Impact of Aortic Cross-Clamping Time on Peripheral Nerves: Experimental Model

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Purpose: The present study investigated the correlation between extend aortic cross-clamping time and peripheral nerve injury on rats.

Methods: 24 male, Sprague Dawley rats were divided into 3 groups; (a) control group: abdomen was directly closed after reached aorta, and followed by 72 hours, (b) short-term ischaemia-reperfusion group: peripheral nerve ischemia was induced in rats by supraceliac aortic occlusion for 20 min followed by 72 h of reperfusion, (c) long-term ischaemia-reperfusion group: peripheral nerve ischemia was induced for 30 min followed by 72 h of reperfusion. Preoperative and postoperative, electromyography (EMG) recordings were done. End of 72 h, the sciatic nerves were harvested from each animal for histopathological and biochemical analysis.

Results: The mean compound muscle action potential (CMAP) amplitude of long-term ischaemia-reperfusion group was statically significant reduced when compared to the control group (p <0.01). However, the mean distal latency value of long-term ischaemia-reperfusion group was statically significant increased (p <0.01). On the other hand, there were statically significant differences between the results of malondialdehyde, edema and ischaemia fiber degeneration grades on control and long-term ischaemia-reperfusion group (p <0.001).

Conclusion: This study demonstrated that the extending cross clamping time directly harms the peripheral nerve of rats.

Keywords: long-term, short-term, sciatic, rat

Introduction

The prevention of paraplegia is still an elusive goal after surgical reconstruction of the descending thoracic or thoracoabdominal aorta. In experimental aortic surgery conducted in the 19th century, a cross-clamping time of 10–15 min was proposed to safely prevent ischemic complications. Following the first clinical application of this technique to aorta aneurysms, it became to known as “clamp and go”.1 Spinal cord injuries such as paraplegia and paraparesis are still the most devastating complications of surgery for descending thoracic aortic aneurysm (dTAA) or thoracoabdominal aortic aneurysm (TAAA).2 Livesay, et al. demonstrated that the risk of paraplegia would increase from 3% to 11% with a clamping time of more than 30 min.3
Ischemia inflicts pathological changes on the nerve tissue, and the return of blood flow results in reperfusion injury. The pathophysiology of ischemia-reperfusion (I/R) injury includes platelet aggregation, release of oxygen radicals, and leucocyte-endothelial cell interactions. The resulting injury of the endothelium and obstruction of capillaries leads to impaired oxygen supply to the nerve tissue. The nerve tissue levels of malondialdehyde (MDA) lactate can be used to evaluate I/R injury.

There are insufficient data in the literature to determine the effects of cross clamping on peripheral nerves. Therefore, this experimental study was planned to illustrate the effects of aortic cross clamping on peripheral nerves.

Materials and Methods

In this study, 24 male Sprague Dawley rats weighing 350–450 g were used. The animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment (22°C ± 2°C) with 12-hour light/dark cycles. The experimental procedures were approved by the Committee for Animal Research of Ege University. All animal studies strictly conformed to the animal experiment guidelines of the Committee for Human Care.

Surgical procedure

All the rats were anesthetized by a combination of ketamine hydrochloride at a dose of 50 mg/kg (Alfamine®; Ege Vet, Alfasan International B.V., Holland) and 7 mg/kg of xylazine hydrochloric (Alfazyne®; Ege Vet, Alfasan International B.V., Holland), which was administered intraperitoneally. Before the rats were randomly divided into 3 groups, EMG recordings were obtained. After abdominal shaving, using a midline laparotomy incision, the aorta was dissected and controlled proximally at the supraceliac portion. In the first group (control group, n = 8), nerve ischemia was not induced, and there was no treatment. The abdomen was closed immediately after reaching the aorta, and the animals were followed for 72 h. In the second group (short-term I/R group, n = 8), peripheral nerve ischemia was induced in rats by supraceliac aortic occlusion for 20 min, followed by 72 h of reperfusion. In the third group (long-term I/R group, n = 8), peripheral nerve ischemia was induced in rats by supraceliac aortic occlusion for 30 min, followed by 72 h of reperfusion. At the end of the 72 h, sciatic nerve EMG recordings were done. Then, following reperfusion, the animals were euthanized, and left sciatic nerve tissues were isolated. A sciatic 2 cm nerve segment located proximally at 2 cm was harvested from each animal for histopathological and biochemical analysis.

Electrophysiological recordings

EMG recording were obtained 3 times from the right sciatic nerve and stimulated supra-maximally (intensity 10 V, duration 0.05 ms, frequency 1 Hz, range of 0.5–5000 Hz, 40 kHz/sec sampling rate) by a Biopac bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Inc., Santa Barbara, California, USA) from the Achilles tendon. Compound muscle action potentials (CMAPs) and changes of sensory and motor nerve conduction velocity were recorded by unipolar needle electrodes located on the 2nd and 3rd interosseous muscles. The distal latency, duration, and amplitude of the CMAP were evaluated with Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc., USA). During the EMG recordings, the rectal temperatures of the rats were monitored with a rectal probe (HP Viridia 24-C; Hewlett- Packard Company, Palo Alto, California, USA), and the temperature of each rat was maintained at approximately 36°C–37°C with a heating pad.

Biochemical assessment

A total of 100 mg ml⁻¹ of tissue was homogenized in buffer at a pH of 7.4. Artefactual production of additional MDA during processing was eliminated by the addition of 2% butylated hydroxytoluene to the homogenized tissue. A total of 20% trichloroacetic acid was then added to this mixture in 0.6 N hydrochloric acid. The mixture was centrifuged at 10000 g for 10 min at 4°C. A total of 0.12-mol l⁻¹ of TBA in buffer (pH 7.0) was added to the supernatant. The pigment was measured spectrophotometrically at 532 nm. The MDA results were calculated as nmol g⁻¹ tissue based on the optical density difference.

Histopathological analysis

The sciatic nerves were fixed in 2.5% glutaraldehyde in phosphate buffered saline (PBS) for 24 h. The tissue samples were then washed in PBS, postfixed for 2 h in 1% buffered osmium tetroxide, and dehydrated in graded concentrations of acetone and embedded in epoxy resin. Semithin sections (1 μm) were taken by Reichert ultramicrotome, stained with 1% toluidine blue, and observed with an Olympus BX51 light microscope. The thickness of the epineurium was measured with an Olympus C-5050 digital camera mounted on an Olympus BX51.
microscope. These sections were graded for edema and ischemic fiber degeneration (IFD) using previously described methods.9,10) The fibers were considered to have undergone IFD if axons appeared 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 undergoing IFD was graded from 0 to 4 as follows: 0 ≤ 2%, 1 = 3%–25%, 2 = 26%–50%, 3 = 51%–75%, and 4 ≥ 75%. Edema was semi-quantitatively graded from 0 to 4 as follows: 0-normal, 1-mild edema, 2-moderate edema, 3-severe edema, and 4-severe and global edema. No distinction was made as to endoneurial, perivascular, or subperineurial edema.

Statistical analysis
Data analyses were performed using SPSS version 15.0 for Windows. The groups of parametric variables were compared by a Student’s t-test and analysis of variance (ANOVA). The groups of nonparametric variables were compared by the Mann–Whitney U test. Results are given as mean ± standard error of mean. A value of p < 0.05 was accepted as statistically significant, and p < 0.001 was accepted as statistically highly significant.

Results
None of the animals died during the study, and no complication was noted in any of the animals.

Electrophysiological assessments
There were no statically significant differences (p > 0.05) in the mean CMAP values of the control and short-term I/R group before or after the operation. However, there were statistically significant differences (p < 0.01) in these parameters in the long-term I/R group. Furthermore, the mean CMAP amplitude of the long-term I/R group showed a statistically significant reduction (p < 0.01) when compared to that of the control and short-term I/R group. There were no statically significant differences (p > 0.05) in the mean CMAP amplitudes between control and short-term I/R group. The mean distal latency value of the long-term I/R group displayed a statistically significant increase (p < 0.01) when compared to that of the control and short-term I/R group. The means of the CMAP amplitude of the control and short-term I/R group showed no statistically significant differences (p > 0.05) before or after the operation (Fig. 1).

Biochemical analysis
The average MDA values of the control, the short-term I/R and the long-term I/R groups were 0.038 ± 0.05 nmol g−1, 0.049 ± 0.09 nmol g−1 and 0.247 ± 0.02 nmol g−1, respectively. The postoperative difference in the average MDA value of the control and long-term I/R groups was statistically significant (p < 0.001). However, there were no statically significant differences (p > 0.05) in the postoperative average MDA values between control and short-term I/R group (Table 1).

Histopathological assessment
In the histopathological analysis, the nerve fibers of the control and short-term I/R groups exhibited a normal structure and normal morphology, whereas those of the long-term I/R group showed an abnormal structure and morphology. In the long-term I/R group, intensive edema and perineural and endoneurial fibrosis were visible (Fig. 2).

Edema and IFD degeneration
The edema grades of the left sciatic nerve of the control group, the short-term I/R group and the long-term I/R group were 0.2 ± 0.2, 0.5 ± 0.3 and 3.6 ± 0.1, respectively. There was a statistically significant difference (p < 0.001) between the edema grades of the control and the long-term I/R group. But there were no statically significant differences (p > 0.05) in the edema grades between control and short-term I/R group. The IFD grades of the left sciatic nerve of the control group, the short-term I/R group and those of the long-term I/R group were 0.1 ± 0.1, 0.2 ± 0.1 and 3.7 ± 0.3, respectively. There was a statistically significant difference (p < 0.001) between the IFD grades of the control and the long-term I/R group. However, there was no statically significant difference (p > 0.05) in the IFD grades between control and short-term I/R group (Fig. 3).

Discussion
Aneurysm or the dissection of the thoracic aorta and thoracoabdominal aorta is induced by several etiologies and is managed by surgical treatment for the best results. But ischemic damage in major organs caused by aortic clamping is unavoidable with such surgical procedures. Ischemic damage can occur in kidney, liver, small and large intestines, and spinal cord. Among these, paraplegia due to the ischemic injury of the spinal cord is the most serious.11) There is insufficient information in the
Clamping Effects on Peripheral Nerves

The literature on how the clamping time of the aorta affects peripheral nerves. The prior investigations, which was about ischemia injury of spinal cord, was guided us to perceive of the influences of the aortic clamping time on peripheral nerves.\(^8,12–14\) Ischemic spinal cord injury (ISCI) causing paralysis is a devastating perioperative complication of surgical repair in aortic surgery.\(^14\) Paralysis can be immediate but is often delayed, with the reported incidence (0%-40%) varying between centers and as a function of the surgery and Crawford classification.\(^6,8,15,16\) Recent reviews focusing on high-volume centers indicate that 5%-11% of cases are accompanied by delayed postoperative paresis or paralysis.\(^15,16\) To understand the mechanisms responsible for ISCI, a number of experimental models have been developed.\(^17,18\) The need to minimize paresis and paralysis caused by ISCI subsequent to aortic cross clamping has stimulated the development of pig, canine, rabbit, and rat models.\(^17\) Lui, et al.\(^19\) evaluated ISCI in descending aortic occlusion in pigs. Based on electrophysiological recordings, they found that the spinal cord neuron amplitude decreased to <50% of baseline values. In addition, the prolongation of latency was >10% of baseline values when the descending aorta was clamped. They reported that if the clamping time is less than 30 min, it protects the spinal cord neurons. Liang, et al.\(^8\) investigated the efficacy of tetramethylpyrazine and deferoxamine in the treatment of ISCI in rats after the aorta had been clamped.

<table>
<thead>
<tr>
<th>Assessments</th>
<th>Control Group (Before Procedure)</th>
<th>Control Group (After Procedure)</th>
<th>Short-Term I/R Group (Before Procedure)</th>
<th>Short-Term I/R Group (After Procedure)</th>
<th>Long-Term I/R Group (Before Procedure)</th>
<th>Long-Term I/R Group (After Procedure)</th>
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</thead>
<tbody>
<tr>
<td>Distal Latency (ms)</td>
<td>1.27 ± 0.06</td>
<td>1.32 ± 0.07</td>
<td>1.33 ± 0.05</td>
<td>1.36 ± 0.04</td>
<td>1.29 ± 0.06</td>
<td>2.18 ± 0.02*</td>
</tr>
<tr>
<td>CMAP Amplitude (mV)</td>
<td>15.12 ± 0.7</td>
<td>15.19 ± 0.7</td>
<td>15.22 ± 0.9</td>
<td>15.14 ± 0.8</td>
<td>15.16 ± 0.9</td>
<td>12.21 ± 0.6*</td>
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<tr>
<td>Tissue Malondialdehyde (MDA) (nmol/µgr)</td>
<td>0.038 ± 0.05</td>
<td>0.049 ± 0.09</td>
<td>0.247 ± 0.02**</td>
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*: p<0.01 Control group and short-term ischemia-reperfusion (I/R) group compared long-term I/R group; **: p<0.001 Control group and short-term I/R group compared long-term I/R group. I/R: ischemia-reperfusion; CMAP: compound muscle action potentials.
for 30 min and reperfused for 72 h. They showed that spinal cord injury was reduced in the treatment group compared with the control, in which all the animals had paraplegia.

Anatomical, physiological, and biochemical changes by oxygen radicals and lipid peroxidation have been suggested as important factors in post-traumatic neuronal degeneration. Lipid peroxidation can have an adverse effect on undamaged neuronal tissue, leading to the collapse of the microcirculation and to irreversible damage to myelin and axons. Similar pathophysiological changes also occur in ISCI, such as calcium influx, eicosanoid production, hypoxic free-radical generation, and the subsequent development of lipid peroxidation. A number of pharmacological methods have been applied to treat traumatic spinal cord injury, reduce ischemic or reperfusion injury of the spinal cord, and prevent paraplegia. The mechanism of I/R injury in peripheral nerves has been described in previous studies. Ischemia, especially severe ischemia, results in the cessation of nerve blood flow, conduction block, and blood-nerve barrier disruption, with resulting endoneurial edema due to the generation of reactive oxygen species and subsequent IFD. These perturbations are amplified during reperfusion, which results in a large increase in lipid hydroperoxide, a dramatic rise in endoneurial edema, and, subsequently, significantly greater IFD than that observed with ischemia alone. Previous studies of I/R injury of the peripheral nerves applied ischemia of more than 2 h. The current study applied ischemia for 20 min and 30 min to assess the effects of the cross-clamping time on the peripheral nerves.

The biochemical analysis of the effect of aortic clamping on the peripheral nerves revealed an increase in MDA, clearly illustrating the effect of long-term I/R. The results of the electrophysiological and histopathological assessments indicate that in the first 72 h, edema leads to axonal injury and myelin damage. The occurrence of IFD and edema indicates that long-term I/R has a devastating impact on the peripheric neurons and that there is a direct link between paraplegia, one of the most common postoperative complications of aorta surgery, and the cross-clamping time (longer than 25 min). The CMAP amplitude in the EMG reflects the number of axons. A reduction in the CMAP amplitude following I/R denotes axonopathy, and the distal latency (ms) reflects functional changes in myelin. In the present study, myelopathy was present in the long-term I/R group as shown by the increase in the distal latency (ms). We found that the structure of the peripheral nerves was damaged during clamping of the aorta when the duration of the ischemia was longer. The results emphasize that the long time ischemia influences peripheral neurons at least as spinal cord injury on rats. This preliminary study was performed in an animal model, and therefore the findings might not be applicable to humans. Future studies are

Fig. 2 (A) Control group showing the natural structure of the sciatic nerve of rat (toluidine blue, × 20). (B) Short-term ischemia-reperfusion (I/R) group showing the natural structure of the sciatic nerve of rat (toluidine blue, × 40). (C) The white arrow shows intensive edema, the black arrow indicates endoneurial fibrosis, and the arrowhead shows axonal degeneration and demyelination in the long-term I/R group (toluidine blue, × 20).

Fig. 3 Comparison of edema and ischemic fiber degeneration grades among the control, the short-term ischemia-reperfusion (I/R) group and the long-term I/R group.
needed to determine the connection between spinal cord and peripheral nerve injuries and the effects of clamping-time intervals on peripheral neurons.

Conclusion

In the present study, the harmful effects of aortic clamping on the peripheral nerves appear to have been attributed to long-term I/R injury. We believe that I/R injury, which is dependent on the duration of the cross clamping, may not only affect the spinal cord but also the peripheral nerves.

Disclosure Statement

There are no conflicts of interest and no funding or grants to declare.

References