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Schwann Cells Transplantation Promoted and the Repair of Brain Stem Injury in Rats[1]

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Objective To explore the possibility of Schwann cells transplantation to promote the repair of injured brain stem reticular structure in rats. **Methods** Schwann cells originated from sciatic nerves of 1 to 2-day-old rats were expanded and labelled by BrdU *in vitro*, transplanted into rat brain stem reticular structure that was pre-injured by electric needle stimulus. Immunohistochemistry and myelin-staining were used to investigate the expression of BrdU, GAP-43 and new myelination respectively. **Results** BrdU positive cells could be identified for up to 8 months and their number increased by about 23%, which mainly migrated toward injured ipsilateral cortex. The GAP-43 expression reached its peak in 1 month after transplantation and was significantly higher than that in the control group. New myelination could be seen in destructed brain stem areas. **Conclusion** The transplantation of Schwann cells can promote the restoration of injured brain stem reticular structure.

Key words: Schwann cell; GAP-43; Brain stem

INTRODUCTION

It is well known that the injured central nervous system is hard to restore itself and might lose its function forever. Since Schwann cells are able to promote axonal renewal and remyelination in peripheral nervous system by secreting a variety of neurotrophic factors, extracellular matrix and cell adhensive factors, they attract more and more attention in repairing and regenerating the central nervous system[1-3]. In the present study, we transplanted Schwann cells derived from sciatic nerves of newborn rats into the rat brain stem reticular structure pre-injured by electric needle stimulus. The survival of transplanted Schwann cells and their effect on repair and regeneration of the central neural system was explored.

MATERIALS AND METHODS

Culture of Newborn Rat Schwann Cells in vitro

The bilateral sciatic nerves of 1 to 2-day-old rat pups obtained under aseptic conditions were placed into L-15 medium. After epineurium and connective tissue were removed under

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microscope, the nerves were cut into 2 mm^3 segments and were digested with 0.25% trypsin and 0.125% collagenase for 15 min at 37℃, then were centrifuged at 1 000 rpm/min for 6 min. The precipitation were re-suspended with DMEM supplemented with 10% fetal bovine serum, 2 μmol/L forskolin (Sigma) and 20 mg/L bovine pituitary extract (Sigma), placed at 37℃ in 5% CO_2. The medium was changed twice a week. When cells were in good growth state, BrdU at a final concentration of 10 μmol/L was added into the medium and was kept for 24 h[4-6].

Electric Needle Injury of Rat Brain Stem Reticular Structure and Schwann Cells Transplantation

The wistar adult rat was anesthetized with pentobarbital (50 mg/kg, IP), its head was fixed in a stereotactic frame, skull was drilled and an electric needle was introduced into the brain according to rat stereotactic coordinates: 5.2 mm posterior to frontal fontanelle, 1.5 mm lateral to midline and 6 mm below the surface of skull. Anodic current of 1.5 mA was applied for 30 seconds. Two minutes later the needle was drawn out, a micro-needle was introduced into the same hole, and 5 μL (1×10^5 cells) Schwann cells suspension was injected into the brain stem very slowly. In the control group, the brain stem reticular structure of rat was electrically injured, but no Schwann cells were transplanted. There were 6 animals in each group.

Preparation of Tissue Section of Injured Brain Stem

In 2 weeks and 1, 2, 4, 6 and 8 months after electrical injury and Schwann cells transplantation, the rat was anesthetized by injecting 10% chloral hydrate into abdominal cavity, and 250 mL physiological saline was perfused into the heart via cannula, followed by 4% paraformaldehyde-phosphoric acid (0.1 mol/L). The rat brain was taken out 1 h later and fixed in the above-mentioned solution at least for 6 h. The brain was transferred to 20% sucrose solution for dissolving fixation fluid, and then was frozen at -70℃. The frozen brain was sectioned using cryoultramicrotome (Leitz, Germany) into 12 μm thick slices 2 mm anterior and posterior to the needle entry site. The sections smeared on the slides were dried with cold wind, and then were stored at -70℃.

Detection of BrdU and GAP-43 Expression

BrdU labelling and GAP-43 expression were measured by immunohistochemical method. After being taken out from freezer, the slides dried at room temperature for 2 h were placed into sodium citrate buffer (0.9 mL of 0.1 mol/L citric acid, 4.1 mL of 0.1 mol/L sodium eitrate and 45 mL of H_2O) and heated at 92℃-98℃ for 10 min. After being washed with PBS buffer, tissue sections on the slides were treated with normal goat serum for 20 min to block nonspecific reaction. The primary antibodies (1:100 mouse anti-BrdU monoclonal antibody; 1:20 mouse anti-GAP-43 monoclonal antibody, Oncogene, USA) were added to the sections and kept overnight at 4℃, and then were rinsed with PBS buffer and exposed to secondary antibody (1:200 goat anti-mouse IgG-biotin, Vector, USA) at 37℃ for 40 min. The final reaction was carried out with HRP-labelled streptavidin at 37℃ for 4 h. DAB was used for color development. The mean optic density (MOD) values were recorded by a color imaging analyzer.

Myelin Staining

Some brain samples were used to make 50 μm thick frozen sections. After BrdU

immunohistochemical staining, the staining positive areas were selected to make semi-thin sections (0.5 μm) for myelin staining with azure methyleneblue, and observed under oil-microscope.

Statistics

The results of BrdU and GAP-43 staining were presented as $\bar{x} \pm s$. Student's t test was used for statistical evaluation.

RESULTS

In vitro Newborn Rat Schwann Cells

Two types of cells were visualized under phase contrast microscope. One type was shuttle-shaped with oval nucleuses and two threadlike processes at two cell body poles, which was Schwann cell. The other one was flat-shaped with round nucleuses and multi-processes, which was fibroblast cell. More than 95% cells were Schwann cells (Fig. 1).

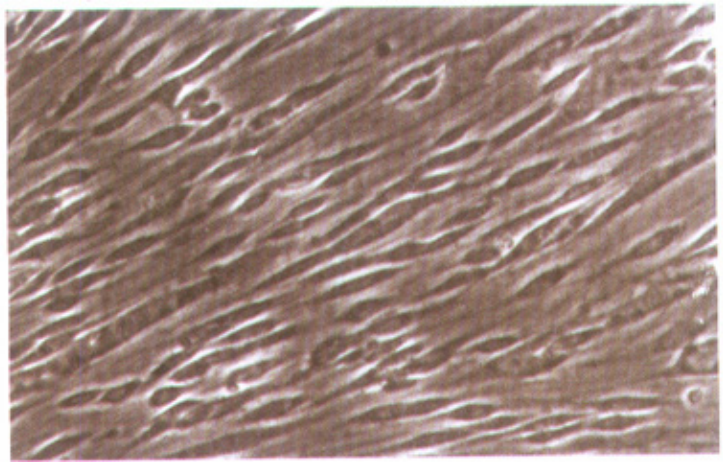

FIG. 1. *In vitro* newborn rat Schwann cells, one kind of cells were shuttle-shaped with oval nucleuses and two threadlike processes at two cell body poles, these are Schwann cells; the other kind of cells were flat-shaped with round nucleuses and multi-processes, these are fibroblast cells. More than 95% cells were Schwann cells under phase contrast microscope (240 ×)

Survival of Transplanted Schwann Cells

Rats were sacrificed at different time after transplantation and BrdU immunohistochemical staining was performed on brain sections. Under microscope, BrdU positive cells were oval and brown. Their number increased gradually in the 8 month observation period. Statistical analysis showed that the number was 23% higher than that in the control group. These cells migrated mainly toward the cortex (Fig. 2).

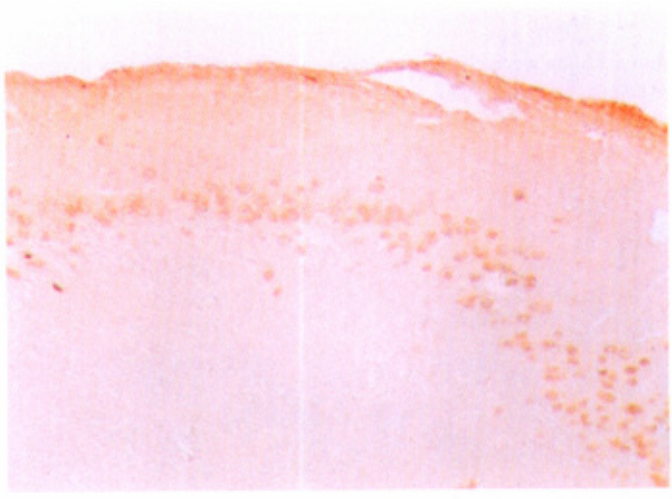

FIG. 2. Immunohistochemical staining for Schwann cells incorporated BrdU and implanted into injured brain stem reticular structure 8 month in rat. BrdU positive cells were oval and brown and mainly migrated toward the cortex (120 ×).

The Axonal Regeneration of Injured Neurons

GAP-43 immunoactivity was present in some neurons of injured stem reticular structure. Some brown granules were scatted or coagulated under cell membrane and in cellular processes. These cells were seen as early as 2 weeks after injury, reaching their quantity peak 1 month later (Fig. 3) and disappearing 4 months later.

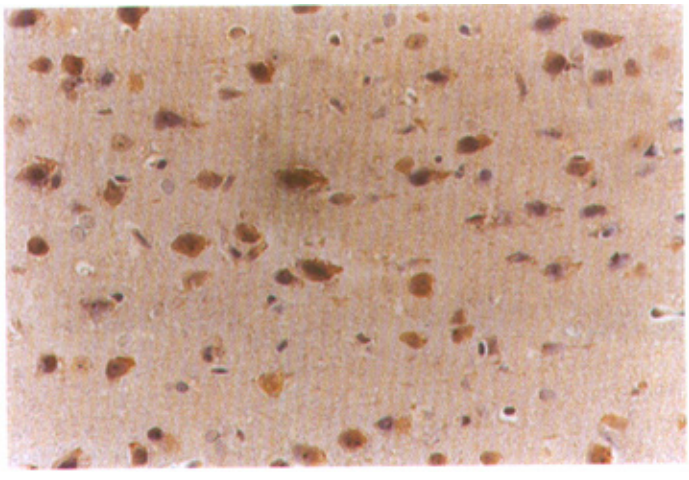

FIG. 3. GAP-43 immunohistochmical staining. It was shown as some brown granules scatted or coagulated under cell membrane and in cellular processes in some neurons 1 month after Schwann cells were implanted into injured stem reticular structure (120 ×).

The Regenerative Myelin

Semi-thin sections were visualized under oil-microscope. Newly generated myelins were multi-layers and stained blue, which enveloped the injured axons. Around them there were proliferative fibrous tissues (Fig. 4).

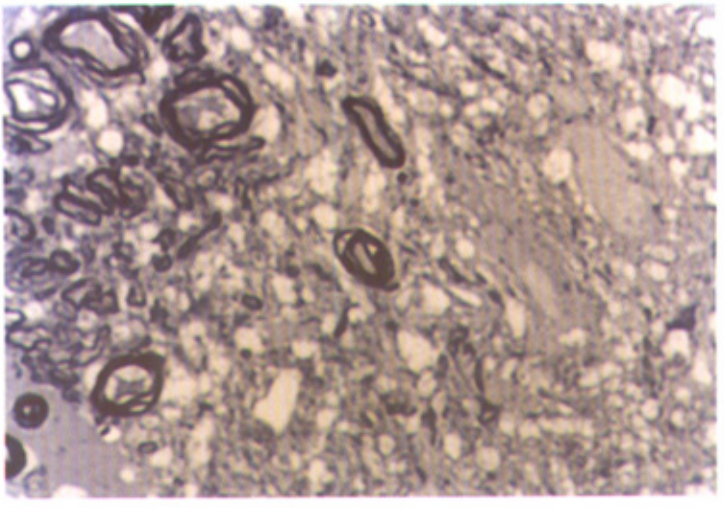

FIG. 4. Semi-thin sections were visualized after Schwann cells were implanted into injured brain stem reticular structure. Newly generated myelins were multi-layers and stained blue which enveloped the injured axons (600 ×, under oil-microscope).

DISCUSSION

The injury of brain stem reticular structure as one of life centers is fatal. To reduce the disturbance of hemorrhage and space occupation and to make the function of Schwann cells more clearly observed, electrical injury animal models were selected. This kind of injury causes coagulative necrosis around the lesion with neurons nearby damaged at different degree and hemorrhage hardly observed. In the present study, we micro-injected Schwann cells in destructed brain tissue and found that Schwann cells labeled with BrdU survived, actively proliferated and distinctly migrated toward the cortex 8 months after transplantation. These findings are coincident with the previous reports[7-10]. It is not yet clear whether the long-distance migration of Schwann cells is due to the electrical wave conduction during electrical injury or not.

GAP-43, which is largely synthesized in nerve tissue during the development and regeneration of neurons, is the molecular substance of nerve reconstruction and regeneration. It takes part in axonal growth and synapse formation[11-13]. In the present study, GAP-43 expression reached its peak 1 month after Schwann cells transplantation, implying that implanted Schwann cells did promote regeneration of neurons and extension of injured axons. Chen *et al.* found that GAP-43 expression reached its peak 2 weeks after spinal cord lesion and then dropped gradually. Its expression in the 12th week was not significantly

different from that in the 1st week[14]. The mechanism how GAP-43 promotes regeneration and reconstruction is not yet clear. Recent studies suggest that GAP-43 is one substrate of protein kinase C (PKC), which is able to mediate the reconstruction of axonal cytoskeleton and to promote the budding of injured axons.

We also found newly generated myelin in the present study. Since transplanted Schwann cells were pre-labeled with BrdU, we could conclude that remyelination was due to implanted Schwann cells.

Till now, the results of Schwann cells transplantation in central nervous system have substantially varied from different experimental methods. Xu *et al.*[15,16] tested the ability of Schwann cells to enhance axonal regeneration in adult rat spinal cord by grafting Schwann cells seeded guidance channel into transected spinal cords. Thirty days later, they found that both myelinated and unmyelinated nerve fibers vigrously threaded into the channel containing Schwann cells, and the axonal regeneration was also closely related with the survival of implanted Schwann cells. As to the outcome of intracerebral transplantation of Schwann cells, however, no consensus was reached so far. Stichel CC *et al.*[17] injected Schwann cells suspension into the transected postcommissural fornix in adult rats and found that Schwann cells were rapidly distributed in extensive cortical areas. The cells survived for 8 months were poorly proliferated. Induced by Schwann cells, the dissected nerve fibers crossed the lesion site and reached their target tissues via their original routes. Regenerated nerve fibers could be remyelinated by oligodendrocytes. However, Yasushi *et al.*[18] achieved different results. They implanted Schwann cells which were transduced with Lac-Z gene into normal white matter of rats. Four weeks later, they found no Schwann cell but hyperplastic glial scar. Wilby MJ *et al.*[19] co-cultured Schwann cells with astrocytes *in vitro* and found that these two kinds of cells formed separate territories with sharp boundaries between them. Schwann cells migrated poorly when they were placed on astrocyte monolayers, implying that N-cadherin-mediated adhesive of astrocytes inhibited the widespread migration of Schwann cells in the central nervous system.

The mechanism of central nervous system injury and its recovery is so complicated that numerous questions involving cellular and molecular changes of pathology and physiology need to be solved. Now it is clear that Schwann cells implantation can promote the regeneration of injured central nervous system, which might become a new therapeutic way for central nervous system injury in the future.

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