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Neuroprotective effect of etomidate on functional recovery in experimental spinal cord injury

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Abstract

Objective: Primary impact to the spinal cord causes rapid oxidative stress after injury. To protect neural tissue, it is important to prevent secondary pathophysiological mechanisms. Etomidate, a strong antiexcitotoxic agent, stimulates the gamma aminobutyric acid (GABA) receptors. The purpose of this study was to investigate neurobehavioral and histological recovery and to evaluate the biochemical responses to treatment of experimental spinal cord injury (SCI) in rats with etomidate or methylprednisolone (MP) or both etomidate and MP.

Material and methods: Seventy-two rats were randomly allocated into six groups: a control group (laminectomy alone), a trauma group (laminectomy + trauma), a methylprednisolone group (30 mg/kg MP), an etomidate group (2 mg/kg), a methylprednisolone and etomidate combined treatment group (30 mg/kg MP and 2 mg/kg etomidate) and a vehicle group. Six rats from each group were killed at the 24th hour after the injury. Malondialdehyde, glutathione, nitric oxide and xanthine oxidase levels were measured. Neurological functions of the remaining rats were recorded weekly. Six weeks after injury, all of those rats were killed for histopathological assesssment.

Results: Etomidate treatment revealed similar neurobehavioral and histopathological recovery to MP treatment 6 weeks after injury. Combined treatment did not provide additional neuroprotection.

Conclusion: Etomidate treatment immediately after spinal cord injury has similar neuroprotection to MP. In spite of different neuroprotection mechanisms, combined treatment with MP and etomidate does not provide extra protection.

Keywords: Etomidate; Methylprednisolone; Spinal cord injury; Functional recovery; Malondialdehyde; Glutathione; Nitric oxide; Xanthine oxidase

1. Introduction

The pathophysiology of acute spinal cord injury (SCI) is complex and not fully understood. The initial mechanical damage to the spinal cord is referred to as primary injury and cannot be avoided. Excitotoxicity is an important mechanism in secondary injury after SCI. Many pathological changes seen after spinal cord trauma are thus secondary to the initial impact and include edema, altered blood flow and changes in microvascular permeability (Tator and Fehlings, 1991). Previous studies showed that one of the most important factors precipitating posttraumatic degeneration in the spinal cord is oxygen free radical-induced lipid peroxidation (Anderson et al., 1985a, 1993; Hall, 1992, 1993).

Pharmacological intervention in the acute phase of spinal cord injury aims to counteract secondary neurotoxic events or to interrupt the progression of this process. To date, relatively little progress has been made in the treatment of SCI and related neurological impairments. Methylprednisolone (MP) is a potent pharmacological agent that has a clinically proven benefical effect on functional recovery after SCI (Bracken et al., 1990). The underlying mechanism is not fully understood, but experimental data point to protection against membrane peroxidation and edema (Braughler and Hall, 1982). Further research has shown that the high dose MP required to inhibit lipid peroxidation also exerts a number of other actions on the

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injured spinal cord such as reduction in posttraumatic lesion area (Cayli et al., 2004; Young and Flamm, 1982), neurofilament degradation (Braughler and Hall, 1984), reversing intracellular calcium accumulation and preserving evoked potentials (Cayli et al., 2004).

Etomidate, a nonbarbiturate hypnotic, is a frequent agent of choice for the induction of rapid sequence intubation in emergency departments (Bergen and Smith, 1987). The pharmacologic effect of etomidate is through the stimulation of the gamma aminobutyric acid (GABA) receptors. Etomidate is strong antiexcitotoxic agent. It is shown that etomidate potentiates potassium chloride-evoked GABA release in rat cortical synaptosomes (Murugaiah and Hemmings, 1998). It is also postulated that it reduces hippocampal neuronal injury in rats subjected to incomplete forebrain ischemia (Watson et al., 1992), blocks ischemia-induced increases in extracellular glutamate and glycine in the hippocampus (Patel et al., 1995) and attenuates posttraumatic functional (motor and cognitive) and histologic (CA3 neuron loss and contusion volume) deficits.

The purpose of this study was to investigate neurobehavioral and histological recovery and evaluate biochemical responses to treatment of experimental SCI in rats with etomidate, MP and both etomidate and MP.

2. Materials and methods

Adult female albino rats weighing 200–250 g were used in this study. The rats were housed four per cage in a temperature-controlled room (18–21 °C) with a 12-h light:12-h dark cycle and were given free access to food and water. Before surgery, all rats were tested and a normal motor function was found. The animals were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and pinned in the prone position. A rectal probe was inserted and the animals were positioned on a thermistor-controlled heating pad. All surgery was done under sterile conditions. Following T5-12 midline skin incision and paravertebral muscle dissection, spinous processes and laminar arcs of T7-10 were removed with the assistance of a surgical microscope. The dura was left intact. Weight-drop trauma modeling was performed for all the animals (Allen, 1911). The apparatus was a 10 cm guide tube positioned perpendicular to the center of the spinal cord with an inner stainless steel rod (weighing 5 g). The animals were subjected to an impact of 50 g/cm to the dorsal surface of the spinal cord. Then, the muscles and incision were sutured with 6-0 vicryl suture (Ethicon).

The rats were randomly allocated in six groups: each having 12 rats. Group 1: control group, underwent laminectomy alone. Group 2: trauma group, underwent laminectomy followed by SCI and received no medication. Group 3: etomidate treatment group, underwent laminectomy followed by SCI and received etomidate. Group 4: methylprednisolone treatment group, underwent laminectomy followed by SCI and received methylprednisolone. Group 5: etomidate and methylprednisolone treatment group, underwent laminectomy followed by SCI and received etomidate in combination with methylprednisolone. Group 6: vehicle group, underwent laminectomy followed by SCI and received lipid emulsion (vehicle).

A 2 mg/kg single dose of etomidate (Etomidate-Lipuro, B. Braun, Melsungen, Germany) for Group 3; a single dose of methylprednisolone 30 mg/kg (Mustafa Nevzat Ilac Sanayi A.S.) for the Group 4; a 2 mg/kg single dose of etomidate and a 30 mg/kg single dose of MP for the Group 5; lipid emulsion 10% (Baxter, UK) (1 ml) for the Group 6 were injected intraperiteonally immediately after SCI. After surgery, the animals were placed in a warming chamber and body temperature maintained at approximately 37 °C until they were fully awake and received 3 ml saline subcutaneously to compensate for blood loss during the surgical procedure and limited water intake in the postoperative period.

Then, each group of rats was subdivided into two subgroups, one of which was killed at the 24th hour (for biochemical analysis) and the other at the 6th week (for neurobehavioral and histopathological assessment) following trauma. All rats in the 6 week group received gentamycin twice daily during the first 3 days as prophylaxis against urinary tract infection. Bladders were emptied manually twice a day during this period. In case of mortality, an additional rat was assigned to ensure a minimum of 6 rats per each study subgroup. Posttraumatic neurological recovery was recorded weekly. Either after 24 h or 6 weeks, rats were killed and 1 cm spinal cord samples were removed for biochemical analysis and histopathological recovery of injured spinal cord area.

2.1. Biochemical analysis

Six rats from each group were killed for biochemical analysis 24 h after injury. The samples were immediately frozen and stored in a −20 °C freezer for assays of malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO) levels and xanthine oxidase (XO) activity.

2.1.1. MDA measurements

The level of lipid peroxides in traumatized spinal cord tissue were measured as thiobarbituric acid-reactive material and determined using the method of Mihara and Uchiyama (1978). MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. The assay procedure for lipid peroxide in spinal cord tissue was set up as follows. Tissues were homogenized in 10 volumes (w/v) of cold 1.5% KCl. One-half a milliliter (0.5 ml) of homogenate was mixed with 3 ml of 1% H_3PO_4 and 1 ml 0.6% thiobarbituric acid. The mixture was then heated in boiling water for 60 min. After cooling, the color was extracted into 4 ml *n*-butanol and the absorbance was recorded at 535 and 520 nm. Using tetramethoxypropane as the standard, tissue lipid peroxidate levels were calculated as nanomole per gram of wet tissue.

2.1.2. GSH level measurements

GSH levels were measured by the method of Elman (1959). GSH is reacted with 5,5-dithiobis-2-nitrobenzoic acid resulting in the formation of a product which has a maximal absorbance at 410 nm. The results are expressed as nanomole per gram wet tissue.

2.1.3. NO measurements

Nitric oxide levels were measured as total nitrite with the spectrophotometric Greiss reaction, because total nitrite is an index of endogenous nitric oxide production. The procedure was partly adapted from the method descrebed by Ozbek et al. (2000). Results were reported as nanomole per milligram protein.

2.1.4. XO activity measurements

XO activity was determined spectrophometrically according to the method of Prajda and Weber (1975), based on the formation of uric acid from xanthine which increases absorbance at 292 nm (ε_M 9.2×10^3). One unit of activity was defined as 1 μmol of uric acid formed per minute and the data are presented as U/g protein.

2.2. Behavioral assessment

2.2.1. Motor function score

A "motor function scale" (Farooque et al., 1999) that is a slight modification of the "motor score" descibed by Gale et al. (1985) was used to evaluate motor function (Table 1). The animals were allowed to move freely in an open field (0.7 m × 0.9 m). An observer who was blinded to treatments observed the rats for at least 1 min and recorded movements in the hip, knee and ankle joints weekly.

2.2.2. Inclined plane score

An inclined plane technique that is defined by Rivlin and Tator (1977) was used in this study. It is an angle board test consisting of measuring the maximum angle at which an animal can support its weight on an inclined plane measured in 0–90°. The animals were placed transversely on the inclined plane and the