Experimental and Clinical Study on Peripheral Nerve Allografting

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Summary

There are numerous factors that affect the success of peripheral nerve grafting. Among these are (1) the type of injury to the nerve; (2) the degree of damage to the surrounding tissue and its complications; (3) the general condition of the patient; (4) the age of the patient; (5) the size, level and location of the nerve defect; (6) the local nutrition and the amount of scar in and around the nerve; (7) the accuracy of repair and the suture method; (8) the time lapse between injury and grafting; (9) the kind, length and thickness of the graft; (10) sterilization and preservation methods of the graft; (11) the problem of histocompatibility; (12) the method of immunosuppression; and (13) the rehabilitation method.

In the authors' clinic, research has been carried out to improve the methods of sterilization and preservation using a Cialit solution and cathode irradiation, the immunosuppressive method using steroid and Imuran, and microsurgical techniques such as sheathing method, full thickness grafting and cable grafting. The results of these experimental studies were applied in some clinical cases.

The experimental and clinical data on the peripheral nerve allografting in our clinic have been reported in this paper.

Key words: Allografting, xenografting, rejection reaction

Introduction

Repair of a large defect in a peripheral nerve caused by injury is one of the most difficult and frustrating problems for surgeons. The purpose of peripheral nerve transplantation is to provide a pathway for the nerve fibers to regenerate through the defect between the proximal and distal nerve stumps. A survey of the literature on the repair of peripheral nerves using grafts emphasizes the difficulty in inhibiting the immune response of the host to the graft. Since the first autogenous nerve transplantation in the dog by Phillipeaux and Vulpian in 1870 and the first homograft in a human by Albert in 1878, various modifications have been tried to improve the transplantation method up to recent years.

In the authors' clinic, research has been performed to improve the methods of sterilization and preservation using a Cialit solution and cathode irradiation, the immunosuppressive method using steroid and Imuran, and microsurgical techniques such as sheathing method, full thickness grafting and cable grafting, and the results of these experimental studies were applied in some clinical cases. The purpose of this paper is to present the results of such experimental and clinical studies in our clinic on peripheral nerve allografting. The experimental work includes xenografts which provide easier investigation of the rejection reaction in animal experiments.

Materials and Methods

Experimental animals used in this study were adult mongrel dogs, albino rabbits and Sprague-Dawley rats. Histological observations were performed with hematoxylin-eosin, Bodian's axon, Masson trichrome and pyronine stains.
A fluorescent microscope and a scanning electron microscope were also used in this study. Cathode irradiation was performed with Van de Graaff generator.

**Results**

**I. Rejection reaction of the host**

Edema and bleeding were observed in and around the graft in histological studies 1 to 3 days after fresh allo- and xenografting in dogs. One week after grafting, round cells were found to have invaded the graft from the suture lines and the epineural tissue. The round cell invasion reached a maximum in the third week after grafting. The axons regenerating from the proximal stump were seen to grow through the inside of the graft, but with severe attacks by round cells. The orientation of the regenerating axons was much distorted by proliferation of fibroblasts, especially three weeks after grafting. The disoriented axons produced a neuroma with proliferated connective tissue. The graft also adhered to the surrounding soft tissue. The cell proliferation was most pronounced at the distal suture line. Only a few axons were observed at the distal nerve trunk, they had been replaced by fibrotic connective tissue after Wallerian degeneration.

**II. Grafting and the regional lymph nodes**

Gallone, Scothorne, et al. pointed out the importance of the regional lymph nodes in the rejection of skin grafts. However, little has been reported regarding nerve grafts. Since the humoral antibody was not observed by the immunodiffusion method during the first four weeks after the nerve grafting, a study was made of cellular changes in the regional lymph nodes draining the implanted nerve. Our study showed a characteristic increase in the number of pyroninophilic large lymphoid cells, especially in the cortex of the regional lymph nodes. This response was not considered to result from the traumatic injury of the incision. The maximal response was observed on the third day after the implantation of the nerve, with a correlated increase in the weight of the lymph nodes in the early period of cell invasion. The cell invasion was at its maximum in the 2nd and 3rd weeks after the implantation of the nerve. In the third week after the implantation, the lymph nodes had almost the same appearance as the control. These findings suggest that some antigenic factors of the nerve graft travel by way of the lymphatic system to the regional ipsilateral lymph nodes. The pyroninophilic large lymphoid cell seems to play an important role in forming the antibody. This antibody is considered to be freed via a lymphatic pathway in the form of cell-bound-antibody and to cause a chronic inflammation at the site of the antibody, i.e., the nerve graft. This cellular response was more prominent in the xenograft than in the allograft. There were no obvious changes in the contralateral lymph nodes, spleen and adrenals. It is interesting to note that the cellular infiltration in the graft was preceded by the reaction of regional lymph nodes.

**III. Study on the fluorescent antibody of the graft**

According to the direct observation method of the fluorescent antibody using rabbit serum with anti-dog y-globulin labeled with FITC, there was accumulation of y-globulin in the myelin and the perineurial tissue with pinpoint fluorescence on the axon substance from the third to seventh day after fresh nerve grafting. The accumulation started to decrease from seventh day and no accumulation was observed in the fourth week after grafting. The accumulation of y-globulin was observed in the myelin substance up to the fourth week, while it disappeared on the perineurial connective tissue in the second week after grafting. This suggests that the myelin substance plays an important role in the rejection reaction.

**IV. Study on regeneration of the autonomous nerve fiber through the graft**

Sympathetic nerve cells synthesize monoamine and send it distally by axoplasmic flow down the nerve fibers. The authors thought that periodic checking of the fluorescent substance (monoamine) which accumulates at the distal stump of a regenerating nerve fiber would give us a useful index as to the amount and the pattern of any nerve fiber regeneration that occurs after nerve allografting. A method to investigate the presence of monoamine by fluorescent microscopy was published by
Eraenkoe\textsuperscript{23} in 1952, and by Falck and Hillarp\textsuperscript{24} in 1962. The method provides a practical means for histochemical investigation of the monoaminergic neuron. The purpose of this study is to investigate the regeneration pattern of monoaminergic nerve fibers in experimental animals after peripheral nerve allografting. The authors' experiment showed a massive accumulation of monoamine, which started to spread distally from the proximal nerve suture into the nerve graft in the first week after transplantation. By the fourth week after transplantation, the monoaminergic nerve fiber extended further distally in the graft—down through the midportion and into the distal portion of the graft. By the 24th week after transplantation, the monoaminergic nerve fiber had regenerated down beyond the portion of the distal suture into the distal nerve stump. There was no accumulation of the monoamine in the nerve fibers there, however, and the monoaminergic nerve fiber showed less fluorescence than in the initial stage of the regeneration. These findings suggested that the monoaminergic nerve fiber had regenerated through the nerve allograft down into the end organ, without any obvious obstruction in the passage of the proximo-distal axonal flow of the nerve fibers. The extensive experimental studies of Weiss\textsuperscript{102,103} (1944) showed that nerve fibers follow the direction of oriented fibrillar units along their contact substratum, taking the course of the least mechanical resistance. The orientation of the regenerating nerve fibers is reportedly effected through the mediation of Schwann cords, or by a pathway structure such as the framework of the endoneural tube. The results of the authors' experiment indicate that the elastic fibers precede the axons, aligning themselves along the linear structures just as Schwann cells and axons do. This finding suggests that the elastic fibers also play a role in the orientation of regenerating connective tissue and nerve fibers.

V. Study on the regeneration of the vascular system through the graft
The blood supply of peripheral nerves was first investigated by Hyrtl\textsuperscript{41} (1859), Adams\textsuperscript{1} (1942) and Sunderland\textsuperscript{93} (1947) showed that each peripheral nerve is vascularized abundantly throughout its entire length by a succession of divisions and anastomosis. Tarlov\textsuperscript{95,96} (1944) insisted on the importance of the blood supply of the regenerating nerve trunk to obtain successful nerve grafting. Smith\textsuperscript{96} (1966) reported that the state of local nutrition, the amount of scar tissue at the site of repair, and the length of the nerve gap are known to be important factors in regeneration of the nerve tissue, and that all are related either directly or indirectly to the blood supply of nerves. The authors studied the development of the vascular system in the grafted tissue. According to the authors' experiment, proliferation of the capillaries around the graft was found to be extreme in the third to sixth week of the transplantation. The network appeared to form a cylindrical tube with fine anastomosis with the intraneural vessels. This proliferation of fine blood vessels from the surrounding tissue became almost complete in the 24th week after transplantation. Concerning the intraneural vessels, on the other hand, vascular proliferation in the longitudinal direction started to enter the inside of the graft from both the proximal and distal suture sites in the third week. In the 24th week, the intraneural vessels were abundant. As mentioned above, there were two types of proliferation of the blood vessels, i.e., intraneural and extraneural vessels, with proliferation coordinated with the degree of the inflammatory condition and the neural regeneration. The bundle-like arrangement of the vessels in the successful nerve graft appeared stereoscopically to be related to funiculi containing the regenerating nerve fibers.

VI. Scanning electron microscopic study of nerve grafting
It has reportedly been very difficult to analyse the mechanism of regeneration of nerve fibers in nerve grafts, because of complicated problems such as autolysis of the graft and the rejection reaction in the host. The authors intended to study the regeneration mechanism of nerve tissue, especially connective tissue and nerve fibers, using an S.E. microscope in experimental nerve homografting in the dog. Examination by S.E. microscope of specimens two years after the grafting in the authors' experiment revealed numerous mature regenerating nerve fibers within the complex capil-
laries and proliferating collagen fibers at the proximal suture site. In the middle of the graft, the remnants of proper nerve elements of the graft such as myelin and axons were hardly detected. They appeared to be replaced by the host tissue. Scarcely-and well-oriented frameworks composed of connective tissue were noted at this stage. On the whole, irregular proliferation of collagen fibers appeared to predominate, with mature regenerating nerve fibers running longitudinally through them. These nerve fibers formed funiculi of various sizes assuming a bundlelike appearance, surrounded by proliferating collagen fibers. The number of regenerating axon was markedly reduced as they passed through the distal suture site. Marked degenerative findings were predominant in the nerve trunk distal to the graft which contained very small numbers of regenerating nerve bundles. These results indicate that the proliferation of connective tissue of the proximal and distal sites caused a decrease and disorientation of the regenerating axons. The canal appearing as a framework consisting of connective tissue and other elements disappeared after a long time following grafting, and was replaced by regenerating nerve fibers and proliferating collagen fibers.

VII. Trials to improve transplantation methods

1. Treatment of the graft with Cialit

Cialit was applied for preservation of bone, artery and tendon. Egawa21) (1973) used it for preservation of the nerve tissue.

Enya22) (1969) employed various concentrations of Cialit for preservation of the nerve graft. According to his experiment, allotransplants which are treated with 5,000 × Cialit and stored frozen in a freezer (−20°C ~ −25°C) reveal less tissue reaction, such as less invasion of round cells, macrophages and fibroblasts with a better preservation of the canal structure of the connective tissue, compared with the fresh allograft and saline treated nerve. He also obtained better axonal growth with nerve allografting in the peroneal nerve of the dog. Cialit, however, is not used now in the authors’ clinic because the mercury compound is prohibited in clinical use because of its toxicity.

2. Cathode irradiation of the graft

Recently, Campbell15,16) (1963) and Marmor56-58) (1963, 1964) used high voltage cathode irradiation of the nerve graft and reported its effect on the sterilization of the graft and some suppression of the rejection reaction. The authors’ experiment revealed that the nerve xenograft implanted in the subcutaneous tissue irradiated with 5 × 10⁶ r.e.p. appeared to suppress round cell and fibroblast invasion and produced less disorganization of the connective tissue than when other doses of irradiation were used. In contrast, irradiation by more than 10 × 10⁶ r.e.p. produced a distorted connective tissue matrix which resulted in a thick, homogenous, poorly vascularized layer around the degenerated myelin substance. Microscopically, the implanted allograft showed obvious tissue damage in the nerves that had been irradiated with over 5 × 10⁶ r.e.p. It appears that allografts irradiated at 2 × 10⁶ r.e.p. or 5 × 10⁶ r.e.p. produce the most inert and favorable conduit for axonal regeneration in the nerve graft. The implanted xenograft was chosen for the time lapse study because no significant foreign body response was observed in the rat receiving an implanted allograft. The relation of the interval of time between irradiation and implantation reveals that there is less reaction if the frozen nerve is implanted within a maximum of 8 weeks after irradiation. Implants used within this period revealed that cell invasion and collagen deposit are minimal and connective tissue is less distorted than in nerve grafts stored for longer periods.

3. Effect of sheathing the nerve graft

Since the 18th Century, various sheathing methods have been devised to protect the suture site of the nerve from cellular infiltration and to prevent neuroma formation. Since 1965, the authors have been developing a sheathing method utilizing a silicone tube and have obtained favorable results. Further improvements have subsequently been added and various experiments were undertaken, such as sheathing with an irradiated xenoartery, with a silastic sheet and with an autogenous vein. All of these methods, however, have advantages as well as disadvantages, and none can be considered the best. Observation of the
sutured portion after sheathing shows that no complete barrier has been formed against cellular infiltration, except for the less complex state of axonal bursting with maintenance of favorable directionality. The paucity of suture thread at the repair site also appears to be a major factor. Another important factor is the alleviation of tension or torsional stress acting across the sutured portion. Finally, in the presence of an extensive soft tissue wound of the extremities, prevention of adhesion between the nerve and the soft tissue also appears to be an important factor. In the authors' experiment, it was also detected that lyophilized human dura stimulates little foreign body reaction and provides sufficient support to the suture site, in spite of its flexibility, indicating that it is highly possible that the sheathing method will be effective. Fibrin membrane, on the other hand, is thin and fragile but has an advantage of not creating perineural adhesion. A secondary operation including the transplant may also be carried out advantageously with the sheathing method.

4. Effect of Imuran administration on the host

Imuran has been shown to be an immunosuppressive agent, although the mechanism involved in suppression of the immunological response remains obscure. The use of antagonists of nucleic acid biosynthesis in experiments with dogs has prolonged the survival of the allografts of the kidney and other organs. According to the experiment of Marmor and Hirasawa\(^5\) (1968), the combination of the irradiation of xenografts with Imuran treatment decreased the cellular infiltration and subsequently decreased fibroblast invasion. Fibrosis was reduced to an extent which allowed axon regeneration through the graft. There is encouraging evidence that satisfactory results could be obtained using irradiation of the xenograft combined with administration of Imuran in minimal doses. A experiment with dogs, however, revealed that the prolonged administration of Imuran (1–2 mg/kg B.W./day) for more than four weeks produces frequent pulmonary infections. These immunosuppressive drugs should be carefully used clinically because of the serious side effects.

5. Trial of enzymatic treatment and lyophilization of nerve grafts

Preservation of the nerve graft is one of the most difficult problems and freezing is not the solution of this problem. The authors considered that lyophilization is one of the ways to overcome this problem. Decreasing the antigenicity of the nerve graft is another problem. The result of the authors' experiment suggested that the myelin substance and the perineurial connective tissue, at least, are antigenic. The authors consider that it is worthwhile to decrease the antigenicity of the nerve graft by removing lipids and some proteins from the nerve tissue using enzymes. The authors intend to provide a lyophilized nerve graft by removing lipids and some proteins by enzymes, combined with the irradiation method using the Van de Graaff generator. This experiment is now on progress. However, disadvantages of this graft are that it is difficult to provide a smooth, mucous pathway for the regenerating axons, and that it does not include Schwann cells which are important for the regenerating nerve fibers. These disadvantages should be overcome by further experiments.

Clinical Study of Nerve Allografts

There are numerous factors that affect the success of a peripheral nerve grafting. Among these are (1) the type of injury to the nerve; (2) the degree of the damage to the surrounding tissue and complications; (3) the general condition of the patient; (4) the age of the patient; (5) the size, level and location of the nerve defect; (6) the local nutrition and the amount of scar in and around the nerve; (7) the accuracy of the repair and the suture method; (8) the time lapse between injury and grafting; (9) the kind, length and thickness of the graft; (10) sterilization and preservation methods of the graft; (11) the problem of histocompatibility; (12) the method of immunosuppression; and (13) the rehabilitation method.

When pieces of nerve are obtained from a donor, there will be comparatively few problems if they are taken directly from the amputated limbs but there may be some ethical and religious problems involved if they taken
from a cadaver. The authors take nerve grafts from amputated limbs as a rule. Moreover, the authors exclude those donors who have malignant tumours, syphilis, tuberculosis and other infectious diseases. In performing a nerve graft, a thorough examination of the indications is essential. Of course, it is not necessary to use only allografting when there

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is a possibility of satisfactory recovery by nerve autografting. In the clinical cases reported so far, the results of nerve grafting have not been satisfactory. This is probably because nerve grafting is performed as the final step after the lapse of a long period of failures in many attempts at various treatments. Even in the simple suture of the nerve, recovery of the motor function becomes difficult when a year and half have passed before surgery, and recovery of sensory functions is also difficult in many cases if there are 2 or 3 bland years. Therefore, it is also necessary to consider this point in nerve grafting. Injury to arteries, tendons, bones, and other soft tissues makes the prognosis of nerve grafting poor and complicated. Unfortunately, cases with nerve allografting indications are mostly complicated with multiple injuries which makes the prognosis poor. Boehler (1966) reported seven cases of useful recovery out of 17 cases. Campbell (1963) obtained 50 per cent recovery with the irradiated nerve allografting. Jacoby (1972) reported about 80 per cent recovery with lyophilized, irradiated nerve allografts. The clinical experience of the authors is shown on Table 1. To obtain better results in nerve grafting, further research should be continued, especially to improve graft preparation.

Conclusion

The experimental and clinical studies which have been performed in the authors' clinic are introduced in this paper. Less antigenic nerve grafts which have a smooth and soft medium for the regenerating nerve fibers should be obtained by further experiments.

References

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