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Original Article

Neuroprotective effect of Coenzyme Q10 against Neurological Disorders induced in an Experimental Model of Epilepsy

Effet Neuroprotecteur de la Coenzyme Q10 sur les Troubles Neurologiques induits chez un modèle Expérimental d'Epilepsie

Ahras-Sifi Nesrine and Laraba-Djebari Fatima*
USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, BP32, El Alia, Bab Ezzouar, 16111 Algiers, Algeria

ABSTRACT

Introduction: Epilepsy is a common neurological disorder characterized by recurrent seizures and a neurodegenerative process. Neuroinflammation, oxidative stress and mitochondrial dysfunction may play an important role in the pathophysiology of seizures. In this study, a neurodegenerative model of seizure was developed using Kaliotoxin (KTx), a potassium channel blocker neurotoxin isolated from scorpion venom. The role of the coenzyme Q10 (CoQ10), which isan essential cofactor of the electron transport chain in mitochondrial against KTx-disorders was investigated. **Subjects and Methods:** Naval Medical Research Institute (NMRI) mice were injected with KTx byintracerebroventricular (i.c.v) route prior or no the use of CoQ10 administered by *per os*(p.o) route. **Results:** Binding of KTx to its biological target the Kv channels, causes seizures, activation of inflammatory response and oxidative stress characterized by an increase of cyclooxygenase 2 (COX-2) expression, Nitric oxide (NO) and Malondialdehyde (MDA) levels associated to a decrease of GSH level. The neuroinflammatory response is accompanied by cerebral alterations. The administration of CoQ10 before KTx injection seems to be able to reduce the observed alterations, probably by reducing oxidative stress characterized by an increase of anti-oxidant markers (GSH level and the catalase activity).**Conclusions:** The developed model using KTx could help to the understanding of the molecular pathway involved in the neuropathological processes related to K^+ channel dysfunctions. The CoQ10 seems to have a neuroprotective effect against the induced disorders by KTx by enhancing the mitochondrial function. These findings suggested the therapeutic potential of the CoQ10 in neurodegenerative disorders. The CoQ10 treatment could be a new therapeutic approach in brain alterations due to seizure.

KEY WORDS: Seizure, neurodegeneration, neurotoxin, mitochondria, coenzyme Q10.

RÉSUMÉ

Introduction: L'épilepsie est un trouble neurologique caractérisé par des crises récurrentes et un processus neurodégénératif. La neuro-inflammation, le stress oxydatif et le dysfonctionnement mitochondrial semblent jouer un rôle important dans la physiopathologie des crises. Dans cette étude, un modèle neurodégénératif de convulsions a été développé en utilisant la kaliotoxin (KTx), une neurotoxine purifiée à partir du venin de scorpion. Le rôle de la coenzyme Q10 (CoQ10), un cofacteur essentiel de la chaîne respiratoire mitochondriale *a* été étudié chez ce modèle.

Sujets et méthodes : Des souris de l'Institut naval de recherche médicale (Naval MedicalResearch Institute, NMRI) ont reçu une injection de KTx par voie intracérébro-ventriculaire (i.c.v) avec ou sans prétraitement en utilisant la CoQ10 administrée par voie*per os* (p.o).**Résultats :** La liaison de KTx à sa cible biologique, les canaux potassiques Kv, provoque des convulsions. Cette fixation induit une activation de la cascade neuro-inflammatoire et du stress oxydatif. En effet, une augmentation de l'expression de la cyclo-oxygénase 2 (COX-2) et des taux de monoxyde d'azote (NO) et de malondialdéhyde (MDA) associés à une diminution du taux de GSH a étéobservée. Cette augmentation se traduit par d'importantes lésions cérébrales. L'administration du CoQ10 avant l'injection de KTx semble pouvoir réduire les lésions cérébrales, en améliorant la balance oxydative.**Conclusions :** Ce modèle pourrait servir comme outil afin d'explorer les mécanismes impliqués dans les processus neuropathologiques liés aux dysfonctionnements du canal Kv. La CoQ10 semble avoir un effet neuroprotecteur en améliorant la fonction mitochondriale. La CoQ10 pourrait constituer une nouvelle approche thérapeutique dans les troubles neurodégénératifs liées aux convulsions.

MOTS CLES: Convulsion, neurodégénérescence, neurotoxine, mitochondrie, coenzyme Q10.

* *CorrespondingAuthor*: Fatima Laraba-Djebari : tel : 00 213 21 24 79 13
fax : 00 213 21 24 72 17 ; e-mail : flaraba@hotmail.com

Received on : 03 juillet 2019
Revisedon: 10 juin 2020
Accepted on : 17 juin 2020

1. Introduction

The epilepsy is considered as one of the most common neurological disorders worldwide, with a prevalence of 0.5–1% of the population [1]. The epilepsy is very complex neurological disorder characterized by recurrent seizures resulting from excessive activation of cerebral neurons due to ion channel dysfunction [2].

Despite the development of conventional and novel antiepileptic drugs, one-third of adult epileptic patients do not respond to antiepileptic drugs or surgical treatment and therefore suffer from intractable epilepsy [3, 4]. Understanding the pathophysiogenesis of seizure relies largely on the use of epileptic models, such as, the use of ion channel modulators [5-7].

The Kaliotoxin is a potassium channel blocker neurotoxin, with high affinity to the voltage-gated potassium channels (Kv1.1 and Kv1.3). This neurotoxin was isolated from *Androctonus australis hector* scorpion venom. It has an excitotoxic effect when it is injected in mice by intra-cerebro-ventricular route [8]. Indeed, the voltage-dependent potassium channels (Kv) are found in all excitable cells; they contribute to the regulation of the membrane potential and they are involved in the repolarization phase [9]. Many studies have shown that dysfunction of Kv channels can lead to various forms of epilepsy [10]. However, the pathological mechanisms involved in the process of epileptogenesis are still poorly understood [11-13].

Various hypotheses have been raised to explain the pathogenesis of epilepsy, in particular the involvement of oxidative stress, mitochondrial dysfunction and neuroinflammation. The oxidative stress is emerging as a mechanism that plays an important role in the etiology of seizure-induced neuronal death [14]. It's plays a crucial role in neuroinflammation and mitochondrial dysfunction induced brain damage during epileptic seizures [15, 16].

In the inner mitochondrial membrane, Coenzyme Q10 (ubiquinone), is essential for electron transfer activities during oxidative phosphorylation [17]. Recent studies reported that lipid peroxidation in epileptic patients is accompanied by a reduction in CoQ10 that aggravates the brain alterations [18, 19]. The CoQ10 was known for its key role in mitochondrial bioenergetics [20]. Indeed, the CoQ10 plays a vital role in ATP production [21]. The CoQ10 also has membrane-stabilizing properties and acts as an antioxidant in both mitochondrial and lipid membranes [22]. The neuroprotective effects of the CoQ10 have been reported in multiple models of neurodegeneration, and were investigated as a promising neuroprotective in Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders; including Huntington's disease and epilepsy [23].

Therefore, the aim of this study was to investigate the potential neuroprotective effect of CoQ10 on brain alteration in an experimental model of seizure induced by a K^+-channel blocker neurotoxin.

2. Subjects and Methods

2.1. Experimental animals

The male NMRI mice (9–10 weeks old) were used for experiments. The animals were housed in plastic perspex cages under controlled conditions (ambient temperature of 21 ± 2°C, natural light–dark cycle) for acclimation. Standard rodent chow and water *ad libitum* were provided. All experiments were done at the same time of day (between 9:00 and 12:00 AM) to minimize circadian influence on seizure susceptibility. Animals were used according to the European Community rules of the Ethical Committee for animal Welfare [24]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Chemicals and drugs

The Kaliotoxin (KTx) was isolated from *Androctonus australis hector*scorpion venom [8]. The CoQ10 was obtained from Nutri-santé Laboratory (France). Both KTx and CoQ10 were dissolved in sterile saline (0.9% NaCl). All other chemicals were of pure analytical grad and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Experimental procedure

Mice were randomly divided into four experimental groups of 7 mice each. The first group (the normal control group) consisted of mice that received only vehicle (0.9% NaCl) by intra-cerebro-ventricularly (i.c.v) route. The second group (KTx group, animal model of seizure) mice were injected by KTx (25 ng/25 g) by i.c.v. route. The third group (CoQ10 group): Mice received CoQ10 (100 mg /kg) *per os* (o.p) route 5 et 1 h before sterile saline (0.9% NaCl) i.c.v. injection. The fourthgroup (KTx and CoQ10-treated group): Mice received CoQ10 (100 mg /kg; o.p) 5 et 1 h before KTx (25 ng/ 25g; i.c.v).

After 24 hours, animals were sacrificed and four brains tissues for each group were obtained and immediately frozen at −80 °C for the different biochemical determinations. The three other brains were fixed in 4% paraformaldehyde in PBS (pH 7.5) during 24 h at room temperature for histological and immunohistochemical studies.

2.4. Estimation of nitrites

The accumulation of nitrites (NO_2^-) in the supernatant is an indicator of the production of nitric oxide (NO). The nitrites were determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid). Equal volumes of the supernatant and the Greiss reagent were mixed. The mixture was incubated for 20 min at room temperature and the absorbance was measured at 540 nm. The concentration of nitrites in the supernatant was determined from a sodium nitrite standard curve and was expressed as micromoles of per milligram of tissue(µmol/mg tissue) [25].

2.5. Determination of lipid peroxides

The amount of malondialdehyde (MDA), a measure of lipid peroxidation in cerebral cortex was determined by thespectrophotometry as thiobarbituric acid-reactive substances (TBARS) at 532 nm according to the method described by byEsterbauer [26]. Molar extinction coefficient of chromophore ($1.56\times 10^5\ M^{-1}\ cm^{-1}$) was used and concentration was expressed as millimoles of MDA formed per milligram of tissue (mmol/mg tissue).

2.6. Determination of glutathione

The level of reduced glutathione (GSH) in cerebral cortex was measured in brain tissue using Ellman method [27]. Tissular GSH levels were expressed as millimoles of per milligram tissue weight (mmol/mg tissue). Molar extinction coefficient of chromophore (1.36 × l04 M−1 cm−1) was used.

2.7. Determination of catalase activit

The catalase activity was assayed according to Aebi [28],Wherein breakdown of hydrogen peroxide (H_2O_2) is measured at 240 nm using spectrophotometer. The results were expressed as the enzyme concentration required for the decomposition of 1 mol of H2O2 per min per milligram of tissue (U/mg tissue).

2.8. Histological Assessment

The haematoxylin and the eosin (H&E)-stained, deparaffinized sections (3 µm thick) were observed through a light microscope equipped with a camera (Leica Microsystems, Germany).

2.9. Immunohistochemical detection brain COX-2 expression

The deparaffinized sections (3% H_2O_2 in PBS for 10 min) were pre-blocked for 30 min with 5% bovine serum albumin (BSA). Then the sections were incubated overnight at 4 °C with primary rabbit antibodies specific to COX-2 (1:100 dilution) (Dako, Denmark).

The sections were washed with phosphate buffered saline (PBS), incubated for 10 min with biotinylated secondary antibody and stained with 3,3′-diaminobenzidine (DAB) solution (Dako, Denmark). Finally, the slides were counterstained with haematoxylin for 5 min and examined using a video camera installed on a light microscope (Leica Microsystems, Germany).

2.10. Statistical analysis

Data were analyzed by unpaired Student's *t* test. P values of less than 0.05 were considered to indicate a statistically significant difference. Microsoft Excel software was used.

3. Results

3.1. Effect of CoQ10 on oxidative stress in the KTx induced seizure

The kaliotoxin (KTx) induced oxidative stress in brain homogenates as reflected by a significant increase in MDA ($p < 0.01$) and NO ($p < 0.001$) concentrations associated with significant reductions in GSH ($p < 0.01$) level compared with the normal control group (**Figure 1**).

These deleterious effects associated with KTx-induced seizures were restored by CoQ10 treatment as compared with the untreated KTx animal group. The CoQ10 appears to contribute to the amélioration of antioxidant status in treated animals with the KTx.

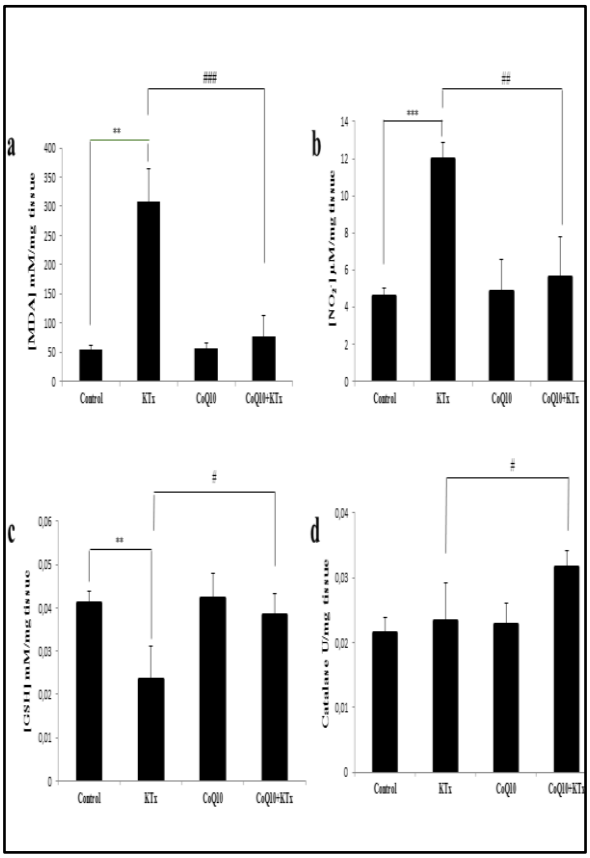

Figure 1: Effect of CoQ10 on KTx-induced oxidative stress parameters: (a) MDA (mM/mg tissue), (b) NO (µM/mg tissue), (c) GSH (mM/mg tissue), and (d) catalase (U/mg tissue) in brain tissue homogenates.
Values are means± SD (n=4);Student's t test; * Compared with normal control group. #Compared with KTx animal group

3.2. Effect of CoQ10 on histopathological alterations induced by KTx

Histological analysis of the cerebral cortex of the group injected animals with KTx showed several alterations characterized by cerebral oedema, inflammatory cell infiltrate and neuronal pyknosis (**Figure 2b**). However, sections obtained from the treated animals with CoQ10 prior to the KTx injection, revealed an attenuation of pathological changes, with an almost normal histological structure comparable to that of the control group (**Figure 2d**).

3.3. Effect of CoQ10 on the brain tissue COX-2 expression induced by KTx

The KTx administration enhanced the expression of COX-2 in the cerebral cortex area compared to that of control group (**Figure 3b**). However, CoQ10 markedly decreased the brain tissue COX-2 expression induced by KTx, thereby restoring normal expression (**Figure 3d**).

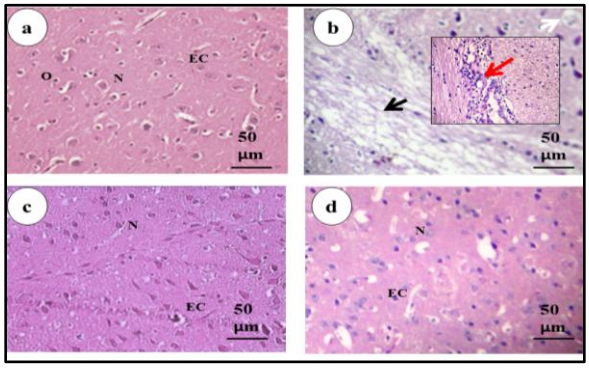

Figure 2:. Effect of CoQ10 treatment on tissular alterations induced by KTx-seizure.(a) Control group showing of the cerebral cortex area. (b) KTx group showing neuronal necrosis (white arrow), oedema (black arrow) and inflammatory infiltrate (red arrow).(c) CoQ10 group displaying normal histological structure as a control group. (d) CoQ10 + KTx treated animals showing mitigated histopathological alterations with almost normal cerebral cortex structure.
The bar = 50 µM. N: neuron; EC: endothelial cell; O: oligodendrocyte (H-E staining).

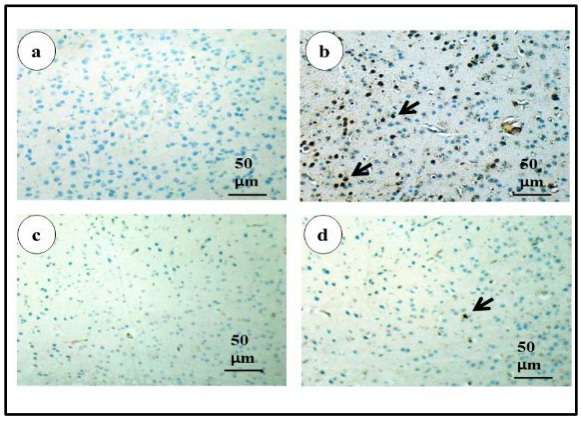

Figure 3: Effect of CoQ10 on KTx-induced changes in immunohistochemical staining of brain COX-2 expression.(a) Control group(b) KTx-treated group(c) CoQ10 group(d) CoQ10+KTx group. Immunohistomarking with the anti-COX-2 antibody is indicated with the black arrow. KTx induces an increase in the expression of COX-2. Treatment with CoQ10 decreases COX-2 expression.

5. Discussion

In the present study, convulsive dose of KTx caused neuroinflammation and oxidative damage [29]. Indeed, a relationship between free radical and seizure has been established. Reactive oxygen species (ROS) have been involved in the development of seizures and seizure-induced neurodegeneration [30]. This study supports the hypothesis that oxidative stress occurs in the cerebral cortex during seizures, which indicates that brain damage induced by the oxidative process that plays a crucial role in the physiological consequences of seizures. We observed in this study, an increase of MDA level and a depletion of the GSH level an antioxidant parameter in brain homogenates. This could be explained by the excitotoxicity associated with excessive neurotransmitter release and oxidative stress leading to free radical damage [31, 32].

This oxidative process can induce damage on all intracellular organelles (e.g., endoplasmic reticulum, mitochondria) leading to cell death [33]. Due to their high content of polyunsaturated phospholipids, the mitochondria are especially sensitive to lipid peroxidation. Mitochondrial dysfunction and neuroinflammation have been well reported to play a critical role in the pathophysiology of epilepsy [34]. Under normal conditions, there is a steady state balance between the production of ROS and their scavenging by the cellular antioxidant system. However, when ROS production is excessive, the intrinsic antioxidant scavenging capacity is submerged, resulting in oxidative stress and cellular oxidative damage [35].

Our results showed that treatment with CoQ10 before KTx-induced seizures reduced lipid peroxidation and increase antioxidant parameters in the brain of animals injected with KTx, resulting in an overall decrease in oxidative stress. CoQ10 is a membrane stabilizer and an essential cofactor of the electron transport chain in the mitochondrial respiratory chain [36]. It accumulates in the mitochondria and restores the loss in mitochondrial transmembrane potential, which results in the reduction of mitochondrial ROS generation and thus protect the mitochondria and cellular components from the oxidative damage [37].

Moreover, pre-treatment with CoQ10 prior to neurotoxin injection induced a decrease in COX-2 expression. This treatment appears to inhibit the expression of the COX-2 [38]. The COX-2 is the

inducible form of cyclooxygenase enzymes, it is the first enzyme involved in the biotransformation of arachidonic acid in response to different stimuli such as pro-inflammatory cytokines that are released by astrocytes and microglial cells [39].Overexpression of COX-2 in the brain has been reported following convulsions induced in epilepsy models and in patients with epilepsy [40]. This positive response is due to the induction of the COX-2 by pro-inflammatory cytokines, in particular IL-1B which induce the activation of NFκB (Nuclear Factor -KappaB) and MAPK (MAPkinases) signaling pathways, which will lead to the gene transcription coding for the most innate immunity proteins, such as pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), chemokines (CCL2, IL-8, etc.), prostaglandins, cyclooxygenase-2 (COX-2) and the inducible NO synthase (iNOS), which can explains the increase in the level of NO and COX-2 expression after KTx convulsions [41, 42].However, in this study we did not investigate whether the treatment with coenzyme Q10 altered the seizures induced by KTx.

Conclusion

In conclusion, these results showed that KTx is able to induce neurological disorders by blocking the Kv ion channel. This developed model using KTx could help to the understanding of the molecular pathway involved in the neuropathological processes related to K^+ channel dysfunctions. The CoQ10 seems to have a neuroprotective effect by enhancing the mitochondrial function. The mitochondrial function may be a new pathway to explore in neurodegenerative diseases and CoQ10 treatment could be a new therapeutic approach in brain alterations due to seizures.

Conflict of interest

The authors declare that they have no conflict of interest.

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