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Neuroprotective effect of Lovastatin on motor deficit induced by sciatic nerve crush in the rat

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ABSTRACT

Following severe peripheral nerve injury (PNI), regeneration is often insufficient and functional recovery is incomplete. Any agents that limit the spread of neural tissue damage may enhance the nerve regeneration. In this regard, statins have been shown to have neuroprotective properties. We investigated the effects of Lovastatin against sciatic nerve crush injury in male Wistar Rats. Lovastatin or vehicle was given parenteraly to rats for 7 days postoperative. In Lovastatin treatment groups, a single dose of agent (2 and 5 mg/kg) was administered daily. The control group was given vehicle in the same manner. The rats were subjected to crush injury in the left sciatic nerve with non-serrated clamp for 30 s. Behavioral, electrophysiological and morphological alterations were evaluated during the experimental period. Results showed that Lovastatin in a dose of 5 mg/kg could significantly ($P < 0.05$) accelerate regeneration process and functional recovery. Also results demonstrated that morphometric parameters such as mean axonal number and myelin thickness were significantly higher in Lovastatin (5 mg/kg) treatment groups compared to controls ($P < 0.05$). These findings suggest that a short-term course treatment with Lovastatin can protect against sciatic nerve injury. Findings indicate that postoperative administration of Lovastatin led to accelerate regeneration process and motor function recovery in nerve crush model in rats. This effect might be due to the anti-inflammatory, immunomodulatory or anti-oxidative properties of Lovastatin. It is clear that more research is needed to confirm these findings.

1. Introduction

Peripheral nerve injury (PNI) is a common traumatic injury. Despite the innate capacity of peripheral nervous system to regenerate, following severe PNI, regeneration is often insufficient and functional recovery is incomplete (Kim et al., 2011). Primary damage initiates a series of secondary events such as oxidative stress, inflammation and excitotoxicity that lead to the expanding damage zone (Tator and Fehlings, 1991; Umebayashi et al., 2014). Therefore, any agents that attenuate above-mentioned mechanisms may enhance the efficacy of nerve regeneration process and improve functional recovery, especially if the continuity of the nerve is still intact.

Statins such as lovastatin, are potent cholesterol-lowering agents that clinically used for prevention of cardiovascular events due to atherosclerosis (Vollmer et al., 2004). Also, statins have pleiotropic effects including anti-inflammatory, antioxidant, immunomodulatory and neuroprotective properties (Hayashi et al., 2005; van der Most et al., 2009; Li et al., 2009). Several studies indicate that statins are effective against a variety of neurological disorders. Some evidences showed that statins may be effective in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD) (Zamrini et al., 2004), Parkinson's disease (PD) (Wolozin et al., 2007) and multiple sclerosis (MS) (Ciurleo et al., 2014). Also, some studies showed that administration of statins can attenuate traumatic brain and spinal cord injury (Pannu et al., 2005; Chen et al., 2007b).

Although several studies have investigated the effects of statins on the central nervous system (CNS), there is little scientific information about their effects on the peripheral nerve regeneration. The results of some studies indicated that Simvastatin and Atorvastatin can protect against sciatic nerve crush injury in rats (Pan et al., 2010; Xavier et al., 2012). Also, Atorvastatin is effective against neuropathic pain in rat neuropathy model (Pathak et al., 2014). In contrast, the results of another study show that Simvastatin delay regeneration as shown in histological studies but still there was no influence on electrophysiological measurements (Daglioglu et al., 2010). In this regard, Lovastatin as a lipophilic statins is capable of crossing the blood–brain barrier (BBB) (Botti et al., 1991). Some studies show that Lovastatin can increase neurite outgrowth (van der Most et al., 2009), attenuate

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glutamate excitotoxicity (Dolga et al., 2009) and has immunomedulatory effects (Paintlia et al., 2006).

Due to the lack of sufficient information about neuroprotective effects of Lovastatin against peripheral nerve injury, this experimental study was designed to evaluate the effect of Lovastatin on behavioral, electrophysiological and morphological parameters during sciatic nerve regeneration process in Wistar rats.

2. Materials and methods

2.1. Chemicals

Lovastatin and dimethyl sulfoxide (DMSO) were purchased from Sigma (USA) while ketamine and xylazine were obtained from Alfasan Pharmaceutical Co. (Holland) in injectable form. Lovastatin was dissolved in DMSO and fresh drug solutions were prepared each day of the experiments. All drugs were injected intraperitoneally.

2.2. Animals

All experiments were performed on adult male Wistar rats (weighing 250–300 g, aged 3 months). Animals were maintained under standardized housing conditions (temperature, 22 ± 2 °C, 12-h light/dark cycle light on from 7 a.m. and 60 ± 5% humidity) in plexiglas cages with free access to food (standard laboratory rodent chow) and tap water *ad libitum*. Experiments were carried out between 9 a.m. and 12 p.m. Ten rats were used for each treatment group. All animal experiments were carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC).

2.3. Surgical procedures

All experiments were performed under an operating microscope in sterile conditions by the same investigator. All animals were deeply anesthetized using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). The skin was shaved and disinfected using 10% povidone iodine. Then, rats were fixed in the prone position on the operating table under sterile conditions. The left sciatic nerve was exposed through a longitudinal incision extending from the greater trochanter to the mid-thigh. Then, a 3 mm-long segment of the sciatic nerve was crushed by maximally clamping the nerve with non-serrated hemostatic forceps (Belge de Gembloux, Belgium) for 30 s at 1 cm below sciatic notch. This procedure causes axonal interruption but preserves the connective sheaths (axonotmesis). For limiting the inter-animal variability in the postoperative outcome followed by microsurgical neurorrhaphy, the crush model was used. The crush model is appropriate to assess the roles of different agents in the nerve regeneration process and for pharmacological investigation (Tos et al., 2009). All surgeries were done using the same forceps. The nerves were kept moist with 37 °C sterile saline solution throughout the surgical intervention. The crush site was marked with a 10-0 nylon suture (Alcon). In the sham-operated group, the left sciatic nerve was treated in the same way except for the crush. Finally, the muscle and skin were sutured with 6-0 nylon and rats were allowed to recover spontaneously from anesthesia. After recovery, each rat has been housed separately per cage. All animals received buprenorphine (1 mg/kg) for three days after surgery in order to control pain. To prevent autotomy, bitter nail polish was applied to each rat's left foot. During the study, animals were examined for signs of autotomy and contracture.

2.4. Experimental groups

Fifty rats with sciatic nerve crush were randomly allocated into five groups (n = 10). In the two experimental groups, the animals were treated daily with Lovastatin at the doses of 2 or 5 mg/kg within 7 days after surgery. These selected doses of Lovastatin had neuroprotective effect in CNS and experimental autoimmune encephalomyelitis (Aguirre-Vidal et al., 2015; Paintlia et al., 2008). Controls were injected with vehicle (DMSO) and the sham-operated group was subjected to the surgical procedure without the nerve crush.

2.5. Functional evaluation

The recovery of motor function was assessed by calculating the sciatic functional index (SFI) at 1, 3, 5, 7 and 9 weeks after crush injury.

2.6. Sciatic functional index (SFI)

The SFI test was performed in a confined corridor (100 × 10 × 20 cm) with a dark box at the end. A white paper was placed on the floor of the corridor. Before the surgery, all rats were trained to walk in the corridor. Once the animals had learned to walk along the runway without stopping, their footprints were recorded. To record the footprints, the hind paws of rats were pressed down onto a finger paint-soaked sponge. Then animals were allowed to walk down the corridor leaving their hind footprints on the paper. The SFI value was calculated by putting the obtained data in the formula: $SFI = -38.3[(EPL-NPL)/NPL] + 109.5[(ETS-NTS)/NTS] + 13.3\ [(EIT-NIT)/NIT] - 8.8$, where EPL: the experimental paw length, NPL: the normal paw length, ETS: the experimental toe spread, NTS: the normal toe spread, EIT: the experimental intermediary toe spread and, NIT: the normal intermediary toe spread (Bain et al., 1989). The SFI value varies from 0 to −100, with 0 corresponding to normal function and −100 indicating total impairment. When no footprints were measurable, the index score of −100 was given (Dijkstra et al., 2000). In each walking track, three footprints were analyzed by a single observer and the average of the measurements was used in SFI calculations.

2.7. Electrophysiological evaluation

At before and immediately after sciatic nerve crush, 5 and 9 weeks postoperative, non-invasive compound muscle action potential (CMAP) recording was performed in all animals following anaesthesia by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). CMAPs were recorded in the gastrocnemius muscle by surface stimulation via the tendon–belly method (Oğğuzhanoğğlu et al., 2010), using an electrophysiological apparatus (CEPTU, England) with PicoScope System Software. The sciatic nerve was stimulated by bipolar stimulating electrodes, which were placed on the skin premoistened with gel electrode, just in between ischial tuberosity and major trochanter and parallel to the sciatic nerve. The active and reference monopolar needle electrodes were inserted into the mid-belly and muscle tendon surface, respectively. A ground electrode was clamped to the skin, between the stimulating and recording electrodes. Stimulations with durations of 0.02 ms were given at gradually increasing intensity until a maximal CMAP response was obtained. The recording was repeated three times, and the amplitude and latency of CMAP were averaged for each rat. Normal CMAPs were measured from the contralateral uninjured sides. All acquired data were entered into the computer to calculate the electrophysiological parameters of the regenerated nerve.

2.8. Histomorphometry analysis

At 9 weeks postoperative, following the electrophysiology study, the animals were deeply anaesthetized with an intraperitoneal injection of ketamine and xylazine cocktail, distal parts of the crushed site of the left sciatic nerves were harvested from every group. Nerve samples were fixed in 4% paraformaldehyde and post-fixed in 1% osmium tetroxide. After dehydration with ascending ethanol passages, the

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