SHORT REPORT

ABSTRACT: We studied the effect of initiation time of nerve expansion after nerve transection on the induction of ODC activity and Schwann cell proliferation in nerve tissue under Wallerian degeneration. The levels of ODC activity and Schwann cell proliferation decreased as the initiation time of nerve expansion was delayed after nerve transection, and peak levels of ODC activity following nerve expansion preceded peak levels of Schwann cell proliferation. © 1997 John Wiley & Sons, Inc. *Muscle Nerve* **20:** 1314–1317, 1997
Key words: ornithine decarboxylase; Schwann cell proliferation; initiation time of expansion; transection; sciatic nerve; rat

NERVE EXPANSION IN NERVE REGENERATION: EFFECT OF TIME ON INDUCTION OF ORNITHINE DECARBOXYLASE AND SCHWANN CELL PROLIFERATION

S. OHKAYA, MD,[1] H. HIBASAMI, PhD,[3] H. HIRATA, MD,[1]* H. SASAKI, MD,[1] A. MORITA, MD,[1] M. MATSUMOTO, MD,[1] A. UCHIDA, MD, PhD,[1] and K. NAKASHIMA, MD, PhD[2]

[1] Department of Orthopedic Surgery, Faculty of Medicine, Mie University, Tsu-city, Mie 514, Japan
[2] Department of Biochemistry, Faculty of Medicine, Mie University, Mie, Japan
[3] College of Medical Sciences, Mie University, Mie, Japan

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Polyamines are intimately involved in the mechanism of cellular growth, and the activity of ornithine decarboxylase (EC 4.1.1.17) (ODC), which catalyzes the rate-limiting step of polyamine biosynthesis, is elevated in proliferating cells. The rapid increase of ODC activity is one of the major responses of cells to growth-promoting stimuli.[13] Several investigators[3,12] have demonstrated a correlation between the cell cycle and the induction of ODC activity. Thus, increased cellular polyamine contents resulting from enhanced polyamine biosynthesis are believed to play an important role in cell proliferation.

Soft tissue expansion was first reported by Neumann[8] in 1957 and then by Radovan[9] in 1982. They designed an implantable and inflatable device, and used it to expand skin and soft tissue. This tissue expander has been widely used in the field of plastic and reconstructive surgery with great success. Because of the low complication rate involving peripheral nerves in the expanded skin area, several investigators[2,11,14] studied the application of the device to peripheral nerve surgery. However, almost all reports concern the expansion of the proximal segment of transected nerves. In a previous study,[4] we reported on the expansion of the distal segment of a transected nerve. In contrast to the normal nerve expansion which mostly depends on the viscoelastic property of the peripheral nerve, we found that expansion of the distal segment was accompanied by accelerated Schwann cell proliferation.[4] We also confirmed that polyamines play a key role in the induction of Schwann cell proliferation.[4]

The purpose of the present study was to examine the effect of initiation time of nerve expansion after nerve transection on the induction of ODC activity and Schwann cell proliferation in nerve tissue under Wallerian degeneration. The earlier the initiation of nerve expansion yielded the more induction of enzyme activity and cellular proliferation.

MATERIALS AND METHODS

Male Wistar rats were used for the experiments. The left sciatic nerve was transected posterior to the major trochanter. The proximal stump was reflected

*Correspondence to: Dr. H. Hirata

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and sutured to the tip of the greater trochanter to avoid axonal regeneration, while the distal stump was sutured to the posterior aspect of the greater trochanter to secure the sciatic nerve. The rats were divided into four groups based on the initiation time of nerve expansion. The sciatic nerve was reexposed on day 5 (group 1), day 12 (group 2), day 19 (group 3), and day 26 (group 4) after nerve transection, and a rubber tissue expander was placed beneath the distal stump of the nerve. A subcutaneous tunnel on the rat back accommodated the tubing and the injection port as shown in Figure 1A. For each group, 5 mL of physiological saline was injected into the tissue expander on the first day, and an additional 2 mL was injected daily from the second day to the fourth day. A total of 11 mL of saline was injected into the tissue expanders in all rats. Two rats from each group were sacrificed each day, and the distal stumps of the transected sciatic nerves were removed. One sciatic nerve was used for assay of ODC activity, and the other specimen was subjected to immunohistochemical analysis. Immunohistochemical double staining was carried out using the labeled streptavidin–biotin system for S-100 protein (DAKO Co., diluted 1:100) and enhanced polymer one-step staining system for proliferating cell nuclear antigen (PCNA) (DAKO Co.). Assay of ODC activity and immunohistochemical analysis were carried out by the methods described in our previous article.[4]

RESULTS

As shown in Figure 1B, in group 1 rats whose nerve tissues were initially to be expanded on day 5, maximal ODC activity was observed in the tissues on the third day of expansion and had reached about fivefold the initial value. In group 2, the transected nerves were expanded from day 12, and maximal ODC activity was observed on the second day of the

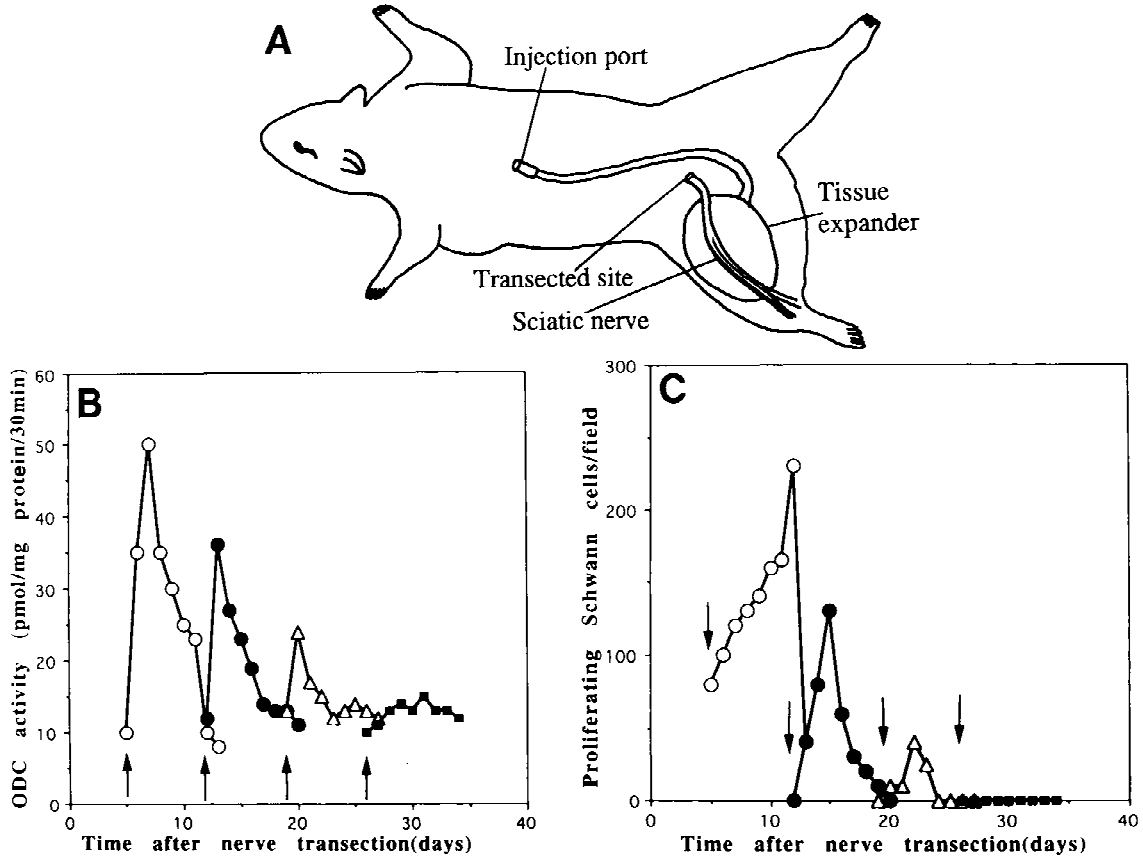

FIGURE 1. (A) Procedure of nerve expansion by a rubber expander. The sciatic nerve was transected posterior to the major trochanter. A rubber tissue expander was placed beneath the distal stump of the nerve. **(B)** Time course of ODC activity alteration in the distal stump of the transected nerve tissue after varying initiation time of nerve expansion. The initiation time of expansion was on day 5 (group 1, ○), day 12 (group 2, ●), day 19 (group 3, △), and day 26 (group 4, ■) after nerve transection. The arrows indicate initiation of nerve expansion in each group. **(C)** Time course of number alteration of proliferating Schwann cells in a field (original magnification: ×100) in the distal stump of the transected nerve after varying initiation time of nerve expansion. The initiation time of expansion was the same as those described in **(B).**

tissue expansion, showing approximately a threefold increase over the initial value. In group 3, nerve expansion was initiated on day 19 and the maximal ODC activity observed after 2 days was only twofold over the initial value. No significant change in ODC activity was observed during tissue expansion in group 4 rats, whose nerve tissues were expanded on day 26.

The proliferating Schwann cells appeared in the nerve tissues of group 1 rats from the first day to the ninth day following nerve expansion, and the number of proliferating Schwann cells peaked on the seventh day after expansion (Fig. 1C). In group 2, the number of proliferating Schwann cells increased to peak on the fourth day after nerve expansion, but the increase was about half that of group 1. In group 3, the number of proliferating Schwann cells peaked on the fourth day after nerve expansion; however, it was only one sixth of that observed in group 1. In group 4, no proliferating Schwann cells were observed in the nerve tissue either during or after nerve expansion, which was consistent with the observation in ODC activity.

The nerve tissues of group 1 rats, whose nerve tissues were initially to be expanded on day 5, were lengthened to 140 ± 5 (%) of the nerve of transected and unexpanded rats.

DISCUSSION

Treatment of peripheral nerve injury with a large gap remains a challenging problem. The only applicable technique at the present time is autologous nerve graft. However, the technique has a number of inevitable disadvantages, including the need to sacrifice cutaneous sensory nerves, the limited amount of graft materials, and the requirement of two coaptation sites. Since there is ample evidence that Schwann cells play a key role in axonal regeneration,[5,7,10] expansion of Wallerian-degenerating nerves can be a promising treatment for peripheral nerve injury with a nerve gap.

Hirata et al.[4] reported that the level of Schwann cell proliferation is closely related to ODC activity, and have suggested that polyamines play a key role in the mitosis of Schwann cells during Wallerian degeneration. They further demonstrated that nerve expansion using a tissue expander markedly increased ODC activity in the Wallerian-degenerating nerve even on day 5 or later after the injury, which is followed by significantly increased mitosis of the Schwann cells.

Fujisawa et al.[1] carried out Wallerian-degenerating nerve expansion using the same expansion protocol as employed in the present study, which yields as much as 47% expansion of the nerve. They observed an excellent functional and morphological recovery of the injured and expanded nerve 54 days after nerve injury. Milner and Wilkins[6] reported that normal nerve expansion by a similar protocol took more than 100 days to recover. Functional recovery following distal nerve expansion takes a shorter time than that of normal nerve expansion. Other advantages of the expansion of Wallerian-degenerating nerves include absence of the pain during nerve expansion that is a usual symptom of normal nerve stretching.

In the present study, we found that levels of ODC activity and Schwann cell proliferation decreased as the initiation time of nerve expansion was delayed after nerve transection, and that peak levels of ODC activity following nerve expansion always preceded peak levels of Schwann cell proliferation. These findings suggested that the amounts of growth factors induced by nerve expansion might have decreased as the initiation time of nerve expansion was delayed, resulting in lowered levels of ODC activity and Schwann cell proliferation.

Therefore, it may be concluded that the expansion of Wallerian-degenerating nerves should be carried out as early as possible after nerve injury to ensure recovery. Further experiments are needed to verify the growth factors involved in the inductions of ODC activity and Schwann cell proliferation in injured and expanded nerve tissue.

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