A Thesis

For The Degree of Master of Veterinary Medicine

Transplantation of olfactory bulb promote locomotor recovery in dogs with spinal cord hemisection

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Abstract

Transplantation of olfactory bulb promote locomotor recovery in dogs with spinal cord hemisection

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Olfactory bulb fragments were transplanted into hemisected spinal cords of dogs to determine the effect of transplants on locomotor recovery after spinal injury. Dogs were rendered hemiparaplegic via hemisection of the spinal cord at the T10 level and received an autologous transplant of a piece of olfactory bulb tissue. After surgery, locomotor recovery was observed and graded on a scale of 0 to 5, weekly for 4 weeks. Dogs that received olfactory bulb transplants showed progressive locomotor recovery for four weeks, from complete paralysis to partial recovery of hindlimb gait. At four weeks after transplantation, some cells from the transplanted tissues were found within the spinal parenchyma upon histopathologic examination. These findings suggest that autologous transplantation of olfactory bulb tissue can contribute to the repair of spinal cord injuries and recovery of locomotion in dogs.

Key word: transplantation, olfactory bulb, spinal cord hemisection

I . Introduction

Traumatic spinal cord injuries are very frequent in our society, with increasing numbers seen every year. This pathologic condition causes permanent and irreversible functional deficits in patients, although many attempts have been made in animal models to regenerate the spinal cord using a variety of transplanted cell types. These include Schwann cells (Paino et al., 1991), oligodendrocyte precursors (Rosenbluth et al., 1997), foetal neural tissue (Iwashita et al., 1994), peripheral nerves (Cheng et al., 1996) and stem cells, which can differentiate into multiple classes of neurons or glia (Liu et al., 2000).

The source of the olfactory ensheathing cells for all the studies in humans and rats, referred to above, was the olfactory nerve layer of the olfactory bulb. The use of olfactory bulb as a neural graft source raises the attractive possibility of autograft, with the ensuing advantages of ease of acquisition, minimal ethical concerns, and no risk of rejection.

In the past, transplantation approaches developed in rodents have presented certain difficulties for introduction as clinical therapies for naturally occurring injury in human patients, including differences in organ sizes and the high variability of natural injuries compared to those produced in controlled experiments. Some of these problems could be resolved by the use of an intermediate model before phase 1 clinical trials in humans. The naturally occurring spinal cord injuries that occur in the domestic dog population have been advocated as having many of the features suitable for such a model (Blight et al., 1991).

To investigate the efficacy of olfactory bulb transplantation in the recuperation of injured spinal cord, we transplanted autologous olfactory bulb fragments into the hemisected spinal cords of dogs, and examined the process of locomotor recovery.

Ⅱ. Material and Methods

1. Animals

Five healthy male and female mongrel dogs, weighing 6 to 9 kg, were used in this study. All of the dogs were considered to be normal following physical and hemodiagnostic (complete blood count) examinations. Dogs were rendered hemiparaplegic via hemisection of the spinal cord at the T9/10 level.

2. Opening of olfactory bulb regions

To harvest a portion of the olfactory bulb for transplantation, the experimental dogs were deeply anesthetized prior to opening of the frontal bone with a bone saw and laungeur, and the olfactory bulb was exposed. The right bulb was excised for transplantation and kept in saline.

3. Laminectomy and transplantation of the olfactory bulb

For the transplantation, a laminectomy was performed at the level of thoracic vertebrae T9/10. In brief, the overlying muscles were bluntly dissected, and the dorsal and lateral parts of the vertebrae were removed to expose the dura. After longitudinal incision of the dura, the spinal cord was hemisected with a fine mass, followed by aspiration to assure complete hemisection. Then two to three pieces of olfactory bulb tissue were introduced into the empty space, and the dura was closed (Fig. 1A). As a control, bone wax was substituted in place of olfactory tissue in three animals (Fig. 1B).

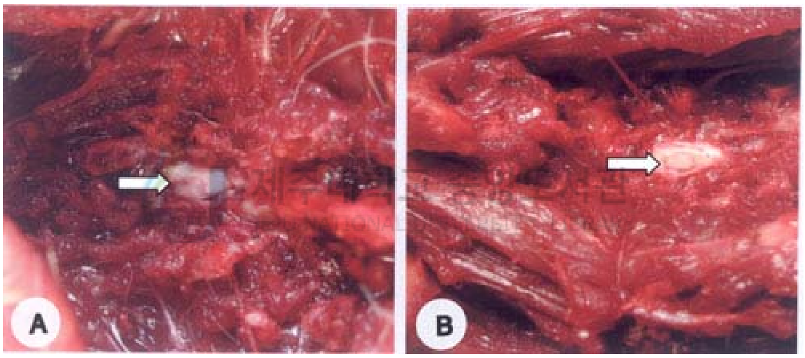

Figure 1. Gross finding of hemisected spinal cord with olfactory bulb transplantation (A) and with bone wax as a control (B) at T10 level.

4. Locomotive behavior assessment

The use of the hind legs was examined in a place where the animal was allowed to move freely, unleashed, and where the surface offered good footing. Locomotion was graded according to a standard locomotor rating scale (Bister et al., 2000). This scale measures pelvic limb movements, with a score of 0 indicating no movement, and with increasing scores for strength, coordinated movement and so on, to a maximum score of 5. Each dog was scored every week by two observers. In brief, the locomotor rating scale for dogs was as follows: 0, absence of purposeful movement: hemiparaplegia; 1, unable to stand to support weight; slight movement when supported by the tail: severe paraparesis; 2, unable to stand to support weight; when assisted, moves limbs readily, but stumbles and falls frequently: moderate paraparesis and ataxia; 3, can stand to support weight, but frequently stumbles and falls: mild paraparesis and ataxia; 4, can stand to support weight: minimal paraparesis and ataxia; 5, normal strength and coordination.

5. Histological assessment

Animals were sacrificed at 4 weeks post-transplantation for the histological study. Spinal segments from the transplantation site were then removed, fixed in 4% paraformaldehyde in phosphate buffer, and processed for paraffin embedding. Five-micron sections were stained with hematoxylin and eosin.

III. Results

1. Locomotive behavior recovery

Animals that underwent hemisection and olfactory bulb transplantation showed recovery of the locomotor system (Fig. 2). As shown in Table 1 as judged by the criteria of the locomotor function test, olfactory-bulb-transplanted animals recovered hindlimb movement at 3 weeks post-transplantation ($p<0.05$), while control animals showed less recovery at that time (Table 1). Although the control group still showed less locomotor recovery after 4 weeks, there was no longer a significant difference between the two groups.

Figure 2. Posture of dogs at 3 days(A, C) and 4 weeks (B, D) after transplantation of olfactory bulb(A, B) and of control(C, D).

Table1. Time course of locomotor recovery.

Weeks after grafting	Bone wax control(n=3)	Olfactory bulb(n=3)
1	0.33±0.58	1.33±0.58
2	1.00±1.00	1.66±0.58
3	1.33±0.58	2.67±0.58*
4	2.33±0.58	3.50±0.71

Values represent mean ± standard deviation.

*p<0.05.indicate statistical significance as compared with bone wax substituted control.

2. Histological study of transplanted lesions

Within the transplantation lesion, the dura was constricted. The remaining, unsectioned half of the spinal cord had extruded into the contralateral space caused by the hemisection surgery, apparently because the transplanted tissue fragments were not voluminous enough to fill the created space. There were cell masses under the dura, typical of neural elements (Fig. 3).

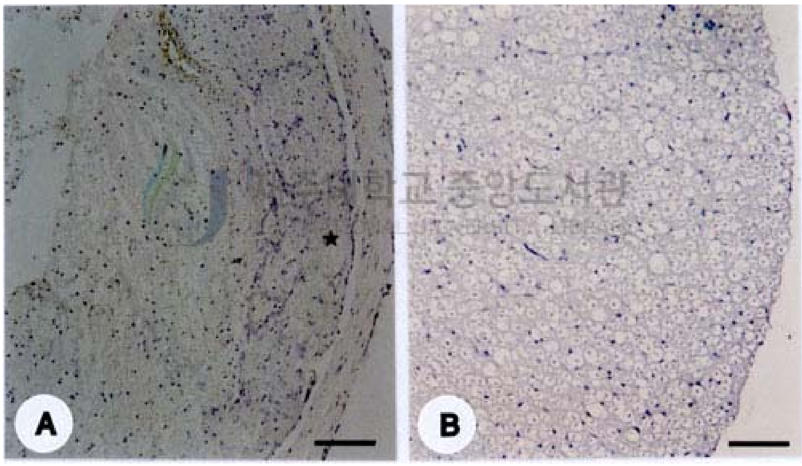

Figure 3. Histology of hemisected spinal cord with olfactory bulb transplantation (A) and normal spinal cord (B). Some transplanted cell masses were found in the spinal cord parenchyma (A). H-E staining. Scale bar represents 100 μm.

Ⅳ. Discussion

This study is the first report demonstrating that autologous transplantation of olfactory bulb tissue promotes locomotor recovery in dogs with spinal cord hemisection. The present study provides evidence that autograft is possible in spinal cord injury and may avoid the detrimental effects of rejection after transplantation.

Significant progress has recently been made with the transplantation of olfactory ensheathing cells into spinal cord injuries. These cells have become popular because of their ability to promote long-distance growth of regenerating axons in vivo (Pascual et al., 2002) and to remyelinate spinal cord axons (Imaizumi et al., 1998) in the adult mammalian CNS. The previous reports also make the claim, with which we concur, that olfactory ensheathing cells are good candidates for transplantation even though they are heterogeneous. In the approach that we chose in the present study, having two cell types, namely olfactory ensheathing cells and neural cells, presents the advantage of possible transdifferentiation into glial cells and neurons when those cells are necessary.

The contribution of the pyramidal tract in the spinal cord of the dog is somewhat different from humans, because only about 20% of pyramidal tract in dogs extends to the hindlimb compared with 100% in humans (Dyce et al., 1996). This discrepancy may account for differences in recovery from spinal cord injury in humans and dogs. In the present study we found that locomotor recovery did occur in control animals with bone wax inserted in the injury site, although the recovery was delayed. It has been postulated that this form of locomotor recovery is dependent on the smaller contribution of the pyramidal tract in dogs to hindlimb locomotion (Dyce et al., 1996).

There are multiple mechanisms by which locomotor recovery may be promoted by olfactory bulb transplantation. One possibility is that many growth factors are secreted from the transplanted tissues and activate the regeneration of damaged neural elements (Liu et al., 2002). This possibility is well supported by evidence that glial-cell-derived neurotrophic factors are beneficial to injured spinal cords, and are possibly secreted from transplanted olfactory bulb cells (Alexander et al., 2002).

Considering the present findings together with the results of previous studies, we postulate that the promotion of functional recovery in dogs with spinal cord hemisection by olfactory bulb transplants is partially due to the beneficial effects of transplanted tissues, and partially to spontaneous recovery, as was seen in the control dogs that received a bone wax insert instead of transplanted neural tissue.

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초 록

척수 절단된 개에서 후각망울 이식의 따른 운동력 회복 증진에 대한 연구

(지도교수 : 신 태 균)

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척수는 중추신경계의 한 부분으로 대뇌로 부터의 신호를 말초에 전해주는 중요한 역할을 한다. 척수의 손상은 이런 신호전달이 차단됨으로써 상행성 신경로 및 하행성 신경로 모두에 기능장애를 초래할 수 있다. 척수가 손상을 받게 되면 1차적으로 조직의 퇴행성 변화가 일어나고 그 후 반흔조직이 형성된다. 척수 손상후 퇴행성 변화를 줄이고 새로운 신경계 세포의 증식은 기능회복에 중요한 영향을 미칠 수 있다. 이 실험에서는 개를 실험동물로 하여 척수 손상을 준 후 자기 후각망울을 손상부위에 이식한 후 행동학적 평가 및 조직학적 소견을 관찰하였다. 척수 손상 실험군은 제 9-10흉추부위에서 추궁을 제거한 후 흉수를 가로로 절반 절단하고 길이 1cm 제거 하였다. 그 후 두개강을 열고 반대쪽 후각망울을 분리한 후 조직

편을 만들고 이들 조직편을 척수가 제거되어 생긴 공간에 이식하였다. 대조군의 개에서는 추궁절제후 후각망울 대신 신경조직이 옆에서 확장되는 것을 차단하기 위하여 고형제인 bonewax를 같은 크기로 만들어 삽입하였다. 이식 후 뇌막을 덮은 후 순서에 따라 봉합하였다. 행동학적 평가를 시행한 결과 수술후 3주째 bonewax 이식 대조군(1.33±0.58)에서 자연적 기능회복이 인정되었으며 후각망울을 이식한 실험군(2.67±0.58)($p<0.05$) 에서 운동기능은 대조군보다 유의성 있게 회복이 촉진되었다. 조직학적으로 이식부위를 관찰한 결과 척수의 실질 및 수막하 공간에서 이식된 것으로 추정되는 세포덩어리가 확인되었다. 결론적으로 개를 활용한 척수 손상모델에서 후각망울의 자가 이식은 척수의 기능회복이 기여할 수 있을 것으로 추정된다.

주요어 : 이식, 후각망울, 척수 절단