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Development of an Improved Rabbit Model of Spinal Cord Compression by Embolectomy Catheter

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ABSTRACT

The aim of this experimental study was to evaluate the efficacy of epidural balloon inflation into the unroofed spinal cord for the creation of a experimental spinal cord injury model in rabbits. Ten New Zealand white rabbits were used for this study. Before operation and after anasthesia with 60 mg/kg ketamine and 6 mg/kg xylazine. A midline skin incision was done on the lumbar skin at the level of L1-L4 lumbar vertebrae. Paravertebral muscles were bluntly dissected bilaterally. A microhemilaminotomy was done in the right L3 lamina close to the midline. An arteial embolectomy catheter was inserted into the spinal column between the bone and dura mater to the level of L1-L2 intervertebral space. The microballoon was gradually inflated by using a volume-controlled microballoon inflation syringe over a period of 3 minutes. The microballoon was deflated 8 minutes later and removed completely from the epidural space. All rabbits were paraplegic after the operation. In conclusion, this experimental study demonstrated that the microballoon inflation technique is a very successful method for the evaluation of spinal cord injury in rabbits. Unroofing of the spinal column is extremely important because decompression may be an effective treatment in spinal cord injury. A suitable spinal injury model was created, that is minimally invasive, uniform and easily reproducible.

Keywords: Microhemilaminotomy, microballoon, arteial embolectomy catheter, rabbit, spinal cord

Spinal cord injury models have been developed for studies related to development of therapeutics and surgical techniques, as well as for prediction of clinical outcome and prognosis (Vanicky *et al.* 2001; Grill, 2005). Rodents, rabbits and dogs are the most common animal models for the study of SCI. Several techniques have been employed, including mechanical and chemical method and the use of electromagnetic devices (Scheff *et al.* 2003). The mechanical methods include contusion models using NewYork University impactor (Satake *et al.* 2004), compression models using epidural balloon compression (Lim *et al.* 2007) and aneurysm clips (von Euler *et al.* 1997) etc. Hemisection, transection, and bridge defect on the spinal cord have also been used to produce an injury for implant therapy (Himes *et al.* 2001; Kuh *et al.* 2005; Lepore and Fischer, 2005). Chemical method of creating spinal injury is by using ethydium bromide, which will create demyelination of spinal cord. Spinal cord ischemia models have been produced by means of occlusion of infrarenal aorta in order to study the neuroprotective effects of various drugs including the antibiotic: minocyclin (Kale *et al.* 2011; Fang *et al.*

2013). The MASCIS Impactor, formerly called the NYU Impactor, was developed in 1991 by Drs. John Gruner, Carl Mason, and Wise Young. It is now used in laboratories throughout the world in their spinal cord injury studies. The device measures the impact velocity (ImpV), cord compression distance (Cd), cord compression time (Ct), and cord compression rate (Cr). Aneurysm clips are used to create compression injuries, which is applied extradurally around the spinal cord. The closing force is measurable and can be adjusted to create uniform injury. These forces can be selected to simulate acute compression injuries of mild to moderate, moderate and moderate to severe degrees.

A computer-controlled distraction (Dabney *et al.* 2004) and a method of sustained cord injury (Carlson *et al.* 2003) are regarded as good methods to provide critical data, however, these spinal cord injury models generally require custom-built lesion-making devices. Hemisection, transection, and bridge defect on the spinal cord have also been used to produce an injury for implant therapy (Himes *et al.* 2001; Lepore and Fischer, 2005), however these methods were different than those used to reproduce spontaneous injury. In addition, all of the models described above require laminectomy to expose the site of spinal cord injury, which could interfere with the delivery of therapeutics due to adhesion with surround tissues (Purdy *et al.* 2004). Percutaneous translumbar angioplasty balloon can be used in large dogs, resulting in an intradural compression of the spinal cord (Purdy *et al.* 2004). More recently, a study by Fukuda *et al.* (2005) introduced a new balloon method without laminectomy that was simple and took only 2 to 3 hours to implement. This had only a few complications, including hemorrhage from a segmental artery and vein running along the spinal nerve root. A simpler way of using embolectomy catheters, introduced through a drilled hemilaminectomy hole has the advantages such as easy exposure of spinal cord, no haemorrhage, and use of fluoroscopically detectable catheter (Lim *et al.* 2007).

In order to make a valid model of spinal injury, application of minimal but sufficient compression to the spinal cord is the best approach (Sykova and Jendelova, 2007). Epidural balloon compression is considered as the minimally invasive and easily reproducible uniform model of spinal injury rabbit model (Baydin *et al.* 2007). Contusion compression models are more appropriate to evaluate the process of functional preservation of axons or de-novo axonal re-growth across the lesion site (Kamada *et al.* 2011).

MATERIALS AND METHODS

Ten clinically healthy New Zealand White rabbits (*Oryclologus cuniculus*) of either sex were used in this study. Prior to the study, all the animals were provided with the standard diet, *ad libitum* water and allowed to

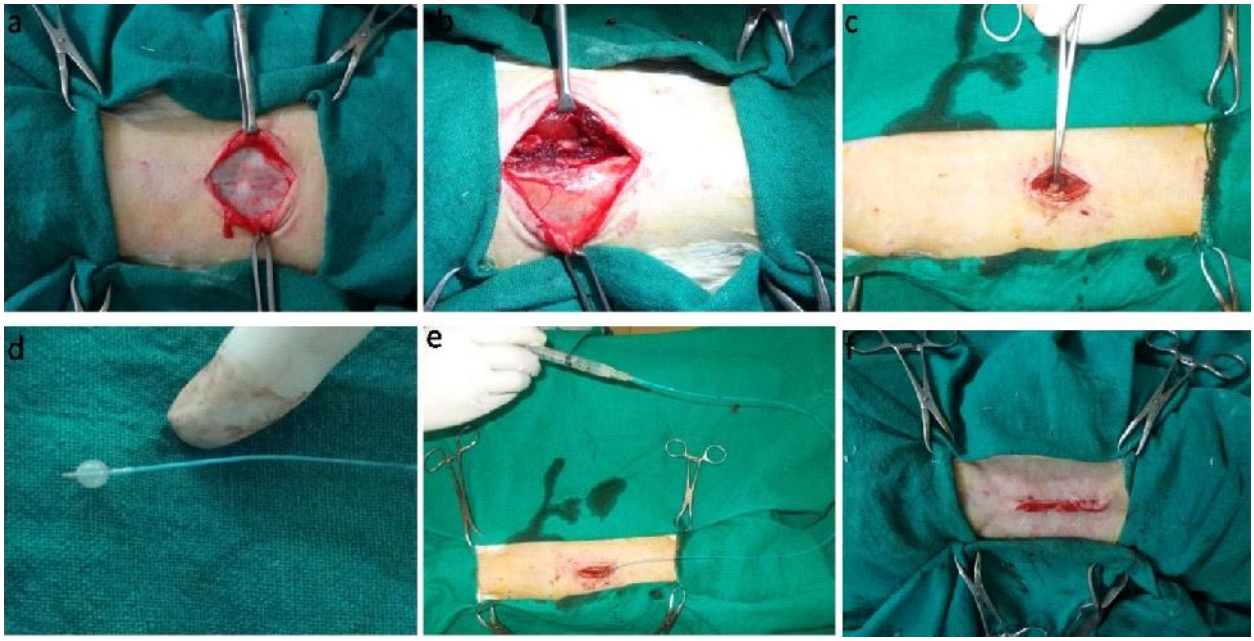

Fig. 1. (a) Midline skin incision was made on the lumber skin at the level of L1-L4, **(b)** Paravertebral muscles were dissected, **(c)** The dorsal laminae of L3 were exposed, **(d)** The maximum capacity of the microballoon when fully infilated is 150ml, **(e)** Microballoon catheter 2F Biosensors embolectomy used to create compression, **(f)** The muscles were sutured with Vicryl 3-0 and skin was closed with Nylon 2-0.

acclimatize for approaching, handling and animal house conditions for a period of 10 days.

Table 1: Olby scoring

Stage	Point	Neurological signs
1	0	No pelvic limb movement and deep pain sensation
	1	No pelvic limb movement with deep pain sensation.
	2	No pelvic limb movement but voluntary tail movement.
2	3	Minimum non weight bearing protraction of pelvic limb.(movement of one joint)
	4	Non weight bearing protraction of pelvic limb with more than 1 joint involved, less than 50% of the time.
	5	Non weight bearing protraction of pelvic limb with more than 1 joint involved more than 50% of the time.
3	6	Weight bearing protraction during less than 10% of the time.
	7	Weight bearing protraction during 10-50% of the time.
	8	Weight bearing protraction during above 50% of the time.
4	9	Weight bearing protraction during 100% of the time, with reduced strength, mistake made is above 90%.
	10	Weight bearing protraction during 100% of the time, with reduced strength, mistake made is 50-90%.
	11	Weight bearing protraction during 100% of the time, with reduced strength, mistake made is less than 50%.
5	12	Ataxic pelvic gait with normal strength. Mistakes made is more than 50%.
	13	Ataxic pelvic gait with normal strength. Mistakes made is less than 50%.
	14	Normal pelvic limb gait.

Behavioural testing: Before injury, each animal was acclimated and scored using Basso, Beattie Bresnahan locomotor rating scale (BBB scoring) (Basso *et al.* 1996) (Table 3). modified Talov scale (Tarlov, 1957) (Table 2) and Olby scoring (Olby *et al.* 2004) (Table 1). The animals showing the normal scores in all the three were selected for the study (Olby score: IV-14, BBB: 21, Modified Tarlov score: 4).

The rabbits were anaesthetized by intramuscular injections of xylazine @ 6 mg/kg followed 10 minutes later, by ketamine @ 60 mg/kg in the thigh muscles as per standard protocol. Anaesthesia was maintained by additional dose of intravenous ketamine hydrochloride when needed. Local analgesic, lignocaine 2% inj. was used on the site for local analgesia, whenever needed. The dorsum of the animal, from T11-L7 was clipped, scrubbed with savlon and painted with povidone iodine solution for aseptic preparation of the surgical site.

Table 2: Modified Tarlov scale

Score	Observations
0	Spastic paraplegia and no movement of the lower limbs
1	Spastic paraplegia and slight movement of the lower limbs
2	Good movement of the lower limbs but unable to stand
3	Able to stand but unable to walk normally
4	Complete recovery and normal gait-hopping

The animals were controlled in dorsal recumbency and a midline skin incision was made on the lumbar skin at the level of L1-L4 (Fig. 1a). Paravertebral muscles were bluntly dissected bilaterally (Fig. 1b) and the dorsal laminae of L3 were exposed (Fig. 1c). A micro-hemilaminotomy was performed after removal of the articular process, in the right side of L3 lamina close to the midline, with great care to avoid any iatrogenic damage to the spinal cord. A microballoon (2F Biosensors embolectomy catheter) was inserted into the spinal column between the bone and dura mater (maximum capacity of the microballoon when fully infilated (Fig. 1d) is 150 µl) to the level of L1-L2 intervertebral space (Fig. 1e). The length of the catheter to be inserted was predetermined by assessing the distance between L3 and the L1-L2 intervertebral space, on a pre operative radiograph. The position of the bulb was confirmed with the help of C-Arm machine. The microballoon was inflated slowly to the full capacity by using a microballoon inflation syringe, over a period of three minutes. For inflation, a positive contrast agent (iohexol) diluted with distilled water in 1:1 ratio. The inflated bulb was kept in position for eight minutes to create spinal compression. The microballoon was deflated 8 minutes later and removed completely from the epidural space. The epaxial muscles were sutured with Polyglycolic acid 3-0 (Vicryl®) in simple continuous pattern and the skin was closed with Nylon 2-0 in horizontal mattress pattern (Fig. 1f).

RESULTS

Before injury, each animal was acclimated and scored using Basso, Beattie Bresnahan locomotor rating scale (BBB scoring), modified Talov scale and Olby scoring. The animals showing the normal scores in all the three were selected for the study (Olby score: IV-14, BBB: 21, Modified Tarlov score: 4). All the experimental animals

recovered from anesthesia uneventfully. The animals were examined for the hind limb function as a measure of the evaluation of spinal cord injury.

Table 3: BBB scoring

Score	Observation
0	No observable movement of the hindlimbs.
1	Slight (limited) movement of one or two joints, usually hip and/or knee.
2	Extensive movement of one joint or extensive movement of one joint and slight movement of the other.
3	Extensive movement of two joints.
4	Slight movement of all three joints of the hindlimbs
5	Slight movement of two joints and extensive movement of the third joint.
6	Extensive movement of two joints and slight movement of the third joint.
7	Extensive movement of the three joints in the hindlimbs.
8	Sweeping without weight bearing or plantar support of the paw without weight bearing.
9	Plantar support of the paw with weight bearing only in the support stage (i.e., when static) or occasional, frequent or inconsistent dorsal stepping with weight bearing and no plantar stepping.
10	Plantar stepping with occasional weight bearing and no forelimb-hindlimb coordination.
11	Plantar stepping with frequent to consistent weight bearing and occasional forelimb-hindlimb coordination.
12	Plantar stepping with frequent to consistent weight bearing and occasional forelimb-hindlimb coordination.
13	Plantar stepping with frequent to consistent weight bearing and frequent forelimb-hindlimb coordination.
14	Plantar stepping with consistent weight support, consistent forelimb-hindlimb coordination and predominantly rotated paw position (internally or externally) during locomotion both at the instant of initial contact with the surface as well as before moving the toes at the end of the support stage or frequent plantar stepping, consistent forelimb-hindlimb coordination and occasional dorsal stepping
15	Consistent plantar stepping, consistent forelimb hindlimb coordination and no movement of the toes or occasional movement during forward movement of limb; predominant paw position is parallel to the body at the time of initial contact.
16	Consistent plantar stepping and forelimb-hindlimb coordination during gait and movement of the toes occurs frequently during forward movement of the limb; the predominant paw position is parallel to the body at the time of initial contact and curved at the instant of movement.
17	Consistent plantar stepping and forelimb-hindlimb coordination during gait and movement of the toes occurs frequently during forward movement of limb; the predominant paw position is parallel to the body at the time of initial contact and at the instant of movement of the toes.
18	Consistent plantar stepping and forelimb-hindlimb coordination during gait and movement of the toes occurs consistently during forward movement of limb; the predominant paw position is parallel to the body at the time of initial contact and curved during movement of the toes.
19	Consistent plantar stepping and forelimb-hindlimb coordination during gait and movement of the toes occurs consistently during forward movement of limb; the predominant paw position is parallel to the body at the instant of contact and at the time of movement of the toes, and the animal presents a downward tail some or all of the time.
20	Consistent plantar stepping and forelimb-hindlimb coordination during gait and movement of the toes occurs consistently during forward movement of limb; the predominant paw position is parallel to the body at the instant of contact and at the time of movement of toes, and the animal presents consistent elevation of the tail and trunk instability.
21	Consistent plantar stepping and coordinated gait, consistent movement of the toes; paw position is predominantly parallel to the body during the whole support stage; consistent trunk stability; consistent tail elevation.

The scoring was done soon after the complete recovery from anesthesia, on the 3rd post operative day, 7th post operative day and from then every week up to 60th post operative day. The animals that had undergone the micro hemilaminectomy and epidural balloon compression showed no motor function of the hind limbs. There was no tail movement observed. Deep pain sensation was present on both the hind limbs. The bladder tone was depressed for an average of 12 days. Massaging of the bladder was done for two weeks for all the animals to minimize the chances of retention of urine in the bladder and post renal uremia. Animals were having a BBB and modified Tarlov score of zero and an Olby score of one throughout the observation period.

DISCUSSION

In the present study the spinal injury model in rabbits was created by using 2F arterial embolectomy catheter, for epidural compression. The compression was given for a period of 8 minutes. This provided a satisfactory compression of the spinal cord, resulting in diminished reflexes, except for deep pain sensation. A minimally invasive spinal cord injury model in rabbit was created

by Baydin *et al.* (2007) by means of epidural micro balloon inflation. Their experimental study demonstrated that the microballoon inflation technique is a very successful method for the evaluation of spinal cord injury in rabbits. In the present study, instead of using epidural micro balloon catheter, an arterial embolectomy catheter was used, for attaining the spinal cord compression. The unroofing of spinal column is extremely important because decompression may be an effective treatment in the spinal cord injury. A similar technique was used to create spinal cord injury in dogs by Ji-Hey Lim *et al.* (2007), the compression was done by means of three french embolectomy occlusion catheter for 6, 12 and 24 hours. They suggested that a spinal cord occlusion > 50% for 24 h, and > 75% for 12 h would be appropriate for use as a severe spinal cord injury model. In the present study, complete occlusion of the spinal cord was done for a relatively short period of time of 8 minutes, which provided a satisfactory compression of spinal cord, resulting in the moderate spinal injury with intact deep pain sensation. The balloon compression technique provides a scale of animal models that mimic human spinal injury with quantifiable trauma that could be correlated with functional recovery and morphology of the cord lesion (Vanicky *et al.* 2001). This experimental traumatic spinal cord injury was also suitable for research on intervertebral disc herniation and vertebral fractures that were seen commonly in canine spinal cord injury (Olby *et al.* 2004). The method using the subarachnoid approach had an advantage for studying the spinal cord itself, as opposed to simple removal of the mass. However, when this approach was used, the introduced catheter was likely to damage the blood-brain barrier of the spinal cord and disturb maintenance of spinal cord homeostasis (Fukuda *et al.* 2005).

CONCLUSION

The microballoon inflation technique is a very successful method for the evaluation of spinal cord injury in rabbits. Unroofing of the spinal column is extremely important because decompression may be an effective treatment in spinal cord injury. A suitable spinal injury model was created, that is minimally invasive, uniform and easily reproducible.

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