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Summary: Differences in neural damage due to different injection needles were investigated in vitro on sciatic nerve specimens of adult rabbits. Three kinds of 22-gauge needles with differently shaped tips were tested : one typical, long-bevelled venepuncture needle; a short-bevelled, typical nerve block needle; and a tapered, atraumatic spinal needle. Both sciatic nerves of 50 adult rabbits were perpendicularly pierced three times by one of the needles mentioned above. The extend of nerve damage was evaluated by electrophysiological methods as well as histological methods based on the fluorescence microscopy technique. The following results were obtained.

1) Nerve funiculi easily slipped away from the tip of the needle and were difficult to injure. This tendency was especially observed with the short-bevelled and tapered needles.

2) Based on the electrophysiological findings, the tapered needle caused the least reduction in the relative amplitude of the compound action potentials. Statistically significant differences were obtained between the tapered needle and the other kinds of needles.

3) With the tapered needle, there was the least leakage of Evans blue albumin which suggested the least damage to the perineurium, and almost no rupture or tearing of the nerve fibers was observed.

4) In the short- and long-bevelled needles, the damage was reduced when the face of the bevel was inserted parallel to the fibers,

These results suggested that the tapered needle without a cutting edge was most ideal for use in nerve blocking and neuro- or radiculo-graphy and the like.

Key Words : peripheral nerve, palsy, mechanical injury, injection needle

Introduction

Numerous reports have appeared describing peripheral nerve paralysis due to injection, ranging from a slight dysesthesia all the way up to motor paralysis. This may be due to a medical mishap. Many studies have attributed the cause of the paralysis as being mainly due to the toxicity of the injection drug. Because careful attention is given to select the correct injection site, these medical mishaps have become less frequent, but still they are known to occur. In performing nerve blocks or neurography and the like, it is necessary to inject drugs into either the trunk or periphery of the nerve¹⁾. Thus clinicians are constantly exposed to the risk of causing injection nerve paralysis. The main causes of injection nerve paralysis include^{2,3)}:1) mechanical damage due to injection needle, 2) injury to blood vessels and hematoma formation in the nerve trunk, 3) effect of injected drug, 4) ischemia, compression and traction. It is surmised that these factors act individually or in combination to cause paralysis.

Our attention was concentrated on mechanical damage due to the injection needle. Differences in neural damage due to different injection needles were investigated in vitro on sciatic nerve specimens of adult rabbits. Three kinds of injection needles were tested. The degree of nerve damage was studied by using electrophysiological and histological methods based on the fluorescence microscopy technique⁴⁾. sciatic nerve at this level ranges from 3 to 4 mm. The nerve consists of thick funiculus of the tibial nerve (diameter: 1.0-1.5 mm) and 2-5 thin funiculi.

1. Injection Needles used to cause Neural Damage

Three kinds of needles, all with an external diameter of 0.7 mm (22 Gauge), were used, namely, a long-bevelled needle, a shortbevelled needle and a tapered needle (Fig.1).

1) The long-bevelled needle (Terumo, 22 G, venepuncture needle) is commercially available and has a bevel angle of 14°. Its external edge has been well sharpened and it is used for stabbing into the skin and vascular walls.

2) The short-bevelled needle with an attached stylet (Hakko, 22 G, nerve block

Materials and Methods

Fifty adult rabbits weighing from 2.5 to 3.0 kg were used as the experimental animals. They were sacrificed by an overdose of pentobarbital and the sciatic nerves were exposed at the middle thigh of the legs, then about 6 cm of the nerves were extracted. The nerves of one side were used for the electrophysiological study and others for the histological study. The diameter of the



Fig. 1 Each nerve trunk was perpendicularly pierced three times by one of three kinds of needles with different face direction.

Group	1	;	long-bevelled needle (venepuncture needle), across.
Group	2	:	long-bevelled needle (venepuncture needle), parallel.
Group	3	;	short-bevelled needle (nerve block needle), across.
Group	4	:	short-bevelled needle (nerve block needle), parallel.
Group	5	:	tapered needle (atraumatic spinal needle).

needle) is commercially available and has a bevel angle of 30°. Its external edge is not as sharp as the venepuncture needle.

The tapered injection needle with an attached stylet is an atraumatic spinal needle mannufactured in West German (Hell & Co. GMBH, 22 Gauge, atraumatische Spinalnadel).
 Its tip has no cutting edge, but is tapered.
 First Study : Electrophysiological Study

An experimental chamber was constructed using a transparent plastic box (acryl-resin) in which two silver-silver chloride electrodes were aligned in parallel at a distance of 10 mm. Two ml of saline solution was infused into the bottom of the chamber to prevent it from drying out. After extracting the sciatic nerves from rabbits as mentioned previously, they were immediately placed onto the silversilver chloride electrodes of the experimental chamber maintained at a constant temperature of 25°C. Both ends were bound by a 4-0 silk thread to the edges of the chamber with wax to maintain the original tension, and the thread was affixed (Fig. 2). The electrodes were connected to the digital memoriscope (Hitachi, VC-801 B) and the compound action potentials (CAPs) were recorded on the XY



Fig. 2 Experimental chamber. R₁₋₄: recording electrodes.

Stim.:stimulation. Indif.:indifferent electrode.

recorder (Watanabe, WX 4301).

Two electrodes attached to the distal ends of the nerve were used for stimulation. The nerves were stimulated by rectangular pulses (6-10 V) of 0.01 msec duration. The CAPs evoked by maximum stimulation intensity were picked up monopolarly from R_1 to R_4 successively, and the amplitudes of the CAPs were measured (Fig. 2).

Then materials were apportioned to 5 groups including 10 nerves each. The abovementioned needles were perpendicularly pierced three times into the middle of the nerve trunk in the midpoint between sites R_2 and R_3 in the manner described below. During this procedure a rubber board with a groove was used to hold the nerves in place.

Group 1 -- The long-bevelled needles was inserted so that the face of the bevel cut across the nerve fibers.

Group 2 -- The long-bevelled needle was inserted so that the face of the bevel entered parallel to the nerve fibers.

Group 3 -- The short-bevelled needle was inserted so that the face of the bevel cut across the nerve fibers.

Group 4 -- The short-bevelled needle was inserted so that the face of the bevel entered parallel to the nerve fibers.

Group 5 -- The tapered needle with no cutting edge was pierced into the nerve fibers.

Then, following the same method used before injury, the CAPs were recorded monopolarly from R_1 to R_4 , and the amplitudes of CAPs were measured.

3. Second Study : Histological Study based on the Fluorescence Microscopy Technique

The following study was carried out in the above-mentioned 5 groups. Immediately after

inserting the needles, the nerves were immersed for two hours in Evans blue albumin (EBA), which consisted of 5% bovine albumin (Sigma Co.) labelled with Evans blue (Merck Co.). Following washing in saline solution, they were fixed in 5% formalin for 12 hours. Then frozen sections ranging in thickness from 10 to $15 \,\mu$ m (including both longitudinal and transverse planes) were prepared with a cryostat (Lipshaw, 1500 N type) and mounted with 50% aqueous glycerin. The damage to the funiculi of the tibial nerves was observed by fluorescent microscope. The details of the above method are as described by Steinwall⁴⁾ and Olsson⁵⁾. Following injury, the nerves were fixed in formalin and stained with hematoxylin and eosin.

Results

Electrophysiological Study

In the control nerves tested before injury, the amplitudes of CAPs recorded from R_1 to R_4 were gradually decreased. At site R_1 the amplitudes were 12.2±4.2 mV (mean±SD), R_2 : 9.8±3.1 mV, R_3 : 6.2±2.0 mV and R_4 : 3.6±1.5 mV, respectively.

After injuring the area between R_2 and R_3 , there was almost no change in the amplitude of the CAP at site R_1 and R_2 , but the amplitude at R_3 and R_4 was reduced in comparison with the controls. This reduction was most marked in group 1 and very slight in group 5. When the relative amplitudes (i.e., the percentages of amplitudes at R_3 after injury compared with control values) were calcu-





lated, it was $42.2 \pm 22.0\%$ (mean \pm SD) in group 1;60.9 \pm 18.2% in group 2;51.0 \pm 22.3 % in group 3;71.0 \pm 18.0% in group 4; and 90.1 \pm 10.9% in group 5. Statistically significant differences (paired t-test) were obtained between the group 5 and the other four groups (Fig. 3).

Study based on the Fluorescence Microscopy Technique

This method is based on the fact that under ultraviolet rays blue EBA emits a red fluorescence and also EBA does not pass through the normal perineurium within 24 hours after death (Lundborg⁶⁾). For this reason, the perineurim except for the injured region is stained by EBA emitting a red fluorescence, but this is not observed in the endoneurium. EBA leaks into the endonerium if there is an injury to the perineurium,.

a) Group 1. In the longitudinal sections,

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Fig. 4 Funiculus pierced by the long-bevelled needle with bevel transverse to nerve fibers (longitudinal section). The endoneurial EBA-fluorescence indicates perineurial damage.

rupture of the nerve fibers was clearly observed at the damage site caused by the needle's tip, and EBA leaking from the damaged site of the perineurium was observed mainly in the rupture site (Fig. 4). In the transverse sections, inflow of EBA was likewise observed mainly around the rupture site. In some of the sections, hernia formation of the nerve fibers was observed in the damaged area.

b) Group 2. Disarray of the fibers was observed, but rupture of the nerve fibers was much less than group 1 and there was a little leakage of EBA.

c) Group 3. Rupture of the nerve fibers was observed to the same degree as in group 1 and disarray of the nerve fibers was also seen. Damage to the perineurium was most severe in comparison with the other groups, and leakage of EBA was quite marked.

d) Group 4. Leakage of EBA and disarray of the nerve fibers were observed but rupture



Fig. 5 Funiculus pierced by tapered needle (longitudinal section). This picture indicates that the tapered needle produced less damage.

of the nerve fibers was hardly observed.

e) Group 5. Only slight disarray of the nerve fibers was observed, and rupture of the nerve fibers and leakage of EBA were very slight (Fig. 5).

Discussion

Up to the present, many reports on peripheral nerve paralysis due to injection have appeared dealing mainly with the toxicity of the drug. Hirasawa³⁾ reported a case of paralysis of the radial nerve after the injection of a drug combination consisting of a steroid and a local anesthetic, which are considered to be relatively safe. He described the mechanism of this paralysis as mechanical damage by the injection needle, vascular damage, the enlargement of the neural damage area due to inflow of the drugs, and constriction of the nerve trunk by the scar formation.

Selander⁷⁾ stated that inflow of a local anesthetic into the nerve trunk may give rise

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to the risk of causing an edema in the nerve trunk. And in 1977⁸⁾ based on an experimental study on inserting injection needles into the nerve trunk, he reported that damage to the nerve fibers was caused by the tip of the needle, and that the least damage was caused when an injection needle with a 45° bevel angle was inserted to cut transversely across the course of the nerve fibers.

Ruch⁹⁾ stated that the amplitude of the CAP corresponded fairly well to the number and size of the nerve fibers. According to our experimental results, the reduction in the amplitude of the CAP was most severe in group 1 and was less severe in group 5. Thus the tapered needle caused the least damage to the nerve fibers, and the venepuncture needle caused the most damage when it was inserted so as to cut across the nerve fibers. These results correlated well with the histological findings.

It is known that the perineurium plays the role of a diffusion barrier for various substances. Damage to this defence mechanism changes the environment of the endoneurium and disrupts the function of the nerve fibers. EBA leaks into the endoneurial space from the damaged diffusion barrier. For this reason, it was thought that there was little damage to the perineurium since there was little leakage of EBA in group 5.

Commercially available venepuncture needles have keenly sharpened bevel edges for easy penetration of the walls of blood vessels. It is dangerous to use such needles for insertion into nerve trunks. Thus tapered needles are considered to be ideal for daily practice such as nerve blocking and neuro- or radiculo-graphy and the like.

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注射針による末梢神経の機械的損傷についての実験的研究

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要旨:注射針の先端による機械的な末梢神経損傷を検討する目的にて、3種類の注射針,静脈針(long bevelled needle),神経ブロック針(nerve block needle),先細り針を用いて家兎の坐骨神経に刺入した. 電気生理学的および fluorescence microscopy technique による組織学的検索を行い、その損傷程度を評価した.

神経束は注射針,とくに神経ブロック針および先細り針から容易に滑脱し,損傷されにくかった.先 細り針は電気生理学的および組織学的検索ではもっとも損傷は少なかった.また静脈針と神経ブロック 針では、ベーベルの面を神経線維と平行に刺入したほうが損傷は少なかった.