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The Influence of Diabetes Mellitus on the Healing of Segmental Defect of Sciatic Nerve of Rat

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= **Abstract** =

To assess the influence of diabetes mellitus on the healing of segmental defect of rat, a defect measuring 5mm was made at right sciatic nerve in thirty-three adult female Wistar rats(control group:17, diabetic group:16). To induce diabetes in rats, Streptozotocin(50mg/kg body weight) was injected into tail vein after dissolution in saline solution. Both proximal and distal nerve ends were connected with 9mm long silicone tube, and the tube was filled with 10μl collagen(Vitrogen 100) solution.

Two and 4 weeks after the operation, electromyographic study(latency period and amplitude) and histologic examination(the number of myelinated axon, non-neuronal cell, and vessel at mid-chamber level, the mid-chamber cross-sectional area) after toluidine blue staining were carried out.

From the results, we concluded that diabetes mellitus retarded the healing process of segmental defect of sciatic nerve in rat. And we might suggest that if we meet this situation in clinical practice, we have to consider some supportive measures to overcome the bad effect of diabetes mellitus on the healing of nerve defect.

Key Words : Sciatic nerve, Diabetes mellitus, Segmental defect, Rat

INTRODUCTION

The occurrence of peripheral neuropathy in patients with diabetes mellitus has been recognized since 1864, and the first comprehensive report on clinical findings in patients with diabetic neuropathy did not emerge until 1945[14].

Clinically, orthopedic surgeons occasionally meet the situation with irreparable segmental defect of the peripheral nerve.

Many authors studied and reported about that situation experimentally and clinically[9-11,23]. They

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said there are useful methods for closure of the nerve gap, such as change of position, nerve graft, even muscle fiber interposition graft[9,23]. Recently some authors used entubulation technique to bridge the gap[3,21,23].

But till now, we couldn't find any report on the nerve regeneration in the situation of diabetes mellitus. We can expect the adverse effect of diabetes mellitus on the regeneration of nerve fibers. So we had done this experiment with entubulation model to clarify the effect of diabetes mellitus on the regeneration of the peripheral nerve fibers.

MATERIALS AND METHODS

Thirty-three adult female Wistar rats weighing between 200 and 250 grams were divided into a control group(17) and a diabetic one(16), and each group was further divided into 2 week and 4 week groups according to the follow-up period(Table 1).

Table 1. Grouping of Experimental Rats

Follow-up period	Control	Diabetes mellitus	Total
2 week	8	7	15
4 week	9	9	18
Total	17	16	33

Whole blood glucose level was tested by Life Scan(Johnson & Johnson Co., USA) from tail vein. Control group was the rats with blood glucose level between 80 and 120mg/100ml.

To induce diabetes, Streptozotocin(Boehringer Mannheim Biochemica, GmbH, Germany) solution was injected into the tail vein at a dose of 50mg/kg body weight. At 3 days after inducing diabetes, the rats with blood glucose level between 300 and 400mg/100ml were used as diabetic group.

All surgical procedures were performed under the loupe magnification(x4). Under the ketamine anesthesia(100mg/kg body weight), a segmental defect measuring 5mm was made at right sciatic nerve. Both proximal and distal nerve ends were connected with 9mm long silicone tube(Silastic Medical-Grading Tube, Dow Corning Asia Ltd., Hong Kong, inner diameter: 1.57mm, outer diameter: 2.41mm) with 10-0 nylon sutures. The defect was filled with 10μl collagen solution(Vitrogen 100, Celtrix Laboratories, Palo Alto, California) using 25μl Hamilton microliter syringe for the induction of early regeneration of nerve fibers[13,20], and the ends of the tube were sealed with petrolatum jelly(Figure 1). The collagen solution was made by mixing 500μl of Vitrogen 100(3mg/ml), 62.5μl of (x10)phosphate buffered saline, and 62.5μl of 0.1M NaOH solution. pH was corrected to 7.5, and maintained in liquid form at 4° C. When injected into the silicone tubes, the collagen formed a gel at body temperature[2]. The rats were housed in a temperature and humidity controlled room and were given food and water ad libitum. At 2 or 4 week after the operation, the rats underwent electromyographic recordings, and after the study, were sacrificed by perfusion of a fixative for histological examination.

Electromyographic examination :

With the rat under anesthesia using ketamine,

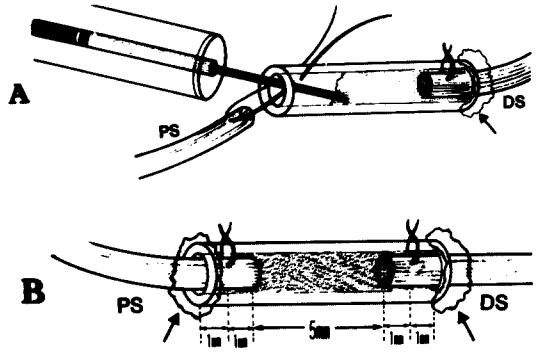

Fig. 1. Schematic drawing of the procedure.
A: After putting the distal stump(DS) into the silicone tube the dollagen solution is injected in liquid form followed by sealing off the distal end with vaseline(↑). Then the proximal stump(PS) of nerve is being introduced.
B: Both ends are sealed with vaseline(↑). At body temperature the mixture gels.

the sciatic nerve was exposed at the sciatic notch proximal to the silicone tube and stimulated supramaximally(CADWELL 7400 Lab., Kennewick, WA) with two silver wire electrodes. A recording needle electrode was placed in the gastrocnemius-soleus muscle group at a level 1 cm below the tibial tubercle. Compound muscle action potentials were recorded, and the distal latency and amplitude were measured[2,7].

Histological examination :

Just after electromyographic recordings, the abdomical cavity was opened, and 18-gauge needle was introduced into the abdominal aorta distally. The needle and aorta were ligated together with silk suture material. The rats were perfused with 100ml of heparinized saline solution(20IU/ml), followed by 200ml of a fixative containing 1% paraformaldehyde and 2% glutaraldehyde and with 100ml of 15% sucrose. All solutions were made up in 0.1M sodium phosphate buffer(pH 7.3).

The silicone tubes containing the regenerating nerve were harvested and cut transversely through the mid-chamber level with a scalpel blade. The regenerated nerve cables were easily separated from their surrounding silicone tubes by cutting the 10-0 nylon sutures. The regenerated nerves were postfixed in buffered 2% osmium tetroxide solution, washed in 0.1M sodium phosphate buffer (pH 7.3), dehydrated, and embedded in Epon 812. Transverse 1-μm-thick sections of the regenerated nerve were mounted on glass slides and stained with toluidine blue dye. The cross-sectional area at the mid-chamber level was measured using an image analyzer(Vidas 2.0, Kontron bildanalyse, GmbH, Germany), and the number of myelinated axon, non-neuronal cell, and vessel at the area were counted using transparent plotting film.

Statistics :

With electromyographic and histological results, statistical analysis was performed using 2-way ANOVA between control and diabetic groups, between 2 and 4 week groups. Statistical significance was accepted at the $P \leq 0.05$ level.

RESULT

Electromyographic examination(Table 2) :

No significant differences in distal latency and amplitude at 2 and 4 weeks were observed between control and diabetic groups($P>0.05$, each). But the latency period of diabetic group was decreased with statistic significance from 2 weeks(1.38 ± 0.09msec) to 4 weeks(1.24 ± 0.16 msec)($P<0.05$).

Table 2. Result of compound muscle action potentials in electromyographic study

(mean $\pm$ SD)

Group	Distal latency(msec)		Amplitude(mv)	
	2 week	4 week	2 week	4 week
Control	1.27 ± 0.15	1.16 ± 0.13	5.65 ± 2.92	8.64 ± 4.17
Diabetes mellitus	1.38 ± 0.09	1.24 ± 0.16	4.66 ± 3.26	7.54 ± 2.68

Histological examination(Table 3, Figure 2,3):

At 2 week after the operation, both groups showed some collagen matrix in the tissue of regenerated nerve and in the lumen of silicone tube.

There was no visible myelinated axons at 2 week, but at 4 week, numerous myelinated axons appeared in both control and diabetic groups. The number of myelinated axon of control group (3135.22 ± 335.44) was greater than that of diabetic one(2017.11 ± 607.64) with high statistic significance($P<0.01$).

The non-neuronal cells(like Schwann's cells, fibroblasts, and inflammatory cells) were scattered through the tissue at 2 week, and the number of non-neuronal cells in diabetic group(3665.73 ± 1586.25) was greater than that of control

Table 3. Result of histologic studies at the midcross-section of the regenerated nerve.

(mean±SD)

Group	Number of myelinated axon	Number of non-neuroal cell	Number of vessel	Area (mm^2)
2 week				
Control	None	1378.75± 874.85	27.00±17.83	0.26±0.01
Diabetes mellitus	None	3665.73±1586.25	36.86±21.71	0.41±0.08
4 week				
Control	3135.22±335.44	820.50± 249.59	33.22±12.57	0.23±0.05
Diabetes mellitus	2017.11±607.64	998.33±150.45	20.22±10.35	0.20±0.06

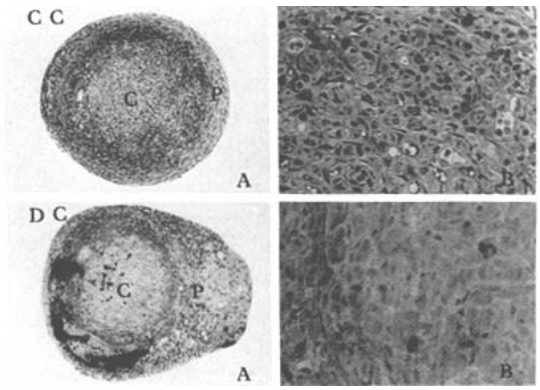

Fig. 2. Transverse sections of chamber midpoint at 2 week. Toluidine blue stain, A: X100. B: X400.
CC: Control group.
A: Perineurium-like circumferential cells(P) surround the central area(C).
B: Magnification of the same section shows scattered Schwann's cells with nonmyelinated axons in their cytoplasms.
DC: Diabetic group.
A: Perineurium-like circumferential cells(P) surround the central core(C).
B: Magnification of the same section shows that scattered Schwann's cells contain very few nonmyelinated axons in their cytoplasm.

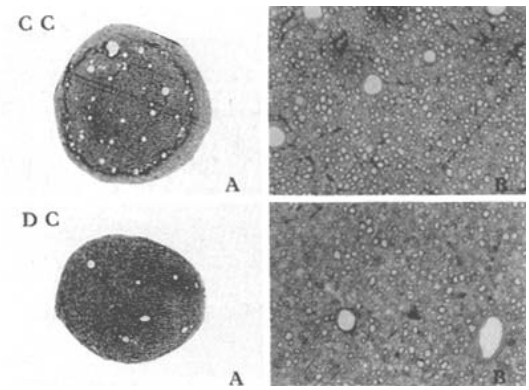

Fig. 3. Transverse sections of chamber midpoint at 4 week. Toluidine blue stain, A: X100, B: X400.
CC : Control group.
A: Diameter is reduced in comparison to that of 2 week.
B: Magnification of the same section shows that myelinated axons appear.
DC: Diabetic group.
A: Diameter is similar to that of control group, but vessels are fewer than in control group.
B: Magnification of the same section shows that myelinated axons are fewer than in control group.

one(1378.75±874.85) with high statistic significance(P<0.01). At 4 week, there was significant statistic difference between the number of non-neuronal cells of diabetic and control groups also(998.33±150.45, 820.50±249.59, each, P<0.05). The number of non-neuronal cells in diabetic group at 4 week was smaller than that at 2 week with high statistic significance(P<0.01).

At 2 week, there were numerous vessels with variable sizes in the regenerate. There was no statistically significant difference between the number of vessel of diabetic group(36.86±21.71) and that of control one(27.00±17.83). But at 4 week, the number was decreased significantly in diabetic group(20.22±10.35, P<0.05), and increased in control one(33.22±12.57, P<0.05), so the number of vessel in control group was greater than that of diabetic one with high statistic significance(P<0.01).

At 2 week, cross-sectional area of the diabetic group($0.41 \pm 0.08 mm^2$) was greater than that of control one($0.26 \pm 0.01 mm^2$)($P<0.01$). But at 4 week, there was no significant difference between those groups because of the high statistically significant decrease of the area of diabetic group ($0.20 \pm 0.06 mm^2$).

DISCUSSION

Diabetic peripheral neuropathy may develop after several years of the disease process, and can be classified as symmetrical sensory neuropathy, cranial or peripheral neuropathy, and autonomic neuropathy[5,19].

Their main morphological changes are segmental demyelination and axonal degeneration of both somatic and autonomic nerves, and many authors reported about their biochemical abnormalities also[5,16,22]. Diabetes mellitus is associated with a generalized defect in connective tissue metabolism, including decreased growth, poor wound healing and osteopenia, and marked alteration in collagen production[12,17,18].

Orthopedic surgeons occasionally meet the clinical situation of segmental defect of peripheral nerve which can not be repaired instantaneously. In such cases various methods, mobilization, positioning of extremity, transposition, and nerve grafting may be used depending on the surgeon's choice[9,23]. But because those methods have some adverse effects or limiting factors in itself, some authours had developed and experimented with interposition materials between nerve gaps[3,6,8]. Among those various methods, entubulation technique using silicone tube is simple to excute, easy to apply to sciatic nerve of rat, and easy to observe the progress or content of the tube[4,21].

In this experiment, we think that the silicone tube constantly guided the direction of the regenerating nerve fibers to prevent the formation of neuroma[9].

In experimental diabetes mellitus, amplitude and conduction velocity are reduced in peripheral nerve and spinal cord[1,15], and Kim and Ahn[7] reported latency period delayed about 20% from 1 week after the induction of diabetes mellitus.

In our experiment, there was no statistically significant difference between diabetic and control groups in latency period and amplitude. Even though the improvement of latency period at 4 weeks was noticed in diabetic group, there was no better result than in control group.

Williams et al.[21] reported the myelination of the regenerating axons is not complete at 4 weeks after the experiment, and the process is dependent on the regeneration process of individual axon. They couldn't find any myelinated axons at 2 week after the experiment at mid-chamber level(5mm from the proximal stump). In our experiment, at 2 week, there was no visible myelinated axon in every group, too. At 4 week, numerous myelinated axons appeared in each group, but the number of myelinated axon in control group was greater than that of diabetic one as the major difference between diabetic and control groups.

The number of non-neuronal cell of control group was smaller than that of diabetic one at 2 and 4 week. The number of non-neuronal cell of diabetic group was so high at 2 week, at 4 week, even after the significant decrease, diabetic group had more non-neuronal cells in its tissue, and was considered as more immature than control one. The number of non-neuronal cells significantly decreased at 4 week in comparison to 2 week in both groups. It was thought that the decrement was not because of the decrease of the number of Schwann's cells, but because of the decrease of the number of fibroblast and / or inflammatory cells.

The number of vessel had no significant difference between diabetic and control groups at 2 week. But at 4 week, even though there was no significant change during the time, control group had more vessels in its regenerated tissue than diabetic one. It was thought that if one vessel grew into the regenerated tissue, it might be existed consistently.

In regard to the cross-sectional area, Williams et al.[21] reported that the 1-week structures had noticeably larger diameter than the 2 - 4-week structures, and there was possibility of a condensation of the matrix material during and shortly after the first week. This implies that from 1 week after operation, the more mature tissue has the smaller cross-sectional area. In our experiment, the cross-sectional area of control group was significantly smaller than that of diabetic one at 2 week. At 4 week, the area of diabetic group was significantly decreased, and there was no difference between control and diabetic groups. It was thought that the maturation process of regenerate in control group was faster than that of diabetic one untill 2-week after the operation, but after that time, the status of maturation was similar.

From these results it would be suggested that diabetes mellitus retarded the healing process of sciatic nerve defect in rat. And we might suggest that if we meet this situation in clinical practice, we have to consider some supportive measures to overcome the bad influence of diabetes mellitus on the healing of nerve defect.

CONCLUSION

We performed this experiment to assess the influence of diabetes mellitus on the healing of segmental defect of sciatic nerve of rat. Although there was no statistical difference in the electromyographic study between control and diabetic groups, the histological result suggested that diabetes mellitus significantly retarded the healing process of sciatic nerve defect in rat. It would be needed some experiments in the future to overcome this harmful effect in clinical practice.

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= 국문 초록 =

당뇨병이 흰쥐의 좌골신경 분절결손 치유에 미치는 영향

가톨릭 대학교 의과대학 정형외과학교실

김 인 · 이인주 · 최남용 · 송석환 · 안익주

당뇨병이 흰쥐의 좌골신경 분절결손 치유에 미치는 영향을 조사하기 위하여 본 실험을 실시하였다. 체중 200-250g의 건강한 Wistar계 암컷 흰쥐 33마리(대조군 17마리, 당뇨군 16마리)를 사용하였으며, Streptozotocin(50mg/kg 체중)을 생리 식염수에 용해후 꼬리정맥에 주사하여 당뇨병을 유발하였다. 우측 좌골신경에 5mm의 결손을 만들고, 근위 및 원위 신경단을 9mm silicone도관에 삽입하여 그 끝을 각각 10-0 nylon실로 봉합, 연결하였다. 도관내에는 10㎕의 collagen으로 채웠다.

수술후 2주와 4주에 원위잠시와 진폭을 포함한 근전도검사를 실시하였고, toluidine blue 염색후 도관 정중간에서의 유수화 축색돌기수, 비신경세포수, 혈관수 및 횡면적을 포함한 조직학적 검사를 실시하였다.

상기 실험의 결과로 보아 당뇨병은 흰쥐 좌골신경 분절결손의 치유를 저해하였으며, 당뇨병 환자에서의 말초신경 분절결손의 치료에는 이러한 당뇨병에 의한 역효과를 극복할 보조적인 치료가 고려되어야 한다고 생각되었다.