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Alpha-lipoic acid provides neuroprotection from ischemia-reperfusion injury of peripheral nerve

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Abstract

Background: Reperfusion aggravates nerve ischemic fiber degeneration, likely by the generation of reduced oxygen species. We therefore evaluated if racemic α-lipoic acid (LA), a potent antioxidant, will protect peripheral nerve from reperfusion injury, using our established model of ischemia-reperfusion injury. *Methods:* We used male SD rats, 300±5 g. Ischemia was produced by the ligature of each of the supplying arteries to the sciatic-tibial nerve of the right hind-limb for predetermined periods of time (either 3 or 5 h), followed by the release of the ligatures, resulting in reperfusion. LA was given intraperitoneally daily for 3 days for both pre- and post-surgery. Animals received either LA, 100 mg/kg/day, or the same volume of saline intraperitoneally. Clinical behavioral score and electrophysiology of motor and sensory nerves were obtained at 1 week after ischemia-reperfusion. After electrophysiological examination, the sciatic-tibial nerve was fixed in situ and embedded in epon. We evaluated for ischemic fiber degeneration (IFD) and edema, as we described previously. *Results:* Distal sensory conduction (amplitude of sensory action potential and sensory conduction velocity (SCV) of digital nerve) was significantly improved in the 3-h ischemia group, treated with LA ($P<0.05$). LA also improved IFD of the mid tibial nerve ($P=0.0522$). LA failed to show favorable effects if the duration of ischemia was longer (5-h ischemia). *Conclusion:* These results suggest that α-lipoic acid is efficacious for moderate ischemia-reperfusion, especially on distal sensory nerves.

Keywords: Ischemia-reperfusion injury; Peripheral nerve; Oxidative stress; α-Lipoic acid; Neuroprotection; Sensory conduction velocity; Ischemic fiber degeneration

1. Introduction

Ischemic injury to peripheral nerve is aggravated by reperfusion, resulting in lipid peroxidation and fiber degeneration [1,2]. In other tissues, including brain, the major mechanism of reperfusion injury is considered to be due to reduced oxygen species [3]. α-Lipoic acid (LA) is a powerful lipophilic antioxidant in vitro and in vivo [4]. It is reported to be efficacious in the experimental ischemia-reperfusion injury in brain [5–7], heart [8,9] and in experimental diabetic neuropathy [10,11], where oxidative stress is present. We therefore evaluated if LA will ameliorate ischemia-reperfusion (IR) injury of peripheral nerve subjected to different durations of ischemia followed by reperfusion. We used our established model of IR injury [12].

2. Materials and methods

2.1. Animals

We used 44 male Sprague–Dawley rats weighing 300±5 g.

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2.2. Surgery

The surgical procedure for producing IR injury has been previously detailed. In essence we ligated each of the supplying arteries to the sciatic-tibial nerve of the right hind-limb for predetermined periods of ischemia (either 3 or 5 h), followed by reperfusion, resulting from the release of the ligatures.

The rat was anesthetized with intraperitoneal pentobarbital (60 mg/kg). Additional doses of pentobarbital were used if anesthesia lightened during experiment. Silk ties with slip-knots were used for ready release for reperfusion. Ischemia was maintained for either 3 or 5 h followed by reperfusion for 1 week prior to harvest for pathological studies. Just before tissue harvest, they underwent standardized behavioral and electrophysiological evaluation. Sixteen rats were subjected to 3 h of ischemia, and 12 rats for 5 h of ischemia. An additional 16 rats, subject to 3 h of ischemia were used for the measurement of monophasic sciatic tibial nerve action potential. The monophasic recordings, done with the nerve immersed in oil with stimulation and recording via hook electrodes, results in morphological artifacts. We deemed these unsuitable for pathological evaluation, hence the additional groups. The experimental paradigm is summarized in Table 1.

2.3. α-Lipoic acid

For the 3-h ischemia-reperfusion group, 16 rats received 100 mg/kg α-lipoic acid (3-LA) and the other 16 rats were given saline (3-CONT). For the 5-h ischemia group, six rats were given α-lipoic (5-LA) acid and the other six rats were given saline (5-CONT). α-Lipoic acid was given intraperitoneally 100 mg/kg daily for 3 days prior to the surgical procedure and the same regimen was repeated for the 3 days after surgery.

2.4. Behavioral score

The function of the limb was scored with the observer blinded to the status of the rats. The score was based on gait, grasp, paw position, and reaction to pinch. Gait was scored from 0 (no function), to 3 (normal function) with 1 and 2 for very and slightly impaired function, respectively. Similarly, grasp was graded from 0 (none) to 3 (normal). Paw position varied from 0 (paw contracted) to 3 (normal). Withdrawal from pinch was either scored as present (2) or absent (0). Increasing function was indicated by a larger score. From the foregoing, a composite score, 0–11, in increasing limb function, was derived.

Table 1
Experimental paradigm

Ischemia	Reperfusion	*N*	Lipoic acid	Endpoints
3 h	7 days	8	No	SAP; CMAP; histology
3 h	7 days	8	No	MNAP (in vivo)
3 h	7 days	8	100 mg/kg	SAP; CMAP; histology
3 h	7 days	8	100 mg/kg	MNAP (in vivo)
5 h	7 days	6	No	SAP; CMAP; histology
5 h	7 days	6	100 mg/kg	SAP; CMAP; histology

2.5. Electrophysiology

We used techniques that are standard for our laboratory [13,14]. We measured the amplitude of compound muscle action potential (CMAP) of sciatic-tibial nerve and sensory nerve action potential in the digital nerve using fine stainless steel near-nerve stimulating and recording electrodes for the initial series of experiments. The CMAP was recorded from the dorsum of the hindpaw while stimulating at the level of the sciatic notch and ankle. The sensory nerve action potential was recorded from the ankle while stimulating the digital nerve at the tip of digit. These recordings did not cause histological damage and the same nerves were studied histologically. For the second series of experiments, we measured the monophasic compound nerve action potential amplitude of the sciatic-tibial nerve using previously described in vivo recording methods [15]. This latter approach was used only for electrophysiology. In brief, the sciatic-tibial nerve was exposed at the hip and midcalf levels, forming two pools, bathed with mineral oil and maintained at 35°C. The sciatic nerve at the hip and the tibial nerve at the midcalf levels were raised by platinum hook electrodes, and the nerve was crushed under the recording inactive electrode. The preparation was grounded between the stimulating and recording electrode pairs. All recordings were done at 35°C and amplified ×1000, stored on computer disk, and analyzed off-line using a Nicolet digital oscilloscope (Nicolet Instruments, Madison, WI). For each animal, identical recordings were done on the contralateral nerve, which served as control.

2.6. Neuropathology

Nerves were fixed in situ for 30 min using 4% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4). The entire length of the sciatic-tibial nerve was harvested, and the following segments were identified and separately dehydrated: the proximal sciatic, distal sciatic, mid tibial, and distal tibial. These nerve segments were separately osmicated, dehydrated, infiltrated, and embedded in epoxy. Transverse sections of 0.5 μm were stained with 1% toluidine blue. Under ×400 magnification, these sections were graded for edema and ischemic fiber degeneration (IFD) using a modification of previously described methods [12,16]. In brief, all slides were coded and read by blinded observers. Fibers were considered to be undergoing ischemic fiber degeneration if axonal changes were visible. The axon may be swollen or shrunken, watery and light, or dark and shrunken. Secondary myelin changes were typically seen, including attenuation, collapse or breakdown. For each section the percent of fibers undergo-

ing IFD was estimated. Edema was graded semiquantitatively as follows: 0=normal; 1=mild edema; 2=moderate edema; 3=severe edema. No distinction was made as to endoneurial, perivascular, or subperineurial edema.

2.7. Statistics

All experiments involving 3 h of ischemia were done on groups of eight rats and values were expressed as mean±S.E.M. For 5 h of ischemia, we used six rats for each group and values were also expressed as mean±S.E.M. Statistical analysis was done using two-tailed unpaired group *t*-tests.

3. Results

3.1. Behavioral score

The behavioral score for 3-CONT and 3-LA were 5.8±0.8 and 7.8±1.0, respectively (Fig. 1A). The behavioral score for 5-CONT and 5-LA were 2.5±0.8 and 3.8±0.7, respectively (Fig. 1B). Behavioral score was consistently normal (11.0) for contralateral side. The improvement with LA did not reach statistical significance.

3.2. Electrophysiology

In the present study, the results of the amplitude of nerve compound action potential was expressed as a percentage of the left side. For SCV, we used absolute values of m/s. The mean SAP amplitude of the right digital nerve was 75.6±11.2% and 116.5±13.8% for 3-CONT and 3-LA, respectively (Fig. 2A). The mean SCV of the right digital nerve was 48.8±1.6 m/s and 53.3±1.3 m/s for 3-CONT and 3-LA, respectively (Fig. 2B). The mean SAP amplitude of the right digital nerve was 6.7±4.7% and 7.1±1.1% for 5-CONT and 5-LA, respectively. Both amplitude and velocities were significantly improved with lipoic acid ($P<0.05$). The mean MNAP amplitude of the right sciatic-tibial nerve was 38.6±10.8% and 49.9±13.4% for 3-CONT and 3-LA, respectively (Fig. 3), lipoic acid-treated nerves showing a nonsignificant improvement. The mean CMAP amplitude of the right sciatic-tibial nerve was 40.0±15.3% and 32.3±8.5% for 3-CONT and 3-LA, respectively. The mean CMAP amplitude of the right sciatic-tibial nerve was 4.1±3.3% and 3.2±1.9% for 5-CONT and 5-LA, respectively.

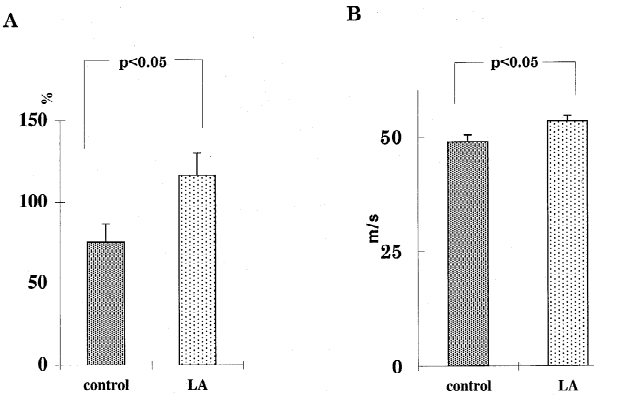

Fig. 2. Sensory conduction study for 3 h of ischemia. The amplitude of sensory action potential (left panel) is expressed as a percentage of the contralateral side following lipoic acid treatment. Sensory conduction velocity is shown on the right panel. Both amplitude and velocity were significantly improved with treatment of LA.

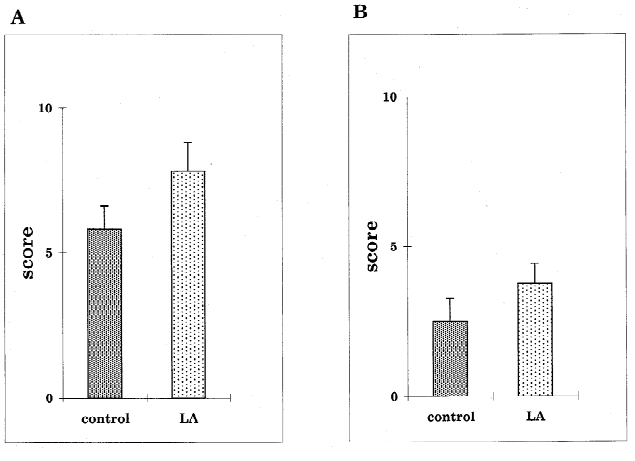

Fig. 1. (A) Behavioral score for 3 h of ischemia. Function was greater for the lipoic acid (LA) group than nontreated group. (B) Behavioral score for 5 h of ischemia. Function was poorer than for 3 h for both groups.

3.3. Neuropathology

The frequency of fiber degeneration varied by duration of ischemia, by segment of peripheral nerve and by the treatment (placebo vs. α-lipoic acid). The pathologic changes of nerves subjected to ischemia-reperfusion consisted of fiber degeneration associated with nerve edema. Ischemic fiber degeneration was most pronounced at the

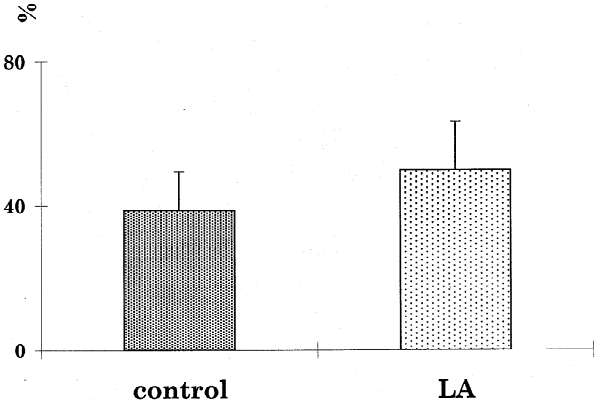

Fig. 3. Monophasic nerve action potential for 3 h of ischemia. The amplitude of muscle nerve action potential (MNAP) is expressed as a percentage of contralateral side.

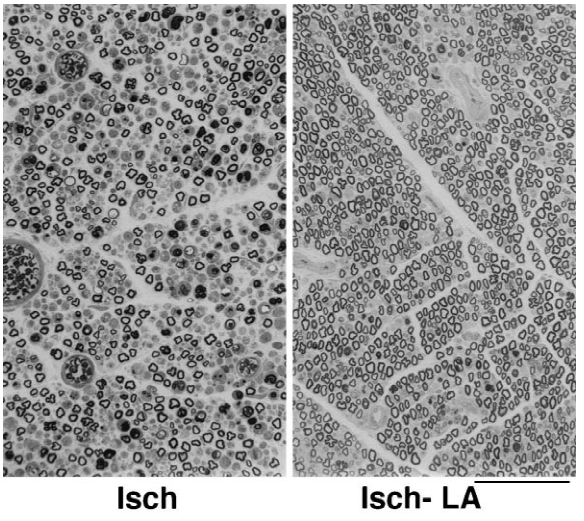

Fig. 4. Light microscopic findings of transverse sections of the mid-tibial nerve after 3 h of ischemia followed by 1 week reperfusion. Degenerated fibers were predominant for nontreatment group, and a small number of degenerated fibers were seen in the lipoic acid group. Scale bar is 100 μm. Isch, ischemia-reperfusion; Isch-LA, ischemia-reperfusion+lipoic acid treatment.

mid and distal tibial level in both 3-h ischemia and 5-h ischemia (Figs. 4 and 5). The pathological findings following 5 h of ischemia (Fig. 5) were consistently more pronounced than those seen after 3 h of ischemia (Fig. 4) for both IFD (Fig. 6B) and endoneurial edema (Fig. 7B). These findings were similar to our previous study [12]. For the 3-h ischemia group, treatment with LA resulted in some improvement in the pathological findings of IFD and endoneurial edema (Figs. 4, 6A and 7A) reaching borderline significance. For the 5-h ischemia group, LA-treated

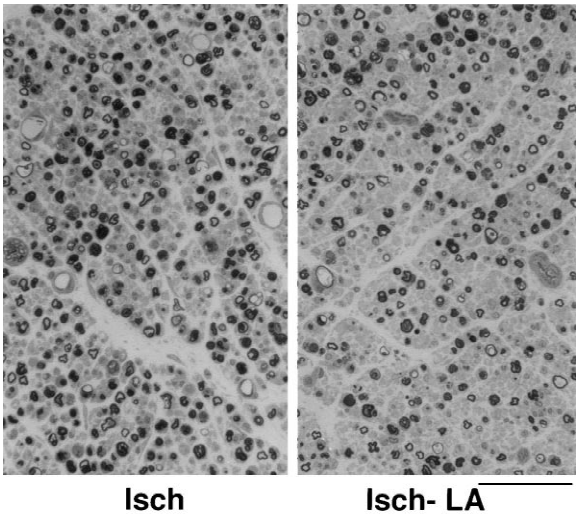

Fig. 5. Light microscopic findings of transverse sections of the mid-tibial nerve after 5 h of ischemia. Most fibers were degenerating and normal fibers were seldom seen in both groups. Scale bar is 100 μm. Isch, ischemia-reperfusion; Isch-LA, ischemia-reperfusion+lipoic acid treatment.

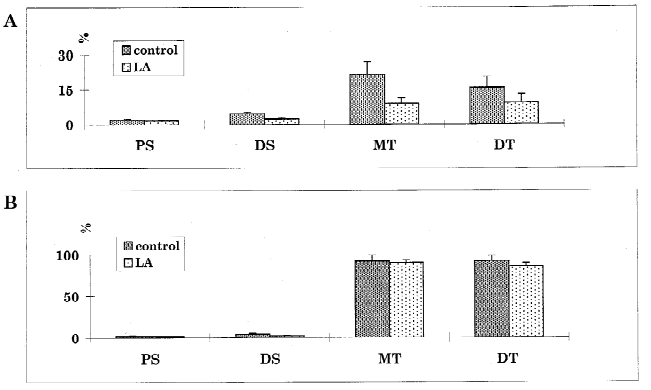

Fig. 6. (A) Ischemic fiber degeneration (IFD) index for 3 h of ischemia. Changes were confined to tibial nerve. For both tibial nerve levels, neuroprotection was seen in the lipoic acid (LA) group. IFD grade of mid-tibial nerve in the lipoic acid (LA) group was higher than no treatment group ($P=0.0522$). (B) IFD grade for 5 h of ischemia. Changes are more pronounced at 5 h than 3 h. Nearly 100% of nerve fibers were affected in both groups. PS, proximal sciatic; DS, distal sciatic; MT, mid tibial; DT, distal tibial.

nerves failed to show any improvement of these pathological changes (Figs. 6B and 7B).

4. Discussion

The main findings of the present study are that LA provided some neuroprotection of peripheral nerve from ischemia-reperfusion injury (IR injury) in nerves subjected to 3 h of ischemia, but failed to do so in those subjected to 5 h of ischemia. Treatment with LA significantly improved distal sensory conduction in the group subjected to 3 h of ischemia. In contrast, LA treatment did not improve CMAP in the 3-h ischemia group (3-LA). In the pathological studies, IFD grade at mid tibial nerve showed some improvement ($P=0.0522$) for nerves subjected to 3 h of ischemia (3-LA).

In nerves, the effects of reperfusion-induced reduced

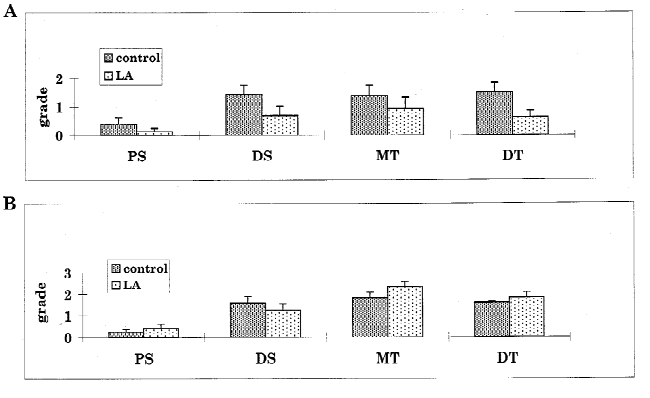

Fig. 7. (A) Edema grade for 3 h of ischemia. Edema was less seen in the LA group at all portions of the nerves. (B) Edema grade for 5 h of ischemia. More pronounced edema is seen at 5 h than with the 3 h group. PS, proximal sciatic; DS, distal sciatic; MT, mid tibial; DT, distal tibial.

oxygen species can be evaluated directly by measuring the permeability surface area product [17], or the passage of molecules of known size morphologically [18], or indirectly by evaluating endoneurial edema [18,19]. Transient nerve ischemia causes no change in permeability, whereas reperfusion results in a significant increase in the permeability coefficient [17]. The footprints of lipid peroxidation in nerve have been demonstrated biochemically by the increase in lipid hydroperoxides with reperfusion [2] or histochemically by the increase in carbonyl formation in vessels following ischemia-reperfusion [20]. In our present study, neuroprotection was demonstrable in nerves subjected to moderate ischemia (3 h) but not in the group with total or near-total fiber degeneration. This observation is consistent with our earlier observations. We reported a breakdown of the blood-nerve barrier by ischemia alone, when it was sufficiently severe and prolonged [17]. We also found [2] that reperfusion resulted in an increase in nerve lipid hydroperoxides, and increases in endoneurial edema and IFD in moderately ischemic nerves, whereas the most ischemic segment, of non-reperfused nerves, underwent both edema and IFD that was as pronounced as those of other segments after reperfusion, providing further evidence that ischemia alone can also cause IFD and edema.

LA and its reduced form, dihydrolipoic acid (DHLA) are potent biological antioxidants [4,21,22]. These compounds are capable of scavenging hydroxyl radicals and singlet oxygen [4]. They also act as a chelator of transition metals such as Cu^{+}, Mn^{+} and Zn^{+} [23]. These actions contribute to reduce oxidative stress. The mechanisms of reperfusion injury are complex. Reperfusion results in a burst of free radicals during reoxygenation of hypoxic tissue [3]. Maximal tissue damage is observed during reperfusion, which is primarily attributed to oxidative injury resulting from production of oxygen free radicals. One of the major consequences of such damage is the depletion of the cellular antioxidant, glutathione (GSH), leading to oxidation of protein thiols to disulfides and the loss of activity of critical enzymes having active thiol group(s) [6]. In addition to oxidative stress (reduced $NADH/NAD^{+}$ ratio), there is also reductive stress (increased $NADH/NAD^{+}$ ratio), and LA has been shown to normalize indices of both oxidative and reductive stress [24]. Indeed studies reporting neuroprotection of brain from reperfusion injury have appeared. Neuroprotection ascribed to LA was reported in the gerbil subjected to experimental forebrain ischemia [5]. Similar benefits were reported in the rat subjected to bilateral carotid ligation and hypotension [6]. Wolz and Krieglstein [7] reported benefits in both rats and mice subjected to focal ischemia and found that the *R*- and *S*-enantiomers were equally effective.

The different response of sensory from motor nerves deserves comment. In our study, LA improved the distal sensory conduction, but failed to improve the motor conduction of the sciatic-tibial nerve. This discrepancy of electrophysiology was also shown in our study with experimental diabetic neuropathy [2]. Part of the explanation may reside with the use of compound muscle action potential to evaluate motor nerve function. Muscle appears to be more vulnerable to ischemia than peripheral nerve [25] so that CMAP, which reflects function of both muscle and nerve, may simply be an indicator of more pronounced muscle damage. This cannot be the sole explanation, since we also recorded the compound nerve action potential in vivo. This method allowed us to evaluate nerve action function in isolation. The results showed some degree of improvement of nerve action potential, but this improvement still was not enough to explain this discrepancy, suggesting some specificity by fiber type.

As previously described, hypothermia showed dramatic neuroprotection on nerve IR injury. Compared with hypothermia, the neuroprotective effect of LA on IR injury is considerably more modest. Hypothermia of 28°C could completely prevent the behavioral, electrophysiologic and pathologic indices of fiber degeneration; this level of neuroprotection was never seen in LA neuroprotection. These results suggest that oxidative stress is a mechanism of nerve injury but likely not the major mechanism, and that therapeutic strategy for nerve neuroprotection from ischemia injury should not be based on antioxidants alone.

In summary, the α-lipoic acid provides neuroprotection from reperfusion injury but the therapeutic window is relatively narrow, definable in terms of severity and duration of ischemia and possibly, by fiber type, with sensory fibers being more susceptible than motor nerve fibers.

Acknowledgements

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