

ARTICLE ORIGINAL/ORIGINAL ARTICLE
**EFFECTS OF MAGNESIUM SULFATE
IN KAINIC ACID-INDUCED STATUS EPILEPTICUS**

Mohamad A. MIKATI, Hiba INJIBAR, Rana M. KURDI, Jimmy EL HOKAYEM, Suha ABOU RIALY
Lina LTEIF, Mona ABDUL JAWAD, Elie FRANCIS, George GEHA, Firas FARHAT

Mikati MA, Injibar H, Kurdi RM, El Hokayem J, Abou Rialy S, Lteif L, Abdul Jawad M, Francis E, Geha G, Fahrat F. Effects of magnesium sulfate in kainic acid-induced status epilepticus. *J Med Liban* 2006 ; 54 (4) : 200-204.

Mikati MA, Injibar H, Kurdi RM, El Hokayem J, Abou Rialy S, Lteif L, Abdul Jawad M, Francis E, Geha G, Fahrat F. Effets du sulfate de magnésium dans les crises d'épilepsie induites par l'acide kainique. *J Med Liban* 2006 ; 54 (4) : 200-204.

ABSTRACT : Because magnesium has antiseizure effects in some animal models of epilepsy, and possible neuroprotective effects in some models of neuronal injury, we aimed to investigate its effects in the kainic acid (KA) model of status epilepticus (SE) in prepubescent rats. This age was chosen because it is a common age for onset of epilepsy and of SE in humans.

Three groups of P35 rats were studied : Group I (MgKA) received magnesium sulfate $MgSO_4$ (270 mg/kg then 27 mg/kg every 20 minutes for 5 hours) and 10 mg/kg KA. Group II (KA) received saline instead of $MgSO_4$ and 10 mg/kg KA. Group III (control) received saline injections only. The dose we used has been shown previously to have anticonvulsant activity in another seizure model.

Rats were recorded for their acute behavioral seizures directly after KA, and underwent the handling and Morris Water Maze (MWM) tests on P96-97 and P102-106 respectively. The MgKA and the KA groups did not differ in their acute seizures and both showed similar histologic lesions in CA3/CA4 and CA1 hippocampal subfields, and were more aggressive on the handling test than control rats. The MgKA group took more time to reach the platform in MWM than controls, while the KA group scores were intermediate between the two groups.

Using the dose of 540 mg/kg $MgSO_4$ and 54 mg/kg every 20 min showed the similar result of lack of protection against impairment in long-term memory.

We conclude that (1) Magnesium did not manifest acute behavioral antiseizure effects in the KA P35 model of SE. (2) Magnesium did not prevent the tested long-term behavioral and histological consequences of SE in this model.

INTRODUCTION

Magnesium has neuroprotective effects in a number of models of neuronal injury including neonatal hypoxia-ischemia [1-2] and head trauma [3]. Magnesium blocks open NMDA channels. This may be the basis for its central neuroprotective and possibly anticonvulsant effects [4]. In some seizure models magnesium is ineffective in altering seizure activity [5-6], while in others it has an anticonvulsant effect [7-9].

Status epilepticus (SE) is defined as more than

RÉSUMÉ : Des études expérimentales animales sur le syndrome épileptique (SE) ont révélé que le magnésium (Mg) a des propriétés anticonvulsives dans certains cas d'épilepsie et de possibles effets neuroprotecteurs dans certaines atteintes neurales. Nous avons étudié les effets du Mg sur les crises épileptiques induites par l'acide kainique (AK) chez des rats prépubères, âge choisi car il correspond à celui où les débuts des manifestations de SE sont les plus fréquents chez l'homme. Trois groupes de rats P35 ont été étudiés : le groupe I (MgAK) a reçu du SO_4Mg (270 mg/kg puis 27 mg/kg chaque 20 min durant 5 heures) et 10 mg/kg de AK ; le groupe II (AK) des injections salines et 10 mg/kg de AK ; le groupe III (contrôle) des injections salines. La dose utilisée s'était révélée anticonvulsive dans un autre modèle d'étude sur l'épilepsie. Les rats ont été filmés durant leurs manifestations convulsives aiguës immédiatement après l'injection de AK, et soumis ensuite à des tests de maniement et au Morris Water Maze (MWM) respectivement pratiqués sur les rats P96-P97 et P102-103. Il n'y a pas eu de différences entre les groupes MgAK et AK, et les lésions histologiques étaient identiques aux niveaux CA3/CA4 et CA1 de l'hippocampe, mais plus prononcées dans le groupe de tests de maniement par rapport au groupe contrôle. Le groupe I a mis plus de temps à parvenir à la plateforme du test MWM par rapport au groupe de contrôle, les scores du groupe II étaient intermédiaires. Doubler la dose à 540 mg/kg de SO_4Mg et 54 mg/kg chaque 20 min, n'a pas amélioré les résultats en ce qui concerne la perte de mémoire à long terme. Nous pouvons donc conclure que (1) Le Mg n'a pas manifesté d'effets anticonvulsifs comportementaux aigus dans le modèle étudiant SE chez les rats P35 injectés avec du AK. (2) Le Mg n'a pas empêché les conséquences comportementales et histologiques à long terme de SE observées dans ce modèle.

30 minutes of either continuous seizure activity or intermittent seizures without full recovery of consciousness between seizures. SE, including kainic acid (KA)-induced SE, is associated with long-term cerebral injury [10-11]. Whether magnesium treatment can attenuate acute seizure activity and the long-term behavioral sequelae of KA-induced SE is not known.

To our knowledge, there is only a single study by Wolf et al. that reported that magnesium sulfate ($MgSO_4$) administered subcutaneously protected against histologic damage induced by intracerebroventricular injection

Adult and Pediatric Epilepsy Program and Department of Pediatrics, American University of Beirut, Beirut, Lebanon.

Corresponding author : *Mohamad A. Mikati, MD. Epilepsy Program and Department of Pediatrics. American University of Beirut, POBox 11-0236 Riad El-Solh. Beirut 1107 2020. Lebanon.*

Phone : +961 1 374374 ext. 5500

Fax : +961 1 744464

E-mail : mamikati@aub.edu.lb

ABBREVIATIONS

| | |
|-------------------|------------------------------------|
| SE | Status epilepticus |
| KA | Kainic acid |
| MgSO ₄ | Magnesium sulfate |
| NMDA | N-methyl-D-aspartate |
| FLC | Forelimb clonus |
| MWM | Morris Water Maze |
| ANOVA | Analysis of variance |
| LSD | Least significant differences |
| BSID | Bayley scale of infant development |

of KA in adult rats [9]. The authors did not report on acute seizure activity after KA and magnesium injections, but reported that rats that received MgSO₄ were "less aggressive."

The goals of this study were, thus, to investigate the potential antiseizure and long-term effects of magnesium in the KA model of SE in prepubescent P35 rats. P35 was chosen because this age corresponds to the prepubescent period that is frequently the time of onset of temporal lobe epilepsy in humans [12], and because early age of seizure onset is one of the reported factors for increased risk of cognitive impairment in childhood epilepsy [10].

MATERIALS AND METHODS

Three groups of P35 Sprague-Dawley rats were investigated :

• **Group I (MgKA, n = 15)** : At time 0, rats were injected with 270 mg/kg MgSO₄, intraperitoneally (ip). Then, they were injected with 27 mg/kg MgSO₄, ip every 20 minutes for 5 hours.

At 60 minutes, they were injected with 10 mg/kg KA, ip.

The dose and timing of MgSO₄ administration were based on the findings of Cotton et al., in 1993, who found anticonvulsant activity of similarly injected ip MgSO₄ on seizures induced by intracranially injected NMDA in rats [7].

• **Group II (KA, n = 20)** : At time 0, rats were injected with saline, ip. Then they were injected with saline, ip every 20 minutes for 5 hours.

At 60 minutes, they were injected with 10 mg/kg KA, ip.

• **Group III (control, n = 8)** : At time 0, rats were injected with saline, ip. Then, they were injected with saline, ip every 20 minutes for 5 hours.

At 60 minutes, they were injected with saline, ip.

Rats were videotaped for 6 hours after receiving KA injections in order to document their behavioral seizures. The handling test was performed on P96-97. The Morris Water Maze test (MWM) was performed on P102-106.

In addition, three parallel similarly treated groups (8 rats each) were subjected to the same treatments described above and were sacrificed at P60 for performance of histological studies.

The Protocol was approved by the Institutional Review Board.

EXPERIMENTAL PROCEDURES

Determination of seizure activity by videotape techniques

In order to determine and quantify seizure activity after KA administration, P35 rats were observed directly and videotaped. Video monitoring consisted of video recordings for 6 hours after KA administration using a wide-angle lens, which allows for monitoring of 6-12 rats simultaneously. Seizure number and duration were assessed by a blinded observer using fast forward. Only seizures with FLC were counted.

Handling test

Emotional responses were systematically studied by observation of the reaction of the rat to non-stressful handling and to stressful handling. A total score was generated as previously described [13].

Water maze test

The test was performed and the latencies to escape onto the platform were recorded as previously described [14]. The latencies to escape onto the platform for each of the three groups on each of the four test days were calculated and compared.

Histology

Animals were sacrificed with a lethal dose of sodium pentobarbital (50 mg/kg). Brains were removed and then cryoembedded. The embedded brains were then frozen sectioned in 7 µm sections at the level of mid-hippocampus and mounted on vectabond-coated slides. Sections were then stained with hematoxylin and eosin. Slides were analyzed for lesions in the hippocampus (CA1 and CA3/CA4 regions), and the total histological damage score was calculated by adding the scores of the above regions for each hippocampus. The severity of the observed lesion was established on a scale of zero to four based on our previously published methodology [13].

Statistical analysis

The duration of FLC in the KA and MgKA groups was compared using the t-test. The two-day handling test scores and the histological lesion severity scores were compared using the Kruskal-Wallis test. The time to reach the platform on each of the consecutive 24 trials in the MWM was compared using the ANOVA of multiple groups with repeated measures with post-hoc analysis with the LSD test.

RESULTS

Acute seizures

All rats that received KA or KA and magnesium sulfate developed SE. There was no difference between MgKA group (mean ± standard error : 548 ± 182 seconds) and KA group (284 ± 43 seconds) in the duration of FLC (p = 0.210).

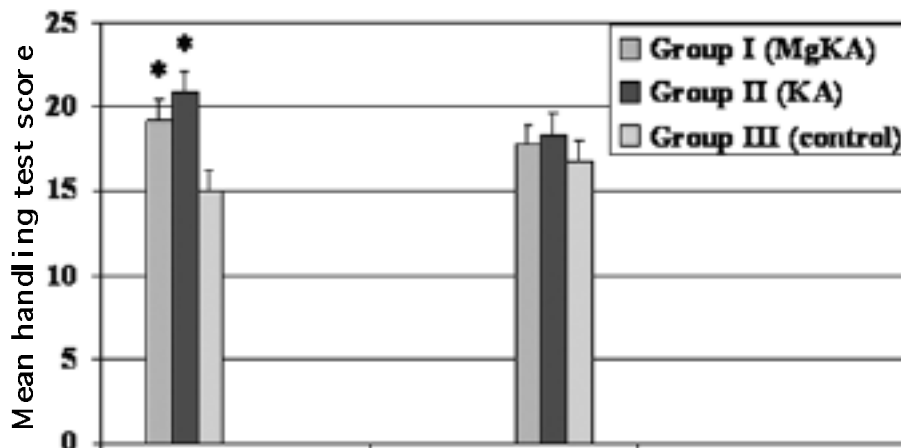


Figure 1
HANDLING TEST
Mean handling scores for the two days of the handling test. Groups I (MgKA) and II (KA) were significantly different from group III (control) when day 1 scores are compared. (See text for further details.)
* $p < 0.05$ as compared to controls.

Handling test (Figure 1)

On comparing the scores of day 1, there was a significant difference among all groups ($p = 0.0105$). Using paired analysis (day 1), the KA and MgKA groups were more aggressive than the control group ($p = 0.0049$, $p = 0.038$ respectively). The KA and MgKA groups were not different ($p = 0.19$). Scores for day 2 were not different ($p = 0.49$).

Mean \pm standard error for MgKA, KA and control groups were the following • day 1 : 19.18 ± 1.26 , 20.90 ± 1.16 , 15.00 ± 1.21 • day 2 : 17.82 ± 1.02 , 18.30 ± 1.29 , 16.75 ± 1.25 respectively.

Morris Water Maze (Figure 2)

There was a significant difference among the groups ($F = 3.41$, $p = 0.0049$). On paired comparisons, the MgKA group was significantly slower in reaching the platform than the control group, ($p = 0.018$). The KA group was not different from controls ($p = 0.196$), or from the MgKA group ($p = 0.228$).

Mean \pm standard error for MgKA, KA and control groups were • day 1 : 468 ± 58 , 324 ± 66 , 202 ± 41 • day 2 : 209 ± 46 , 147 ± 35 , 84 ± 16 • day 3 : 119 ± 34 , 94 ± 17 , 71 ± 18 and • day 4 : 130 ± 39 , 116 ± 40 , 76 ± 22 , respectively.

The dose of 540 mg/kg $MgSO_4$ and 54 mg/kg every 20 min showed the similar result of lack of protection against impairment in long-term memory. As compared to the control group ($p = 0.006$) and as compared to KA group ($p = 0.18$). The mean \pm standard error for this group ($n = 5$ rats) were • day 1 : 387 ± 28.15 • day 2 : 267 ± 20.6 • day 3 : 297 ± 48.61 and • day 4 : 306 ± 81.74 .

Histology (Figure 3)

Histologic lesions in the hippocampus (observed in CA1 and CA3/CA4 subfields) were documented in both the KA and MgKA groups. Magnesium supplementation did not prevent or ameliorate those lesions ($H = 0.03$; $p = 0.855$ for comparison of the two groups, both groups were different from the control group $p < 0.05$).

Mean \pm standard error for MgKA, KA and control groups were the following • CA3 : 1.55 ± 0.34 ,

1.33 ± 0.14 , 0.00 ± 0.00 • CA1 : 2.77 ± 0.27 , 2.91 ± 0.29 , 0.00 ± 0.00 . Total combined scores for the CA3 and the CA1 subfields : 4.33 ± 0.47 , 4.25 ± 0.37 , 0.00 ± 0.00 respectively.

DISCUSSION

Magnesium is a drug of choice for the treatment of eclampsia associated seizures. Recently, a number of studies have suggested that the use of magnesium in eclampsia may be associated with neuroprotective effects in the fetus and newborn [15-16]. However, these findings were not reproduced by other studies [17]. Thus, it is not known whether the above reported beneficial effects of magnesium were due to definite neuroprotective effects, or due to other unrecognized confounding variables.

SE often results in long term deleterious effects in prepubescent and adult rats [10-11]. Neuronal injury after SE is probably mediated by activation of NMDA glutamate receptors that causes necrosis as well as programmed cell death [18].

Magnesium has had variable anticonvulsant effects on acute seizures. It was ineffective in altering seizure discharges in pentylenetetrazol-induced SE in rodents [6], or EEG discharges in myoclonic SE in a 22-year-old man [5]. Animal data indicating anticonvulsant effects of magnesium have come from essentially one laboratory and have been published in the obstetrical literature [8].

We found that magnesium did not protect against KA-induced aggressivity and memory deficits. This finding differs, somewhat, from that of Wolf et al., in 1991, who found in adult rats histological neuroprotection with a high dose (600 mg/kg) of $MgSO_4$ given simultaneously with intracerebroventricular injection of 0.1% KA [9]. The authors did not report on the acute seizures, but did report that they observed increased aggressiveness in all groups except in those that received the 600 mg/kg dose. Lower doses had intermediate (300 mg/kg) or no effects (150 mg/kg) on the histology. The differences with our study may be related to the following factors : (1) Different dose and time of administration of $MgSO_4$. The dose we used (total of 648 mg/kg over 5 hours)

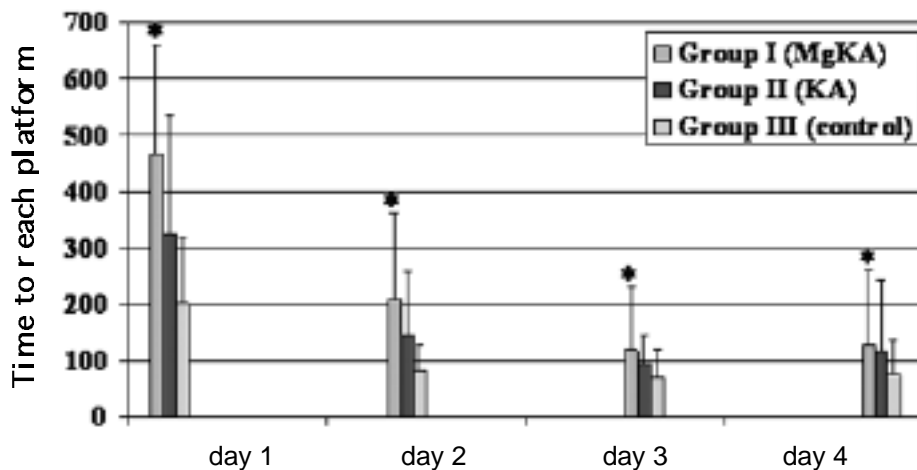


Figure 2
MWM TEST

Mean time to reach the platform in the four days of the water maze test. Group I (MgKA) was significantly different from group III (control). (See text for further details).
* $p < 0.05$ as compared to controls.

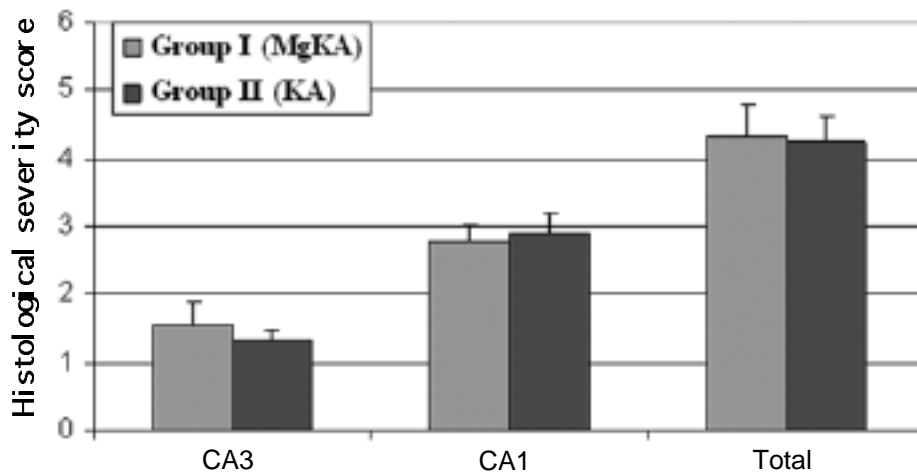


Figure 3
HISTOLOGY

Mean scores given for assessment of hippocampal neuronal damage in the CA3, CA1 and both subfields combined.

has been reported to be pharmacologically active in prior studies in other models of seizures and neuronal injury [7-8]. (2) Different ages at the time of testing. (3) Different outcome measures (histology versus behavioral testing). (4) The number of rats we used may have not been large enough to detect differences that may have existed between the groups given the standard deviations of our data. However, it is noteworthy that the one difference we noted (in the water maze test) was for a deleterious and not beneficial effect of magnesium.

One limitation of our study is that we only recorded behavioral seizures and not EEG. Despite this limitation, our data did not show a beneficial effect of magnesium. This is consistent with the findings of other studies in which postasphyxial treatment with magnesium worsened brain damage [1, 19]. The reasons for this warrant further investigation, but may be related to several factors: (1) Magnesium may not be crossing the blood brain barrier in this model. (2) Magnesium blockade of NMDA channels is voltage-dependent and is probably not operating under conditions of sustained depolarization such as SE. (3) Magnesium may be causing vasodilatation in areas other than those involved in seizure activity which could potentially result in relative

ischemia to areas involved in seizure activity or even hypotension [1, 19]. Further studies of EEG and other behavioral consequences of Mg therapy are needed to further understand the effects of Mg on seizures, behavior and memory.

ACKNOWLEDGMENT

The work of this study was supported by a grant from Parke-Davis DCR 114170-25305.

REFERENCES

1. Sameshima H, Ota A, Ikenoue T. Pretreatment with magnesium sulfate protects against hypoxic-ischemic brain injury but postasphyxial treatment worsens brain damage in seven-day-old rats. *Am J Obstet Gynecol* 1999 ; 180 : 725-30.
2. Sameshima H, Ikenoue T. Long-term magnesium sulfate treatment as protection against hypoxic-ischemic brain injury in seven-day-old rats. *Am J Obstet Gynecol* 2001 ; 184 : 185-90.
3. Feldman Z, Gurevitch B, Artru AA et al. Effect of magnesium given 1 hour after head trauma on brain edema and neurological outcome. *J Neurosurg* 1996 ; 85 : 131-7.

4. Gathwala G. Neuronal protection with magnesium. Indian J Pediatr 2001 ; 68 : 417-19.
5. Fisher RS, Kaplan PW, Krumholz A, Lesser RP, Rosen SA, Wolff MR. Failure of high-dose intravenous magnesium sulfate to control myoclonic status epilepticus. Clin Neuropharmacol 1988 ; 11 : 537-44.
6. Link MJ, Anderson RE, Meyer FB. Effects of magnesium sulfate on pentylentetrazol-induced status epilepticus. Epilepsia 1991 ; 32 : 543-9.
7. Cotton DB, Hallak M, Janusz C, Irtenkauf SM, Berman RF. Central anticonvulsant effects of magnesium sulfate on N-methyl-D-aspartate-induced seizures. Am J Obstet Gynecol 1993 ; 168 : 974-8.
8. Hallak M, Irtenkauf SM, Cotton DB. Effect of magnesium sulfate on excitatory amino acid receptors in the rat brain. II. Kainate and -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Am J Obstet Gynecol 1996 ; 175 : 582-7.
9. Wolf G, Fischer S, Hass P, Abicht K, Keilhoff G. Magnesium sulphate subcutaneously injected protects against kainate-induced convulsions and neurodegeneration : in vivo study on the rat hippocampus. Neuroscience 1991 ; 43 : 31-4.
10. Holmes GL. The long-term effects of seizures on the developing brain : clinical and laboratory issues. Brain Dev 1991 ; 13 : 393-409.
11. Mikati MA, Tarif S, Lteif L, Jawad MA. Time sequence and types of memory deficits after experimental status epilepticus. Epilepsy Res 2001 ; 43 : 97-101.
12. Weiser HG. Temporal lobe epilepsies. In : Vinken PJ, Bruyn GW, Meinardi H, eds. Handbook of Clinical Neurology, Ch 3, Vol 73 (29), The epilepsies, Part II. Amsterdam : Elsevier Science, 2000 : 53-96.
13. Mikati MA, Holmes GL, Chronopoulos A et al. Phenobarbital modifies seizure-related brain injury in the developing brain. Ann Neurol 1994 ; 36 : 425-33.
14. Morris RG. Synaptic plasticity and learning : selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. J Neurosci 1989 ; 9 : 3040-57.
15. Schendel DE, Berg CJ, Yeargin-Allsopp M, Boyle CA, Decoufle P. Prenatal magnesium sulfate exposure and the risk for cerebral palsy or mental retardation among very low-birth-weight children aged 3 to 5 years. JAMA 1996 ; 276 : 1805-10.
16. Mittendorf R, Pryde PG. An overview of the possible relationship between antenatal pharmacologic magnesium and cerebral palsy. J Perinat Med 2000 ; 28 : 286-93.
17. Mittendorf R, Dambrosia J, Dammann O et al. Association between maternal serum ionized magnesium levels at delivery and neonatal intraventricular hemorrhage. J Pediatr 2002 ; 140 : 540-6.
18. Mikati MA, Abi-Habib RJ, Sabban ME et al. Hippocampal programmed cell death after status epilepticus : evidence for NMDA receptor and ceramide mediated mechanisms. Epilepsia 2003 Mar ; 44 (3) : 282-91.
19. Blair JL, Warner DS, Todd MM. Effects of elevated plasma magnesium versus calcium on cerebral ischemic injury in rats. Stroke 1989 ; 20 : 507-12.

تأثير سلفات المنيبيزيوم وحمض كايينك على تحريض نوبات الصرع

موجز : بما ان للمنيبيزيوم تأثيراً مضاداً لنوبات بعض حالات الصرع عند الحيوان ولإمكانية الحماية العصبية لبعض حالات الاصابة العصبونية فالتنا نحاول ان نبحت تأثير المنيبيزيوم على بعض اشكال نوب الصرع الناتجة عن حامض الكايينك عند الجرذان قبل حالة البلوغ وقد اخترنا هذه السن لانها هي كثيراً ما تكون عمر البدء للصرع وحالات الصرع عند الانسان . درست ثلاث مجموعات من الجرذان من عمر ٣٥ يوماً.

المجموعة الاولى : اعطيت منيبيزيوم وحمض الكايينك بمقدار سلفات المنيبيزيوم ٢٧٠ ملغ / كغ ثم ٢٧ ملغ / كغ كل ٢٠ دقيقة لمدة خمس ساعات و ١٠ ملغ / كغ من حمض الكايينك، المجموعة الثانية : اعطيت المصل الملحي عوضاً عن سلفات المنيبيزيوم و ١٠ ملغ / كغ حمض الكايينك المجموعة الثالثة للمقارنة اعطيت فقط المصل الملحي.

سجل التأثير على الجرذان في الثلاث مجموعات للتصرفات الصرعية راساً بعد اعطاء حمض الكايينك وأجري تدبير اختبارات « موريس ووترماز » في اعمار ٩٦-٩٧ و ١٠٢-١٠٦ يوماً على التوالي. مجموعة المنيبيزيوم مع حمض الكايينك ومجموعة الكايينك لا يختلفان في النوبات الحادة والمجموعتان تبديان افات نسجية متشابهة في CA1, CA3/CA4 في منطقة الحصين (جزء من الدماغ) وهاتين المجموعتين اكثر « هجومية » في اختبار التدبير نسبة لمراقبة الجرذان. مجموعة المنيبيزيوم مع حمض الكايينك اخذت وقتاً اطول للوصول الى تعلم اختبار « موريس ووترماز » نسبة الى مقارنتها مع مجموعة المصل الملحي. اما مجموعة حمض الكايينك فكانت متوسطة الحالة بين المجموعتين. ان استعمال المقادير ٥٤٠ ملغ / كغ لسلفات المنيبيزيوم مع ٥٤ ملغ / كغ كل ٢٠ دقيقة اظهرت نتيجة مشابهة تتكون عن عدم وجود حماية ضد افساد الذاكرة على المدى الطويل ونستنتج بان : (١) - المنيبيزيوم لا يبدي فعالية ضد النوبات الصرعية الناتجة عن استعمال حمض الكايينك . (٢) - المنيبيزيوم المعطى في هذه الطريقة لا يقي من خلل السلوك والذاكرة الناتجين من المدى الطويل أو من النتائج النسجية للحالة الصرعية في عمر ٣٥ يوماً .