博士論文

論文題目　Paraplegia prevention by oral pretreatment with memantine in a rabbit model
（ウサギモデルにおけるメマンチン術前経口投与の対麻痺予防効果）

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Paraplegia prevention by oral pretreatment with memantine in a rabbit model

（ウサギモデルにおけるメマンチン術前経口投与の対麻痺予防効果）

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ABSTRACT:

Objective: Spinal cord injury following thoracic and thoracoabdominal aortic surgeries is a dreadful complication. Its incidence ranges from 2 to 32 % despite the use of various adjunct strategies. Aiming at reducing the risk of paraplegia after thoracic and thoracoabdominal aortic surgeries, I evaluated the role of memantine (N-Methyl-D-Aspartate receptor antagonist) pretreatment for prevention of spinal cord ischemia following infrarenal aortic clamping in a rabbit model.

Methods: Thirty New Zealand White rabbits were divided into 5 different groups of 6 each. Groups 60-7 and 60-5 received oral memantine 60 mg once daily for 7 and 5 days, respectively, prior to surgery. Groups 30-5 and 30-3 received oral memantine 30 mg once daily for 5 and 3 days, respectively, prior to surgery. Group C (control) received normal feeds without memantine. Paraplegic model was created by clamping both aorta and inferior vena cava (IVC) at infrarenal and just proximal to their bifurcations for 45 minutes. Intraoperatively, vitals and motor evoked potentials (MEP) were monitored. Serum memantine level was measured by obtaining 5 ml of blood sample at the end of the surgery. Paraplegia was clinically assessed by Modified Tarlov score (0= no movement of lower limbs, 1= slight movement of lower limbs, 2= sits with support, 3= sits alone, 4= weak hop, 5= normal hop) at 6, 24, 48, and 72 hours. At 72 hours, rabbits were sacrificed by intracardiac injection of 10 mEq KCl, lumbar segments of spinal cords were harvested and histopathology was evaluated by using Hematoxylin and Eosin (H &E) stain.

Results: Mean modified Tarlov scores were 4.2±1.3, 4.3±1.0, 4.2±1.3, 4.3±1.2, and 0.8±1.6 in group 60-7, 60-5, 30-5, 30-3, and C, respectively at 6, 24, 48, and 72 h ($p<0.009$ for individual groups vs control; and p=not significant among groups 60-7, 60-5, 30-5, 30-3). Percentage loss

of MEP amplitude by the end of surgery compared with baseline amplitude was 29.5±46.3, 11.9±28.0, 30.0±46.8, 16.7±40.8, and 81.8±40.3 % in 5 groups, respectively (p=0.049). After declamping, MEP reappeared in 83, 100, 83, 83, and 33 % cases in 5 groups, respectively (p=0.005). Serum memantine level was 4.0±2.1, 6.4±2.5, 6.8±2.4, and 7.5±6.3 ng/ml in group 60-7, 60-5, 30-5, and 30-3, respectively (p=0.421). Spinal cords were normal in majority of group 60-7, 60-5, 30-5, and 30-3; but severely ischemic in majority of group C (p=0.016).

Conclusions: Oral memantine pretreatment is protective against spinal cord ischemia, and can be an additional strategy for prevention of paraplegia during thoracic and thoracoabdominal aortic surgeries.

INTRODUCTION:

Spinal cord injury remains a devastating complication after thoracic and thoracoabdominal aortic aneurysm (TAAA) repairs. Its incidence has been reported to range from 2 to 32 % depending on series.[1-14] Paraplegia following TAAA repairs carries a huge burden of physical disability; and is associated with decreased survival.[3] Intraoperative clamping of the aorta to carry out anastomosis of the native aorta with tube graft is an essential component during surgeries involving thoracic and thoracoabdominal aorta. This maneuver essentially leads to impaired blood supply to the spinal cord, which is dependent on branches of thoracic and thoracoabdominal aorta for its blood supply, thereby posing a risk of postoperative paraplegia. In addition, intraoperative and perioperative hypotension is also associated with increased risk of spinal cord injury following thoracic and thoracoabdominal aortic repairs.[15] Multimodality approach with a number of surgical adjuncts has long been adopted aiming at preventing paraplegia, which include:

Maintenance of perioperative high normal blood pressures,[7]

Moderate to profound hypothermia,[7,16]

Topical spinal cord hypothermia,[7]

Cerebrospinal fluid (CSF) drainage,[17]

Left heart bypass,[18]

Segmental intercostal or lumbar arteries reattachament.[1,2,4]

Role of pharmacological protection has also been considered in most adjuncts which includes use of steroids,[7] naloxone,[15] and free radical scavengers,[19] among many others.

Memantine:

Memantine is a drug that was first synthesized in the 1960s as an agent to lower elevated blood sugar levels but it was completely devoid of such activity. The fact that it affects central nervous system (CNS) was discovered in the 1970s; and it was found to inhibit N-methyl-D-aspartate (NMDA) receptors in the 1980s.[20,21] Chemical structure of memantine is 1-amino-3,5-dimethyl-adamantane (Figure 1).

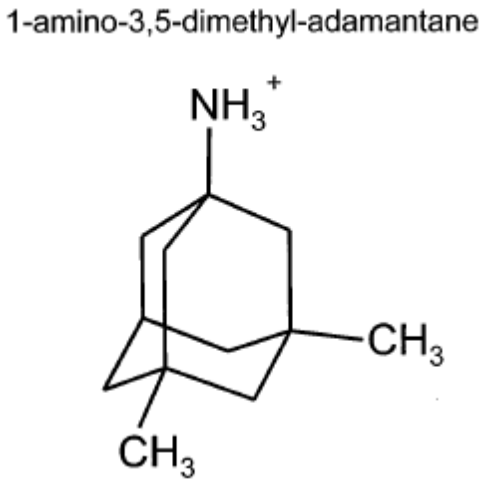

Figure 1. Chemical structure of memantine (Adapted from Parsons CG, et al. Neuropharmacology 1999;38:735-767).[21]

NMDA receptor blocking activity of memantine is being continuously explored for potential newer therapeutic applications since last several years. Memantine has already been approved for its clinical use in the treatment of moderate to severe Alzheimer's disease. However, memantine might have far broader therapeutic utility, and is effective in the treatment of other forms of dementias and neurodegenerative diseases, including Parkinson's and Huntington's diseases.[20] Physiological activation of NMDA receptors is essential for normal neuronal function. Memantine has a unique property that it blocks NMDA receptors only during its overactivation;

but allows their physiological activity. This property of memantine results into favorable clinical side effect profile of this drug compared with other NMDA receptor antagonists which block both physiological and pathological activation. Thus, memantine may have a huge potential for a variety of neurological diseases, including a role to prevent ischemic injury during thoracic and thoracoabdominal aortic surgeries.

Memantine has an absolute bioavailability of approximately 100 % after oral intake. There is no indication that food influences the absorption after oral intake. About 45 % of memantine is bound to plasma proteins within the body. Its main route of elimination is via kidneys with elimination terminal half life of 60 to 100 hours. Common side effects of memantine include dizziness, headache, constipation, and somnolence.[22]

N-Methyl-D- Aspartate (NMDA) receptors:

NMDA receptors play a crucial physiological role in various forms of synaptic plasticity such as those involved in learning and memory. Neuroprotective agents which completely block NMDA receptors also impair normal synaptic transmission and thereby causing intolerable side effects. The challenge has therefore been to develop antagonists that prevent the pathological activation of NMDA receptors but allow their physiological activity.[21]

N-Methyl-D-Aspartate (NMDA) receptors have been shown to have an important role in mediating calcium mediated injury in neuronal tissue after a wide variety of insults, including ischemic [23] (Figure 2). NMDA receptors are composed of NR1, NR2, NR3A or NR3B subunits, and activation of these receptors requires dual agonists, glutamate and glycine.[24,25] During ischemia, glutamate and glycine are co-released by reverse operation of Na- dependent transporters. Glutamate and glycine released by the ischemic trigger lead to subsequent

activation of NMDA receptors [26] with subsequent injury of neuronal tissue. NMDA receptor activation secondary to ischemia-induced release of glutamate is a major mechanism of neuronal death in gray and white matter of spinal cord after transient ischemia.[27] Some authors looked at the potency of memantine against cultured hippocampal, cortical, superior colliculus, striatal, or spinal cord neurons under otherwise identical conditions; and found no difference in the potency based on the neuronal site.[21] Memantine is a non-competitive antagonist of NMDA receptor already approved clinically for treatment of Alzheimer's dementia and its further clinical application is constantly being explored. In this study, I evaluated the neuroprotective role of oral memantine pretreatment following infrarenal aortic clamping in a rabbit model.

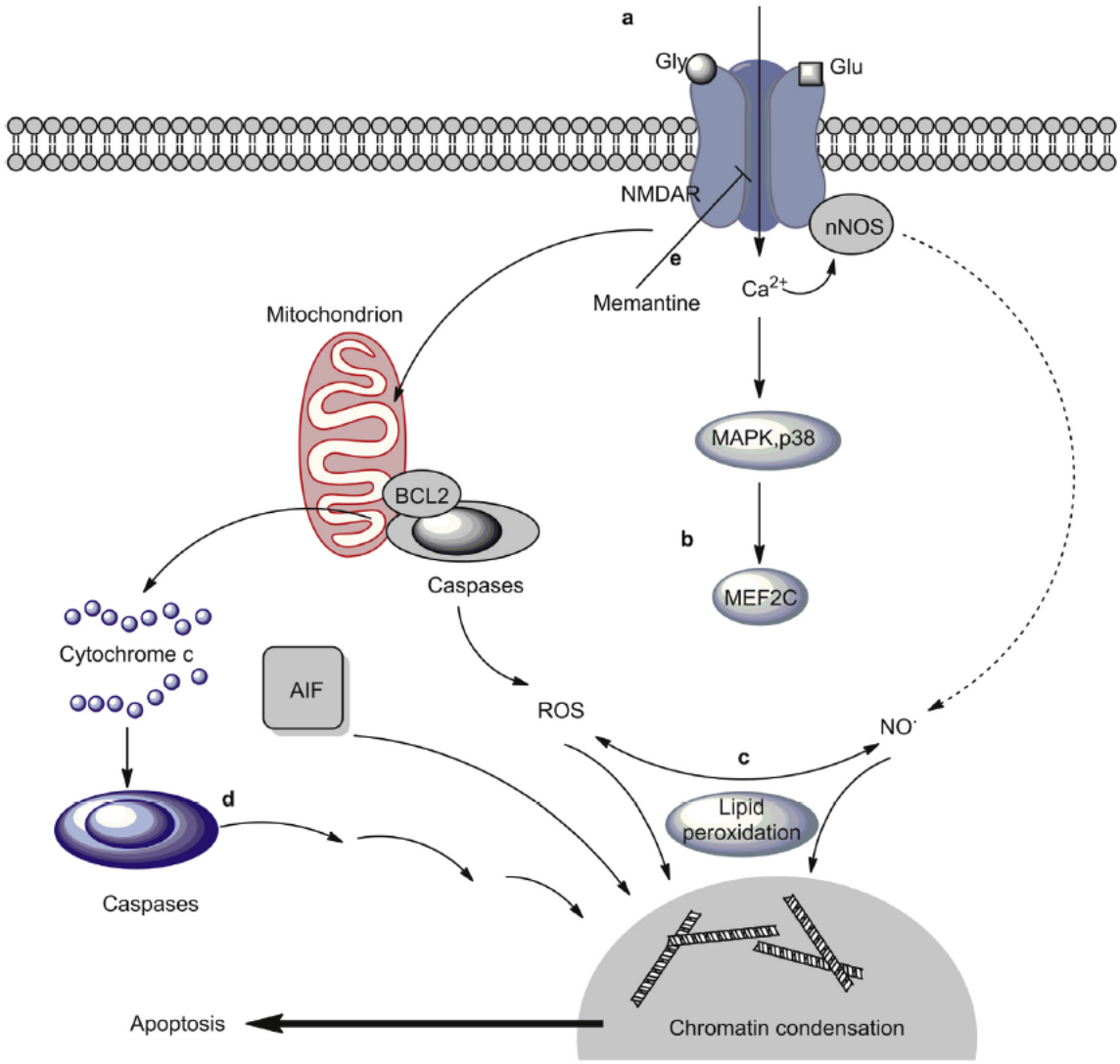

Figure 2: Schematic illustration of the apoptotic-like cell injury and death pathways triggered by excessive NMDAR activity and its prevention by memantine. The cascade includes **(a)** NMDAR hyperactivation; **(b)** activation of the p38 MAPK-MEF2C (transcription factor) pathway (MEF2 is subsequently cleaved by caspases to form an endogenous dominant-interfering form that contributes to neuronal cell death); **(c)** toxic effects of free radicals such as NO and ROS; **(d)** activation of apoptosis-inducing enzymes including caspases and AIF; and **(e)** blockade of NMDAR by memantine preventing Ca^{2+} influx into neuronal cell with subsequent inhibition of steps (a) to (d). AIF: apoptosis-inducing factor; MAPK: mitogen-activated kinase; MEF: myocyte enhancer factor; NMDAR: N-methyl-D-aspartate receptor; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; ROS: reactive oxygen species; Glu: glutamate; Gly: glycine. (Adapted with permission from Nature publishing group: Lipton SA. Nature Reviews 2006;5:160-170).

Spinal cord blood supply:

Spinal cord receives its blood supply from three longitudinal arteries that run across the length of spinal cord from the medulla to the conus medullaris: an anterior spinal artery running along the anterior median fissure; and a pair of posterior spinal arteries running along the lateral sulcus of the spinal cord. Anterior two thirds of the cord is supplied by anterior spinal artery; and posterior one third is supplied by the posterior spinal artery. Central artery from anterior spinal artery supplies the central region of the cord. Radiculomedullary arteries run along the spinal roots and contribute blood supply to both anterior and posterior spinal arteries. Adamkiewicz artery usually arises from T10 thoracic intercostal to L2 lumbar arteries and contributes blood supply in the anterior circulation (Figure 3).

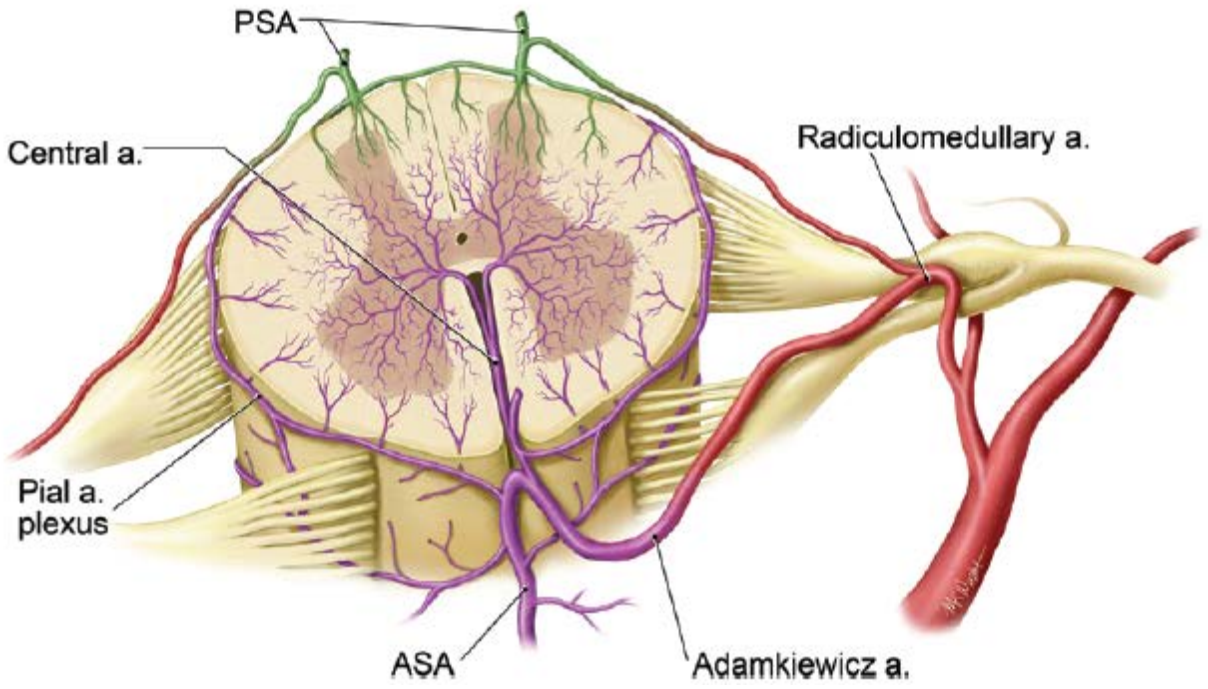

Figure 3. Cross section of the spinal cord showing its arterial supply. Anterior two thirds of the cord is supplied by anterior spinal artery; and posterior one third is supplied by the posterior spinal artery. ASA: anterior spinal artery; PSA: posterior spinal artery; a. artery. (Adapted from Martirosyan NL, et al. Journal of Neurosurgery Spine 2011;15:238-251).[28]

By themselves, the anterior and posterior spinal arteries can supply only the short superior part of the spinal cord. The circulation to much of the spinal cord depends on segmental radiculomedullary arteries which are branches of intercostal arteries arising from aorta. Of major importance is the great anterior segmental medullary artery, which is also called arteria radicularis magna (ARM) or Adamkiewicz artery. This ARM is much larger than the other segmental medullary arteries; it arises from an inferior intercostal or upper lumbar artery and ascends in the anterior median fissure; and it contributes a large portion of blood supply to the spinal cord (Figure 4). Surgery of the descending thoracic aorta, or thoracoabdominal aorta puts the intercostal arteries along with artery of Adamkiewicz at risk; thus risking postoperative paraplegia.

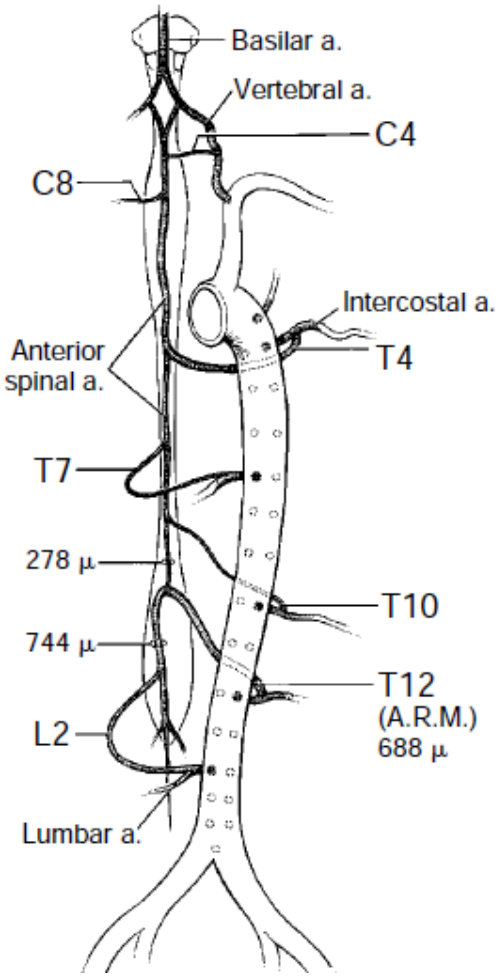

Figure 4. Anterior spinal artery is formed by the union of branches from right and left vertebral arteries. Intercostal arteries arising from thoracic and lumbar aorta contribute blood supply to the spinal cord. The arteria radicularis magna (ARM) is the largest of the intercostals and it joins the anterior spinal artery. The sizes of the arteries are shown. The corresponding cervical, thoracic, and lumbar vertebral levels are shown. (C: cervical; L: lumbar; μ: micron; T: thoracic) (Adapted from Svensson LG. Descending thoracic and thoracoabdominal aortic surgery. In: Sellke FW, del Nido PJ, Swanson SJ, editors. Sabiston and Spencer Surgery of the Chest. Philadelphia: Saunders Elsevier Publishing; 2010. p. 1063-1087).[29]

Spinal cord blood supply in rabbits:

Rabbits receive spinal arteries from thoracic and lumbar segments of aorta.[30] There are 13 pairs of thoracic spinal and 6 pairs of lumbar spinal arteries in rabbits. Majority of cord blood supply is segmental in rabbits (each spinal cord segment is supplied with one corresponding radicular artery with minimal or none collateral bloodstream).[30] Anatomical evaluation of spinal cord

blood supply in 10 rabbits showed that artery of Adamkiewicz originated from sixth lumbar artery; 50 % of times originating from left side and 50 % of times originating from right side.[30] Because of the more caudal origin of this artery of Adamkiewicz and spinal cord receiving segmental blood supply, clamping of infrarenal aorta usually results into paraplegia in rabbits.

METHODS:

Animals and feeding:

Thirty New Zealand White rabbits weighing 3.2 kg (range; 2.8-3.4 kg) were used for the experiment. Rabbits were acquired from rabbit farm about 10 days prior to surgery and were allowed to adapt to the new environment of my animal laboratory, with full access to food, water, and movement inside the cage. All animals received full humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' established by the United States National Institute of Health and the study was approved by the Animal Ethical Committee at the University of Tokyo (approval ID: P12-86). I purchased memantine from Daiichi Sankyo Co. Ltd, Tokyo, Japan. Final memantine food was prepared by Oriental Yeast Co. Ltd, Tokyo, Japan to achieve a concentration of 0.048 % (w/w). Gross appearance of memantine food was similar to that of normal food offered to rabbits in my laboratory (Figure 5).

Figure 5. A. Normal regular food offered to rabbits in my laboratory. B. Memantine food offered to rabbits during my experiment. Each 125 g of this memantine food contained 60 mg of memantine achieving a concentration of 0.048 % (w/w). Note the similar gross appearance of these two types of feeds.

Rabbits were then divided into five different groups of 6 each. Groups 60-7 and 60-5 received oral memantine 60 mg once daily for 7 and 5 days, respectively, prior to surgery. Groups 30-5 and 30-3 received oral memantine 30 mg once daily for 5 and 3 days, respectively, prior to surgery. Group C (control) received normal feeds without memantine.

Surgery:

All rabbits were anesthetized with initial dose of intramuscular ketamine 100 mg and xylazine 20 mg without endotracheal intubation. Repeat dose (ketamine 100 mg and xylazine 10 mg) was given intramuscularly after 45 minutes of the initial dose. Maintenance dose of ketamine was given as continuous intravenous infusion at 5 µg/kg/min. Oxygen was administered via facemask at 2 L/min. Arterial and venous access was obtained using 24 gauge cannula from central auricular artery and marginal auricular vein, respectively. Ringer's lactate solution (8 ml/kg/h) was infused as maintenance fluid intraoperatively. Core body temperature was measured by using a rectal probe. Temperature was maintained as normal as possible by using heating pad, halogen light, and infusion of lukewarm maintenance fluid.

Ten cm long midline laparotomy was made. Bowels were reflected towards right, and abdominal aorta and inferior vena cava (IVC) were exposed by incising the retroperitoneum. Taping of both aorta and IVC was done and bulldog clamps were applied to both aorta and IVC at the infra-renal and just proximal to their bifurcations. Clamping was continued for 45 minutes. At the end of 45 minutes, both aorta and IVC were declamped and abdomen was closed in two layers. Bolus heparin 100 units/kg was injected intravenously 3 minutes prior to aortic clamping. Activated clotting time was not measured and heparin was not reversed at the end of the procedure. Rabbits were observed for about 6 hours in the operating room, and then transferred to the cage. They

were allowed free access to feeds, water, and mobility inside the cage. Although rabbits do not have collateral network of spinal circulation, I clamped aorta at two places to make sure that there is no retrograde flow to the cord ensuring creation of paraplegic model. Unlike in humans, paraplegia in rabbits can be induced by clamping infrarenal aorta alone without the need for clamping thoracic aorta. Previously, various groups of authors have utilized clamping or occluding infrarenal aorta to create a paraplegic model in rabbits.[31,32]

Motor Evoked Potentials (MEPs):

Motor evoked potentials reflect the functional integrity of motor pathways in the spinal cord. Once an electrical stimulus is applied to the motor cortex by an electrical stimulator, the impulse passes through the corticospinal tracts in the spinal cord. In the anterior horn of the spinal cord, the impulse is transmitted to the α-motor neurons which ultimately leads to the contraction of skeletal muscles; and in my experiment, the tibialis anterior muscle (Figure 6).

During ischemia, motor neurons in the anterior horn of the spinal cord are particularly vulnerable to the insult and result into loss of these potentials. Monitoring MEPs serves as a tool to detect spinal ischemia during thoracic and thoracoabdominal aortic surgeries. MEPs were monitored by using multiple electrical transcranial stimulator (Neuropack MEB-9400, Nihon Kohden, Tokyo, Japan).

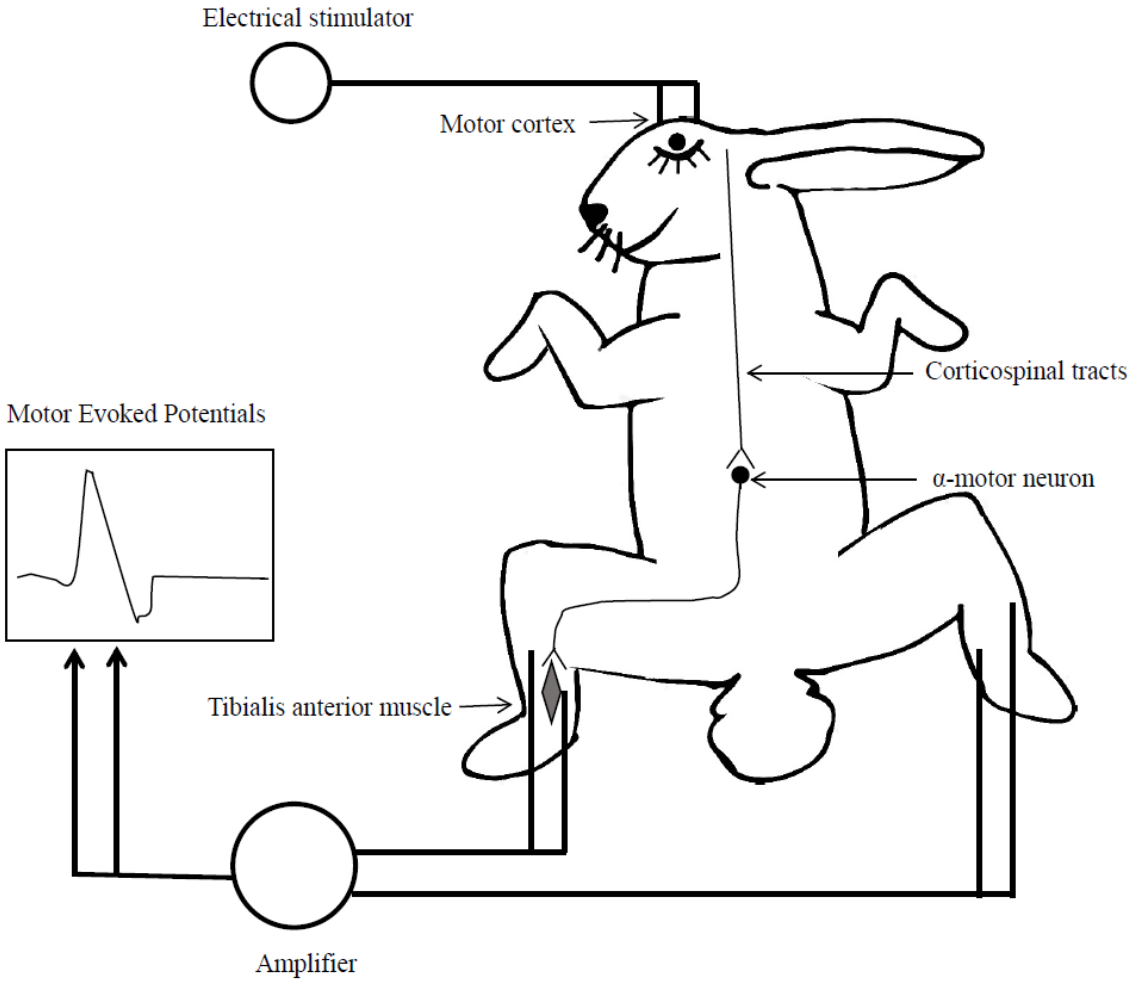

Figure 6. Schematic representation of monitoring of motor evoked potentials (MEPs).

Stimuli consisting of a train of five pulses were applied to the skull with anode placed at frontal midline and cathode at central midline position. Stimulation was applied at the rate of 1 Hz with duration of 0.2 ms, and intensity of stimulation ranged between 20 to 40 mA. Compound action potentials were recorded from bilateral tibialis anterior by using needle electrodes. A typical waveform of compound action potential after stimulation is shown in figure 7 with amplitude defined as the vertical distance between the crest and trough of the waveform (Figure 7).

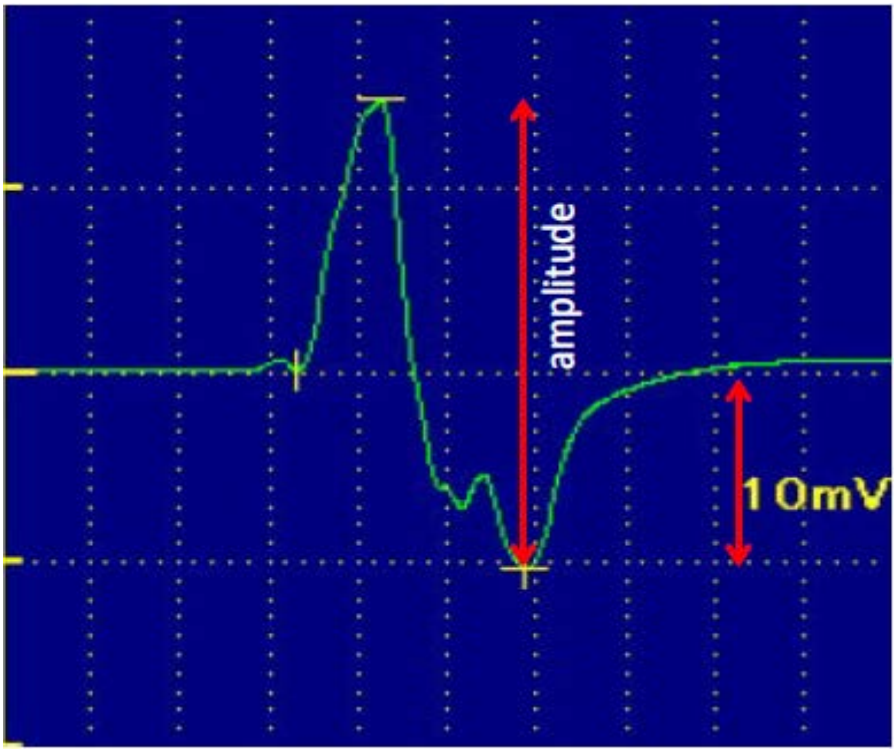

Figure 7. A typical motor evoked potential of tibialis anterior after a transcranial stimulus is applied. MEP is a compound action potential in response to cranial stimulation and amplitude is the height of the waveform (from crest to trough).

MEPs were recorded at baseline, preclamp, clamp 0 min, then every 2 min until 10 min, then every 5 min until 45 min, at the time of declamp, then every 2 min until 5 min, every 5 min until 20 min, followed by every 10 min until 1 hour of declamp. Amplitude of MEP, time to flat, and time to reappearance were analyzed. Flat MEP was defined as loss of spike of MEP bilaterally after clamping. Reappearance of MEP was defined as any MEP waveform which is not flat (unilateral or bilateral) after the release of clamp.

Measurement of serum memantine concentration:

At the end of surgery, 5 ml blood was centrifuged at 3000 rpm for 10 minutes to obtain serum for measurement of memantine concentration. Serum was stored at -80°C for 2-3 weeks before final

analysis. Measurement of serum concentration was done by validated liquid chromatography-tandem mass spectrometry using 4-hydroxychalcone as internal standard. The concentrations were expressed as ng/ml of memantine free base.

Evaluation of paraplegia:

Paraplegia was evaluated by modified Tarlov score at 6, 24, 48, and 72 hours. The scoring was carried out as:

0= no movement of lower limbs

1= slight movement of lower limbs

2= sits with support

3= sits alone

4= weak hop

5= normal hop

Histopathological examination:

At 72 h, rabbits were sacrificed by intra-cardiac injection of 10 mEq KCl. Lumbar segments of spinal cords were harvested and stored in 10 % formalin solution for 2-3 weeks prior to histopathological examination by a neuropathologist who was blinded to the treatment model. Sections were cut 3 µm thick and stained with Hematoxyin and Eosin (H& E) stain. In each section, I looked for normal neurons with polygonal cell body having cytoplasmic extension, centrally located round nuclei with prominent nucleolus; degenerated neurons (red neurons, ghost neurons, chromatolytic neurons, and neurons with vacuolization); and neuronal loss.

Grading of the severity of necrosis was done by dividing the gray matter, excluding the posteriormost part containing sensory neurons, into 4 quadrants: right anterior, left anterior, right posterior, and left posterior. Histopathology was reported as follows (Figure 8):

Normal: none of the 4 quadrants showed evidence of ischemia

Mild ischemia: only 1 quadrant ischemic

Moderate ischemia: 2 quadrants ischemic

Severe ischemia: 3 or all 4 quadrants ischemic

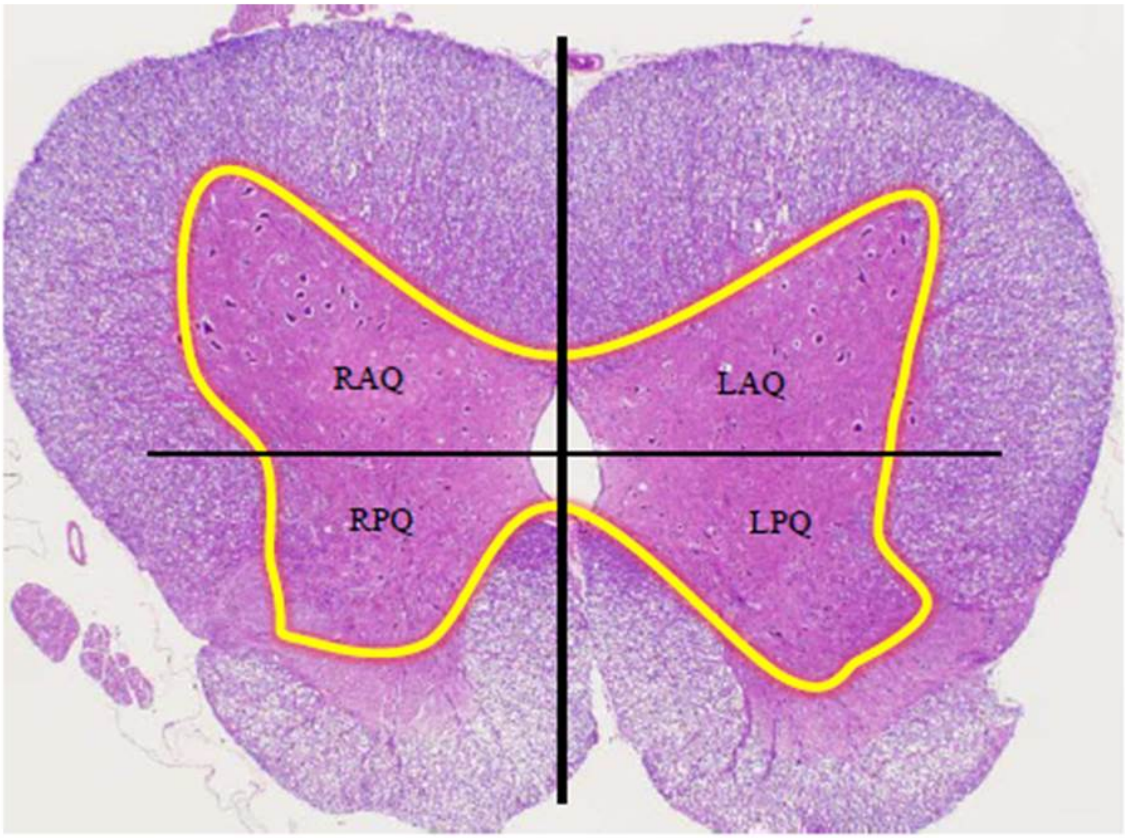

Figure 8. Basis of grading of severity of histopathological findings of spinal cord. RAQ: right anterior quadrant; RPQ: right posterior quadrant; LAQ: left anterior quadrant; LPQ: left posterior quadrant.

Statistical analysis:

Statistical analysis was done using SPSS version 20, Chicago, IL, USA. Data were expressed using mean± SD, median, range, and percentage wherever appropriate. Mann- Whitney U test, analysis of variance (ANOVA), and chi-square tests were utilized depending on the variables. Level of significance was fixed at a p value of <0.05.

RESULTS:

Baseline and intraoperative characteristics:

Baseline and intraoperative characteristics of all five groups were similar (Table 1).

Table 1. Baseline and intraoperative characteristics

Variables	**Group 60-7**	**Group 60-5**	**Group 30-5**	**Group 30-3**	**Group C**	**p-value**
Baseline characteristics						
Body weight, mean±SD (kg)	3.2±0.1	3.2±0.2	3.2±0.1	3.1±0.2	3.2±0.2	0.617
Systolic BP, mean±SD (mmHg)	86±11	92±3	87±5	87±4	92±9	0.383
Heart rate, mean±SD (bpm)	158±13	159±11	163±10	170±29	151±9	0.372
Temperature, mean±SD (°C)	39.2±0.3	39.5±0.4	39.3±0.5	39.3±0.7	39.2±0.3	0.781
Intraoperative characteristics						
Total operating time, mean±SD (min)	86±6	88±4	87±5	89±7	96±10	0.132
Systolic BP, mean±SD (mmHg)	67±17	78±8	78±5	69±15	74±9	0.391
Heart rate, mean±SD (bpm)	171±13	168±11	166±7	169±27	175±18	0.877
Temperature, mean±SD (°C)	37.7±0.4	38.4±0.5	38.1±0.7	37.9±0.5	38.4±0.4	0.108

Intraoperative trends of vitals were also comparable among the five groups (Figures 9, 10, and 11).

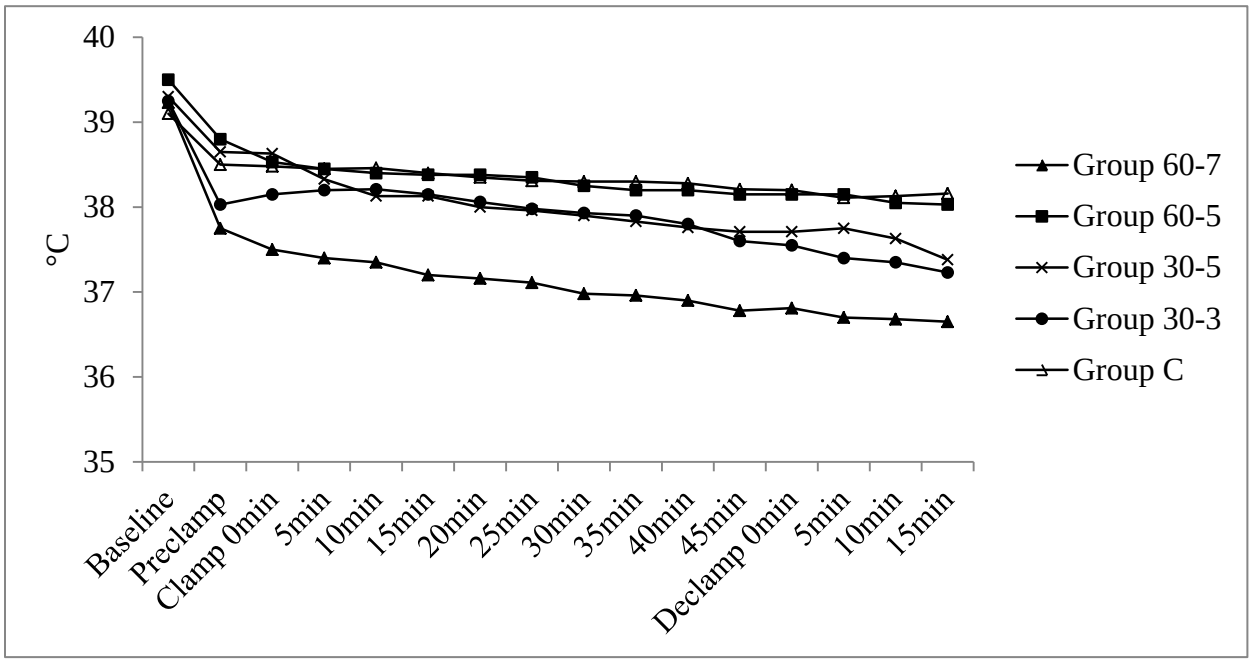

Figure 9. Intraoperative trend of rectal temperature among five groups.

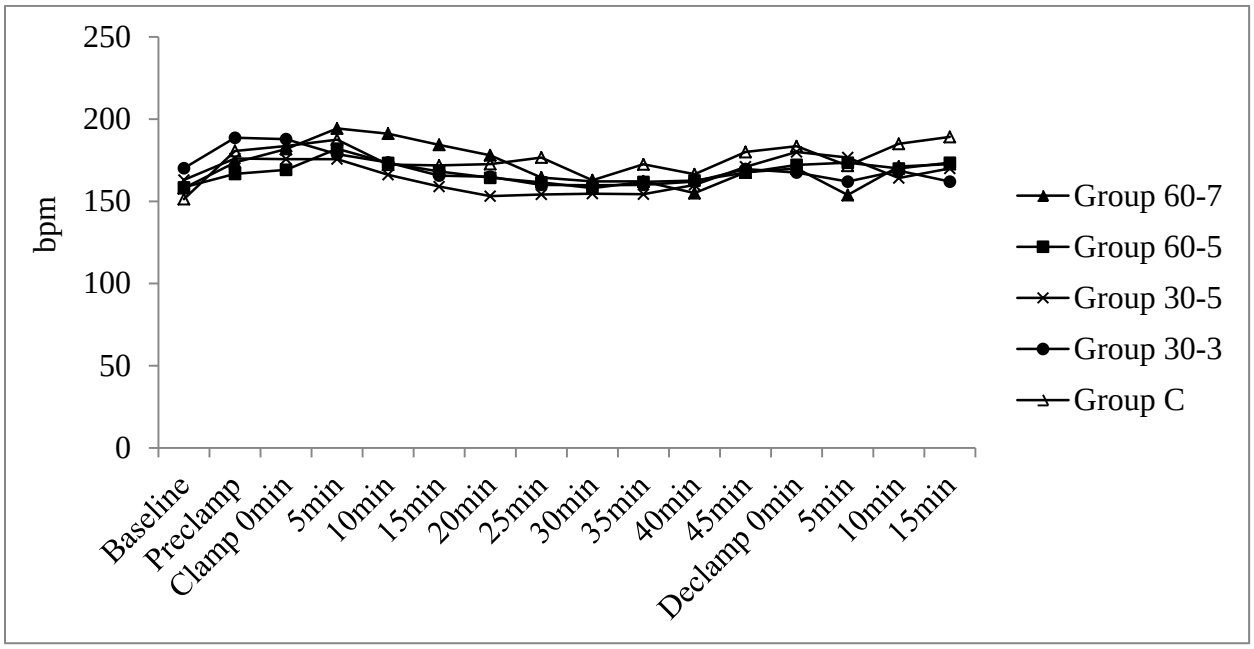

Figure 10. Intraoperative trend of heart rate among five groups. bpm: beats per minute.

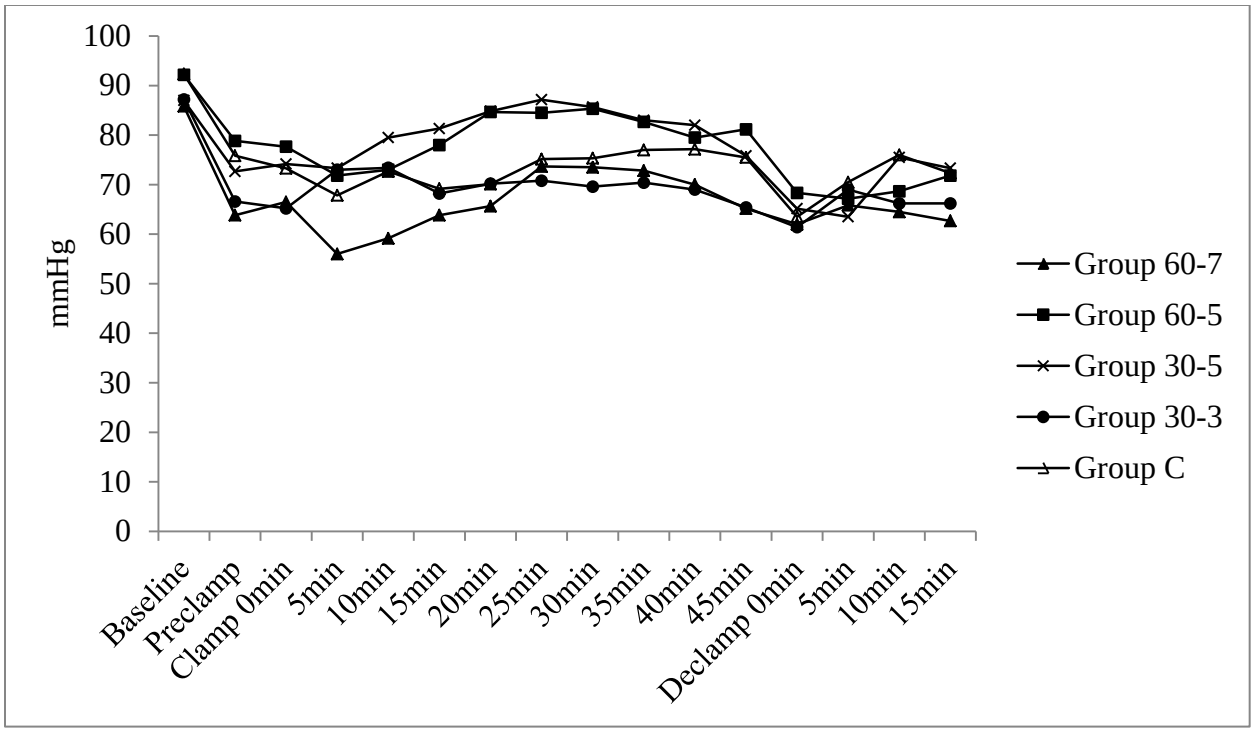

Figure 11. Intraoperative trend of systolic blood pressure among five groups.

Evaluation of paraplegia:

Paraplegia was evaluated clinically at 6, 24, 48, and 72 h by using modified Tarlov score. Mean modified Tarlov scores were 4.2±1.3, 4.3±1.0, 4.2±1.3, 4.3±1.2, and 0.8±1.6 in group 60-7, 60-5, 30-5, 30-3, and C, respectively at 6, 24, 48, and 72 h ($p<0.009$ for individual groups vs control; p=NS among groups 60-7, 60-5, 30-5, and 30-3) (Figure 12). Tarlov score remained the same throughout the observation period and I did not see any case of delayed onset paraplegia.

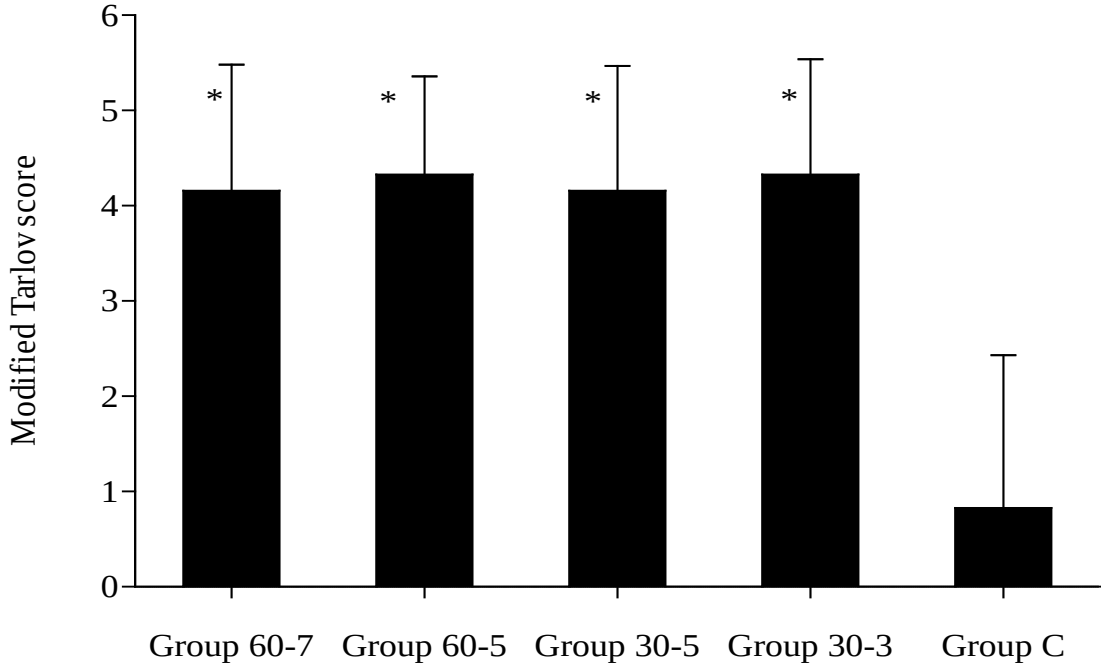

Figure 12. Modified Tarlov score at 6, 24, 48, and 72 h for five groups. * p<0.009 for individual group vs control; p=not significant among groups 60-7, 60-5, 30-5, and 30-3 (Mann-Whitney U test).

Motor evoked potentials:

Baseline amplitudes of MEP were 16.8±8.1, 17.9±6.5, 18.5±4.7, 16.6±6.3, and 17.5±5.8 mV in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.981). Median time to flat MEP after clamping was 17, 15, 12, 15, and 5 minutes in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.048) (Figure 13). After declamping, MEP reappeared in 83, 100, 83, 83, and 33 % cases in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.005) (Figure 14). Mean values of percentage amplitude loss by the end of surgery from baseline values were 29.5±46.3, 11.9±28.0, 30.0±46.8, 16.7±40.8, and 81.8±40.3 % in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.049) (Figure 15). Among cases who had reappearance of MEP after declamping, median (range) time to reappearance of MEP was 5 (2-50), 2 (2-20), 2 (2-10), 2 (2-2), and 2 (2-2)

minutes in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.183). Memantine groups revealed a gradual decrease in amplitudes of MEP waveforms before finally getting flat; and immediate reappearance of the waveforms after aortic declamping (Figure 16). However, control group had almost immediate flattening of MEP after application of aortic clamp; and no reappearance of waveforms after aortic declamping (Figure 17).

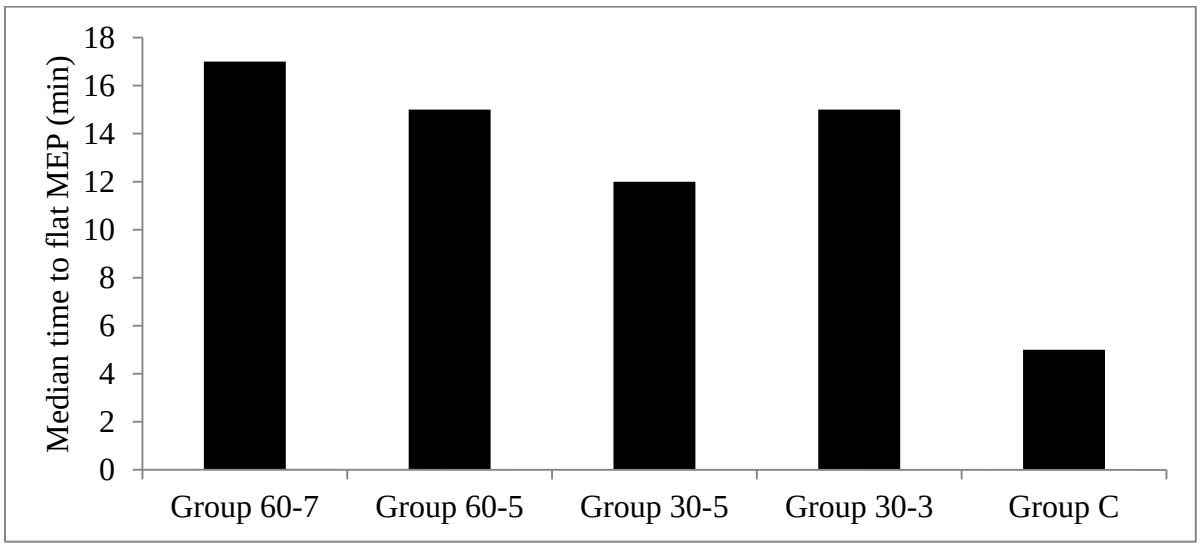

Figure 13. Median time to flat MEP after application of aortic clamp among five groups (p=0.048; nonparametric test for median).

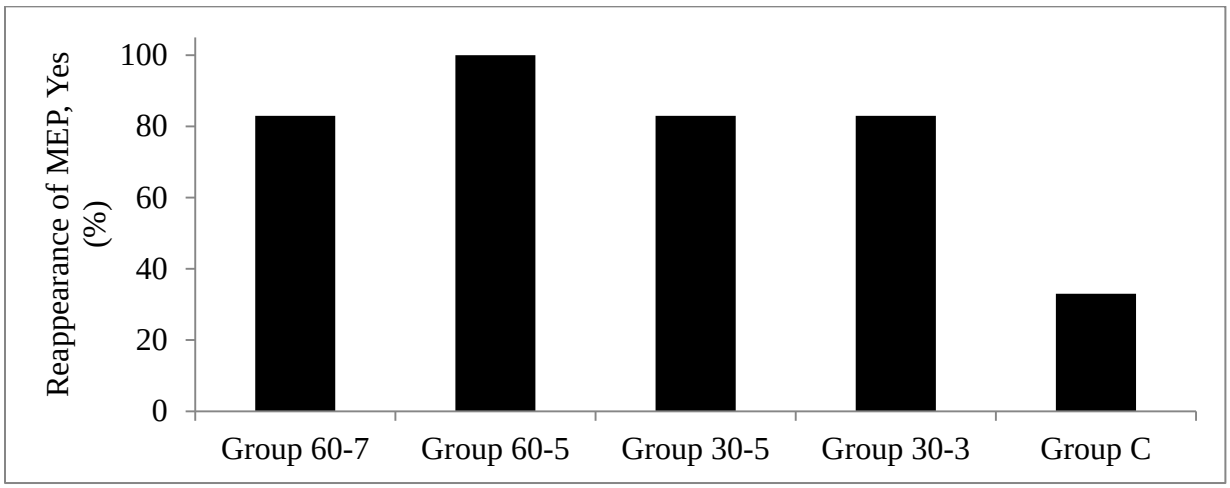

Figure 14. Reappearance of MEPs after release of aortic clamp in five groups (p=0.005; 2x2 Chi-square test).

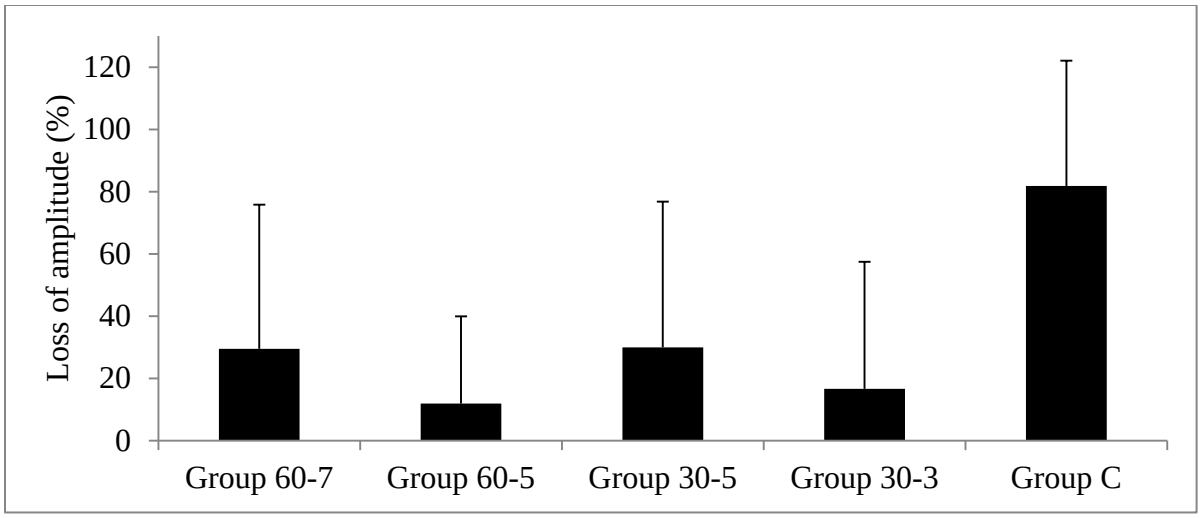

Figure 15. Percentage loss of MEP amplitude by the end of surgery compared with baseline values in five groups (p=0.049; ANOVA test).

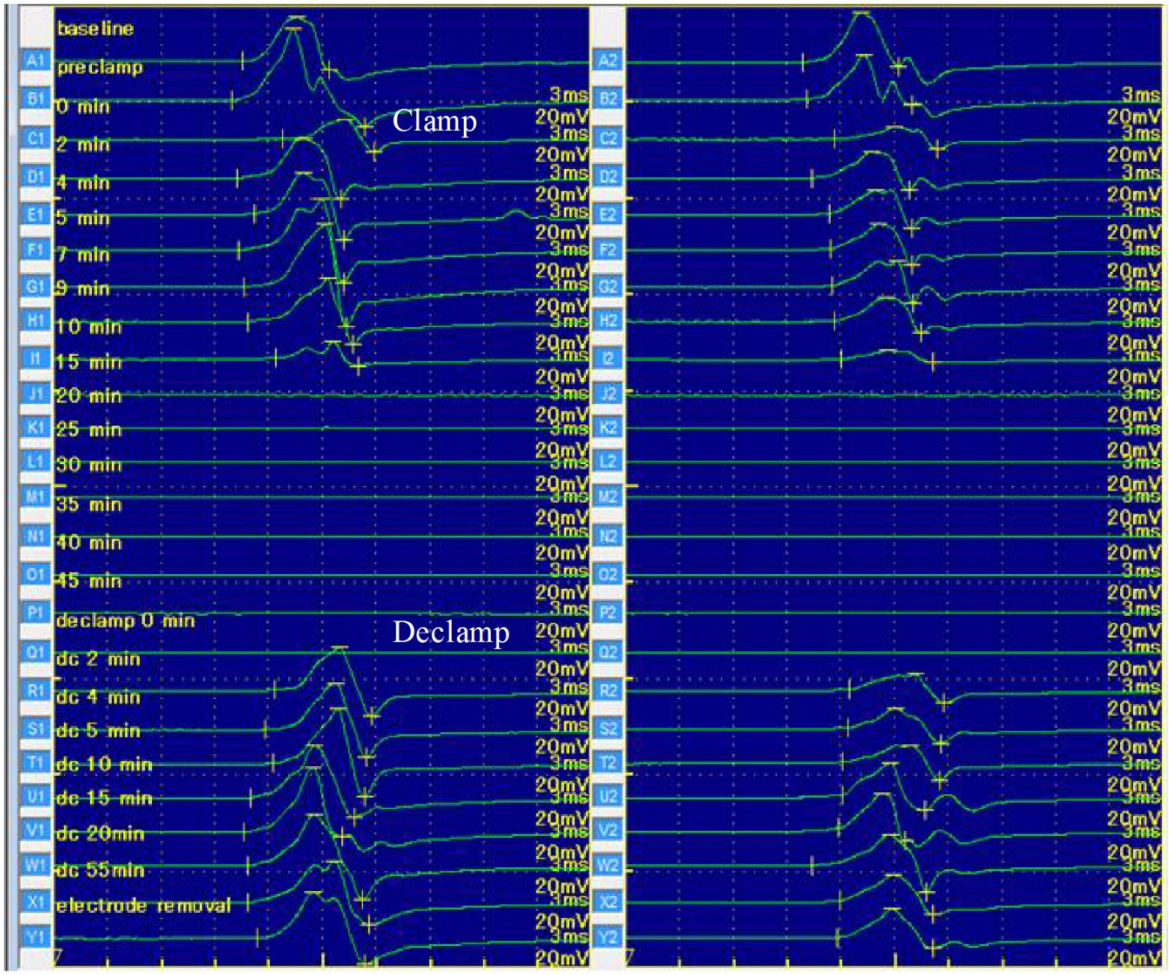

Figure 16. Typical MEP waveforms in memantine group (Note that after clamping, MEP persisted until 15 minutes, and after declamping, MEP re-appeared in 2 minutes and persisted thereafter.

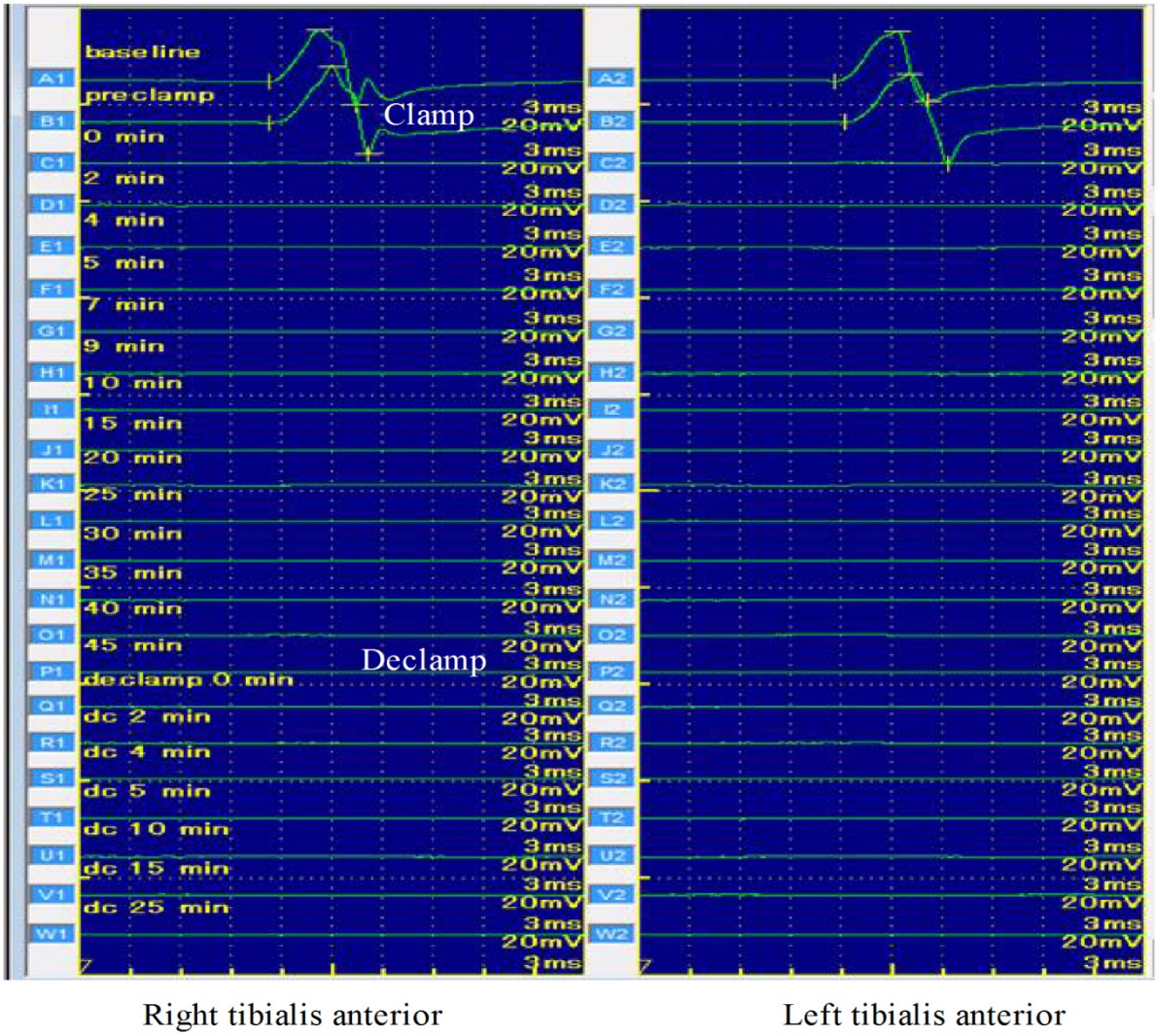

Figure 17. Typical MEP waveforms in control group (Note that after clamping, MEP disappeared almost immediately, and after declamping, MEP did not re-appear at all.

Serum memantine level:

Serum memantine level was 4.0±2.1, 6.4±2.5, 6.8±2.4, and 7.5±6.3 ng/ml in group 60-7, 60-5, 30-5, and 30-3, respectively (p=0.421). There was a relatively wide range of serum level (1.55-19.31 ng/ml) which resulted in a Tarlov score of 5 (Figure 18).

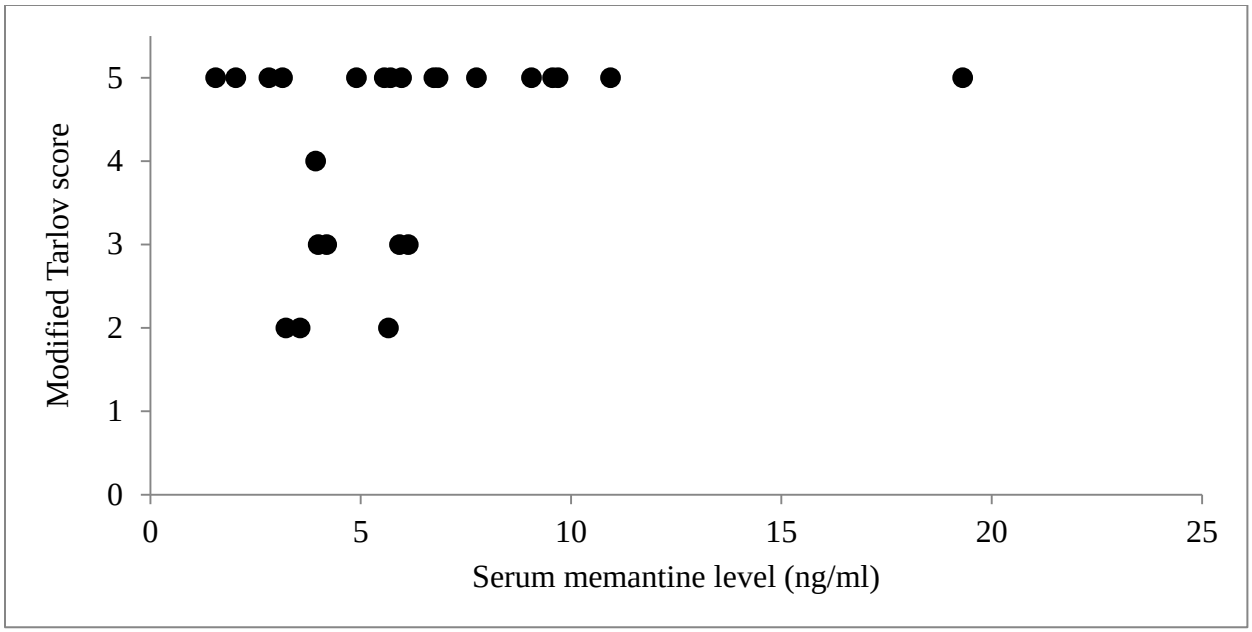

Figure 18. Scatter plot of serum memantine level vs modified Tarlov score.

Histopathology:

The percentages of normal cords, cords with mild, moderate, and severe ischemia were 50, 0, 17, and 33; 67, 0, 17, and 17; 67, 0, 33, and 0; 50, 33, 0, and 17; and 17, 0, 0, and 83 % in group 60-7, 60-5, 30-5, 30-3, and C, respectively (p=0.016) (Figure 19). Evaluation of gray matter revealed normal neurons with polygonal cell body having cytoplasmic extension, centrally located round nuclei with prominent nucleolus in memantine groups (Figure 20); while control group lacked these features; and had degenerated neurons and neuronal loss (Figure 21). For comparison, a representative sample of spinal cord of a rabbit without aortic cross clamp (sham model) revealing normal neurons is shown in Figure 22. Majority of memantine treated rabbits had spinal cords similar to that of rabbit without aortic clamping (Figures 20 and 22).

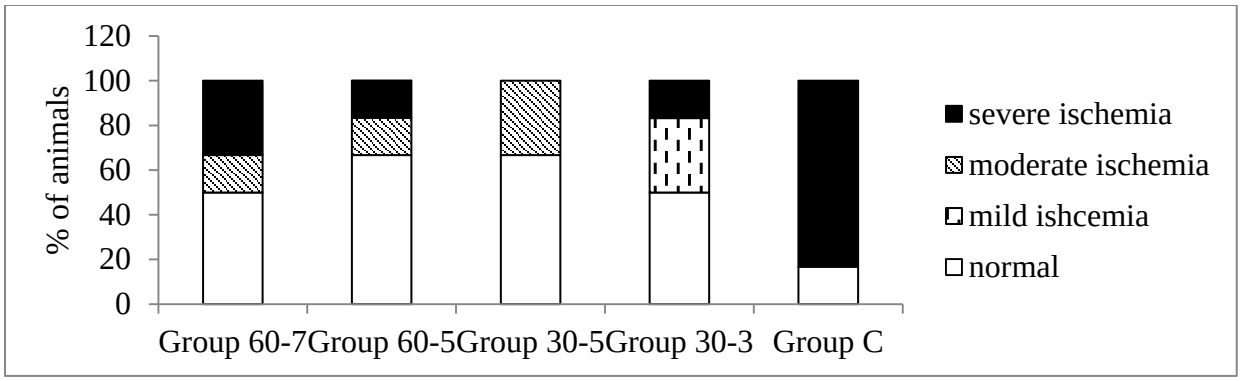

Figure 19. Distribution of normal cords, cords with mild, moderate, and severe ischemia in five different groups (p=0.016; 2x4 Chi-square test).

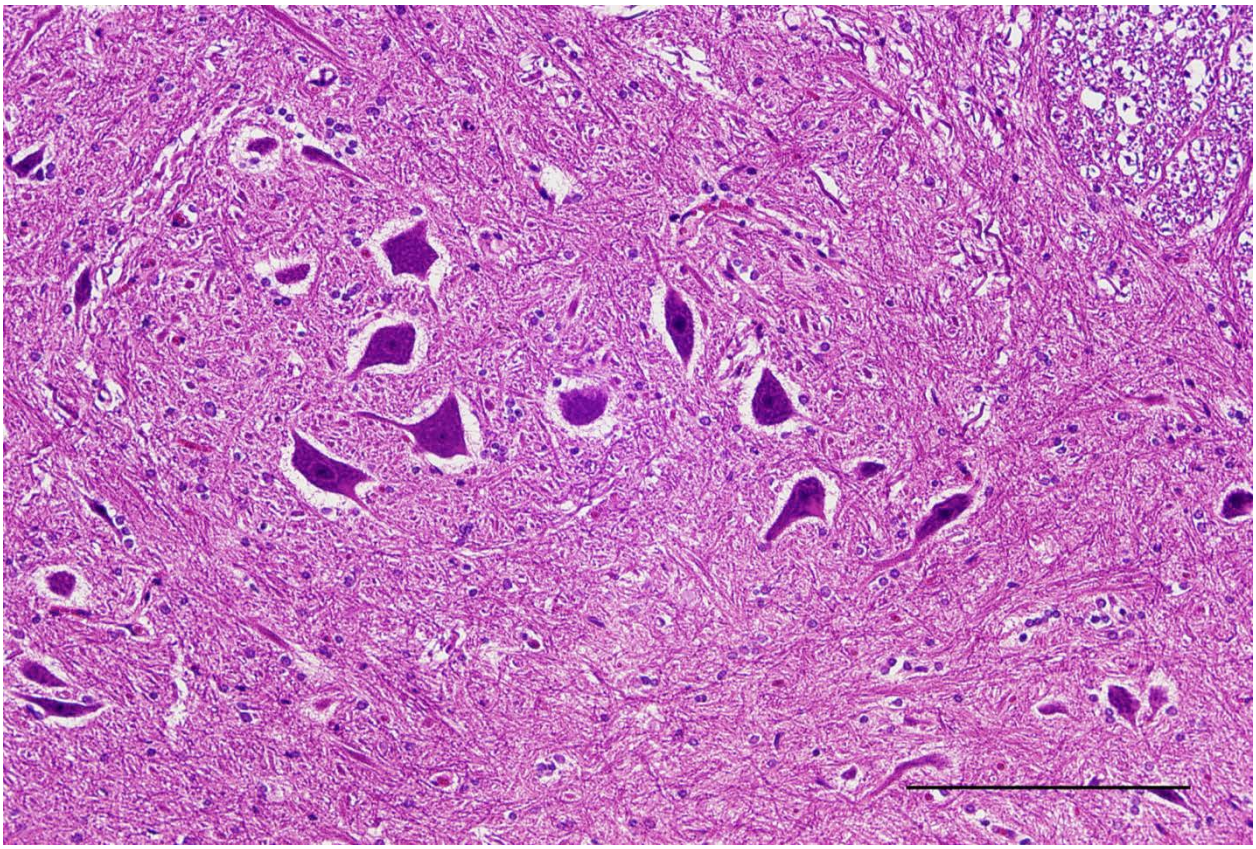

Figure 20. Representative sample of a spinal cord (H & E x20) in memantine group showing normal neurons with polygonal cell body having cytoplasmic extension, centrally located round nuclei with prominent nucleolus. Bar measures 200 µ.

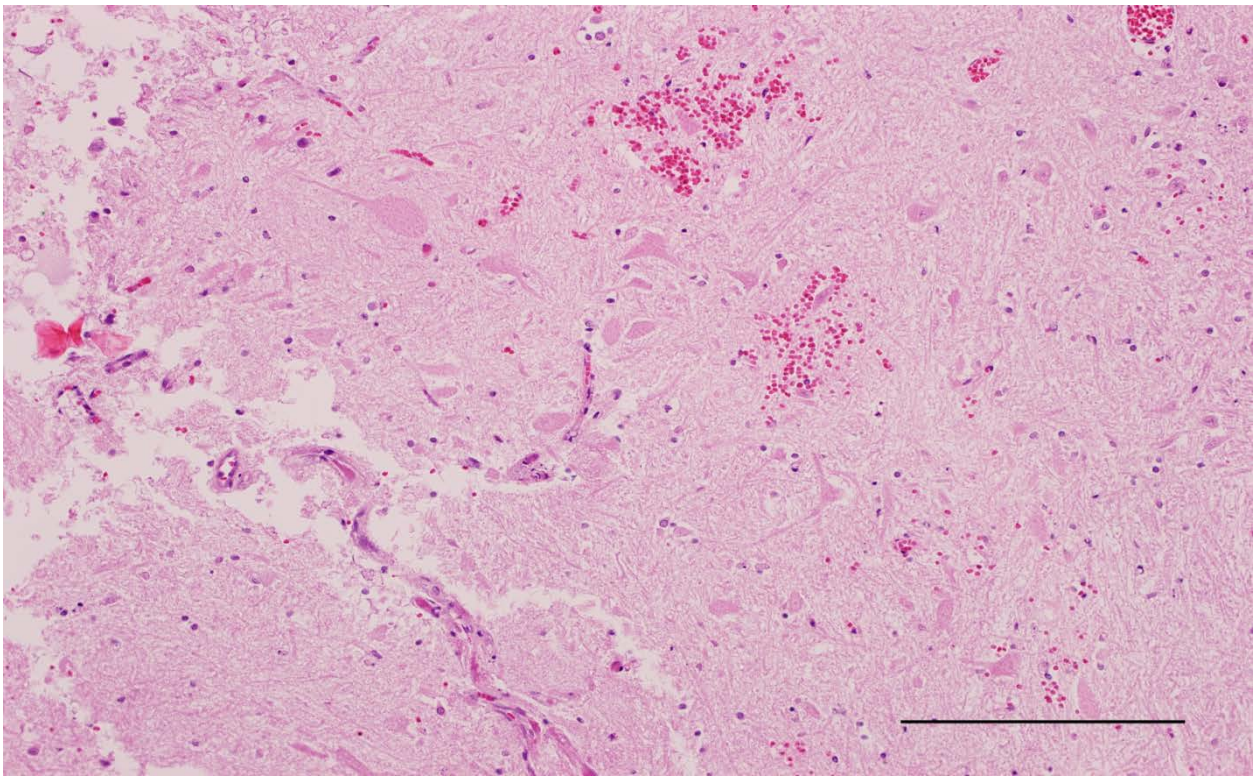

Figure 21. Representative sample of spinal cord (H & E x20) in control group showing degenerated neurons. Bar measures 200 µ.

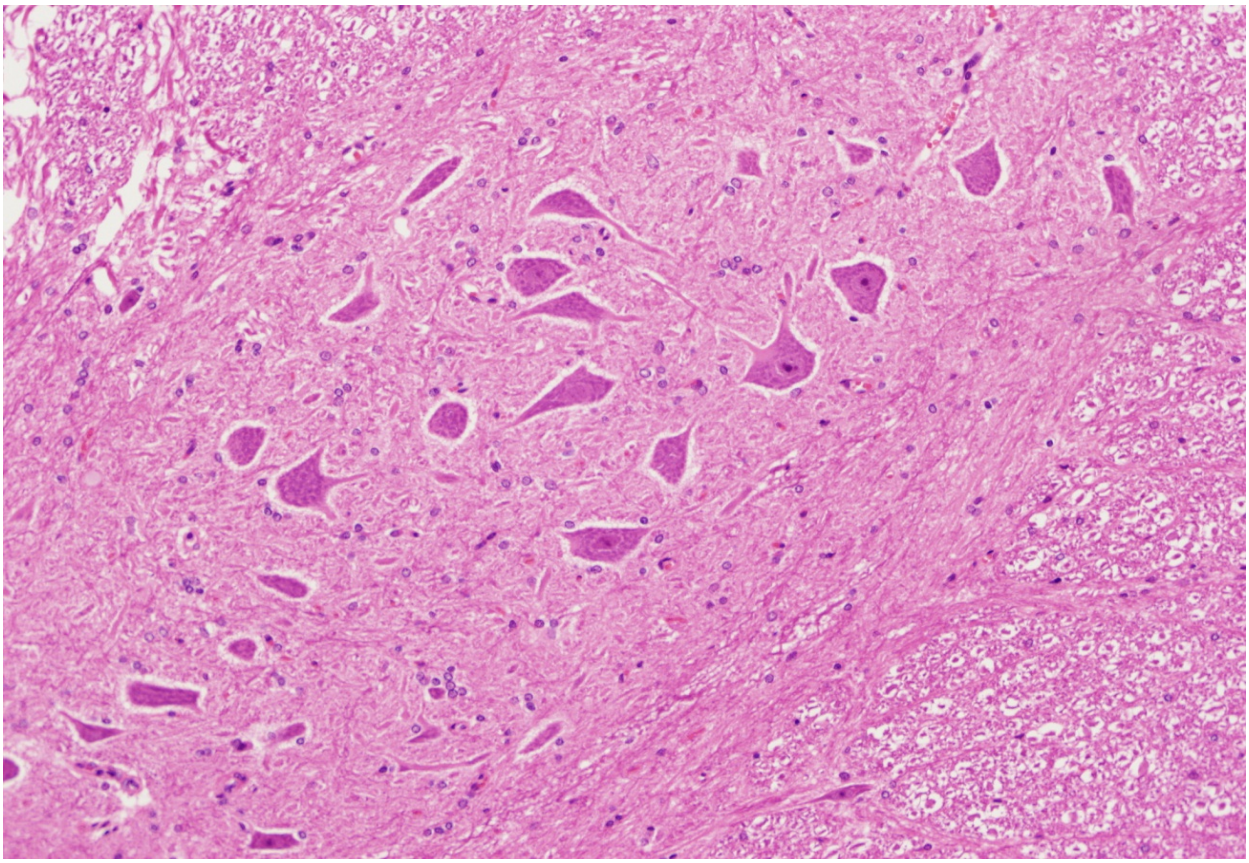

Figure 22. Representative sample of a spinal cord (H & E x20) of a rabbit without application of aortic clamp (sham model) showing normal neurons with polygonal cell body having cytoplasmic extension, centrally located round nuclei with prominent nucleolus. Note the similarity of this figure with figure 20 (rabbit who received memantine).

DISCUSSION:

Thoracoabdominal aortic aneurysms develop in patients with atherosclerotic disease, chronic aortic dissection, and connective tissue disorders (Marfan syndrome, Ehlers-Danlos syndrome, Loeys-Dietz syndrome). The risk of rupture increases rapidly when diameter exceeds 5.5 or 6 cm. Medical therapy includes the reduction of risk factors of aneurysmal expansion (smoking, hypercholesterolemia) and tight control of blood pressure hoping that it would reduce the risk of rupture. Surgical intervention includes either open graft repair; or endovascular stent graft repair. Both of these approaches carry a real risk of spinal cord injury. The surgical repair of TAAAs is an undertaking that requires substantial preoperative planning and consideration of a wide array of techniques.

Spinal cord injury after aortic surgery carries a significant risk of physical disability and increases the risk of mortality.[3] Total aortic cross clamp time, extent of aorta repaired, aortic rupture, patient age, proximal aortic aneurysm, and history of renal dysfunction are the significant predictors of paraplegia.[3] Extent of the aneurysm has been consistently shown as a major determinant of paraplegia after TAAA repair.[3,4,7] Svensson and colleagues reported 31 % incidence of paraplegia after Crawford type II repairs compared with 6 % after Crawford type I repairs; and incidence further increased if accompanied by dissection.[3] Dissection itself is a risk factor for paraplegia.[15] Cross clamp time exceeding 30 min and emergency procedures also significantly increase the incidence of paraplegia.[6]

After so many years of basic and clinical research, thoracic vascular surgeons have been able to reduce the risk of paraplegia to outstandingly low levels at least in expert hands;[8] however, total elimination of this complication is almost impossible due to unavoidable interruption of spinal

perfusion during the procedure. Recognition of predictors of paraplegia and implementation of various strategies including hypothermia,[16] CSF drainage,[17] preoperative detection of artery of Adamkiewicz by magnetic resonance angiography,[33,34] reimplantation of critical intercostal and lumbar arteries, and use of pharmacotherapeutic agents as adjuncts has contributed to better outcomes. In our institute, CT guided identification of artery of Adamkiewicz by Adamkiewicz protocol preoperatively and its reattachment during surgery; avoidance of opioids; use of mild hypothermia and partial cardiopulmonary bypass; MEP monitoring; CSF drainage, use of steroids, and naloxone in case of noticeable MEP changes has persistently resulted in good results for prevention of paraplegia over the last several years. We insert spinal drainage catheter in all patients; but institute CSF drainage at 15 ml/h maintaining CSF pressure at 10 cmH_2O only if we notice MEP changes.

N-Methyl-D-Aspartate (NMDA) receptors have an important role in mediating ischemic neuronal injury.[23] Physiological NMDA receptor activity is essential for normal neuronal function,[35] and therefore must be preserved, even in the face of excessive pathological activity in other areas of the central nervous system. However, excessive NMDA receptor activity is harmful and therefore should be prevented for maintaining the integrity of nervous system in the face of variety of insults.[23] Potential neuroprotective agents that manifest a high affinity for NMDA receptors block virtually all activity including physiological signaling, and will therefore probably have unacceptable clinical side effects.[23] Memantine has a unique property that it preferentially blocks excessive (pathological) NMDA receptor activity without disrupting normal (physiological) function.[36] Memantine does this through its action as a low affinity but still highly selective uncompetitive, open- channel blocker with a relatively rapid off-rate from the channel.[36] Moreover, the relatively fast off-rate of memantine prevents the drug from

accumulating in NMDA receptor-operated channels, so subsequent physiological neurotransmission can proceed in a normal fashion.[37,38] This fast off-rate property contributes to the favourable profile of memantine in terms of its clinical tolerability with lower side effect profile compared with other NMDA receptor antagonists (MK-801, and CGS19755) described prior to the discovery of memantine.[39,40]

Memantine has already been clinically approved for treatment of Alzheimer's dementia. Bioavailability after oral administration is approximately 100 %, and food does not alter its absorption.[22] Clinically used oral doses of 20 mg once daily result in a wide range of serum level measuring 72-182 ng/ml,[41,42] with single oral dose of 20 mg resulting into a serum concentration of 22.08 ng/ml.[43] My treatment regimens resulted into serum concentration ranging between 1.55 and 19.31 ng/ml, majority falling between 4 and 11 ng/ml. Although my treatment dosing (60 mg or 30 mg once daily) seemed to be higher compared with the clinically used dosing regimen, I achieved serum concentration well below the level of toxicity. Although serum memantine level among four treatment groups did not show statistically significant difference, I noted a peculiar finding that the group which received highest dose of 60 mg for longest duration of 7 days (group 60-7) resulted into relatively low values of serum levels. Modified Tarlov score of 5 was achieved with a wide range serum concentration of 1.55 to 19.31 ng/ml. Pharmacokinetics of memantine is different in animals from that in humans. A group of authors has shown that in pigs, following a single oral dose of 200 mg memantine, peak serum concentration of 150 ng/ml was achieved after 1.5 hours of oral intake; and the concentration decreased to a level close to 30 ng/ml following 12 hours of oral intake.[44] They showed that half life of memantine in pigs is just 2 hours as opposed to more than 60 to 100 hours in humans; and bioavailability of memantine in pigs is only about 34 % after oral intake as opposed to 100 % in humans; thus requiring a higher

dose in pigs to achieve the same drug plasma levels as in humans.[44] Although I did not find pharmacokinetic studies with oral memantine in rabbits, I believe that pharmacokinetics of memantine in rabbits is different from that in humans; and possibly might be close to that in pigs. This might possibly explain the lower concentrations of memantine in my experiment compared with clinical studies.

von Euler and colleagues reported that memantine is not protective against spinal cord injuries in rat model.[45] Two years later, Ehrlich and colleagues showed that intravenous and intra-arterial memantine is protective against spinal cord ischemia following aortic clamping in a rabbit model.[46] After a careful search of the currently available literature, I did not find further work on memantine for spinal cord protection beyond rabbits, despite its clinically favorable side-effect profile. Since Ehrlich and colleagues [46] first showed its effectiveness in late 1990s, this topic remained dormant for more than a decade. I demonstrated that oral pretreatment with memantine is effective for prevention of paraplegia in a rabbit model. My treatment model is important because memantine is available only in oral form in Japan; and as little as 3 days of oral treatment before surgery is effective for prevention of spinal cord injury, thereby having a huge potential for its easy clinical application.

Other NMDA receptor antagonists that have been studied in animal models and have shown effectiveness for spinal protection include MK-801, and CGS19755.[31,47] However, they have clinically intolerable side effects. Following ischemic insult, glutamate and aspartate not only activate NMDA receptors but also activate α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-kainate receptor subtypes.[48] AMPA-kainate receptor antagonists have also shown protection against spinal cord ischemia in animal models.[27] However, these drugs are not still in clinical use. Unlike AMPA-kainate receptor antagonists and other NMDA receptor antagonists,

safety profile of memantine is already proven and it is in clinical use for treatment of Alzheimer's dementia favoring its choice over others.

Motor evoked potentials have been routinely used for detection of spinal cord ischemia during aortic surgeries. In my laboratory, I showed that in rabbits who have received memantine pretreatment, MEPs tend to persist longer after aortic clamp is applied and reappear more frequently upon declamping. Moreover, the amplitude loss by the end of surgery compared with the baseline value was significantly more in the control group compared with the treatment groups. Of those who showed reappearance of MEP in the control group, there was no significant difference in the time to reappear compared with memantine groups. This is because of the very small number of rabbits (n=2) in control group who had reappearance of MEP. Memantine groups showed persistence of MEP for longer period with gradual decrease of amplitude until it finally became flat after aortic clamping; while control group showed abrupt flattening of MEP immediately after or within a few minutes of aortic clamping.

Modified Tarlov score was significantly higher in treatment groups compared with the control group at all times of observation. Modified Tarlov score at 24, 48, and 72 h was the same as that of 6 h for all five groups; and I did not witness any case of delayed onset paraplegia. Clinical evaluation closely correlated with the results of histopathology. Histopathological grading was done by dividing the entire gray matter, excluding the posteriormost part containing sensory neurons, into 4 segments. By doing so, I was able to take even subtle changes of ischemia into consideration. My findings open the possibility for another potential strategy in the armamentarium of thoracic and thoracoabdominal aortic aneurysm repair; however translation of these findings to large animal models or to clinical application yet needs to be explored.

Measurement of CSF concentration of memantine would have been the best way to define its role in spinal protection. However, obtaining CSF sample in this small animal model was technically challenging; therefore I chose to measure serum level as a surrogate of CSF level. Previous studies have shown CSF levels of memantine to be highly correlated with serum levels with mean CSF/serum level ratio of 0.5.[49,50] Therefore, I believe that CSF concentration of almost half of the serum concentration would have been achieved in my treatment model had I measured CSF concentration. Even in clinical settings, measurement of serum level is very convenient which avoids invasive lumbar puncture to obtain CSF sample.

Maintenance of high normal blood pressure intraoperatively is a routine practice during TAAA repairs in the clinical settings, as is routine monitoring of MEPs and institution of CSF drainage. In my experimental model, I tried to maintain blood pressure to as normal as possible although all five groups showed transient decrease of blood pressure after release of aortic clamp. Histopathological examination included staining with H&E, and the results of histopathology were strongly correlated with the clinical scoring with modified Tarlov score. However, I believe that looking for the evidence of apoptosis by Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay, and genetic studies for hypoxia inducible factors (HIF) would have further reinforced my histopathological findings. The findings of my research suggest that memantine could play its potential role as a new adjunct in the currently available adjuncts for spinal protection during TAAA repairs; however, translation of these findings to large animal models or to clinical settings needs to be explored.

CONCLUSION:

Memantine oral treatment is effective for prevention of spinal cord injury during infrarenal aortic clamping in a rabbit model. Once daily oral pretreatment with memantine for as little as 3 days prior to surgery was shown to have spinal protection. As we move towards the path of excellency in aortic surgery, memantine can play its role as an additional adjunct strategy for prevention of spinal cord injury.

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