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Effects of Inducible NOS Inhibition on Motor Function Recovery After Sciatic Nerve Ischemia and Reperfusion in Rats

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Purpose: **Introduction: Nitric oxide (NO) is known to play an important role in ischemia and reperfusion (I/R) injuries in the peripheral nerve. However, among various NO synthases (NOS), the effects of inducible NOS (iNOS) on peripheral nerve I/R injuries are still under debate. This study presents the influence of iNOS on the regeneration of crushed peripheral nerve in rats.**
Materials and Methods: **One hundred and seventy female Sprague-Dawley rats (body weights 175-200 g) were randomly divided into two groups to receive an injection of 1400W (iNOS specific inhibitor, 3 mg/kg) or the same volume of purified water. One sciatic nerve of each rat (10 mm in length) was subjected to a 100-g crush load for 2 hours to induce ischemia. Motor functional recovery by walking track test and histology of the crushed nerve in the two groups were evaluated at different reperfusion time (3 hours, and 1, 3, 7, 11, 14, 21, 28, 35, and 42 days). mRNA and protein levels for iNOS in the nerve were measured.**
Results: **Sciatic functional index (SFI) for motor functional recovery in the 1400W treated group improved at a significantly faster rate than that in the control group from day 11 after the ischemia. The difference peaked at day 18 (-46.8 in the 1400W treated group and -29.4 in the control group) and lasted until day 28. Histologic results were comparable with motor functional results. Histological observation revealed less axonal degeneration and earlier regeneration of nerve fibers in the 1400W treated rats than in the control rats. iNOS mRNA and protein were upregulated during the first 3 days of reperfusion, and iNOS inhibitor significantly attenuated the increased iNOS during the early phase of the reperfusion until 7days.**
Conclusion: **Inhibition of iNOS during the regeneration of the ischemic nerve influenced mRNA and protein level early in the reperfused period and assured faster functional recovery faster than in the untreated group. These results suggest that early administration of 1400W has therapeutic potential for the treatment of I/R injury.**

Key Words: ***Peripheral nerve, Ischemia and reperfusion, Inducible nitric oxide synthase, 1400W***

Microcirculatory insufficiency and compromise of reperfused tissue function followimg ischemia is defined as ischenia and reperfusion (I/R) injury. I/R injury contributes to pathological changes in many tissues including the heart, brain, and skeletal muscles[19,37]. Compared to the critical organs, where ischemic effects are fatal such as in cerebral ischemia and myocardial infarction, there have been relatively few investigations of the reperfused peripheral nervous system (PNS).

The peripheral nerves are known to be resistant to ischemic injuries because of the blood-nerve barrier, well-developed anastomoses and low energy demands[16]. However, PNS injury resulting from I/R remains a common clinical problem associated with acute trauma (such as replantation, transplantation, tourniquet injury, compartment syndrome, and "Saturday night palsy") and chronic conditions (such as carpal tunnel syndrome, tumors, and callus). This injury poses a challenge because the degree of injury and time course of recovery are considerably varied. Many studies have revealed that multiple factors, including leukocytes, cytokines, adhesion molecules, PNS I/R injury[2,22].

NO is synthesized from L-arginine by NO synthase (NOS)[6]. NO is an endogenous messenger molecule and plays important roles in both PNS physiology and pathology. There is

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no consensus on the role of NO in the injured peripheral nerve and NO metabolism is influenced by many factors[5,6]. NO plays important roles in physiological function including vasodilator, smooth muscle relaxation, neurotransmission, and anti-aggregatory functions[7,8,17]. NO is also involved in multiple pathological events in many organs and diseases. All 3 major NOS isoenzymes have been found in the nervous system[7,30]. Inducible NOS (iNOS) is induced by endotoxins and cytokines and is found in numerous cell types[38]. When NO release is excessive, iNOS causes significant cellular toxicity[26]. Upregulated iNOS has been implicated in I/R-associated pathology in skeletal muscle[39], myocardial infarction[37], brain[19], liver[20], and kidney[9]. A few investigations have shown that inhibition of iNOS significantly improves microcirculation and contractile function in I/R skeletal muscle[28,31,39]. iNOS knockout mice exhibit reduced I/R injury in the kidney[25], skeletal muscle[29], and brain[19]. However, the exact role of iNOS in I/R injury remains controversial and contradictory data have also been published[21]. In the PNS, Qi et al.[30] have shown that I/R regulates NOS in the rat sciatic nerve, suggesting a potential role for NO in PNS I/R injury. Among the 3 NOS isoforms, iNOS upregulation leads to excess NO production to exacerbate I/R injury. These findings are supported by investigations from rat I/R sciatic nerve[33] and focal ischemia of the CNS[32], but differ from the results of studies of injured peripheral nerve in mice lacking iNOS[23,34]. Thus, the role of iNOS in PNS I/R injury needs further clarification.

This study investigated the role of the highly selective iNOS inhibitor N-[3 (aminomethyl) benzyl) acetamidine] (1400W) in the recovery of motor function and the dynamics of mRNA and protein levels of iNOS in the reperfused rat sciatic nerve.

MATERIALS AND METHODS

One hundred and seventy female Sprague-Dawley rats (weight 175-200 g) were randomly divided into two groups. The rats in the experimental group received 1400W while those in the control group received the same volume of purified water. One sciatic nerve of each rat (10 mm in length) was subjected to a 100-g crush load for 2 hours to induce ischemia using a specially designed compression device (Fig. 1).

To evaluate the effects of the dosage and duration of 1400 W administration on motor functional recovery until 42 days after the reperfusion, eighteen rats were used in pilot study.

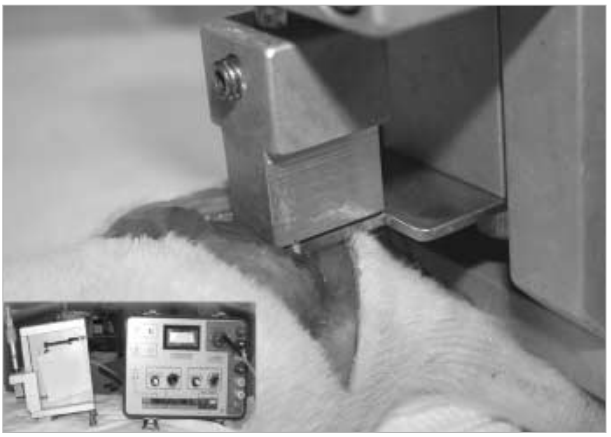


Fig. 1. Special designed device (inlet) produces ischemic injury in the 10-mm length sciatic nerve.

Low dose (3 mg/kg) and high dose (10 mg/kg) of 1400W administered 10 min before reperfusion were compared, with 3 rats in each group. Additionally, twelve further rats were used to compare the effects of longer administration durations of 3 mg/kg 1400W of 1, 3, 7, and 14 days on motor functional recovery, with 3 rats for each duration. Every 12 hours, 3 mg/kg of 1400W was administered after the initial administration until the end of the administration period. After the optimal 1400W dosage and administration duration were determined in the pilot studies, 1400W and the same volume of sterile purified water were administered to the remaining 140 rats.

1. Surgical procedures

Rats were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body weight, Abbott Laboratories, North Chicago, IL). The gluteal area was shaved and swabbed with Betadine solution. Through a gluteal-splitting approach, the right sciatic nerve was exposed and isolated from the surrounding soft tissue under an operating microscope. Using a specially designed compression device[10], a 10-mm segment of the nerve proximal to the bifurcation of the sciatic nerve into the tibial and peroneal nerves was placed between a 10 mm wide anvil and a matching 10 mm wide metal surface mounted on the tip of non-rotating micrometer spindle. The spindle was advanced until the desired 100 g load was achieved, as measured by an integral load cell. The load was maintained for 2 hours. This procedure has been demonstrated to establish an ischemic condition in the sciatic nerve[10,11]. During the procedure, the nerve was kept moist with 37℃

saline. Following ischemia, the proximal and distal ends of the ischemic segment were marked with 10-0 nylon sutures (Ethicon, Somerville, NJ) and the wound was closed with 4-0 silk running sutures. The rats were allowed to recover from the anesthesia and move freely in the cage.

2. Assessment of motor function

Using a walking track test, motor function was evaluated at days 1, 7, 11, 14, 18, 21, 25, 28, 35, and 42 of reperfusion, with 10 rats for each group. The procedure has been described previously[10,11]. Briefly, the rats were allowed to walk down a corridor with a dark shelter at the end. An 8×42 cm piece of photocopying paper impregnated with a 0.5% solution of the anhydrous form of bromphenol blue (Sigma Chemical, St. Louis, MO) in absolute acetone was placed on the floor of the walking corridor. The hind paws were moistened on a water-soaked sponge and the rats were allowed to walk down the corridor, leaving blue footprints on the paper. The distance between the 1st and 5th toes (TS), the distance between the 2nd and 4th toes (IT), and the print length (PL) were measured (Fig. 2). The sciatic functional index (SFI) was calculated according to the Bain's equation[3].

3. Quantitative real-time RT-PCR

Six I/R nerve segments and the opposite non-I/R normal nerve segments (as a normal control) from each group were harvested at 3 hours, and 1, 3, and 7 days after ischemia, placed in RNA later (Ambion, Austin, TX), incubated at 4℃ overnight, and then stored at -20℃. The harvested samples were pooled, 2 nerve segments for each. Total RNA from each pooled sample was extracted using an RNeasy Mini kit plus DNase I digestion (Qiagen, Valencia, CA) and quantitated using RiboGreen (Molecular Probes Inc. Eugene, OR). Reverse transcription was performed in a total volume of 20 μL containing 0.4 μg total RNA. First strand cDNA was synthesized by a SuperScript™ II Reverse Transcriptase kit (Invitrogen, Carlsbad, CA) and quantified using PicoGreen (Molecular Probes, Inc). Quantitative real-time PCR was performed utilizing the iCycler Thermal Cycler (Bio-Rad, Hercules, CA) and using the iQ™, SYBR Green Supermix real-time PCR kit (Bio-Rad). The primer sequences and product size for iNOS and 18S rRNA (as the endogenous reference) were as follows; iNOS 5′ CACGACACCCTTCACCACA AG-5′ TTGAGGCAGAAGCTCCTCCA, 18S 5′ GAGGT GAAATTCTTGGACCGG-5′ CGAACCTCCGACTTTC GTTCT. The relative amounts of the target genes were calculated by the comparative CT (threshold cycle) method.

4. Western blot analysis

Six I/R nerve segments and the opposite non-I/R normal nerve segments (as a normal control) from each group were harvested at each of 3 hours, and 1, 3, 7, 14, 21, and 42 days after ischemia, frozen in liquid nitrogen immediately, and then stored at -80°. The harvested samples were pooled, 2 nerve segments for each, homogenized in boiling lysis buffer (1% SDS, 1.0 mM sodium ortho-vanadate, 10 mM Tris pH 7.4), and microwaved for 10-15 sec. The insoluble material was discarded after centrifugation of the homogenate, according to the manufacturer's instructions (BD Biosciences San Diego, CA). Protein concentrations were determined using a BCA kit (Pierce, Rockford, IL). Proteins (20 μg) were loaded onto NuPAGE 3-8% Tris-Acetate Gel (Invitrogen), then transferred onto polyvinylidene difluoride membrane (PVDF). The membrane was blocked with 5% non-fat dry milk in TBS-T at room temperature for 1 hour, followed by incubation with polyclonal primary antibody for iNOS (1:750, Upstate cell signaling solutions) at 4℃ overnight. After washing in TBS-T three times, the blot was incubated with 1: 12,000 horseradish peroxidase-labeled goat anti-rabbit IgG for 1 hour at room temperature. Following washing in TBS-T three times, Immunoreactivity was detected with a Super-Signal West Pico Chemiluminescent Substrate detection kit (Pierce, Rockford, IL). Kodak film was used to visualize the chemiluminescent signals. Actin control was used to ensure equal loading.

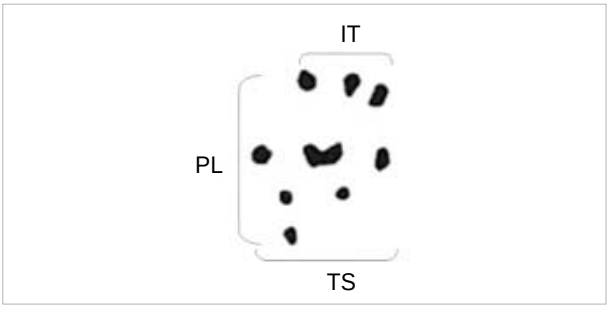


Fig. 2. Variable dimensions in footprint for the sciatic functional index. TS, distance between the 1st and 5th toes, IT, the distance between the 2nd and 4th toes, PL, the print length.

5. Histologic evaluation

Following walking track testing, the ischemic segments of the sciatic nerve were harvested for histological examination, 2 samples from each group, at days 7, 14, 21, and 42. The samples were fixed on a thin wooden stick and stored in 4% glutaraldehyde for at least 24 hrs at 4℃. The nerve sample was then postfixed in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol, and embedded in Epon 812. Sections were stained with toluidine blue.

6. Statistical analysis

Statistical analysis was performed for the SFI measurements using two-way analysis of variance (ANOVA). Changes in mRNA and protein levels in I/R nerves were expressed as a percentage of the level of the opposite non-ischemic normal nerves. All values were represented as means±standard error of the mean (SEM). One-way ANOVA was used for the post hoc analysis of specific values at each time point of the SFI measurement, and for the comparison of RT-PCR and Western blot results. A p-value of <0.05 was considered to be statistically significant.

RESULTS

1. Gross observation during reperfusion

Immediately after ischemia, the compressed segments of all nerves were slightly flattened. Nerve continuity, however, was not interrupted grossly (Fig. 3). At day 1 after ischemia, all right limbs were paralyzed. All rats survived with no wound infection or self-mutilation at nerve sample harvest. The morphology of the ischemic nerves returned to normal appearances from day 21 after reperfusion.

2. Pilot study

The pilot study showed that all 1400W-treated groups had an earlier recovery of the SFI measurement than the water-treated control group between day 11 and day 25. The time course of motor functional recovery was not significantly different between the lower dose (3 mg/kg 1400W) and higher dose (10 mg/kg) groups. Similarly, there was no significant difference in the SFI measurement between the various durations of 1400W administration from the initial administration to consecutive administration for 14 days (Fig. 4). Accordingly, a dosage of 3 mg/kg 1400W and duration of one day were selected for administration in the following experiment.

3. Assessment of motor function

At day 1 after ischemia, there were no measurable hind paw prints from the ischemic limbs in either experimental or control group (Fig. 5). The motor functional recovery started at day 11 in both groups. At this point and at all subsequent time points up to and including day 28 the 1400W group had a superior degree of functional recovery than the control group, with a significantly improved SFI between days 11 and 28 ($p<0.05$ to <0.001) (Fig. 6). At day 35, motor function was recovered to near normal in both groups.

4. iNOS mRNA during reperfusion.

During reperfusion, the expression of iNOS mRNA was significantly upregulated by 43-, 13-, and 6-fold from the normal level in the controls at 3 h, 24 h, and 3 d reperfusion, respectively (Fig. 7). These values in the 1400W group were significantly ($p<.05$ to <0.01) attenuated to 13-, 4, and 3-

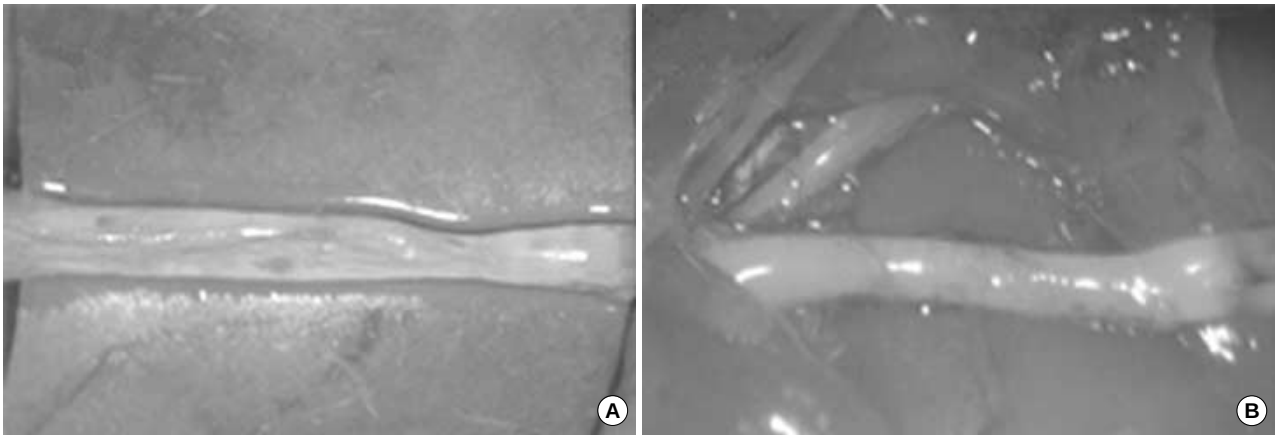


Fig. 3. Gross appearance of the sciatic nerve. (A) immediately after ischemia, (B) on reperfusion day 21.

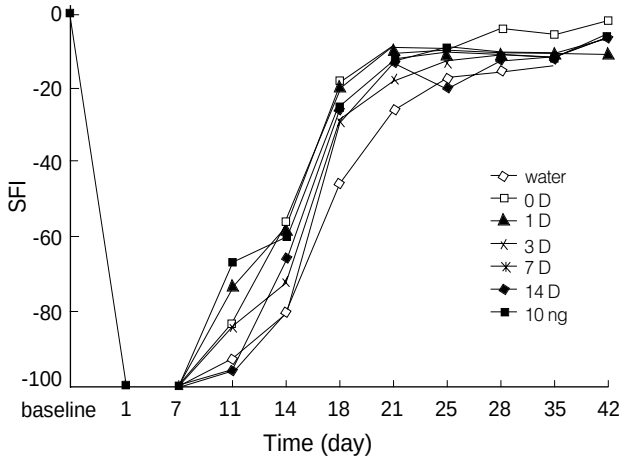


Fig. 4. Sciatic functional index (SFI) changes according to dosage and duration of 1400W administration. The motor functional recovery was not significantly different in the two dosages and variable durations of the 1400W administration.

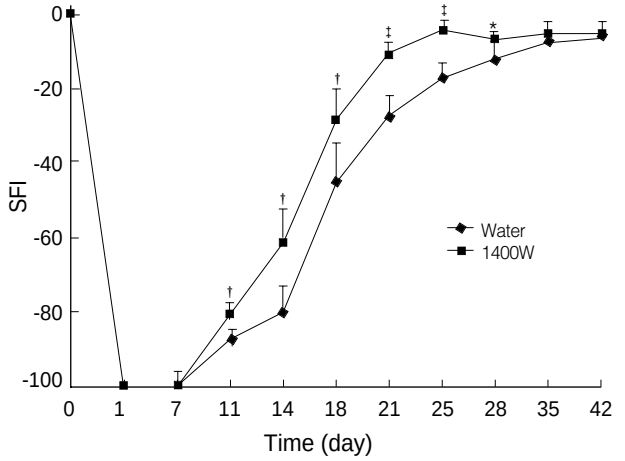


Fig. 6. Time course of the sciatic functional index (SFI) measurement following 2 h ischemia of rat sciatic nerve with 1400W or water administration. The error bars represent the standard error of the mean (SEM). Significant improvement of SFI was found in 1400W-treated rats over water-treated rats between days 11 and 28. *p<0.05; †p<0.01; and ‡p<0.001 compared to the water group.

fold increases, respectively. At day 7, iNOS mRNA declined to close to normal, with no significant difference between the two groups.

5. iNOS protein during reperfusion.

Western blot identified bands were consistent with iNOS protein expression in both groups (Fig. 8A). Definite stainings were observed in both groups from 3 h to 7 d reperfusion, with a maximum at 24 h, compromising a 12-fold increase in controls and a 6-fold increase in the 1400W group (Fig. 8B). Compared to controls, iNOS protein in the 1400W

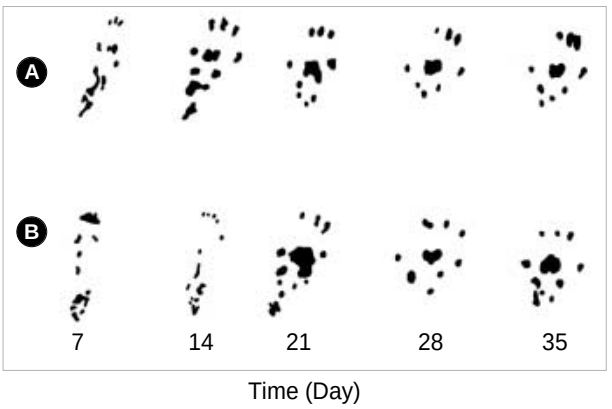


Fig. 5. Serial changes of footprints during the reperfusion period. (A) 1400W group, (B) Control group.

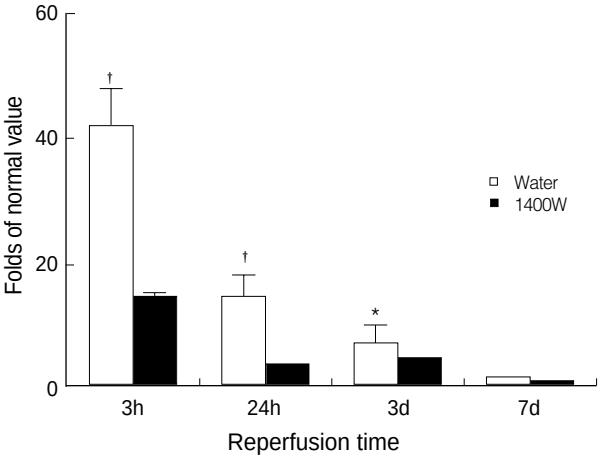
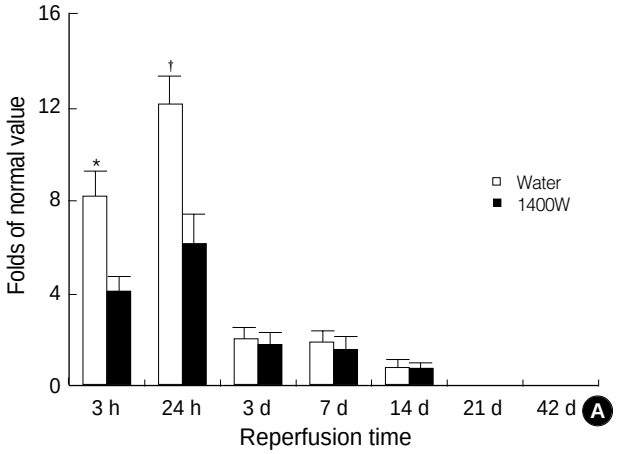


Fig. 7. The effects of 1400W on the expression of iNOS mRNA in the rat sciatic nerve following 2 h ischemia and up to 7 days of reperfusion. The error bars represent the standard error of the mean (SEM). *p<0.05 and †p<0.01 compared to the control group.

group was significantly lower at 3 h ($p<0.05$) and 24 h ($p<0.01$). At 14 d, iNOS protein returned to normal in both groups. Subsequently, iNOS bands were either not detectable or present only in trace amounts.

6. Histologic evaluation

Diffuse edema, vacuole formation, thickening of the vessel wall and degeneration of the majority of the axons were observed in both groups at day 7 of reperfusion. Phagocytosis of the injured myelin by the macrophages was found in both groups. By day 14, the samples contained a mixture of degenerating and regenerating axonal sprouts in both groups (Fig. 9A, B). Although quantitative evaluation was not performed, axon degeneration in the control group was 2.5 times more severe than that in the 1400W group. A small num-



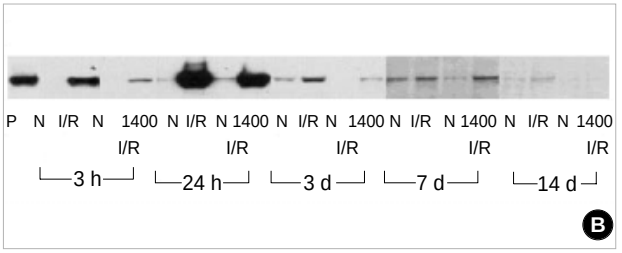


Fig. 8. (A) The effects of 1400W on the expression of iNOS protein in the rat sciatic nerve following 2 h ischemia and up to 42 days of reperfusion. The error bars represent the standard error of the mean (SEM). (B) Representative immunoblotting band of iNOS. * $p<0.05$ and † $p<0.01$ compared to the control group.

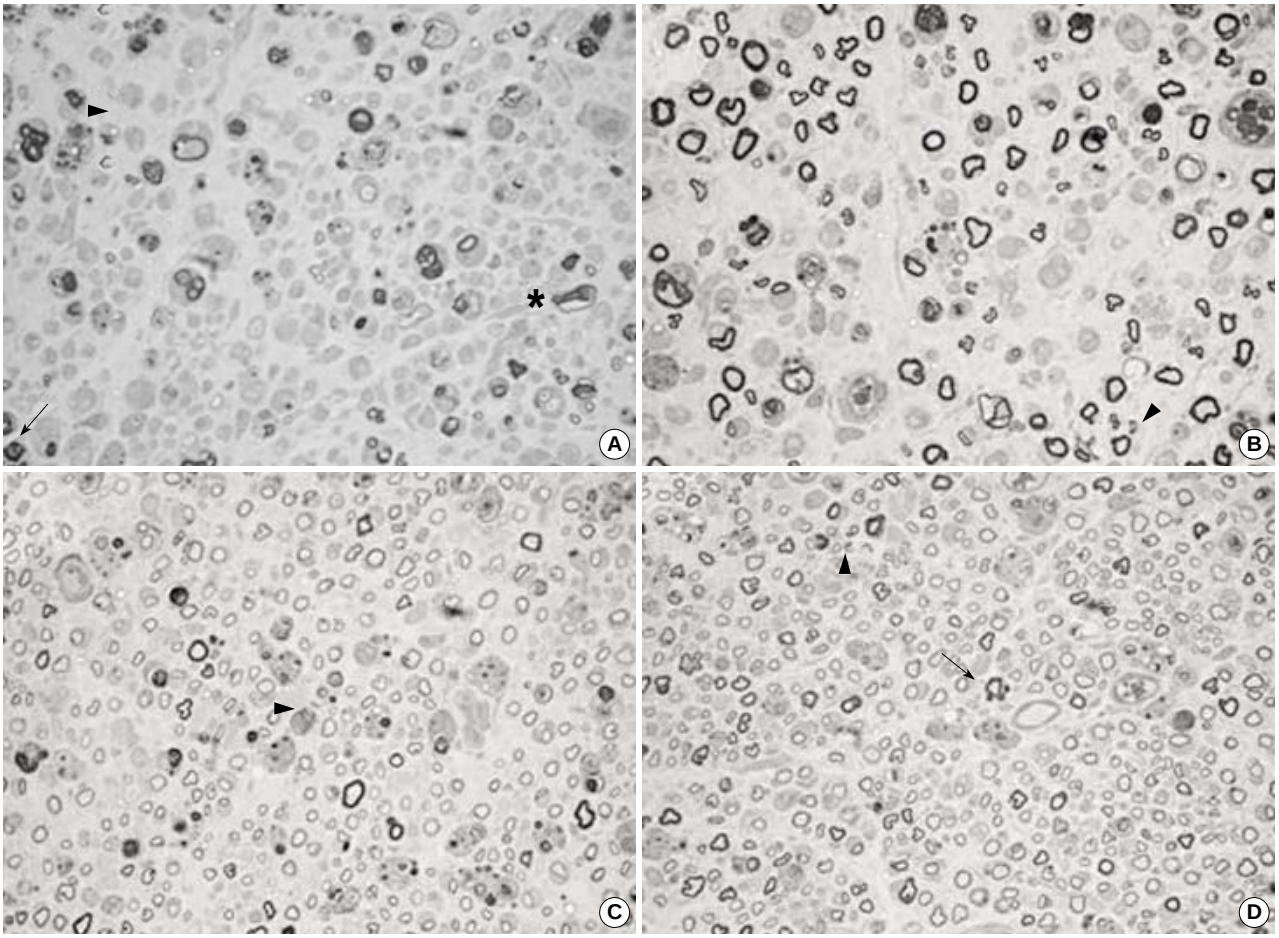


Fig. 9. Photomicrographs of cross-sections of rat sciatic nerves taken from the middle of the ischemic segments (toluidine blue, ×400) (A) Water-treated nerve sample at 14 d reperfusion ►: phagocytosis of the injured myelin by the macrophages, →: swelling of the myelin sheath, *: vacuole formation. (B) 1400W-treated nerve sample at 14 d reperfusion. ►: regenerated axon. (C) Water-treated nerve sample at 21 d reperfusion. ►: regenerated axon. (D) 1400W-treated nerve sample at 21 d reperfusion. Regenerating axons (►) with greater diameter and thicker myelin (→).

ber of regenerating axons surrounded by Schwann cells was found in the 1400W group. Twenty-one days after reperfusion, a higher density of regenerating axons with greater diameter and thicker myelin was found in the 1400W group compared to in the control group, although regenerating axons were also noted in the control group (Fig. 9C, D). At

day 42, there were no appreciable differences between the two groups and the axon population showed obvious recovery.

DISCUSSION

Inhibition of iNOS effectively promoted motor functional recovery as evaluated by walking track test following 2 hours of rat sciatic nerve ischemia and up to 42 days of reperfusion. Earlier axonal regeneration was also demonstrated in the 1400W group by histological examination. 1400W significantly attenuated the I/R-induced increase of iNOS mRNA and protein, and this beneficial effect occurred even at a relatively low dosage and short administration duration. Molecular biological results demonstrated that iNOS mRNA and protein levels were upregulated during the first 3 days of reperfusion. Thus, our results support the involvement of NO/NOS in I/R injury and the critical influence of early iNOS inhibition in the reduction or prevention of I/R injury in PNS.

This investigation indicated that inhibition of iNOS reduces I/R injury. Motor functional recovery in the 1400W group occurred approximately one week earlier than that in the control group, thereby reducing the time required for axonal regeneration of rat sciatic nerve by 25%. These results concur with those of others who reported that NOS inhibitor markedly diminished the I/R-mediated, lipid peroxidation and the decreased superoxide dismutase level, thereby protecting nerve fibers from I/R injury in rat sciatic nerve[33]. It has also been reported that cerebral I/R injury is exacerbated by excessive NO production through iNOS and is efficiently reduced by green tea catechins through inhibition of iNOS [19,36]. Our results are also in agreement with those found in skeletal muscle I/R injury treated with 1400W[28,31,39], and in iNOS knockout mice[29]. Thus, inhibition of iNOS appears to be a potential therapeutic strategy for the treatment of peripheral nerve I/R injury.

The finding that I/R upregulated the expression of iNOS mRNA and protein is in agreement with the results in reperfused rat PNS[30], and brain[36] and is supported by data from I/R skeletal muscle[28,29,31]. However, the peak time of increased iNOS varied according to the tissue. In the ischemic brain, expression of iNOS mRNA began at 12 hours, peaked at 48 hours, and returned to baseline at 7 days of reperfusion[18]. The catalytic activity of iNOS was parallel to the time of iNOS mRNA induction. Takimoto et al.[37] showed that the level of iNOS expression increased on day 1, and peaked on days 14 and 28 after myocardial infarction. The induction of iNOS activity in reperfused skeletal muscle was reported from 10 minutes to 3 hours[15,35].

It is known that iNOS leads to excess NO production. NO reacts with superoxide anion to generate toxic production of peroxynitrite, which in turn causes cell damage by lipid peroxidation, tyrosine nitration, sulfhydryl oxidation, nitrosylation, DNA damage, and blocking of mitochondrial oxidative phosphorylation[5,27]. Indeed, the toxicity of NO affects many cellular functions, subsequently leading to destruction of myelin and axons, collapse of growth cones, and inhibition of neurite outgrowth in the PNS[4]. Our functional results and histological findings showing that inhibition of iNOS led to less severely degenerated axons and earlier axon regeneration during reperfusion appear to support this concept. iNOS may influence Schwann cell-axon relationships, causing axonal damage and regulating endoneurial cell fate[12]. Excessive local elaboration of NO formed from iNOS may also impair microvascular function and disrupt the integrity of the blood-nerve barrier, resulting in edema formation and enhanced entry to the PNS of inflammatory mediators[16]. Therefore, excessive NO production from enhanced iNOS appears to be a critical and detrimental factor in PNS I/R injury.

To the best of our knowledge, this is the first study to observe the serial dynamics of iNOS in the PNS over a long period of reperfusion. Our data showed that both iNOS mRNA and protein were upregulated mainly during the first 3 days of reperfusion, and that this upregulation was significantly attenuated by 1400W administration. This early upregulation affects axonal degeneration and later regeneration. The mRNA and protein levels of iNOS returned to normal levels at 7 days and the protein band was undetectable after 14 days. However, iNOS protein bands were undetectable at 3 hours of reperfusion in our previous study[30]. The difference might be related to nerve sample handling as pooled samples were used in the present study, compared to single samples in the previous study, in which the small size of the nerve sample may not have been able to detect the iNOS protein because of the limited sensitivity of the measurement method. Taken together, our data suggest that prevention of the early upregulation of iNOS is the key point in the treatment of I/R injury in the peripheral nerve.

Although there is convincing evidence that increased iNOS activity deteriorates the PNS I/R, the role of NO in I/R injury is controversial. Understanding the role of NO and the molecular to lead to therapies to treat I/R injury of the peripheral nerves. In nerve injuries, although evidence supported contribution of NO to nerve degeneration, conflicting results have also been reported[14,40]. This may be the result of different responses of individual NOS isoforms after the destruction of tissue homeostasis and the influence on NO metabolism by an altered microenvironment including relative NO concentration, intracellular calcium levels and cellular sources of NO[1]. At present, it is generally assumed that the balance between the beneficial and detrimental effects of NO depends on its concentration and the balance of NO production[24]. Consequently, eNOS and nNOS produce NO in small and highly regulated bursts that are well suited for the molecular messenger function of NO. iNOS produces large amounts of NO continuously for long periods, a feature that is responsible for the cytotoxicity of NO. Future research in our lab will attempt to demonstrate different responses of the three NOS isoforms during the PNS I/R injury. Considering that each NOS has a distinct source, distribution, and function, the dynamic expression of individual NOS and the balance between them in I/R injury needs to be precisely characterized during the course of nerve degeneration and regeneration.

1400W is a highly selective iNOS inhibitor (5,000-fold over eNOS and 200-fold over nNOS)[13]. We found that 1400 W significantly reduced iNOS expression to half or less than half of that seen in controls during the first 3 days of reperfusion, compared to control levels. 1400W may be effective only at higher iNOS levels, because there was no significant difference in iNOS expression between the 1400W group and controls after 3 days of reperfusion. In addition, the finding that motor functional recovery did not differ significantly between 3 mg/kg and 10 mg/kg 1400W and between one initial injection and two injections per day for up to 14 days suggests that the selected dosage is adequate and that early administration of 1400W is critical because the first dose of 1400W was administered 10 min before reperfusion. Thus, 1400W has potential as a treatment agent in the prevention of I/R injury in the PNS.

In summary, NO/NOS is involved in the mechanisms of peripheral nerve I/R injury and iNOS contributes to nerve dysfunction. A highly selective iNOS inhibitor, 1400W, significantly promoted motor functional recovery and axonal regeneration, and decreased iNOS mRNA and protein expressions in the rat sciatic nerve following 2 hours ischemia and up to 42 days of reperfusion. Our results suggest that early administration of 1400W may have therapeutic potential for the treatment of I/R injury, and indicate that precisely characterizing the dynamics of each NOS isoform and the balance between them during reperfusion is essential to understand the mechanisms of I/R injury in the PNS.

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백서 말초 신경의 허혈-재관류 손상 후 기능 회복에 관한 iNOS 억제 효과

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목 적: 말초 신경의 허혈-재관류 손상에서 산화질소 역할에 대한 연구가 활발히 진행되고 있다. 그러나 세 종류의 산화질소 생성효소 중 유발 산화질소 생성효소의 말초 신경 손상에서의 작용은 여러 가지 다양한 결과들이 보고되고 있다. 본 논문은 백서 좌골 신경의 허혈-재관류 손상에서 유발 산화질소 생성효소 억제가 운동 기능 회복에 미치는 영향에 관하여 알아보고자 하였다.

대상 및 방법: 170마리의 백서를 유발 산화질소 생성효소 특이 억제제인 1400W를 투여한 실험군과 증류수를 투여한 대조군으로 나누었다. 각 군 백서의 좌골 신경에 2시간 동안 특별히 고안된 기계로 압박을 가해 허혈을 유발한 후 42일까지의 재관류 기간 동안 운동 기능 회복, 허혈 신경의 조직학적 검사, 유발 산화질소 생성효소의 mRNA와 단백질 발현 변화를 관찰하였다.

결 과: 좌골 신경 운동 기능 지수로 측정한 운동 기능은 재관류 후 11일부터 실험군에서 의미 있게 빠르게 회복되었다. 특히 재관류 18일에 두 군 사이에 가장 큰 차이를 보였으며(실험군; -46.8, 대조군; -29.4) 재관류 28일 후에는 차이가 없었다. 전체 회복 속도는 실험군이 약 일주일 정도 빠른 회복을 나타내었다. 허혈 신경의 조직학적 소견은 실험군에서 대조군보다 빠른 신경 섬유 회복 및 축삭 퇴행 지연이 관찰되었다. 유발 산화질소 생성효소의 mRNA와 단백질 발현은 재관류 첫 3일 동안 정상 발현량보다 의미있게 증가하였다가 감소하였다. 1400W는 증가된 유발 산화질소 생성효소 mRNA와 단백질 발현을 재관류 7일까지 통계학적으로 의미 있게 감소시켰다.

결 론: 말초 신경 허혈 후 유발 산화질소 생성효소의 재관류 초기 억제가 유발 산화질소 생성효소 mRNA와 단백질 발현을 감소시켜 재관류 전 기간 동안 신경을 빠르게 회복시켰다. 유발 산화질소 생성효소의 억제가 말초 신경 허혈-재관류 손상 예방 및 치료의 한 방법이 될 수 있을 것으로 사료된다.

색인 단어: 말초 신경, 허혈-재관류 손상, 산화질소, 산화질소 생성효소, 1400W