Nerve-regenerating effect of 15-deoxyspergualin

Peripheral nerve allotransplants in the rat

Keiichi Muramatsu, Kazuteru Doi, Tatsuaki Akino, Mitsunori Shigetomi, Hisashi Yamamoto and Shinya Kawai

We studied the effect of two immunosuppressive agents, 15-deoxyspergualin and cyclosporine A, on various nerve allografts in inbred rats whose major histocompatibility complex was mismatched. As allografts, we used the sciatic nerve (20 mm) and the saphenous nerve (20 mm). We found that 1) fresh peripheral nerve allografts with a short course of 15-deoxyspergualin and cyclosporine A therapy induced more regenerated axons than autografts did, 2) a short course of 15-deoxyspergualin therapy provided better nerve regeneration than cyclosporine A therapy in all forms of nerve allografts and large caliber nerve allografts induced more regenerated axons.

Department of Orthopedics, Yamaguchi University School of Medicine, 1144 kogushi, Ube-shi, Yamaguchi 755, Japan
Tel +81 836 22-2268. Fax -2267
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The potent immunosuppressant cyclosporine A (CsA) has been studied in nerve allografting. Bain et al. (1988) and Midha et al. (1993) reported that nerve conduction could recover while the rejection reaction could not be prevented. Zalewski and Gulati (1981) claimed that nerve conduction could not recover. We have previously reported results like those of Mackinnon and co-workers (Muramatsu et al. 1994, 1995b). A temporary decrease in nerve conduction could not be avoided and therefore more effective immunosuppressants were desired. 15-deoxyspergualin (DSG) has a unique immunosuppressive action (Takeuti et al. 1981, Umezawa et al. 1981) and Walter et al. (1987) reported induction of donor-specific immunotolerance in kidney allotransplantation. We used this drug in nerve allotransplantation. Although allografts were rejected after a short course of therapy, allografted nerve conduction was more successfully preserved than with CsA therapy (Muramatsu et al. 1995a). To establish the effectiveness of DSG in nerve allotransplantation, we now investigated whether differences in donor nerve form influenced the protection of regenerated axons from rejection.

Animals and methods

Genetically inbred rats were used. The donors were DA rats ($RT1^a$) weighing 300 g, and the recipients were LEW rats ($RT1^l$) weighing 350 g. Orthotopic allotransplantation of 20 mm-long sciatic nerve was performed in 102 rats as a large caliber (800 μm) nerve graft and allotransplantation of the great saphenous nerve in 102 rats as a small caliber (200 μm) graft. The rats were operated on aseptically and, after the donor nerve graft was interposed in the recipient animal, nerve repair was performed at both ends with 11-0 nylon epineural sutures, using an operating microscope. The rats were divided into the following three groups; DSG group, CsA group and non-immunosuppressive group (Table 1). In the DSG group (n 72), a short course of therapy (2.5 mg/kg/day for 6 weeks) and continuous therapy (daily for 6 weeks and then twice a week at the same dose) subgroups were established. In the CsA group (n 36), the drug was administered in a dose of 3 mg/kg/day for 6 weeks. One group with autografts (n 48) was also established.

The grafted nerves were evaluated using histological and histomorphometric parameters. After the rats were killed, both the grafted and contralateral nerves were removed and the tissues were then transversely cut at the center of the nerves stained with standard hematoxylin and eosin, azan (for evaluation of the rejection reaction), toluidine blue, Kluver and Barrera stain and Bodian stain (for counting the myelinated fibers) for examination with light microscopy. Morphometric assessment was based on the following four parameters: 1) total regenerated axon count in the grafted nerve (AC), 2) total regenerated axon count in the grafted nerve/axon count in the contralateral nerve = axon count ratio (ACR), 3) total regenerated axon count/$10^4 mm^2$ = axon count density (ACD), and 4) the grafted nerve ACD/contralateral

Table 1. Observation in 204 nerve grafted rats. Values are mean

Graft drug p.o. weeks	Sciatic nerve (n 102)					Saphenous nerve (n 102)				
	n	AC	ACR	ACD	ACDR	n	AC	ACR	ACD	ACDR
Autograft none										
6	8	12.3	1.50	393	1.92	8	2.61	3.57	435	1.87
10	8	11.2	1.36	462	2.26	8	1.88	2.57	482	2.07
12	8	12.2	1.49	398	1.96	8	1.51	2.06	416	1.79
Allograft none										
6	8	1.56	0.18	53	0.26	8	–[a]	–	–	–
10	8	2.36	0.28	206	1.01	8	–	–	–	–
12	8	3.89	0.47	346	1.69	8	–	–	–	–
DSG 6 weeks										
6	6	20.0	2.43	298	1.46	6	1.61	2.20	236	1.01
8	6	18.4	2.23	467	2.28	6	1.88	2.57	382	2.61
10	6	28.1	3.41	404	1.98	6	2.28	3.12	276	1.18
12	6	29.8	3.61	335	1.64	6	1.82	2.49	267	1.15
DSG continuous[b]										
8	6	14.3	1.73	333	1.63	6	2.86	3.92	320	1.38
12	6	19.4	2.36	553	2.71	6	3.26	4.45	505	2.17
CsA 6 weeks										
6	6	18.6	2.26	326	1.59	6	1.62	2.21	363	1.56
10	6	18.4	2.23	259	1.27	6	1.49	2.04	230	0.99
12	6	13.9	1.69	268	1.31	6	0.97	1.33	261	1.13

AC axon count, ACR axon count ratio, ACD axon count density (axon count/10^4mm^2), ACDR axon count density ratio, DSG 15-deoxyspergualin, CsA cyclosporine A
[a] uncountable because of severe rejection.
[b] continuous DSG therapy group.

nerve ACD = axon count density ratio (ACDR). Axons 1 µm or more in diameter were counted, but minute nerve fibers smaller than 1 µm were not. Nerve fibers with multiple branches were also excluded. The data were analyzed using the Student' t test. A p-value less than 0.05 was considered significant.

Results

Autograft

Both sciatic and saphenous nerve autografts induced excellent nerve regeneration early after surgery, but the mean AC and ACD decreased after week 6. The grafted nerve showed no evidence of rejection. Although the mean ACR and ACDR of the two nerve autografts were not significantly different, the mean ACR of sciatic nerve autografts was significantly higher than that of the saphenous nerve autograft at weeks 6 and 10 (Table 1).

Allograft without immunosuppression

Both sciatic and saphenous nerve allografts were strongly rejected early after surgery and nerve regeneration was very poor. At week 6, sciatic nerve allografts showed severe rejection, with lymphocyte infiltration around the graft. At week 12, lymphocyte infiltration had decreased (Figure 1) and the mean AC had gradually increased. At week 10, half of the saphenous nerve allografts had disappeared due to rejection necrosis.

Allograft with DSG immunosuppression

Short course of therapy. At week 6, when administration was stopped, both sciatic and saphenous nerve allografts showed no evidence of rejection histologically, but at week 8 infiltration by many lymphocytes

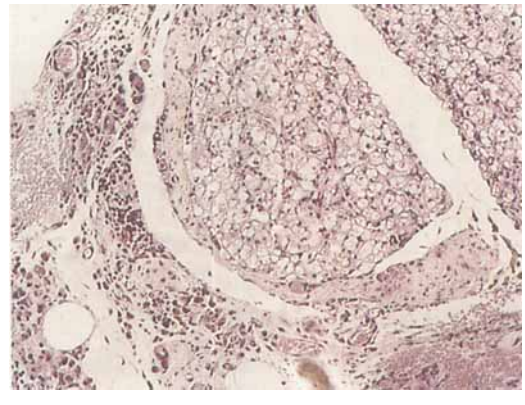

Figure 1. Sciatic nerve allograft without immunosuppression at week 12. The grafted nerve showed no rejection and vacuolar degeneration (arrow). Nerve regeneration was very poor (HE, ×40).

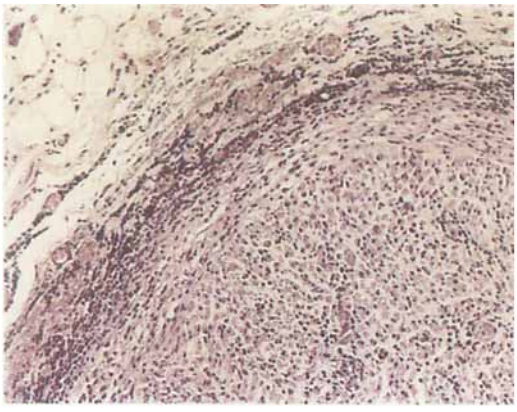

Figure 2. Sciatic nerve allograft with short-course 15-deoxyspergualin immunosuppression at week 12, showing rejection. Lymphocyte infiltration was observed around and within the grafted nerve (HE, ×100).

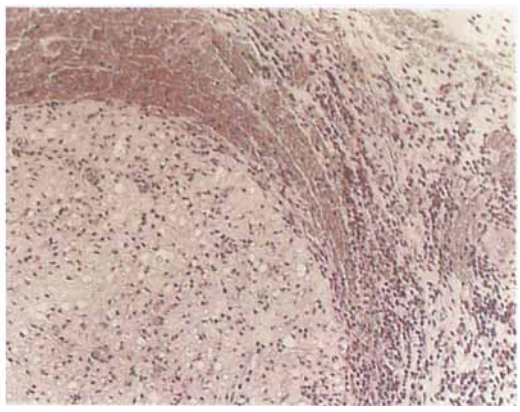

Figure 3. Sciatic nerve allograft with short-course cyclosporine A immunosuppression at week 9. Much lymphocyte and red cell extravasation was observed around the epineurium and nerve regeneration was poor (HE, ×100).

was observed around the epineurium, indicating rejection. At week 10, lymphocyte infiltration had extended within the grafted nerves and this evidence of rejection persisted until week 12 (Figure 2) and partial vacuolar degeneration was observed at week 12. The mean ACD and ACDR at week 10 and the mean ACR at week 12 were significantly higher in the sciatic nerve allografts than in the saphenous nerve allografts (Table 1).

Continuous therapy. Until week 6, during the period of daily administration, the rats showed deterioration of their general condition. After week 6, however, when DSG was intermittently administered, they gradually gained body weight. Histologically, both sciatic and saphenous nerve allografts showed excellent nerve regeneration, and intraneural fibrosis was very mild. Morphometric evaluation showed that the mean ACR in the saphenous nerve allografts at weeks 10 and 12 was significantly higher than in the sciatic nerve allografts.

Allograft with CsA immunosuppression

During administration, the rats' general condition was good. Histologically, the grafted nerve showed good regeneration at week 6, when CsA was withdrawn, but at week 8, lymphocyte infiltration around the epineurium and partial extravasation were observed, indicating a severe rejection reaction. At week 12, the signs of rejection had almost disappeared (Figure 3) and some regenerated axons remained. The mean AC and ACR of the short-course DSG therapy group were significantly higher than in the CsA group in both the sciatic and saphenous nerve allografts at weeks 10 and 12.

Discussion

Nerve regeneration with different forms of autografts have been compared by many authors. Maccabruni (1911) compared autografts of different diameters and found that thin autografts successfully survived as a result of early revascularization from transplant beds. On the other hand, Sanders (1942) studied an allograft using no immunosuppressant and reported that thick allografts were severely rejected because they included many antigen-presenting cells. We believe that similar studies should be performed using temporary immunosuppressant therapy.

Methods for comparing nerve regeneration in nerves differing in diameter involve certain problems. Comparison of motor function recovery is not always possible—e.g., the saphenous nerve is a pure sensory nerve. In our study, the 4 histomorphometric parameters appeared relevant to assess nerve regeneration.

It was interesting to find that, during immunosuppression, both the sciatic and saphenous nerve allografts showed better nerve regeneration autografts. Bain et al. (1988) reported that CsA itself might have a nerve regenerating effect, but others have not confirmed this. After 6 weeks of treatment with DSG, the histologically-determined rejection and the time at which rejection appeared did not differ between the two allograft groups. However, the regenerated axon count after the rejection was higher in sciatic nerve allografts. In the experiment using short-term CsA treatment, we obtained the same results. This suggests that large-diameter nerve allografts, which had great healing capacity and could provide many more regenerated axons, were advantageous in protecting axons against the rejection reaction after withdrawal of the immunosuppressants.

Comparison of short-term therapy with DSG and CsA showed evidence of rejection in both cases after withdrawal of the drug, but the duration of rejection was different. The rejection reaction had already disappeared at week 12 in nerve allografts with CsA, but was still present in the DSG group. Both the sciatic and saphenous nerve allografts showed better nerve regeneration with DSG than with CsA. The rejection reaction might be prolonged because of the residual incomplete immunosuppressive effect of DSG which was considered advantageous for early migration of recipient-derived Schwann cells and protecting regenerated axons.

In peripheral nerve allotransplantation, our results suggest that a short course of DSG treatment could provide better nerve regeneration than CsA treatment. Concerning the type of donor nerve allografts, large caliber nerve allografts were more advantageous in terms of residual regenerated axons than small caliber nerve allografts.

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