Evaluation of effects of memantine on cerebral ischemia in rats

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ABSTRACT

الأهداف: تقييم آثار عقار ميمانتين على حجم الإحتشاء في نقص تروية المخ والنتائج العصبية بعد إجراء عملية إغلاق مؤقت للشريان وإعادة التغطية لدى الجرذان (MCAO) الدماغي الوسطي.

الطريقة: استخدام 30 جرذاً بالغاً من نوع سبراكيو– داولي في هذه الدراسة. تم إجراء نقص التروية في الدماغ بواسطة طريقة اللهيب داخل اللمعة باستخدام غرز النايلون 0–4. تمت إعادة التغطية بعد ساعتين من إجراء عملية الإغلاق للشريان الدماغي الوسطي. تم تقسيم الجرذان بشكل عشوائي الى مجموعتين (مجموعة التحكم) و(المجموعة التي تلقت عقار ميمانتين). تلقت المجموعة المحلول الملحي 0.9% (0.5ml/kg) وعقار ميمانتين (30mg/kg) عن طريق الأنبوب المعدي. تم الحصول على ثلاث شرائح تاجية بسماكة مليمترين من المخ والمخيخ وجذع الدماغ وتم صبغها بمحلول 2% من كلوريد تريفينيلتيترازوليوم. تم وضع الصفحات الشفافة على كل جزء وتم قياس مناطق الدماغ والاحتشاءات.

النتائج: تم الحصول على 45 شريحة من كل مجموعة (المجموع 90). نسبة منطقة نقص التروية بالدم في المخ والمخيخ وجذع الدماغ في مجموعة ميمانتين أعلى من مجموعة التحكم ($p<0.0001$) بالإضافة إلى ذلك فقد حددنا نقاط التحسن العصبية عند 24 و72 ساعة في الجرذان التي تلقت عقار ميمانتين. ظهر على مجموعة عقار ميمانتين شفاء أفضل وذو ($p<0.0001$) دلالة إحصائية من مجموعة التحكم.

خاتمة: إن عقار ميمانتين قد يخفض من منطقة نقص تروية الدم للدماغ لدى الجرذان التي أجريت عليها التجربة ويبدو أنه قد يتم الاستفادة من عقار ميمانتين في نقص تروية الدماغ.

Objective: To evaluate the effects of memantine on infarct size in cerebral ischemia and on neurological outcome after temporary middle cerebral artery occlusion (MCAO) and reperfusion in rats.

Methods: In this study, performed between 2002-2004 in Dicle University School of Medicine, Diyarbakir, Turkey, 30 adult Sprague-Dawley rats were used. Cerebral ischemia was constituted by the intraluminal filament method with a 4-0 nylon suture. Reperfusion was started after 2 hours of MCAO. The rats were randomly divided into 2 groups as control and memantine. Saline 0.9% (0.5 ml/kg) and memantine (30 mg/kg) were administered via nasogastric intubations. Three coronal slices of 2 mm thickness were obtained from cerebrum, cerebellum, and brain stem, and were stained with a 2% solution of triphenyltetrazolium chloride. Transparent sheets were placed over each section and the areas of the brain and infarct were measured.

Results: Forty-five slices from each group (total 90) were obtained. Percent of ischemic area (%) in cerebrum, cerebellum, and brain stem level in memantine was lower than those of the control group ($p<0.0001$). In addition, we determined an improvement in neurological score at 24th and 72nd hours in the rats that have been given memantine. The memantine group showed significantly better recovery than the control group ($p<0.0001$).

Conclusions: We concluded that memantine may decrease ischemic area in experimental cerebral ischemia in rats and it seems that memantine may be beneficial in cerebral ischemia.

Neurosciences 2008; Vol. 13 (2): 113-116

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Received 25th June 2007. Accepted 23rd October 2007.

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The so-called "stroke," meaning the sudden occlusion of one or more brain vessels resulting in an insufficient perfusion of the associated brain area, presents, together with cardiovascular diseases, and cancer.[1] Treatment of stroke is still limited to the optimal supportive measures. Any therapeutic approach in stroke promising to

favorably influence the course of this disease, therefore, merits to be followed up emphatically. This has been carried out in recent years with several substances hoping to positively influence the course of recovery after a stroke due to their neuroprotective properties. Pharmacotherapy of ischemic stroke is a promising treatment option although several neuroprotectants with various mechanisms of action failed to improve neurological symptoms of patients.[1,2] There is increasing evidence that excitatory amino acids (EAA), such as glutamate and aspartate, play an important role as mediators of brain injury during cerebral ischemia.[3] Excessive release of the EAA mediated by N-methyl-d-aspartate receptors (NMDA-R) may trigger severe functional deficits and neuronal necrosis after cerebral ischemia.[4,5] This situation has led investigators to study various NMDA-R antagonists to prevent the neurotoxic effects of EAA. Among them, memantine (1-amino-3, 5-di-methylaminoadamantane hydrochloride) seems to be an uncompetitive N-methyl-d-aspartate (NMDA) open-channel blocker. Memantine is an antagonist of the NMDA subtype of the glutamate receptor and has been clinically used for the treatment of various cerebral disorders such as Parkinson's disease, spasticity, and chronic brain syndrome for many years.[6-8] Previously published reports demonstrating beneficial effects of memantine were limited to focal permanent or global cerebral ischemia.[7,9] However, it was also reported to have a protective effect against ischemic injury, which results from excessive stimulation of NMDA-R after cerebral ischemia, and exhibits neuroprotective activity, which blocks Ca^{++} influx through the NMDA-operated Ca^{++} channel.[4,7] In this study, we aimed to compare the effectiveness of memantine on percent ischemic area in experimental ischemic brain injury and on neurological outcome after temporary middle cerebral artery occlusion (MCAO) and reperfusion in rats.

Methods. ***Experimental protocol and groups.*** All the experimental procedures were performed in accordance with the guidelines of the Experimental Research Institute of Dicle University (DUSAM), Diyarbakir, Turkey after approval of Dicle University Ethic Committee (#02-224) between 2002 and 2004. Thirty adult male Sprague-Dawley rats, weighing 300-350 gr, obtained from the DUSAM were used in this study. The rats were kept in a room with an interior temperature of 21-23˚C. The room was ventilated continuously by fans and illuminated 12 hours in a day. The rats were randomly divided into 2 groups; control (C) (n:15), and memantine group (MM) (n:15), (Memantine, Ebixa® solution, Lundbeck, Denmark). The rats were fed with ad-libitum standard pellet chow and daily fresh tap water during the experimental procedure. The groups were anesthetized with ketamine hydrochloride (90 mg/kg) intraperitoneally, and cerebral ischemia was constituted by using the intraluminal filament method.[10,11] The method consists of introducing a 4-0 nylon intraluminal suture into the cervical internal carotid artery (ICA) and advancing it intracranially to block blood flow into the MCA; collateral blood flow was reduced by interrupting all branches of the external carotid artery (ECA) and all extracranial branches of the ICA. The intraluminal filament was withdrawn after 2 hours of MCAO, and reperfusion started again and passed to therapeutic stages for all the groups. Saline 0.9% (0.5 ml/kg) to the C group, and memantine (30 mg/kg) were administered to the MM group via nasogastric intubations. All animals were sacrificed under pentobarbital by decapitating 72 hours after MCAO. Afterwards the whole brains were immediately removed, briefly cooled in ice-cold saline, and 3 coronal slices in 2 mm thickness were obtained from the cerebrum, cerebellum, and brain stem. The sections were stained with a 2% solution of triphenyltetrazolium chloride (TTC) in a warm water bath at 37°C for 30 minutes. The TTC is a marker for the ischemic areas for up to 3 days after ischemia.[12] The stained sections were immersed in 10% phosphate-buffered formalin, and the infarct size examined after one week. Transparent sheets were placed over each section, and the areas of the brain and of the infarct (as outlined by TTC staining) were traced on the overlay. The tracings were digitalized, and total pixel counts of the ischemic area and the whole brain in all 3 surfaces were determined. The sums of the 3 surfaces were calculated, and the ischemic area was expressed as a percentage of the whole brain area.

Neurological evaluation. The neurological scores were determined at the 24th and 72nd hours after reperfusion by using the modification described by Bederson et al.[12] In neurological evaluation, the worst score was determined as 12 and the best score as 0.

Statistical analysis. EpiInfo 2000 (CDC-Atlanta-USA) was used for statistical analysis. Percent of ischemic area of the 3 groups was presented as mean ± standard deviation (SD). The ANOVA test was used to compare the measurements of the 3 groups. Post hoc Tukey analyses were used. Values of $p<0.05$ were accepted as statistically significant.

Results. Infarction volumes were very different after MCAO. Percent of ischemic area (%) in the cerebral level of the MM group was lower than control group (15.9±2.23% versus 19.6±2.67%, $p<0.0001$). Percent of ischemic area (%) in the cerebellar level of the MM group was also lower than control rats (17.6±2.23% versus 24.2±4.75%, $p<0.0001$). Again, percent of ischemic area (%) in the brain stem level of MM group was also lower than control rats (13.5±2.73% versus

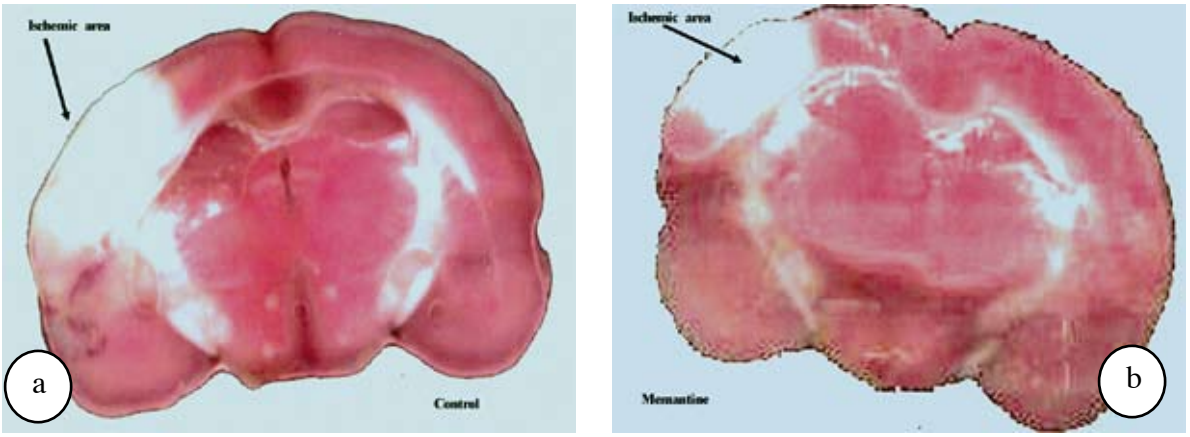

Figure 1 - Infarction samples in brains of **a)** control group and **b)** memantine group. The viable brain tissue is red in the figure.

Table 1 - Neurologic scores[12] of control and memantine (MM) groups.

Rat number	8th hour		72nd hour	
	Control group	MM group	Control group	MM group
1	9	9	9	7
2	8	7	8	6
3	9	9	9	8
4	7	7	8	6
5	7	6	7	5
6	6	6	7	5
7	8	7	8	5
8	9	9	9	7
9	8	8	8	6
10	7	7	8	7
11	9	8	9	6
12	10	9	9	7
13	8	8	8	6
14	9	7	8	5
15	9	8	9	7
Means of groups	8	7.6*	8	5.8*

*p<0.0001 MM group versus control

18.1±2.29%, p<0.0001). **Figures 1a & 1b** demonstrate the sample of whole brain and ischemic area. After 2 hours of MCAO, we determined an improvement in neurological score at the 24th and 72nd hours in the rats that were given memantine (**Table 1**). The memantine group showed significantly better recovery than the control group (p<0.0001).

Discussion. In this study, we have shown that memantine has positive effects on experimental cerebral ischemia in rats, as the mean infarct size of rats treated with memantine was significantly lower than that of the control group. The effects of memantine on cerebral ischemia are still unclear. Some studies with EAA receptor antagonists (particularly with the NMDA-R) in different animal models of cerebral ischemia support our findings.[3,4,7,13] The NMDA-R antagonists may prevent the increased neuronal damage caused by ischemia-reperfusion injury by decreasing such excitotoxic damage. Memantine has been used clinically for the treatment of ischemia in different animal models.[7,12] However, using memantine and other NMDA antagonists in models of ischemia have some contraindication debate. The effects of memantine on cerebral ischemia are probably due to its binding to the site of the NMDA-R, which results in blockage of excess Ca^{++} entry into the cells.[7,8,13,14] In addition, memantine binds to Mg^{2+} in the NMDA-R channel also. However, it has a somewhat less pronounced voltage-dependency than Mg^{2+} and is therefore, more effective in blocking tonic pathological activation of the NMDA-R at moderately depolarized membrane potentials. On the other hand, following strong synaptic activation, memantine, like Mg^{2+}, leaves the NMDA-R channel due to its voltage-dependency and fast unblocking kinetics.[15] Memantine may also provide additional protective effects against ischemic brain damage by induction of hypothermia.[7] It was claimed that memantine-induced hypothermia could further exceed cerebroprotection by the combination therapy demonstrated here in normothermic animals.[16] The other possible mechanisms of memantine have been reported to induce brain-derived neurotrophic factor and its receptor in brain tissue.[17] In another study, it

was claimed that the infarct size was further reduced by a combination of memantine with clenbuterol therapy as compared with the effects of the respective neuroprotectants alone. This combination may provide a synergistic neuroprotection and considerable extension of the individual therapeutic windows at safe doses.[16] It was pointed out that energy depletion is among the frequent initiating conditions leading to excitotoxicity, and mitochondrial dysfunction is believed to be one of the most generalized causes favoring the development of neurodegenerative diseases.[18] Memantine was evaluated in animals against primary insults dependent on mitochondrial impairment and energy depletion and provided protection from inhibition of mitochondrial function.[19,20] There is a close relationship between energy deficiency and excitotoxicity in the stroke/ischemia model.[21]

Although the currently approved agents for treating ischemic stroke are thrombolytics, such as tissue plasminogen activator (tPA), thrombolytic therapy improves long-term functional recovery of patients if treatment is initiated not more than 30 hours after the start of symptoms. Combined analysis demonstrated that patients treated within 90 minutes of stroke onset had an odds ratio of 2.83 (95% confidence interval [CI], 1.77-4.53) of achieving a modified Rankin outcome of 0 to 1, whereas patients treated from 91 to 180 minutes had an odds ratio of 1.53 (95% CI, 1.11-2.11).[2] Whereas, we determined that using of memantine bring in therapeutic windows, our results showed that reperfusion extended the time window with memantine treatment by 2 hours, and this treatment may provide important therapeutic advantage. The main limitation of our study is the absence of histopathological evaluation, and we could not evaluate probable mechanisms of memantine in protecting the brain due to technical difficulties. Therefore, these results need further investigation for determining the exact mechanism of memantine.

In conclusion, memantine can produce a neuroprotective action if treatment is initiated up to 2 hours after the start of cerebral ischemia in rats. We concluded that: 1. Memantine may decrease the ischemic area in experimental cerebral ischemia. 2. Memantine may extend the time window for thrombolytic therapy in cerebral ischemia.

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