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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (23): 4256-4260, 2007
ISSN 1028-8880

The Effects of Simvastatin on Functional Recovery of Rat Reperfused Sciatic Nerve

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Abstract: This study evaluated the effectiveness of simvastatin in protecting sciatic nerve from ischemia-reperfusion (I/R) injury using the model of experimental nerve ischemia. Sixty adult male Sprague-Dawley rats weighing 250-300 g were used. They were divided into ten groups (N = 6 per group). We used ischemia model in these groups. All ischemia groups were rendered ischemic for 3 h. Then followed by reperfusion durations of zero time (0 hR), 3 h (3 hR), 7 days (7 dR), 14 days (14 dR). The treatment group received intravenous simvastatin (1 mg kg^{-1}) 1 h before ischemia, while the control group received an equal volume of intravenous vehicle at the same time schedule and route. Behavioral data were obtained immediately before euthanasia. The score was based on coordination, racing reflex, toe spread and reaction to pinch. In simvastatin treated I/R rats we had increase in functional recovery. In conclusion, pre-ischemic administration of simvastatin exhibits neuroprotective properties in I/R nerve injury.

Key words: Simvastatin, functional recovery, ischemia-reperfusion, peripheral nerve, rat

INTRODUCTION

Ischemia plays a major role in the development of pathological alterations in many different neuropathies. Diabetes mellitus, vascular occlusive diseases, necrotizing vasculitides and trauma are only a few pathologic conditions which result in neuropathy associated with ischemia of peripheral nerves (Kihara *et al*., 1996). Severe ischemia to nerve results in energy rundown (Zollman *et al*., 1991) followed by conduction failure (Schmelzer *et al*., 1989) and fiber degeneration (Haruyasu *et al*., 2003). Reperfusion results in oxidative injury to endothelial cells with ensuing endoneurial edema and augments fiber degeneration (Anderson *et al*., 1997; Nagamatsu *et al*., 1996). Pathological and physiological effects of anti-oxidants, such as vitamin E, alprostadil, α- lipoic acid and melatonin, have been demonstrated in reperfusion nerve injury (Hale *et al*., 2004; Mitsui *et al*., 1999b; Abtullah *et al*., 2004). HMG-CoA reductase inhibitors (statins) are lipid-lowering drugs that also exert peliotropic vasculoprotective effects via activation of the endothelial NO synthesis. Retarding properties of β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitors, or statins, in both the coronary and carotid arterial beds are well established (Carl and Delanty, 1999). Previous experimental studies of statins in ischemia-reperfusion injury have clearly demonstrated cardio protective (Allan *et al*., 1999; Honjo *et al*., 2002) and neuroprotective (Carl and Delanty, 1999) effects despite unaltered serum cholesterol levels. Experimental studies have revealed that acute simvastatin therapy increases the half-life of endothelial NO synthase mRNA and activates the protein kinase Akt pathway, resulting in enhanced NO production. The protective effects of NO have been demonstrated in previous experimental studies in the heart and other organs (Carl and Delanty, 1999; Allan *et al*., 1999; Honjo *et al*., 2002). Statins preserve endothelial function and have anti-inflammatory, antioxidant and antithrombotic effects that may be neuroprotective during cerebral ischemia and reperfusion (Carl and Delanty, 1999). Statins may be neuroprotective through potential antioxidant effects (Carl and Delanty, 1999). In the general, simvastatin seems to have several beneficial effects that may ameliorate damaging outcomes of I/R injury. To date, no study has been performed to examine directly simvastatin possible beneficial effects on I/R of the sciatic nerve. We investigated the effect of simvastatin in a sciatic nerve I/R model of rats and discuss the possible cytoprotective mechanisms of simvastatin against ischemic fiber degeneration. The main goal of the present study was to examine if simvastatin would have protective effects on peripheral nerves subjected to I/R injury. We monitor the influence of simvastatin on functional recovery from a sciatic nerve after ischemia-reperfusion in adult rat.

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MATERIALS AND METHODS

All animals were obtained from experimental research laboratory of Tehran university school of medicine. Sixty adult male sprague-dawley rats weighing 250-300 g were used. The animals were acclimatized for 1 week to the condition of our laboratory before the commencement of the experiment. The animals were exposed to 12 h light and 12 h dark cycle at a room temperature of 22°C. The animals had free access to standard laboratory chow and water ad libitum. The rats were divided into ten groups (n = 6 per group). The group 1 received sham surgery only. The group 2 received simvastatin (Shahre daru Co. Iran) only and four control (Ischemia-reperfusion) and four experimental simvastatin treatment groups. Rats were anesthetized once intraperitoneally with ketamine HCl (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}) (Abtullah *et al*., 2004). The animals were placed in supine position on a heated mat during the operation and recovery. Right femoral vessels were exposed through an inguinal incision and were dissected free the femoral nerve under operating microscope. Sciatic nerve ischemic by occluding the femoral artery and vein with a silk suture 6-0 using slipknot technique (Haruyasu *et al*., 2003) for rapid release and reperfusion was achieved by release of these ligatures. In all groups the vascular ligature was removed after 3 h of ischemia (Saray *et al*., 2003) and the sciatic nerve was allowed to reperfuse for zero time (0 hR), 3 h (3 hR), 7 days (7 dR), 14 days (14 dR) in both the control and experimental simvastatin treatment groups. Simvastatin was administered in all experimental simvastatin treatment groups with a single dose of 1 mg kg^{-1} prior vascular ligature via tail vein (IV) (Allan *et al*., 1999; Wayman *et al*., 2003). The animals were placed under heating lamps until they recovered from anesthesia. Details of the groups in (Table 1). Animals were kept under accordance of protocols approved by the institutional animal care and use committee.

Behavioral score: The function of the limb was scored with observer blinded to the status of the rats Fig. 1. The score for each index was based on a scale of 0 (no function) to 3.0 (normal function). The score was based on gait, racing reflex, toe spread, pinch sensitivity. Gait was scored from 0 (no function), to 3 (normal function) with 1 and 2 for very and slightly impaired function, respectively. Withdrawal from pinch sensivity was scored either as present (2) or absent (0). Racing reflex (0 = foot completely removed to 3 = normal), toe spread with measurement foot print length defined. Increasing function was indicated by larger score. From the foregoing, a composite score, 0-11, in increasing limb function, was derived (Mitsui *et al*., 1999a).

Fig. 1: Ischemic side different from the countralatral side. Attention to position of limb

Table 1: Details of groups, pre-ischemia simvastatin (+) administration of simvastatin before ischemia

Groups	Reperfusion time	Ischemia	Pre-ischemia simvastatin
1	-	-	-
2	-	-	+
3	0 h	+	-
4	3 h	+	-
5	7 day	+	-
6	14 day	+	-
7	0 h	+	+
8	3 h	+	+
9	7 day	+	+
10	14 day	+	+

Statistics: Kurskal-Wallis test and Dunn's multiple comparison tests were chosen for analysis variables.

RESULTS

None of the animals died during the study. No complication was noted in any of the animals. In the present study, the behavioral mean rank for groups I/R1 and I/R2 was 48.50 although there was no significant difference between two groups. The behavioral mean rank for groups IR3, IR4, IR5 and IR6 was 14.42, 11.08, 10.58

Table 2: Experimental paradigm

Groups	N	Mean rank
1.00	6	48.50
2.00	6	48.50
3.00	6	14.42
4.00	6	11.08
5.00	6	30.58
6.00	6	35.58
7.00	6	9.33
8.00	6	15.17
9.00	6	43.33
10.00	6	48.50
Total	60	

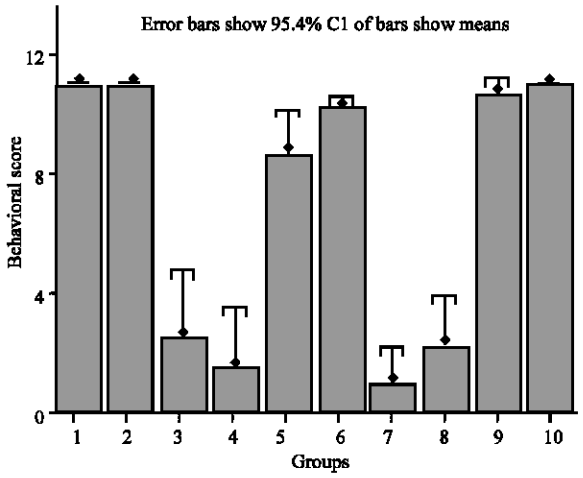

Fig. 2: Result of Kurskal-Wallis test and Dunn's multiple comparison tests for behavioral score. Behavioral score (y-axis) grade for 3h ischemia followed by reperfusion durations of zero time(0 hR), 3 h (3 hR), 7 days (7 dR),14 days(14 dR).Half of the groups (x-axis) had experimental simvastatin IV injection treatment via tail vein 1 h before ischemia (7 (0 hR), 8 (3 hR), 9 (7 dR), 10 (14 dR)). The other half experienced only I/R as control groups (3(0 hR), 4 (3 hR), 5 (7 dR), 6 (14 dR)). Group 1 is normal group without I/R and group 2 only administration simvastatin without I/R. Function acquired was greater for the simvastatin groups (7, 8, 9, 10) than nontreated group (3, 4, 5, 6). Means edema of groups 7, 8, 9, 10, respectively comparison with groups 3, 4, 5, 6

and 35.58 (Table 2). The behavioral mean rank for treatment groups IR7, IR 8, IR 9 and IR 10 was 9.33, 15.77, 43.33 and 48.50, respectively (column).

Animals in the treatment groups showed a better time course in the recovery. Function was greater for the simvastatin group than no treated group. The improvement with simvastatin did not reach statistical significance in the group ischemia alone ($p<0.012$) (Fig. 2). The improvement with simvastatin did not reach statistical significance in the group (IR 8) compared with control group (IR4), $p<0.012$) compared with controls there was significantly recovery seen as long as 7 days and 14 days of reperfusion ((IR 9 and IR 10 compared with IR5 and IR6 respectively), ($p<0.012$). At day 14 motor functions was recovered to near-normal levels in simvastatin treatment group.

DISCUSSION

Peripheral nervous system injury resulting from I/R, however, is still a common clinical problem associated with acute trauma (e.g., replantation, transplantation, tourniquet injury, compartment syndrome, Saturday night palsy) and Chronic conditions (carpal tunnel syndrome, tumors, callus, etc.) (Shin *et al*., 2005). This injury poses a challenge because the degree of injury and time course of recovery vary considerably (Shin *et al*., 2005). Effects of different antioxidant studied in ischemia-reperfusion peripheral nerve. The improvement with α-lipoic acid (LA), a potent antioxidant, did not reach statically significance (Mitsui *et al*., 1999a). Because satisfactory functional recovery is the ultimate goal of the treatment of peripheral nerve injuries, administration of simvastatin has a potential role in the treatment of nervous system injury. Simvastatin (statins) are effective lipid-lowering agents. Simvastatin have anti-inflammatory, antioxidant and antithrombotic effects. Recent experimental studies suggested that the beneficial effects of simvastatin in I/R rats not only a result of an improved lipid profile but also mediated by antioxidant actions.

Simvastatin administration to rat increases functional recovery of sciatic nerve. Two phases were identifiable in the treatment simvastatin group: phase one (0-3 h) the improvement with simvastatin did not reach statically significance compared with control group. Phase two (7 and 14 days) compared with controls there was significantly recovery. Present results have shown that simvastatin treatment effectively promoted motor functional recovery in reperfused rat sciatic nerve after 7 days of ischemia. Our studies have shown that behavioral function can recover to near normal during the 14 days of reperfusion in the treatment group.

Inhibition of inducible nitric oxide synthase promotes recovery of motor function in rats after sciatic nerve ischemia and reperfusion (Shin *et al*., 2005). This study (Shin *et al*., 2005), showed that all 1400 W-treated groups had an earlier recovery of the motor function than the water-treated control group between days 11 and 25 after ischemia. At day 35 motor functions was recovered to

near-normal levels in 1400 W treatment group (Shin *et al*., 2005). In the simvastatin treatment group, at day 14 motor functions was recovered to near-normal levels.

It is well known that simvastatin provides protection against I/R injury of the heart (Allan *et al*., 1999; Honjo *et al*., 2002), lung (Babu *et al*., 2003), brain (Carl and Delanty, 1999) and retina (Honjo *et al*., 2002).

Studies have repeated revealed the neuroprotective action of simvastatin in cerebral I/R model. Retarding properties of simvastatin in the both coronary and arterial beds well established (Carl and Delanty, 1999). Simvastatin improve neurological deficiets in rats, in parts, by reducing reactive oxygen species in endothelial cell (Haendeler *et al*., 2004), inhibiting leukocyte-endothelial interaction through the release of nitric oxide from the endothelium (Honjo *et al*., 2002).

Statins both upregulate endothelial nitric oxide synthase (eNOS) and inhabit inducible nitric oxide synthase (iNOS), effects that potentially neuroprotective. The preservation of eNOS activity in cerebral vasculature, particularly in the ischemic penumbra, may be especially important in preserving blood flow and limiting neurological loss (Carl and Delanty, 1999; Allan *et al*., 1999).

Haendeler *et al*. (2004). demonstrates a novel antioxidant mechanism by which statins reduce reactive oxygen species (causes neural and endothelial damage) in endothelial cells. Statin-mediated S-nitrosylation of thioredoxin enhanced the enzymatic activity of thioredoxin, resulting in a significant reduction in intracellular reactive oxygen species.

Current evidence leads to at least two major hypotheses concerning their antioxidant mechanism:

- Inhibiting leukocyte-endothelial interaction through the release of nitric oxide from the endothelium (Honjo *et al*., 2002).
- Statin-mediated S-nitrosylation of thioredoxin enhanced the enzymatic activity of thioredoxin, resulting in a significant reduction in intracellular reactive oxygen species (Haendeler *et al*., 2004).

In general two mechanism mentions above reduce reactive oxygen species via release of Nitric Oxide (NO). In summary, we conclude that Simvastatin therapy causes physiological improvement on sciatic nerves which have been subjected to I/R. We postulate that the neuroprotective effects of Simvastatin are attributed to its direct and indirect antioxidant actions. We believe that pre-ischemic administration of simvastatin exhibits neuroprotective properties in I/R nerve injury.

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