CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

THE *N*-METHYL-D-ASPARTATE ANTAGONIST MEMANTINE HAS NO NEUROPROTECTIVE EFFECT DURING HYPOTHERMIC CIRCULATORY ARREST: A STUDY IN THE CHRONIC PORCINE MODEL

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Background: Glutamate excitotoxicity has an important role in the development of brain injury after prolonged hypothermic circulatory arrest. The goal of the present study was to determine the potential efficacy of memantine, an *N*-methyl-D-aspartate receptor antagonist, to mitigate cerebral injury after hypothermic circulatory arrest.

Methods: Twenty pigs (23–33 kg) were randomly assigned to receive memantine (5 mg/kg) or placebo in a blinded fashion before a 75-minute period of hypothermic circulatory arrest at 20°C. Hemodynamic, electroencephalographic, and metabolic monitoring were carried out. The intracerebral concentrations of glucose, lactate, glutamate, and glycerol were measured by means of enzymatic methods on a microdialysis analyzer. Daily behavioral assessment was performed until the animals died or were put to death on day 7. Histologic analysis of the brain was carried out in all animals.

Results: In the memantine group, 5 of 10 animals survived 7 days compared with 9 of 10 in the placebo group. The median behavioral score at day 7 was 3.5 in the memantine group and 7.5 in the placebo group ($P > .2$). Among the surviving animals, medians were 9.0 and 8.0 on day 7 ($P > .2$), respectively. The medians of recovered electroencephalographic bursts were equal in both groups. The median of total histopathologic score was 16 in the memantine group and 14 in the placebo group ($P > .2$). There was a negative correlation between glutamate levels and electroencephalographic burst recovery ($\tau = -0.377$, $P = .043$). A positive correlation was found between the highest individual glutamate value and histopathologic score ($\tau = 0.336$, $P = .045$).

Conclusions: The present study demonstrates that memantine has no neuroprotective effect after hypothermic circulatory arrest in the pig. In addition, we have shown the accuracy of cerebral glutamate measurements to predict histopathologic injury after hypothermic ischemia. (J Thorac Cardiovasc Surg 2001;121:957–70)

Hypothermic circulatory arrest (HCA) is a frequently used method during operations on the aortic arch.^{1,2}

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However, the efficacy of hypothermia is limited and the safe duration of HCA is 40 to 50 minutes at brain temperatures of 10°C to 15°C.³ The importance of the failure of neurotransmitter transport as a common pathway in the pathogenesis of ischemic cerebral injury has been well demonstrated in the past few years.^{4–6} Under conditions of depleted cellular energy, including hypoxia and ischemia, glutamate is accumulated in the intercellular space, acting as a neurotoxic substance. It opens calcium channels, leading to an influx of calcium, which initiates the catastrophic cascade that eventually leads to neuronal autodigestion and cell death.^{6,7} Excitatory amino

acid antagonists are used to prevent the development of ischemic brain injury, but their clinical use is frequently limited because of neurotoxic side effects.⁷

Memantine is an uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. It is widely used as a very well tolerated drug in the treatment of chronic neurodegenerative diseases such as Parkinson disease, Alzheimer disease, and acquired immunodeficiency syndrome. It is also used as a symptomatic treatment for epilepsy and depression.⁸⁻¹⁰ Memantine ameliorates NMDA receptor-mediated neurotoxicity in vitro and in vivo in normothermia.^{11,12} Memantine has also proved to be a promising drug in the treatment of retinal ischemia.^{13,14} The results of experimental studies using memantine for prevention of spinal cord injury induced by ischemia have been controversial.¹⁵⁻¹⁷

We have been studying the means to ameliorate brain injury after HCA in a chronic porcine model.^{18,19} The aim of this study was to test the neuroprotective efficacy of memantine during HCA with this model.

Materials and methods

Twenty juvenile (8- to 10-week-old) female pigs of a native stock, weighing 23 to 33 kg, were randomly assigned to receive either memantine (5 mg/kg) or placebo (saline solution) before a 75-minute period of HCA at 20°C.

Preoperative management. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 85-23 revised 1985). The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Pharmacokinetic pilot study of memantine. Therapeutic range of plasma levels in patients treated with memantine (10-30 mg per day) is 0.2 to 1.0 $\mu\text{mol/L}$ and free brain interstitial concentration is 20% to 30% lower than in plasma.¹⁰ Plasma concentrations of 1 $\mu\text{mol/L}$ are achieved in laboratory animals after acute injection of 2.5 to 5.0 mg/kg.²⁰ To determine the appropriate dose of memantine in the pig, we performed a pharmacokinetic pilot study in 4 pigs weighing 25 kg. Memantine doses of 3, 5, 7, and 10 mg/kg were given and blood samples were obtained 30 minutes, 1 hour, 2 hours, and 5 hours after the start of drug administration. A plasma level of 1.49 $\mu\text{mol/L}$ was recorded 30 minutes after a dose of 3 mg/kg. Animals in this model are known to be exposed for rather severe global cerebral ischemia^{18,19} and, on the other hand, plasma is diluted by cardiopulmonary bypass (CPB). Therefore, a dose of 5 mg/kg providing the highest blood level of 2.81 $\mu\text{mol/L}$ 30 minutes after drug administration was selected for the study.

Drug administration. Memantine was diluted in sodium chloride 0.9% to obtain a solution containing memantine at

10 mg/mL, and this was packed in 15 mL ampules in the Pharmaceutical Laboratory of Oulu University Hospital. Saline placebo ampules were prepared similarly. A dose of 5 mg/kg was measured and diluted to 50 mL in saline solution. This volume was given intravenously over a period of 20 minutes, starting 75 minutes before HCA. Randomization was carried out by the chemist in the pharmaceutical laboratory, and the codes were broken after the entire series of experiments were accomplished. Therefore, all observers in every data recording point were unaware of whether the animal was a memantine subject or a control subject. Pharmacokinetic analyses, generously performed by Merz & Co, demonstrated that memantine concentrations in the study group were sustained at a therapeutically relevant level throughout the critical period of the experiment. The median (interquartile range) plasma concentrations of memantine showed a slow tapering after the period of rewarming as follows: 1.23 $\mu\text{mol/L}$ (0.98-1.46 $\mu\text{mol/L}$) at the end of cooling at 20°C, 1.47 $\mu\text{mol/L}$ (1.35-1.65 $\mu\text{mol/L}$) during rewarming at 30°C, 1.12 $\mu\text{mol/L}$ (0.96-1.19 $\mu\text{mol/L}$) 2 hours after the start of rewarming, 0.66 $\mu\text{mol/L}$ (0.56-0.79 $\mu\text{mol/L}$) 4 hours after the start of rewarming, and 0.46 $\mu\text{mol/L}$ (0.43-0.62 $\mu\text{mol/L}$) 7 hours after the start of rewarming.

Anesthesia and hemodynamic monitoring. Anesthesia was induced with medetomidine hydrochloride (0.4 mg/kg intramuscularly), and muscular paralysis was maintained with pancuronium bromide (0.1 mg/kg intravenously). After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 35% oxygen; anesthesia was maintained with isoflurane (1.1%-1.2%). The arterial catheter was positioned in the left femoral artery. A thermodilution catheter (Criticath, 7F; Ohmeda GmbH & Co, Erlangen, Germany) was placed through the femoral vein to allow blood sampling, pressure monitoring in the pulmonary artery, and recording of cardiac output. The intracranial temperature probe was placed through a drill hole in the epidural space. The drill hole was positioned 1 cm to the left side from a sagittal joint above a parietal line. Other temperature probes were placed in the esophagus and rectum, and a 10F catheter was placed in the urinary bladder to monitor urine output.

Electroencephalographic (EEG) monitoring. Cortical electrical activity was registered from 4 stainless steel screw electrodes (5 mm in diameter) implanted in the skull over the parietal and frontal areas of the cortex with a digital EEG recorder (Nervus Company, Reykjavik, Iceland) and an amplifier (Magnus EEG 32/8; Nervus Company). Sampling frequency was 1024 Hz, and bandwidth was 0.03 to 256 Hz. All EEG recordings are referenced to a frontal screw electrode, which, together with a ground screw electrode, is implanted over the frontal sinuses. Isoflurane level was adjusted so that the EEG showed a steady burst suppression pattern. After this, isoflurane end-tidal was kept at this steady level until the end of monitoring. The EEG was recorded for 10 minutes to get a baseline recording of steady burst suppression activity before the cooling period. After HCA, EEG recording was restarted and continued until the first postoperative day. The durations of EEG were measured from 5-

minute EEG samples at fixed time points, first with half-hour intervals, later with 1-hour intervals. From each 5-minute sample artifact periods were excluded, and from the rest the sum of bursts was counted as percentage of the sum of artifact-free bursts and suppressions. This percentage was used as a measure of EEG activity in the analysis.

CPB. Through a right thoracotomy in the fourth intercostal space, the right thoracic artery was ligated, and the heart and great vessels were exposed. A membrane oxygenator (Midiflow D 705; Dideco, Mirandola, Italy) was primed with 1 L of Ringer acetate and heparin (5000 IU). After heparinization (300 IU/kg), the ascending aorta was cannulated with a 16F arterial cannula and the right atrial appendage with a single 24F atrial cannula. Nonpulsatile CPB was initiated at a flow rate of $100 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and the flow was adjusted to maintain a perfusion pressure of 50 mm Hg. A 12F intra-cardiac sump cannula was positioned in the left ventricle for decompression of the left side of the heart during CPB. A heat exchanger was used for core cooling. The pH was maintained, using alpha-stat principles, at 7.40 ± 0.05 with an arterial PCO_2 of 4.0 to 5.0 kPa, uncorrected for temperature. All measurements were performed at 37°C.

A cooling period of 60 minutes was carried out to attain a rectal temperature of 20°C. The ascending aorta was crossclamped just proximal to the aortic cannula. Cardiac arrest was induced by injecting potassium chloride (1 mEq/kg) via the aortic cannula, and topical cardiac cooling was then begun and maintained throughout the aortic cross-clamp period.

Experimental protocol. After cooling to 20°C rectal temperature and crossclamping of the aorta, the animals underwent a 75-minute interval of HCA with the head packed in ice. After this 75-minute period, CPB rewarming was initiated. The left ventricular vent cannula was removed. Weaning from CPB occurred approximately 60 minutes after the start of rewarming with administration of furosemide (40 mg), mannitol (15 g), methylprednisolone (80 mg), and lidocaine (40-150 mg). Cardiac support was provided by dopamine (1-5 mg/h). The animals were kept under isoflurane anesthesia until the following morning, extubated, and moved into a recovery room.

During the experiments, hemodynamic and metabolic measurements were recorded at 5 time points as follows: at baseline; at the end of cooling (at 20°C, immediately before institution of the intervention); during rewarming (at 30°C); 2 hours after the start of rewarming; and 4 hours after the start of rewarming.

Postoperative evaluation. Postoperatively, all the animals were evaluated daily by an experienced observer who was blinded to the study group. The observer used a species-specific quantitative behavioral score as reported earlier.²¹ The assessment quantified *mental status* (0 = comatose, 1 = stuporous, 2 = depressed, 3 = normal), *appetite* (0 = refuses liquids, 1 = refuses solids, 2 = decreased, 3 = normal), and *motor function* (0 = unable to stand, 1 = unable to walk, 2 = unsteady gait, 3 = normal). Numerical summing of these functions provides a final score: the maximum (score of 9)

reflects apparently normal neurologic function, whereas lower values indicate substantial brain damage. A score of 8 means that the animals were able to stand unassisted and were likely to recover fully. Each surviving animal was electively killed on day 7 after the operation. The entire brain was immediately harvested and weighed for subsequent histologic analysis.

Histopathologic analysis. During autopsy the brain was excised immediately and the hemispheres were separated. One half was immersed in 10% neutral formalin solution and allowed to fix for 1 to 2 weeks en bloc. Thereafter, 3-mm thick coronal samples were sliced from the frontal lobe, thalamus (including the adjacent cortex), and hippocampus (including the adjacent brain stem and temporal cortex), and sagittal samples were obtained from the posterior brain stem (medulla oblongata and pons) and cerebellum. The pieces were fixed in fresh formalin solution for another week. After the fixation, the samples were processed as follows: rinsing in water for 20 minutes, immersion in 70% ethanol for 2 hours, in 94% ethanol for 4 hours, and in absolute ethanol for 9 hours. Thereafter, the pieces were kept 1 hour in absolute ethanol-xylene mixture, 4 hours in xylene, and were embedded in warm paraffin for 6 hours. The samples were sectioned at 6 μm and stained with hematoxylin and eosin. The sections of the brain samples of each animal were screened by a single experienced senior pathologist (J.H.), unaware of the experimental design and of the identity and fate of individual animals. Each section was carefully investigated for the presence or absence of any hypoxic or other damage.

Visual estimation of the injuries in the sampled regions was made as follows: 0 = no morphologic damage; 1 = edema or eosinophilic dark neurons or dark/shrunk cerebellar Purkinje cells; 2 = at least 2 small hemorrhages, and 3 = clearly infarctive foci. Total score is the sum of scores in each specific brain area (cortex, thalamus, hippocampus, posterior brainstem, and brainstem). To allow semiquantitative comparisons between the animals, we calculated a total histologic score by adding all the regional scores. A score more than 4 means that the animal had a distinct brain injury.

Microdialysis. The microdialysis catheter (CMA 70; CMA/Microdialysis, Stockholm, Sweden) was placed into the brain cortex. A drill hole was positioned 1 cm to the right side from a sagittal joint above a parietal line. The shaft was introduced free hand through a bolt to a depth of 15 mm below the dura. The microdialysis catheter was connected to a 2.5-mL syringe placed in a microinfusion pump (CMA107; CMA Microdialysis) and perfused with Ringer solution (Perfusion Fluid; CMA Microdialysis). Samples were collected every 30 minutes. The concentrations of glucose, lactate, glutamate, and glycerol were measured immediately after collection by means of ordinary enzymatic methods on a microdialysis analyzer (CMA 600; CMA Microdialysis).

Other measurements. Systemic arterial and venous blood samples were obtained to determine pH, PO_2 , PCO_2 , oxygen saturation, oxygen content, hematocrit value, hemoglobin concentration, and glucose level (Ciba-Corning 288 Blood Gas System; Ciba-Corning Diagnostic Corp, Medfield,

Fig 1. Epidural temperatures of 20 pigs receiving either memantine (5 mg/kg) or placebo before a 75-minute period of HCA. Values are shown as medians with interquartile ranges.

Mass). Lactate was analyzed by means of a YSI 1500 analyzer (Yellow Springs Instrument Co, Yellow Springs, Ohio).

Statistical analysis. Summary statistics for continuous or ordinal variables are expressed as the median with interquartile range (25th and 75th percentiles) or means with standard deviation (SD). In the figures, values are shown as medians with interquartile ranges.

The analysis was performed by analysis of variance for repeated measurements. Comparison between relevant time points and baseline (reference category) was performed by paired sample *t* test or Wilcoxon matched pairs signed rank test. Differences between groups were determined by *t* test or by the Mann-Whitney *U* test. The 2-tailed Fisher exact test was used to determine the significance of mortality rates between groups. The area under the curve (AUC) was calculated for microdialysis measurements, and Kendall's rank correlation was used to estimate correlation coefficients. Analyses were performed with a standard commercially available statistical program (SPSS version 9.0; SPSS Inc, Chicago, Ill).

Results

Physiologic data

Comparability of experimental groups. The mean ($\pm$ SD) weight of the animals was 28 ± 3 kg in the memantine group and 29 ± 2 kg in the placebo group. The mean ($\pm$ SD) CPB cooling time was 66 ± 6 minutes in the memantine group and 66 ± 5 minutes in the control group. Rewarming times were 69 ± 7 minutes and 76 ± 7 minutes, respectively. Temperatures during the

experiments did not differ between the groups (Fig 1).

Hemodynamic data. All animals were in stable condition before, during, and after CPB. As seen in Table I, mean arterial pressure decreased in both groups compared with baseline at the end of cooling, during rewarming at 30°C , 2 hours after the start of rewarming, and 4 hours after start of rewarming ($P < .001$). Cardiac output decreased when compared with baseline in the memantine group 4 hours after start of rewarming ($P = .033$) (Table I). Vascular resistance decreased in the memantine group compared with the baseline group at the end of cooling and during rewarming at 30°C ($P = .001$ and $P < .001$). Vascular resistance decreased also in the placebo group at the same time points compared with baseline ($P = .003$ and $P = .025$) (Table I). The minor differences in hemodynamic data between the groups could be due to chance ($P > .2$) (Table I).

Blood gas and hematocrit measurements are shown in Table I. Both in memantine and placebo animals a decrease in pH was detected at the end of cooling ($P < .001$) and 2 hours after the start of rewarming compared with baseline ($P = .034$ and $P = .006$), but the difference between the groups could be due to chance ($P > .2$). Arterial PCO_2 was higher in both groups at the end of cooling than baseline ($P = .002$ and $P < .001$). In the placebo group, arterial PCO_2 was lower during rewarming at 30°C ($P = .024$) and higher 2 hours and 4 hours after the start of rewarming

Fig 2. EEG burst recovery of 20 pigs receiving either memantine (5 mg/kg) or placebo before a 75-minute period of HCA.

Fig 3. Daily scores indicating behavioral recovery among 20 pigs receiving either memantine (5 mg/kg) or placebo before a 75-minute period of HCA. A score of 8 or 9 indicates essentially complete recovery, lower scores indicate substantial impairment, and 0 indicates coma or death.

($P = .034$ and $P = .001$), but no significant differences between the groups were detected; the difference between the groups could be due to chance ($P > .2$). Hematocrit was lower at the end of cooling and during rewarming at 30°C in both groups ($P < .001$) and in the placebo group 2 hours after the start of rewarming ($P = .011$), but the difference between the groups could be due to chance ($P > .2$).

Metabolic data. The venous lactate level after baseline increased at all recording points among control animals ($P < .001$). The same increase was also seen in

memantine animals ($P = .029$, $P < .001$, $P = .001$, and $P = .064$), respectively. Oxygen extraction and consumption decreased in memantine animals ($P = .058$ and $P = .038$) and in controls ($P < .001$ and $P = .010$) at the end of cooling. The differences in the oxygen extraction and consumption between the groups could be due to chance ($P > .2$) (Table II).

EEG. The pattern of EEG outcome was similar in both groups, and minor differences in median rate of EEG burst recovery could be due to chance ($P > .2$) (Fig 2).

Table I. Hemodynamic data, blood gases, and hematocrit values in 20 pigs undergoing either memantine (5 mg/kg) or placebo administration before a 75-minute period of HCA

Group	N	Baseline (37°C)	End of CPB cooling (20°C)
MAP (mm Hg)			
Memantine	10	95 (88-105)	53 (50-58)‡
Placebo	10	96 (91-103)	56 (49-59)‡
Cardiac output/CPB flow (L/min)			
Memantine	10	2.3 (2.2-2.8)	2.5 (2.3-2.8)
Placebo	10	2.5 (2.5-3.0)	2.6 (2.4-3.0)
Vascular resistance (dyne · s ⁻¹ · cm ⁻⁵)			
Memantine	10	3055 (2273-3331)	1686 (1336-1809)‡
Placebo	10	2890 (2359-3158)	1761 (1538-1821)‡
Arterial pH			
Memantine	10	7.5 (7.4-7.5)	7.2 (7.2-7.3)‡
Placebo	10	7.5 (7.4-7.5)	7.2 (7.2-7.3)‡
Arterial PCO ₂ (mm Hg)			
Memantine	10	6.3 (5.9-6.5)	8.4 (8.1-8.4)*
Placebo	10	6.2 (5.9-6.2)	8.5 (8.3-8.7)‡
Hematocrit (mg/dL)			
Memantine	10	31 (29-33)	18 (16-19)‡
Placebo	10	30 (29-31)	20 (17-21)‡

HCA, Hypothermic circulatory arrest; MAP, mean arterial pressure; CPB, cardiopulmonary bypass; Arterial PCO₂, arterial carbon dioxide tension. Values are shown as medians with interquartile range.

**P* < .05 compared with baseline.

†*P* < .01 compared with baseline.

‡*P* < .001 compared with baseline.

Morbidity and mortality. All animals were in stable condition during the surgical procedures and survived at least to the first postoperative day. Fourteen of the 20 animals survived 7 days after the operation and were electively killed. In the memantine group, 5 of 10 animals survived 7 days compared with 9 of 10 in the placebo group (*P* = .14).

Behavioral outcome. The results of behavioral scoring for both groups are shown in Fig 3. Scores of 8 and 9 indicate an essentially complete neurologic recovery. Animals that died early were given a score of 0 beginning at the time of death. The median behavioral score on day 7 was 3.5 in the memantine group and 7.5 in the placebo group (*P* > .2). Among the surviving animals, medians were 9.0 and 8.0 on day 7 (*P* > .2), respectively.

Histopathologic results. Some representative histopathologic slides from memantine and placebo animals depicting severe and minor ischemic injury are given in Fig 4. In terms of histopathologic findings, there were no statistically significant differences between the groups (Table III). The total histopathologic score was calculated by adding the scores of histopathologic findings in the investigated regions of the brain for each of the animals. The median of the total histopathologic score was 16 in the memantine group and 14 in the placebo group (*P* > .2) (Fig 5).

Microdialysis. The intracerebral glucose concentration decreased during cooling and HCA and returned to baseline with no statistically significant differences between the groups (Fig 6). Lactate concentration increased 30 minutes after HCA in both groups, the highest peak being found 1 hour after the start of rewarming. After this recording point, lactate decreased to the level slightly higher than baseline in both groups (Fig 6). AUC was calculated from the start of HCA to 7 hours after the start of rewarming, but the difference between the groups could be due to chance (*P* > .2). As seen in Fig 6, median glutamate levels exhibited peak values 30 minutes after the start of rewarming and decreased after this recording point to approximately zero. The median glutamate concentration was higher in the memantine group (49 μmol/L) than in the placebo group (23 μmol/L) 30 minutes after the start of rewarming, but the difference could be due to chance (*P* > .2). The difference of glutamate's AUC between the groups could be due to chance (*P* > .2). Glycerol also showed a peak after HCA, but the highest point occurred a bit later than the glutamate peak (Fig 6). The difference of AUC for glycerol between the groups could also be due to chance (*P* > .2).

Because there were no statistically significant differences in AUC values between the groups in microdial-

After start of rewarming		
30°C	2 h	4 h
67 (58-70)‡ 67 (58-69)‡	69 (68-73)† 69 (67-71)‡	74 (70-82)† 67 (67-82)‡
2.6 (2.2-2.8) 2.6 (2.2-2.7)	2.6 (1.8-2.8) 2.2 (2.0-2.3)	2.0 (1.9-2.3)* 2.2 (2.0-2.8)
1901 (1814-2034)‡ 1943 (1742-2360)*	1968 (1915-2514) 2326 (2047-2584)	2772 (2326-3069) 2570 (2093-3122)
7.4 (7.4-7.5) 7.4 (7.3-7.5)	7.4 (7.3-7.4)* 7.4 (7.3-7.4)†	7.4 (7.4-7.4) 7.4 (7.4-7.4)
5.5 (5.3-5.8) 5.5 (5.2-5.8)*	6.5 (5.9-6.9) 6.7 (6.5-6.7)*	6.4 (6.3-6.8) 6.7 (6.5-6.8)†
21 (17-23)‡ 22 (20-23)‡	29 (26-31) 27 (26-28)*	28 (27-31) 29 (27-30)

Table II. Venous lactate levels, oxygen extraction, and oxygen consumption during the experiment in 20 pigs undergoing either memantine (5 mg/kg) or placebo administration before a 75-minute period of HCA

Group	N	Baseline (37°C)q	End of CPB cooling (20°C)	After start of rewarming		
				30°C	2 h	4 h
Venous lactate (mmol/L)						
Memantine	10	1.0 (0.9-1.3)	1.8 (1.5-2.2)*	5.2 (4.7-5.7)‡	4.3 (4.0-6.0)‡	1.7 (1.4-2.5)*
Placebo	10	1.0 (0.9-1.2)	1.6 (1.3-2.3)‡	4.7 (4.5-6.5)‡	4.3 (3.9-5.2)‡	2.0 (1.9-2.2)‡
Oxygen extraction (mmol/L)						
Memantine	10	3.8 (2.5-4.2)	1.8 (1.0-2.2)*	3.0 (2.9-3.4)	4.1 (4.0-4.4)	4.5 (4.0-5.5)
Placebo	10	3.7 (3.3-4.1)	1.9 (1.7-2.2)‡	3.1 (2.7-3.4)	4.6 (4.1-5.1)	4.6 (3.9-5.5)
Oxygen consumption (mL/min)						
Memantine	10	89.6 (73.9-94.1)	39.1 (25.2-57.4)*	80.1 (77.0-89.5)	102.2 (93.4-117.6)	99.4 (82.0-102.3)
Placebo	10	104 (99.9-112.8)	49.9 (42.8-54.8)*	81.4 (71.8-85.8)	100.1 (87.0-107.6)	109.5 (100.3-112.8)

Abbreviations are as in Table I. Values are shown as medians with interquartile range.

* $P < .05$ compared with baseline.

‡ $P < .001$ compared with baseline.

ysis data, the correlation coefficients were calculated using all animals. A positive correlation of AUC was found between glutamate and glycerol ($\tau = 0.474$, $P = .004$), but there was no correlation between glutamate and lactate ($P > .2$). A 1-hour lag was found in correlation between glutamate peak (30 minutes after the start of rewarming) and glycerol (from 1.5 hours after the start of rewarming onward), giving highest correlation with a lag of 1.5 and 2 hours ($\tau = 0.537$, $P = .001$). There was an inverse correlation between the

highest individual glutamate levels and EEG burst recovery 3 hours after the start of rewarming, the highest correlation being ($\tau = -0.377$, $P = .043$) (Fig 7). In addition, there was a positive correlation between the highest individual glutamate value and histopathologic score ($\tau = 0.336$, $P = .045$) (Fig 8).

Discussion

In the present study we have evaluated the efficacy of memantine to protect the brain after experimental

Fig 4. **A**, A 7-day-old local necrosis (infarction) in the frontal cortex in a memantine-treated pig. The arrow depicts a zone of new capillaries at the border of the necrosis. In the lesion (center of figure) neurons are not seen and glial cells and macrophages are accumulated in this area. The finding was classified as a third-degree lesion. **B**, A similar local necrosis as above in the frontal cortex of a control pig. The arrow depicts new capillaries. Gliosis and macrophages are also seen. **C**, A staining from hippocampus indicates that injured neurons have turned red, but surviving neurons have normal color. Edema is seen around the small vessels. The finding was classified as a first-degree lesion. **D**, Hemorrhage in medulla oblongata, classified as a second-degree lesion in our scale. (A, B, C, and D, Hematoxylin and eosin stain, original magnification 60 $\times$.)

hypothermic ischemia in terms of histopathologic and various neurologic end points. The major finding was that pigs receiving memantine before a 75-minute period of HCA at 20°C tended to have an even poorer outcome than control animals given a placebo. The mortality rate tended to be higher in memantine animals and no differences in terms of neurophysiologic,

Fig 5. Total histopathologic scores among 20 pigs receiving either memantine (5 mg/kg) or placebo before a 75-minute period of HCA. The total histopathologic score was calculated by adding the quantitative assessment of histopathologic findings in the investigated regions of the brain for each of the animals.

behavioral, histopathologic, and microdialysis data were seen.

The most frequently used method for brain protection during operations on the aortic arch is hypothermia. It has been demonstrated very recently that even in deep hypothermia (systemic temperature 10°C–15°C) a significant degree of cerebral metabolic rate of oxygen consumption remains, and the length of safe HCA is limited to 40 to 50 minutes.³ The importance of the failure of neurotransmitter transport as a common pathway in the pathogenesis of many neurologic disorders, including ischemic cerebral injury, has been well demonstrated in the past few years. Excitatory amino acids, including glutamate and aspartate, are the primary messengers used for interneuronal communication.⁶ After ischemia, glutamate accumulates in the intercellular space, where it acts as a potent neurotoxic substance. It opens calcium channels, leading to an influx of calcium, which starts the catastrophic intracellular activation of several enzymes.⁶ This knowledge has opened up new avenues of research to enhance current protective methods by the addition of appropriate pharmacologic agents.^{6,22} The most studied antagonists are the glutamate receptor blockers and Ca²⁺ and Na⁺ channel antagonists. Despite promising results in experimental studies with various specific interventional agents, most of these have turned out to be neurotoxic in the clinical setting.²² Only a few potentially neuroprotective pharmacologic agents are clinically used. Lamotrigine is one of them, and we demonstrated recently using the same animal model

Fig 6. Intracerebral concentrations of lactate, glycerol, glutamate, and glucose of 20 pigs, either memantine (5 mg/kg) or placebo, before a 75-minute period of HCA. Values are shown as medians.

that this antiepileptic drug substantially improved brain protection during HCA.¹⁸ Memantine is an uncompetitive NMDA receptor antagonist, and it is well tolerated clinically.¹⁰ Previous studies have shown that memantine carries neuroprotective effects in chronic neurodegenerative diseases and in acute ischemia.^{10,12,14,23} On the contrary, memantine failed to improve neurologic outcome after ischemic and traumatic spinal cord injury.¹⁵

The finding of equal hemodynamic and physiologic data between the groups in the present study indicates that memantine was well tolerated and the setup of this

demanding model was appropriate. In the present study, there were no differences in total histopathologic scores between the study groups, medians being 16 in the memantine group and 14 in the placebo group. In our previous research endeavor studying leukocyte filtration during 75-minute periods of HCA at 20°C, the median histopathologic score in control animals was 15.5, which indicates that the degree of brain injury in control animals is stable. In leukocyte-depleted animals of the same study the median score was 6.5, indicating that the leukocyte filter was a powerful tool to mitigate brain injury after HCA.¹⁹

Fig 7. Correlation between glutamate levels and EEG burst recovery 3 hours after the start of rewarming of 20 pigs undergoing a 75-minute period of HCA.

Fig 8. Correlation between highest individual glutamate value and histopathologic score of 20 pigs undergoing a 75-minute period of HCA.

In the present study the mortality rate tended to be higher in the memantine group (5/10 vs 1/10; $P = .14$), but the explanation for this remains obscure. Glutamate receptor blockers can upregulate the number of glutamate receptors.²⁴ This phenomenon may account for the paradoxically enhanced excitatory amino acid-mediated injury.²⁵ Perhaps this was the reason that the animals

receiving memantine did worse. As seen in Fig 4, early deaths were very likely due to brain damage, because there were no major differences in hemodynamic data and specific comorbid findings were not found during autopsies. Among the animals that survived for 7 days, the behavioral scores were equal. As seen in Fig 2, there were no differences in EEG burst recovery. The EEG data

Table III. Histopathologic scores after the experiment in 20 pigs undergoing either memantine (5 mg/kg) or placebo administration before a 75-minute period of HCA

Protocol	Pig No.	Cortex	Thalamus	Hippocampus	Posterior brain stem	Cerebellum	Total score
Memantine	1	7	1	2	4	4	18
	2	4	4	2	3	3	16
	3	4	2	2	4	4	16
	4	4	1	2	2	1	10
	5	4	2	4	2	2	14
	6	7	1	7	4	4	23
	7	4	2	2	4	4	16
	8	4	2	2	4	4	16
	9	7	2	2	3	3	17
	10	4	4	4	4	3	19
Placebo	1	4	1	2	3	1	11
	2	4	4	4	3	4	19
	3	7	4	4	3	4	22
	4	7	1	2	3	3	16
	5	4	2	2	2	1	10
	6	5	1	2	2	2	12
	7	4	4	2	4	4	18
	8	2	2	2	1	2	9
	9	4	1	2	4	3	14
	10	4	1	2	3	4	14

Scores: 0 = No morphologic damage; 1 = edema or eosinophilic dark neurons or dark/shrunk cerebellar Purkinje's cells; 2 = at least mild hemorrhage; 3 = clearly infarctive foci. *Total score* is the sum of scores in each specific brain area (cortex, thalamus, hippocampus, posterior brain stem, and brain stem). To allow semi-quantitative comparisons between the animals, we calculated a total histologic score by adding all the regional scores.

are in line with the other data and confirm that memantine did not protect the brain in this model.

Our microdialysis facilities provided us with an opportunity to look at brain continuous biochemistry during global hypothermic ischemia. As seen in Fig 6, curves were similar in both groups, demonstrating that memantine had no effect on the intracerebral concentrations of glutamate and glycerol after this dose. There were strong correlations between glutamate levels and glycerol levels, the glycerol peak following a bit later. This is in line with previous findings demonstrating that release of glutamate starts the biochemical cascade and cell damage will follow later.⁶ The finding that glutamate levels were not increased in the brain is not surprising because memantine is an NMDA receptor blocker and should not influence the quantity of glutamate. There was also a correlation between highest individual glutamate levels and histopathologic scores. In addition, as depicted in Fig 6, a negative correlation between glutamate levels and EEG burst recovery was found. These findings support the knowledge that the accumulation of glutamate in the intercellular space is neurotoxic.²⁶

The finding of failed neuroprotection of memantine is surprising, and it can be speculated that the dosage used in the current study was too low. However, side effects

such as ataxia, myorelaxation, and amnesia can be encountered after higher short-term doses such as 20 mg/kg of memantine.¹⁰ This was the first study to test the efficacy of memantine during HCA, and non-neuroprotective efficacy of memantine in this context can be related to hypothermia per se. Because the mortality rate tended to be higher in the memantine group, we believe that this report is of particular importance inasmuch as memantine has been suggested to improve neurologic outcome during operations on the thoracic aorta.¹⁶ On the basis of these data, we conclude that memantine does not have the potential to mitigate cerebral injury in patients undergoing HCA. The present study also supports the previous findings indicating the pivotal role of glutamate in the pathogenesis of ischemic brain injury. In addition, these findings shed more light on the current concept of perioperative detection of neuronal injury, demonstrating that monitoring of intracerebral glutamate levels is an accurate method to predict ischemic insult.

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Commentary

In this study Rimpiläinen and colleagues have tested the hypothesis that an *N*-methyl-D-aspartate (NMDA) receptor blocking agent, memantine, might have a neuroprotective effect during deep hypothermic circulatory arrest (HCA). Their results, based on somewhat small experimental groups, suggest that this agent does not have the hoped for effect. My comments are focused on neurohistologic assessment and grading, which is a major instrument in this study and similar to

procedures used by other groups with experimental deep HCA protocols.

Reliable and refined assessment and grading can only be as good as the initial histologic preparations, or at least this is a limiting factor. Benchmark studies in the 1960s and 1970s established that perfusion fixation followed by a period of *in situ* fixation before any mechanical manipulation of brain and spinal cord is markedly superior in terms of histologic results to the immersion fixation technique used in the present study.¹ Removal and handling before fixation, however gentle, produces artifacts, specifically vacuolation and dark neurons (so-called “blue-neuron” artifact). This happens even in neurosurgical biopsy specimens. A postmortem interval before fixation may also produce varying degrees of autolytic change depending on the precise conditions of time and temperature. In defense of immersion fixation, it should be acknowledged that this is essentially the method, whatever its limitations, routinely used in human autopsies, but this only underscores the handicap under which the clinical neuropathologist must function. Also, some studies, although not the present one, may combine histologic findings with techniques that are incompatible with fixation, and working with “suboptimal” material is unavoidable.

Awareness of some of the histologic vagaries indicates the pitfalls in constructing a histologic scoring system. For one thing, a satisfactory system must not mix effects that are probably artifact and genuine hypoxic-ischemic injury. In the present study it is possible that grade 1 lesions represent artifactual change—vacuolation and dark neurons that result from removal and bisection of unfixed brain and subsequent immersion fixation.

A grading scheme should probably consist of steps that are reliably distinguishable and are accepted or can be demonstrated to result from increasingly severe degrees of the same underlying pathologic process or insult. Presumably one does not arrive at the more severe stages without passing through the milder stages. A clue to the reliability of the grading scheme may be that the milder grades are present at the periphery of more severe lesions. It adds considerable complexity to have an element in this sequence that is an “independent” feature. Systems like this are used, for example, in some brain tumor grading schemes. In those cases, typically each feature is graded as present or absent and a “severity score” is the sum of the features present. Scoring this way raises its own questions: Are all features equally significant?

For hypoxic-ischemic injury several groups have adopted schemes that give progressively higher scores to more severe lesions.^{2,3} The present authors’ system varies somewhat from the usual practice. Their grades are as follows: 1 = edema; 2 = hemorrhages, more severe; and 3 = infarct, most severe. At the same time, Table III, in which given areas have scores greater than 3, seems to imply treating these as “features” rather than “grades.” Some of the underlying assumptions are arguable or at least not clear.

Specifically, an unusual intermediate grade, “2 = hemorrhages,” has been tucked in. Hemorrhages are not typically a part of hypoxic-ischemic injury. They may result from effects related to the bypass procedure, altered coagulation status, and, of course, there can be hemorrhage into infarcted tissue. However, this last should be subsumed under the infarct in terms of grading. It is possible that they are an acute phenomenon, but introducing a mix of acute (hours) and subacute (days) changes complicates interpretation. That an acute change should be present at 1 week is surprising.

My suspicion is that the histologic material supports only 2 grades, “no damage” and “definite hypoxic-ischemic injury,” the authors’ grades 0 and 3, respectively. Fortunately, it appears that restricting evaluation to this more coarse level would not alter the authors’ conclusions. Within the sensitivity of the methods, no significant neuroprotective effect of memantine was discerned. Still, the authors previously found their evaluation sufficiently sensitive to recognize a positive neuroprotective effect of a different agent in a technically similar study.⁴

It should be recognized that there are difficulties facing any investigator using neurohistologic assessment in large animal models. Many parameters are well studied in rodents and in paradigms where the aim is to produce hypoxic-ischemic injury, but these are not so well worked out in the intentionally milder conditions used in experimental surgical models of deep HCA or in dogs and piglets. Using numbers of large animals to work out baseline patterns for variables that may affect damage, such as time after injury and age of animals, is not practical. Manipulations such as placement of indwelling microperfusion catheters, as in the present study, or thermal probes further perturb intracranial pressure, cerebral blood flow, and the final histologic damage. And the experimenter is trapped in the “Catch-22” that damage that is survivable is only discernible by means of standard techniques after several days and will be relatively mild; damage sufficient to be readily identified at short intervals (such as hemorrhages

in this study or acute vacuolation described in other studies⁵) is likely to preclude survival and is hardly a realistic model with which to refine surgical procedures. The best neurohistology achievable can be some help with this.

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