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Pre-ischemic treatment with memantine reversed the neurochemical and behavioural parameters but not energy metabolites in middle cerebral artery occluded rats

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ABSTRACT

In the present study, Memantine (MN) an uncompetitive N-methyl-D-aspartate (NMDA) open channel blocker has been investigated for its suitable therapeutic time-window on the basis of its influence on behavioural and biochemical changes in rats subjected to transient focal ischemia. MN (20 mg/kg, ip) was administered at pre, during and post ischemic state and the extent of neuroprotection was compared to ascertain its therapeutic time-window in stroke treatment. Neuroprotective effect was assessed by measuring glutamate, glutamine synthetase, glutathione, Na^+K^+ATPase, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD), lactate and pyruvate levels. Middle cerebral artery occlusion produced neurological deficits, anxiogenic behaviour, histological changes, increased glutamate levels along with depletion of Na^+K^+ATPase, energy stores such as ATP, NAD, lactate, and antioxidant glutathione. MN significantly restored glutamate, glutamine synthetase, Na^+K^+ATPase and lactate levels on preischemic administration. In addition, MN reversed the altered neurological and behavioural paradigms significantly and prevented the neurodegeneration on preischemic treatment. However, it failed to exert any effect on energy metabolite (ATP and NAD) levels irrespective of the treatment phase. Based on the present data, it is summarized that the suitable therapeutic time window of MN is preischemic phase in stroke and it possesses only a subjective role in reversing ischemic brain biochemical alterations preferentially in favor of neuronal homeostasis.

1. Introduction

Cerebral ischemia, one of the leading causes of medical morbidity and mortality in geriatric population often results in irreversible brain damage. Focal impairment of cerebral blood flow restricts the delivery of substrates, particularly oxygen and glucose. The biochemical alterations following cerebral ischemia starts with depletion of energy phosphates and disruption of ion homeostasis with a consequent increase in the extracellular potassium and glutamate levels rendering over activation of N-methyl-D-aspartate receptors (NMDARs), intracellular Ca^{2+} over load and finally cell death (Arundine and Tymianski, 2004) as time progresses. It will be more appropriate if the therapeutic interventions are based on the bio- and neurochemical status following ischemic-reperfusion (IR).

Various therapeutic strategies are employed in the treatment of stroke, with NMDA blockers gaining greater interest in the recent past. NMDAR antagonists have often failed as therapeutic agents because of their debilitating side effects (Lipton, 2004). Memantine (1-amino-3,5-dimethyladamantane), an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist reduces glutamatergic excitotoxicity. Memantine (MN) has been approved in Europe in 2002 and United States in October 2003 for treatment of moderate to severe Alzheimer's disease. Unlike other NMDA receptor antagonists, memantine possesses fast on/off kinetics, low-moderate affinity and it also blocks the effects of excessive glutamate while preserving the physiologic activation of NMDA receptors (Johnson and Kotermanski, 2006).

Earlier results were paradoxical with NMDA agents administered at different time points of IR (Macleod et al., 2004; Ikonomidou and

Abbreviations: ANOVA, analysis of variance; ATP, adenosine 5′ triphosphate; DNA, deoxyribose nucleic acid; GLAST, glutamate astrocytes transporters; GS, glutamine synthetase; ip, intraperitoneal; IAEC, Institutional Animal Ethical Committee; IR, ischemic-reperfusion; LDH, lactate dehydrogenase; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MN, memantine; NAD, nicotinamide adenine dinucleotide; NMDA, N-methyl D-aspartate; NMDAR, N-methyl D-aspartate receptor; PARP, Poly (ADP-ribose) polymerase; PDH, pyruvate dehydrogenase; SO, sham operated.

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Turski, 2002). In some failed stroke clinical trials, treatments were administered outside the temporal window of efficacy (Labiche and Grotta, 2004) of the drugs. Hence, a study evaluating the correlation of the efficacy of the drugs with different time episodes of ischemia might yield a meaningful result in the treatment of stroke. The present study demonstrates the suitable therapeutic time window of MN and its role on behavioural and biochemical alterations in rats subjected to middle cerebral artery occlusion (MCAO).

2. Materials and methods

2.1. Chemicals

Memantine and L-glutamic acid were purchased from Sigma, US; adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) were purchased from SISCO Research Laboratories, Mumbai, India. 4-0 nylon monofilament Ethicon® was procured locally. All other chemicals, reagents and solvents used were of analytical grade.

2.2. Animals

Male Sprague Dawley rats (290–340 g) were used in this study. Animals were housed in individual polypropylene cages in a well ventilated room (air cycle: 15 per min; 70:30) under an ambient temperature of 23 ± 2 °C and 40–65% relative humidity, with a 12-h light/dark cycle. They were provided with food (Nutrilab Rodent, Tetragon Chemie Pvt Ltd, India) and purified water *ad libitum*. All the animals were acclimatized at least for 7 days to the laboratory conditions prior to experimentation. Guidelines of "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India approved the study.

2.3. Surgical procedure

Focal cerebral ischemia was induced by middle cerebral artery occlusion as described by Longa et al. (1989) with minor modifications. Rats were anesthetized with chloral hydrate (350 mg/kg, ip) and right common carotid artery was exposed at the level of external and internal carotid artery bifurcation. 4-0 nylon monofilament was used and its tip was made round headed by exposing it to flame. Filament was coated with 0.01% poly-L-Lysine and inserted into the external carotid artery and advanced to the internal carotid artery for a length of about 20–21 mm until a slight resistance was felt. On achieving occlusion, the filament was held in place with ligature and external incision was sutured temporarily. After 2 h of ischemia the rats were anesthetized, suture was opened, the filament was pulled out and reperfusion in internal carotid artery was ensured visually. Throughout the surgical procedure, body temperature was measured by inserting a thermometric probe into the rectum of rat and it was maintained at 37 ± 0.5 °C using thermostatically controlled heating blanket. Animals were then kept in a cage with a heating lamp, which maintained the cage temperature between 29 ± 1 °C for another 1 h to counteract any possible hypothermic effect. In the sham-operated group, external carotid artery was surgically prepared for insertion of filament, but the filament was not inserted.

2.4. Experimental design and drug treatment

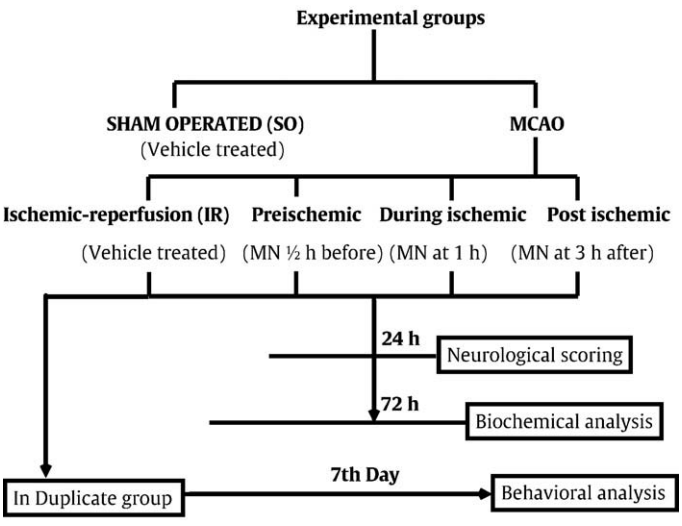
Following the occlusion of MCA with nylon filament the ischemic episode begins. Experimental animals were divided into five groups of 6–9 each. Group 1 served as sham-operated controls (SO) and received 0.9% sterile saline as vehicle. Group 2 animals were subjected to MCAO and received 0.9% sterile saline i.e., vehicle treated (IR) (vehicle was administration in the pre, during and post ischemic phases [$n = 3$/group]; having observed no significant changes in the biochemical and behavioural parameters we have pooled up and presented the data). Group 3 animals were pretreated with MN 30 min before MCAO. Group 4 animals received MN during the ischemic episode i.e., 1 h following MCAO and Group 5 animals were treated with MN 3 h after the onset of reperfusion. MN dose was fixed based on the findings of Block and Schwarz (1996a). MN (20 mg/kg) was dissolved in 0.9% sterile saline and administered intraperitoneally. Behavioural experiments were performed in another set of animals with the same groupings and dose schedule ($n = 6$–7/group) on day 7 following IR, since marked alterations in behaviour were observed only after that period (our unpublished data).

2.5. Steady state experiment

Vehicle or drug treated groups were subjected to neurological deficit scoring after 24 h of IR. Seventy two hours after IR, experimental animals were anesthetized; blood was collected through retro orbital puncture and centrifuged to separate plasma. Earlier reports revealed alterations in the antioxidant, biochemical and neurochemical substances in different brain regions of rats subjected to MCAO. Hence to support the neuroprotective activity of MN the biochemical parameters such as ATP, NAD, lactate, pyruvate, Na^+K^+ATPase and GS along with antioxidants were measured to understand the cell functions and homeostasis. The neurochemical glutamate was measured to elucidate the excitatory activity during the ischemic state. After collection of blood, the animals were euthanized, brains were quickly removed and different brain regions were immediately dissected over an ice-cold plate using the atlas of Paxinos and Watson (1986) as reference. In the duplicate group, behavioural study was performed on the 7th day following IR. Neurobiochemical and behavioural studies were performed by individuals unaware of the treatment schedule to avoid bias.

2.6. Assessment of neurological deficit

Neurological deficits were scored as described by Bederson et al. (1986), with minor modifications as follows: Score 0 — no apparent neurological deficits; Score 1 — contralateral forelimb flexion; Score 2 — decreased resistance to lateral push; Score 3 — spontaneous movement in all directions and contralateral circling when pulled by tail; Score 4 — spontaneous circling.



Experimental group and treatment schedule layout