THE EFFECTS OF NITRIC OXIDE DONOR, SNAC ON MOTOR FUNCTIONAL RECOVERY OF REPERFUSED PERIPHERAL NERVE
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INTRODUCTION
Peripheral nerve ischemia/reperfusion (I/R) injury is an inevitable consequence of limb re plantation, transplantation, and free flap surgery. Accumulated evidences showed Nitric Oxide (NO) is involved in the mechanism of I/R. NO is synthesized by three major NO synthase (NOS) isoenzymes named neuronal (n-), inducible (i-), and endothelial (e-) NOSs. NO as a messenger molecule is involved in many physiologic functions. The PNS functions regulated by NO include neurotransmission, blood flow, nonvascular-smooth muscle relaxation, gastrointestinal motility, and penile erection and so on. However, limited, but controversial data exist regarding the role of NO in peripheral nerve I/R injury. While excessive NO formation during early reperfusion shows to accelerate lipid peroxidation and axonal degeneration in rat sciatic nerve, neuroprotection of NO is also reported in acute ischemic retina. Our previous study shows that I/R downregulates mRNA and protein expressions of eNOS and nNOS in rat sciatic nerve. Based on our earlier data, we hypothesized that I/R reduces NO production from constitutive NOS (cNOS i.e. nNOS and eNOS) isoforms, and supplementation of NO production may attenuate peripheral nerve I/R injury. To verify it, we infused a NO donor, S Nitroso-N-Acetylcycteine (SNAC) into the rats in which the sciatic nerve underwent I/R and evaluated the motor functional recovery during various reperfusion periods. The results were compared to that following methylprednisolone (MP) treatment.

MATERIALS & METHODS
Seventy-eight female Sprague-Dawley rats weighing 176~200 g were divided into SNAC-, MP-, and PBS-treated groups. The sciatic nerve in 66 rats underwent 2 h ischemia (22 rats for each group). The remaining 12 rats were used to determine systemic effects of SNAC-, MP, and PBS (n=4 for each). A 10 mm segment of rt. sciatic nerve was compressed with 100g load to achieve ischemia and maintained this load for 2 h. Agent administration SNAC (100nmol/ 100g/min), MP (30mg/kg/15min-45min pause 5.4mg/kg/hr), and PBS (0.2ml/100g/h) were infused continuously starting 30 min before the start of reperfusion and lasted for 2.5 h through the external jugular vein catheterization. Walking track test was performed on days 1, 7, 11, 14, 18, 21, 25, 28, 35, and 42 of reperfusion in all rats. The animals were allowed to walk down a walking corridor with a dark shelter at the end, leaving blue footprints on the floor paper impregnated with a 0.5% solution of the anhydrous form of bromphenol blue in absolute acetone. The sciatic functional index (SFI) was calculated by measuring the footprints. Muscle contractile test was performed on days 5, 11, and 21 of reperfusion, 4 rats for each group, and on day 42, 10 rats for each. The extensor digitorum longus (EDL) muscle was removed and suspended in a standard organ bath system filled Krebs-Henseleit solution. The maximal twitch force was measured at the optimal contractile length of the muscle. The tetanic contractile test consisted of three 1.5 sec stimulations at 70, 100 and 120 Hz. The contractile force of each experimental muscle was expressed as a percentage of that of the normal maximal twitch force. Histological examination for the sciatic nerve was performed in all groups on days 5, 11, 21, and 42 with 4 rats from each group at each time point. The cross section at 5mm distal from the distal end of the 10 mm I/R area was stained with toluidine blue.

RESULTS
Systemic effect of the agents During 2.5 h of agent infusion, MAP decreased slightly in all groups, without statistically significant differences. The HR remained stable over the 2.5 h infusion period in both SNAC and PBS-treated groups, while significant bradycardia was found in MP-treated group. Walking track test The feet in all rats were completely paralyzed at day 1. By day 7, SNAC-treated rats showed earlier signs of recovery with the SFI of ~84.2±3.7, while the MP- and PBS-treated rats were still paralyzed. From day 7 to day 28, the SNAC-treated rats showed significant improvement in SFI measurement when compared to the other groups (Fig. 1). Muscle contractile test The maximal twitch force of the EDL in SNAC-treated rats remained at around 80% of normal until day 21 and improved to 94±2.2% of normal at day 42. Meanwhile, MP- and PBS-treated rats showed decreasing tendency in the twitch force from day 5 to 21 and recovered to 75.9±4.8% and 60±5.6% at day 42, respectively (Fig. 2). The average isometric tetanic contraction forces decreased in all groups at all three stimulation frequencies at day 5 and 11. After then, the tetanic force in SNAC-treated rats started to recover and reached to 85~90% of normal by day 42. However the tetanic forces in MP and PBS-treated rats progressively decreased until day 21.

Figure 1. Time course of the SFI Figure 2. Twitch contraction of EDL

DISCUSSION
This study shows that systemic infusion of NO donor, SNAC results in significantly earlier and more complete recovery of the SFI, greater muscle contractile forces, and earlier weight gain following 2 h of ischemia when compared to MP and PBS treatment. Histology reveals less severe degeneration and earlier regeneration of axons in reperfused peripheral nerve. Our results indicate that SNAC treatment not only promotes motor functional recovery after peripheral nerve I/R injury, but also attenuates the progression of muscular atrophy that is an inevitable process after denervation induced by I/R. Supplementation of exogenous NO may protect the peripheral nerve from I/R injury through its potent vasodilating action, and NO may attenuate the harmful effect of reactive oxygen species (ROS) that are one of the major causes of reperfusion injury. Although MP-treated rats showed relatively earlier functional recovery compared to controls, the difference was not statistically significant. When considering the potential life threatening side effects of steroid therapy, we hesitate to accept steroid as a routine treatment option for the peripheral nerve I/R injury. In conclusion, the treatment with NO donor, SNAC in rat sciatic nerve I/R injury is effective in improving motor functional recovery and enhancing nerve regeneration. Because the satisfactory functional recovery is the ultimate treatment goal for the peripheral nerve injury, supplementation of NO appears to be a therapeutic potential that could replace the traditional treatment of steroid in the field of nervous system injury.

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