Assessment of Citicoline protection against seizure induced in the rabbits

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الخلاصة

الصرع هواحد المشاكل الصحية الكبيرة في الجملة العصبية حيث يصيب تقريبا 1% من عموم السكان مسببا اعباء صحية واجتماعية واقتصادية مما يستدعي تقييما لعلاجات اكثر فاعلية لهذا المرض.

وفي محاولة لتقييم الاثر الواقي ضد التداعيات العصبية والايضية للصرع للدواء المشتق من الفوسفولبد وهو السايتوكولين فقد تم تحضير نموذج الصرع المحدث بالارانب عن طريق حقن الزايلوكين في البطن ومفارنته عند اعطاء السايتوكولين معه.

فقد تمت ملاحظة وتقييم معيار التشنج السريري وسجلات تخطيط الدماغ وانزيم ال دي اج وانزيم سي بي كي في مصل دم الارانب وقد كان هنالك اثر معتد لوقاية السايتوكولين ضد نوبات التشنج بالاضافة الى اثره ضد التزامن الكهربائي للصرع المبين في تخطيط الدماغ حيث اعطى السايتوكولين موجة أي أي جي تقدر ب 30 +\- 7 مايكروفولت عند الحد الاعلى لموجة الفا (10-11.5 هرتز) والتي تعتبر ضمن الحدود الطبيعية بالمقارنة مع الزايلوكين لوحده حيث ادى الى حدوث موجة 60 +\- 11 مايكروفولت عند حزمة ثيتا 4-7.5 هرتز و 55 +\- 8 مايكروفولت عند حزمة كاما 20-45 هرتز. كما اظهر السايتوكولين انخفاضا معتدا في كل من ال دي اج وكذلك سي بي كي مقاؤنة بالزايلوكين لوحده عند$P<0.05$: وبالاستنتاج فان للسايتوكولين اثار مفيدة واقية ضد الصرع في الجرع العلاجية المنخفضة

Abstract

Seizure is a big neurological health problem affect about 1% of the general population causing significant social, health, and economic burdens that necessities further evaluations of more effective treatment for this disorder. In a trial of assessing the phospholipid derived citicoline protection against neurological and metabolic sequalaes of seizure, a rabbit model was prepared by intraperitoneal (i.p.) injection of xylocaine in comparison with xylocaine given together with citicoline in another group of rabbits.

Clinical monitoring of convulsions together with physiographic electroencephalogram (EEG) recording and serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) parameters were evaluated. There was a significant protection against development of convulsion obtained in citicoline given group. Moreover citicoline significantly protected against EEG synchronization in that just 30 +/- 7 microV at upper alpha band(10-11.5 Hz) has been obtained which was within normal limits in comparison with xylocaine alone : 60 +/- 11 microV at theta 4-7.5 Hz band and 55 +/- 8 microV at gamma 20-45Hz ; $P<0.05$. Significant reductions in serum CPK and serum LDH were also attributed to administration of citicoline, $P<0.05$ .In conclusion : citicoline has beneficial protective effects against seizures and convulsion in a lower therapeutic dose.
Keywords **:** Citicoline, Seizure, Xylocoaine and EEG.

Introduction

Epilepsy means a tendency to have seizures [1] . Seizure is a widely distributed electrical manifestation of underlying brain lesions [2] and it is a symptom of brain disease rather than a disease itself [1] . Epilepsy is widespread among the general population & it is the second most common neurologic disorder after stroke . Usually there is no identifiable cause for epilepsy, although the focal areas that are functionally abnormal may be triggered into activity by change in any of a variety of environmental factors, including alteration in blood gasses, PH, electrolytes or glucose availability [3].Types of epilepsy : Partial epilepsy : in this type, consciousness is preserved . It is divided into : simple and complex . Also generalized epilepsy : in this type, consciousness is lost . It is divided into Tonic – clonic (Grand mal), Absence (Pitit mal), myoclonic, febrile seizures and status epilepticus . Epilepsy affects approximately 3 percent of individuals by the time they are 80 years old. About 10 percent of the population will have at least one seizure in their

lifetime [4] . The worldwide incidence of epilepsy is roughly in the range 5–10 per 1000 people . Epilepsy's approximate annual incidence rate is 40–70 per 100,000 [5] . so that it has a massive health and economic burdens costs [6] which necessitates a farther evaluation and new effective antiepileptic agents, since failure to respond to the first line anti epileptics stand, for up to 25% or more [7] . Many anti epileptics target the ion channels and enhance gabamenergic neuronal stabilizing activity[8] , however many phospholipids like lecithin found to stabilize and optimize cell membrane activity [9] . This action may augment anti epileptic activity of neurons against the pathological spontaneous firing which is the main promoter of progression and synchronization of seizure [10] . Many models of epilepsy had been prepared for investigation [11,12,13] , however, rabbits could also be induced with seizure as an animal model of epilepsy [14,15,16] .

Xylocaine : lidocaine is the most frequently employed amide local anaesthetic agents[17]. Lidocaine alters signal conduction in neurons by blocking the fast voltage gated sodium (Na^+) channels in the neuronal cell membrane, thereby achieve local anesthesia[18] .

xylocaine is generally applied locally by injection into the area of the nerve fibers to be blocked . Also do has additional uses for example, the anti arrhythmic effect of lidocaine and it is then administered by other routes [19] .The absorption and distribution are not as important in controlling the onset of effects as in determining the rate of offset of local analgesia[20]. Two major forms of toxicity are recognized, direct neurotoxicity from the local effects and systemic effects, since, ultimately local anaesthetic agents are absorbed from the site of administration . If blood levels rise too high, effects on several organ systems may be observed including CNS effects at low doses with sleepiness, headache and restlessness . At higher doses with nystagmus, muscle twitching and finally tonic clonic convulsions, CNS depreesion and death. Other toxicity include cardiotoxicity

with hypotension, bradycardia, arrhythmias and cardiac arrest [21] . A.Dereymaeker L.S. conducted that lidocaine has major epileptogenic effect when injected systematically but no such effect when applied directly to the cerebral cortex[22].
citicoline also known as cytidine 5'-diphosphocholine (CDP-Choline) . It is a complex organic brain molecule that occurs naturally in the body which functions as an intermediate in the biosynthesis of cell membrane phospholipids particularly phosphatidylcholine which is a brain chemical [23] . This chemical is important for brain function and brain metabolism [24] . Following administration by both the oral and parenteral routes, citicoline releases its two main components, cytidine and choline. Once absorbed, citicoline is widely distributed throughout the body, crosses the blood-brain barrier and reaches the central nervous system (CNS), where it is incorporated into the membrane and microsomal phospholipid fraction[23]. Citicoline has been shown to have beneficial effects in a variety of CNS injury models and neurodegenerative diseases in humans as hypoxic and ischemic conditions, traumatic brain injury [24], parkinson's, cognitive, behavioral [25] and attention deficit disorders [26]. No serious side effects have occurred in any series of patients treated with citicoline, which attests to the safety of treatment with citicoline [23] . Despite the beneficial effects of citicoline in a variety of neuronal injury models and clinical studies, no systematic data are available showing beneficial effects of citicoline in seizure . We report here the effect of citicoline in a rabbit model of seizure .

Materials and Methods

Animals : Six male rabbits Oryctolagus-Cuniculus species of 4-5 months age and 1.5 kg average body weight . They were healthy on examination and bred with standard cages with ad libitum water and oxoid diet .

Animal model of epilepsy : Six rabbits were divided into two groups in postgraduate lab of pharmacology and therapeutics in Kufa College of Medicine . Both groups were given 4 ml of 2% xylocaine (Obarcaine, manufactured by Oubari-Pharma, Aleppo-Syria) intraperitoneally (i.p) .

Group one were given 100 mg/kg citicoline(Somazina, manufactured by Ferrer International,S.A, Spain) i.p directly and regarded as treated group, where as group two were given 1 ml distilled water i.p and regarded as a control induced treated group .

A prior and continuous EEG was monitored for both groups, in addition to repeated assessment of rabbits, behavior, posture, trunk tone and if any clonic or tonic clonic convulsive manifestations .

EEG monitoring :Electroencephalographic monitoring of the model and response process was measured with physiograph (MKIII.UK) . The instrument was adjusted to EEG channels with sensitivity of 20 microvolts / cm and 5 cm / S chart speed. Peripheral two steel electrodes were implanted through the scalp to monitor the frontal motor area and different EEG frequency zones were considered[27] .

Figure(1) Physiographic settings for EEG recording for rabbits.

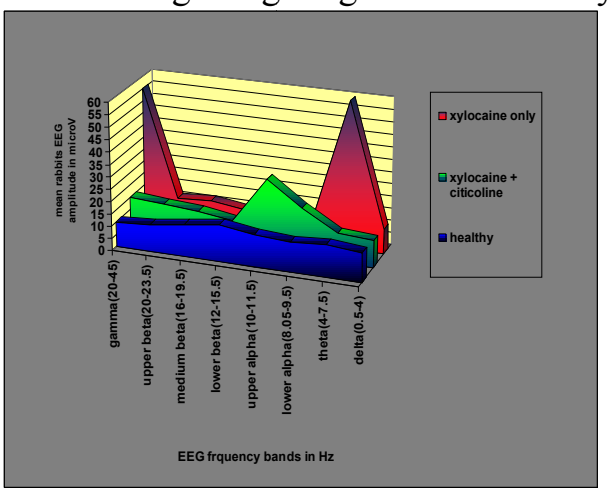
Serum CPK were calculated by Human kit, Germany[28] .

Serum LDH were measured by Human kit, Germany[29] .

Statistic analysis: Continuous data like LDH and CPK had been assessed by t test and chi-sqare for clinical assessment of convulsions where as EEG zones were assessed by Mann-Witnne test for scoring significance . The statistic analysis were done by SSPS version 10 with $p < 0.05$.

Results

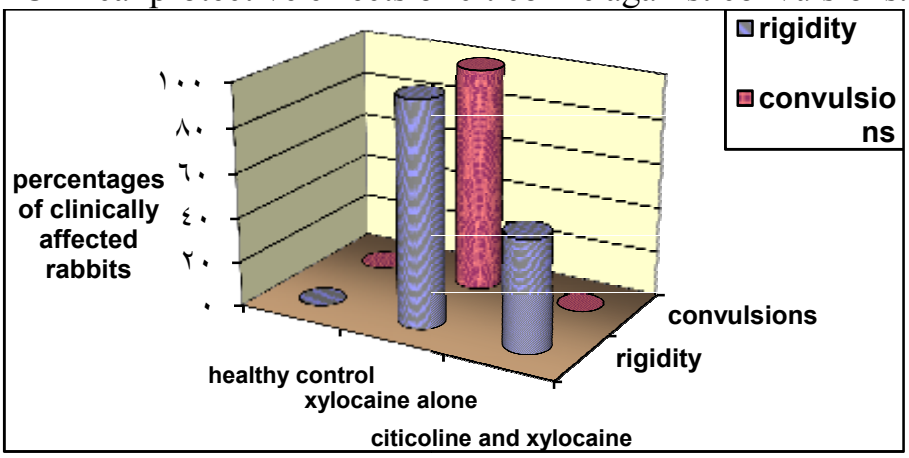
A- Rabbits EEG findings on giving citicoline and xylocaine



Figure(2): The influence of citicoline on development of seizure EEG waves in comparison with xylocaine alone group after 60 minutes of adminstration.

Citicoline caused significant desynchronization at upper alpha band(10-11.5 Hz) in comparison with xylocaine alone at theta 4-7.5 Hz band and at gamma 20-45Hz ; P<0.05.

B-Clinical protective effects of citicoline against convulsions.



Figure(3) Shows the clinical findings of protective effects of citicoline against convulsion in comparison with xylocaine alone .

There was a significant protection against development of convulsion obtained in citicoline given group at P<0.05 .

Figure(4) Shows the tonic attitude of rabbit with xylocaine only .

C- Metabolic markers findings of convulsion protected by citicoline

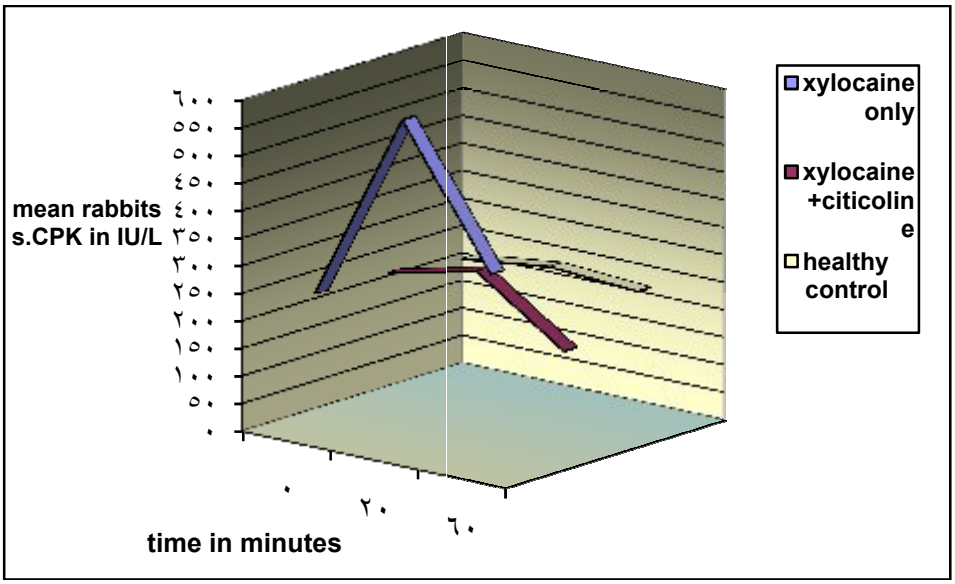


Figure (5): Shows CPK response to protection with citicoline in comparison to xylocaine only induced seizure .
There was a significant reduction in s. CPK level noticed with citicoline in comparison with a sharp rise in CPK due to neuronal insult induced by xylocaine only $P < 0.05$.

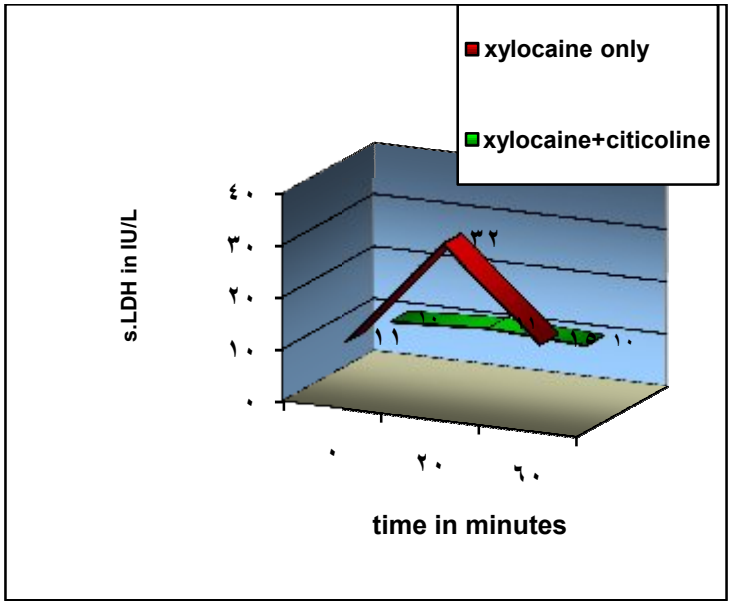


Figure (6): Shows the response of LDH to protection with citicoline in comparison to xylocaine only induced seizure .
Serum LDH level significantly reduced with citicoline in comparison with a sharp rise in LDH due to neuronal insult induced by xylocaine only P < 0.05 .

Discussion

Assessment of brain electrochemical events in lower animals include an accurate experimental and instrumental setting, since this requires a highly sophisticated physiograph filters and amplifiers in order to detect the microvolt scale with the invasive scalp electrodes.

Xylocaine possesses a potent neurotoxic effects to cerebral cortex which correlates strongly with the dose to induce corticospinal excitation, rigidity and convulsive seizure. This stands for the reliable model of seizure in rodents[30].

Different phospholipid derived compounds like citicoline are valuable candidate for modifying neuronal activity in stressful conditions like ischemia and seizures. Citicoline regulate anion channels in cerebral cortex[31].

The results of citicoline protective effects against seizure (figure 2) was significantly suggestive for its beneficial effects against epilepsy in specific situations. Citicoline maintained

EEG amplitude around 30 +/- 7 microV at upper alpha band(10-11.5 Hz) that was within normal limits. This result was significantly different when xylocaine given alone : 60 +/- 11 microV at theta 4-7.5 Hz band and 55 +/- 8 microV at gamma 20-45Hz ; $P<0.05$.

Although citicoline caused a significant increase in EEG amplitude, but it was accepted as a normal excitation to upper alpha band. This effect may be encountered with many CNS stimulants like caffeine[32] . These stimulatory effects are although beneficial but should be used under closed supervision in cases of spontaneous neural excitation like seizures, however citicoline not only modulated rabbits brain electrical events , it had a significant clinical preventive effects against development of convulsions at $P< 0.05$ as well . Clinical assessment of muscle tone and convulsions showed a dramatic reduction in convulsion, muscle fasciculation and rigidity during ictal phase of induced seizures to the tested rabbits . Co administration of citicoline with xylocaine caused 100% of disappearance of convulsions(apart from mild muscular fasciculations and rigidity as shown in (figure 3) in comparison with xylocaine only group in which there was 100% occurrence of convulsions and rigidity; Chi sqare at $P<0.05$. The mechanisms behind neuroprotective effect of citicoline may include: (i) restoring Na^+/K^+-ATPase activity; (ii) preserving the arachidonic acid content of phosphatidylcholine and phosphatidylethanolamine; (iii) partially restoring phosphatidylcholine levels ; (iv) stimulating glutathione synthesis and glutathione reductase activity; (v) attenuating lipid peroxidation; and (vi) preserving cardiolipin (an exclusive inner mitochondrial membrane component) and sphingomyelin . These observed effects of citicoline could be explained by the attenuation of phospholipase A_2 activation . Citicoline also provides choline for synthesis of neurotransmitter acetylcholine, stimulation of tyrosine hydroxylase activity and dopamine release[24,33,34] .

Muscle relaxant and anticonvulsive effects of citicoline were assessed with CPK and LDH (figure 5 and 6) respectively .
Brain CPK could be depressed in seizure attacks, however serum CPK could reflect the muscular excitation and strain during convulsions that renders it a good indicator for assessment of convulsive event during induction of seizure to rabbits[35] . Citicoline showed a protective effects against muscular strain during seizure and concomitant convulsions in that, s.CPK was 599 +/- 15 IU/L after 20 minutes of giving xylocaine alone, but significant reduction occur in rabbits that co administered citicoline with xylocaine , s.CPK 283 +/- 13 IU/L after 20 minutes; t value at $P<0.05$.
Similar changes were noticed in s.LDH with citicoline when given to rabbits with xylocaine induced seizure in that just 11 +/-2 IU/L occurred with citicoline in comparison with 32 +/- 5 IU/L for xylocaine alone after the 20 minutes of adminstration; $P<0.05$. Serum LDH could reflect a metabolic exhausted state due to repeated convulsions . Different other studies assessed concomitant s.LDH in epilepsy and convulsions[36] .

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