Early Protective Effects of Iloprost after Experimental Spinal Cord Ischemia in Rabbits

K. Coskun¹, A. Attar¹, H. Tuna¹, M. F. Sargon², N. Yüceer¹, R. K. Türker³, and N. Egemen¹

¹ Department of Neurosurgery, Ankara University Medical School, Ankara, Turkey
² Department of Anatomy, Hacettepe University Medical School, Ankara, Turkey
³ Department of Pharmacology, Ankara, Turkey

Summary

The potential role of Iloprost, a stable analogue of prostacyclin, in treating spinal cord ischemia was investigated in rabbits subjected to aortic occlusion for 15 minutes. Ten adult rabbits weighing 2–2.5 kg received an intravenous infusion of saline (SF) as a control group and 14 rabbits received an intravenous infusion of Iloprost, 25 µg/kg/h. Iloprost infusion was started immediately after clamping of the aorta and continued 60 minutes thereafter. Cortical somatosensorial evoked potentials (CSEP) were recorded during the pre-ischemic period as a baseline and post-ischemic readings were taken at 15, 30 and 60 minutes. There was no statistically significant difference between CSEP of the saline and Iloprost treated groups (p < 0.05). All animals were examined neurologically by using a modification of Tarlov scale and all subjects were then deeply anesthetized and their spinal cords were removed for light and electron microscopic examinations at 24 h after spinal cord ischemia. In order to obtain an accurate comparison of ultrastructural changes between saline treated and Iloprost treated groups, a grading scale was performed. The light microscopic and ultrastructural analysis of the Iloprost treated group revealed that there was moderate protection of the myelin and axons and edema was attenuated. Findings of this study suggest that Iloprost exerts a protective effect on spinal cord ischemia. However, further studies are needed to reveal possible mechanisms of protection provided by Iloprost.

Keywords: Cortical somatosensorial evoked potentials; Iloprost; prostacyclin; spinal cord ischemia.

Introduction

Non-traumatic spinal cord ischemia (SCI) is one of the major concerns in spinal cord surgery, especially spinal vascular malformations and spinal cord tumors. Moreover, aortic reconstructive procedures may result in SCI. Neuronal cell death in SCI is the result of a cascade of events mediated by deleterious effects of free radical generation, lipid peroxidation, and the accumulation of intracellular calcium [38, 46, 47]. Experimental studies have proved that tissue destruction is caused by the release of vasoactive/chemotactic substances after lipid peroxidation of cell membrane has occurred [2, 37]. Some of the proposed neuroprotective agents are synthetic glucocorticoids, antioxidant vitamin E, selenium, dimethylsulfoxide, opiate antagonists, naloxane and thyroid releasing hormone (TRH). All agents except TRH have antioxidant and/or antilipid hydrolysis properties [17, 24, 27, 29].

Iloprost is one of the stable analogs of PGI2 having a similar profile of action to natural PGI2 in various pharmacological preparations [16, 17]. Cytoprotection is an important but less clear pharmacodynamic effect of Iloprost. Although effects of Iloprost have been widely studied by many researchers in different fields of medicine including cardiac and cerebrovascular pathologies [15, 16, 18, 45], its effects on spinal cord ischemia has not been investigated previously.

Materials and Methods

The animal ethics and research committee of Ankara University approved all protocols. Animals were randomly assigned to experimental groups and were operated on in random order. Investigators blinded to the experimental groups made outcome assessments.

Preparations of Animals

This study was carried out on 24 adult male rabbits (New Zealand albino, weight 2–2.5 kg). Rabbits were anesthetized by ketamine hydrochloride 10 mg/kg and Thiazin hydrochloride 0.15 mg/kg intramuscularly. For muscle relaxation, atracurium besylate 0.3 mg/kg was used. The ear and femoral arteries and the ear vein were cannulated for injections and to monitor the arterial blood pressure (Hawlett Packard 783835, Germany). Core temperature was monitored with a rectal probe to maintain temperature within physiological ranges (37–38°C).
Surgical Procedure

With careful aseptic technique a tracheotomy was performed and animals were ventilated with a mechanical ventilator (B. Braun Aparatebau Melsungen, Germany). The left ischiatric nerve was exposed for cortical somatosensory evoked potential stimulation (CSEP). A left thoracotomy was then performed. Under the magnification of an operating microscope, a thymectomy was performed and the aorta along with the subclavian artery was exposed. The aorta proximal to the left subclavian artery was transiently occluded by using an aneurysm clip (Yasargil FE 752) for 15 minutes. This method has been introduced by others and has been used to induce spinal cord ischemia previously [21]. Fifteen minutes of time has been shown to be the minimal duration of aortic occlusion within which spinal cord will present with ischemia [13]. The blood supply to the spinal cord of the rabbit originates from the segmental branches of the aorta. Obstructing the blood flow at a given level in the aorta can cause a reproducible lesion. Easily recognizable neurological deficits result and the systemic effects of the lesion are not so severe as to prevent the survival of most subjects [26]. Clamping of the aorta proximal to left subclavian artery allows preservation of blood flow to both carotid and right subclavian arteries [21].

Cortical Somatosensory Evoked Potential (CSEP)

Upon completion of surgical procedures, pre-ischemic CSEP was recorded by stimulation of left ischiatric nerve. A point 2.5 mm to the left of the sagittal suture and 17.5 mm away from the lambdoid suture was chosen as recording point at the skull surface corresponding to sensory projection area. For CSEP, Nicolet Compact Four Electrodiagnostic System, and Ag/AgCl needle electrodes were used. Electrical impedance was kept below 0.1 Kohm. An electrical stimulator was placed onto the perineurium of the nerve and applied at 30–1500 Hz, 100 mVolt and 2 miliampers for 1/sec. Analysis time was 250 msec. Results were stored on a floppy diskette. Evoked potentials were recorded at 15, 30 and 60 min after ischemia. All surgical incisions were closed upon completion of the recordings.

Neurological Evaluation

A neurological examination was performed by an observer unaware of the group assignments at 24 h after ischemia. The animals were graded according to a modification of the Tarlov classification [36], as follows; grade 0, spastic paraplegia and slight movement of the lower limbs; grade 1, spastic paraplegia and slight movement of the lower limbs; grade 2, good movement of the lower limbs but unable to stand, grade 3, able to stand but unable to walk normally; and grade 4, complete recovery.

Electron and Light Microscopic Evaluation

A transcardiac perfusion was performed with 1000 ml of fixative (2% paraformaldehyde buffer with 2 mmol/l calcium chloride). The pressure head of fixative was maintained at 100 mmHg, which is equivalent to the mean arterial pressure for the rabbits. Upon completion of fixation, the whole spinal cord of animals from thoracic 1 to lumbar 1 was removed for histopathological examination. The tissues were immediately placed in 5% gluteraldehyde buffered at pH 7.5 with Milonig phosphate buffer for 3 h. The tissue pieces were subsequently fixed in 1% osmic acid for 2 h. Tissue samples were then dehydrated in graded ethanol, embedded in araldite, and processed for electron microscopy using conventional methods.

In order to obtain the accurate comparison of ultrastructural changes between saline treated and Iloprost treated group, a grading system was used [23, 39]. We used grading system for quantitating ultrastructural findings as shown in Table 5. For the comparison of neurons, mitochondrial changes were detected in 100 mitochondria for each group. Perineuronal edema was examined in 100 neurons for each group. For the comparison of myelinated axons, 100 large sized (>20 μm), 100 medium sized (10–20 μm) and 100 small sized (<10 μm) myelinated axons were examined. Additionally, 100 unmyelinated axons were examined and the scores were noted (Table 6).

Experimental Protocol

Since pulmonary edema was encountered in some animals (n = 4) in our preliminary studies, dosage of Iloprost was modified to avoid further morbidity and mortality. Ten rabbits were treated with intravenous saline and 14 were treated with Iloprost at 25 μg/kg rate. Infusions were started immediately after ischemia and continued for 1 h. Postoperative neurological examination of each rabbit was performed at 24 h after ischemia and classified according to Tarlov’s Scale. Arterial blood gases were maintained within physiological ranges. At 24 h post-ischemia, all animals were reanesthetized and sacrificed.

Statistical Analysis

CSEP parameters in the same groups were analyzed with multiple analysis of variance test (ANOVA) and in the different groups were analyzed with Analysis of Variance Test (ANOVA). Data are expressed as the means ± SEM.

Statistical analysis of data in the ultrastructural studies was performed using Student’s t-test. A probability value (p value) less than 0.05 was considered statistically significant. All values are expressed as the mean ± standard deviation.

Results

Physiological Variables

There were no statistically significant differences between groups regarding temperatures, mean arterial blood pressure in the femoral and ear arteries, blood gases and hematocrit levels (Table 1).

Cortical Somatosensory Evoked Potentials

CSEP recorded from the Iloprost treated animals are better than those from saline treated animals. Analysis of the CSEP recordings from the Iloprost and saline treated groups showed that the decline in the amplitude began immediately after occlusion of the aorta and the recordings began to recover partially at 30 minutes after reperfusion. Amplitude values decreased almost 50% of base line at 15 min recordings and then slightly increased to pre-ischemic values. But none of the recordings from animals returned to the base-line values. There was no statistically significant difference between two groups (p < 0.05). Results of CSEPs are shown in Tables 2 and 3.