caused a backward bending reaction. The tube was then deflated and removed, and the muscle fascia and skin were closed.

The position and shape of the balloon were confirmed through preliminary radiographic examination by substituting contrast medium (Iomeprol; Bracco-Eisai, Tokyo, Japan) for saline during injury in one dog subjected to 1.0 ml inflation. Plain radiographs taken with the dog in right lateral recumbency (Fig. 1C) confirmed that the balloon was located at the center of L1.