The effect of sub-anesthetic doses of ketamine on plasma oxidative stress in pilocarpine-induced epilepsy in mice

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INTRODUCTION

Epilepsy is a prevalent neurological disorder worldwide (Devinsky et al., 2018). It is characterized by generating seizures and its development is accompanied by alterations in many brain and plasma reactions (Gao et al., 2017). Oxidative stress plays an important role in disease progression. However, the mechanisms underlying the pro-oxidant/antioxidant system of blood plasma this disease are poorly understood. There is evidence that plasma reactive oxygen species (ROS) play an important role in the pathogenesis of many diseases neurological (Fang et al., 2017). Oxidative stress occurs when the body is unable to eliminate free radicals. The maintenance of oxidative balance in the brain is tightly regulated by antioxidants. In recent years, the role of oxidative stress in epilepsy has been attracting considerable attention (Ambrogini et al., 2019). With the background knowledge, the brain is vulnerable to oxidative stress that is one of the factors causing epileptogenesis (Aguir et al., 2012).

Animal models are very useful for exploring this type of neurological condition since they have the advantage of being able to study in detail this neuropathology, as well as to evaluate potential anti-epileptogenic drugs and their protection capacity during the crisis (Lösch, 2002). The chemo-convulsant models are the closest to epilepsy models in humans (Rubio et al., 2010). Pilocarpine has been used to induce a status epilepticus-like condition in an animal model to study this condition. Pilocarpine produced a sequence of behavioral alterations including staring spells, limbic gustatory automatisms and motor limbic seizures that developed over 5-20 min and build up.
progressively into a limbic status epilepticus lasting for several hours (Turski et al., 1984; Mazzuferi et al., 2012). The pilocarpine-induced epilepsy model is very useful to study the development and neuropathology of temporal lobe epilepsy (Treiman, 1995). Neurochemical, as well as enzymatic activity studies, suggest that excitotoxic stimulation in status epilepticus induces oxidative stress in the brain (Barros et al., 2007). However, less is known about the effects of pilocarpine on plasma oxidative stress in animals.

Although, Ketamine proven to be effective in various neurologic disorders has proven useful in the treatment of refractory status epilepticus (Sabharwal et al., 2015) and is neuroprotective in the pilocarpine model (Loss et al., 2012). Ketamine must be administered within minutes of seizure to avoid the harmful neurological effects induced by pilocarpine (Fujikawa, 1995). Consequently, ketamine produces clinical antidepressant effects with a different time course and different neurochemical mechanisms than conventional antidepressants. Furthermore, ketamine can act as a multifunctional neuroprotective agent by inhibiting oxidative stress in the brain, cellular dysfunction, and apoptosis. Through the regulation of reactive oxygen species by production and maintenance of oxidative phosphorylation, the ketamine acts as an antioxidant and protects neuronal cell membranes (Ayyildiz et al., 2006). However, the mechanisms underlying ketamine in epilepsy is not fully understood. In the present work, we evaluated the effect of the administration of ketamine on the pro-oxidant/antioxidant system of blood plasma in pilocarpine-induced epilepsy in mice.

MATERIALS AND METHODS

Animals and treatment

Forty adult Swiss mice (age of 5 and 6 weeks and 25 to 30 g) from Pasteur Institute of Tunis were used. To minimize pain and discomfort for animals all measures were taken with a regular veterinary control. Animals were used along in this study following Tunisian Chart on Ethics of Animal Experiments and compliance with ethical standards. Experimental animals were provided with food and water ad libitum and maintained at controlled temperature (22 ± 2°C) with a 12 hours light/dark cycle. These conditions follow the recommendations reported in the "Guide for the care and use of laboratory animals". The experimental protocol was approved by the Bio-Medical Ethics Committee (Pasteur Institute, Tunis), Meeting No.: 102/18, Ref: 2018/19/E/NVSM.

The treatment protocol was described in our recent study (Tannich et al., 2019). Animals were divided into four groups of ten animals: Control group (C): Mice were injected intraperitoneally (IP) with saline solution NaCl (0.9%). Pilocarpine group (P): Mice received 3 injections of pilocarpine hydrochloride (100 mg/kg; IP; Sigma-Aldrich, Germany) every 20 min until the beginning of status epilepticus as recommended in the literature (Gröticke et al., 2007 and De Oliveira et al., 2008). To avoid peripheral cholinergic effects, atropine (1 mg/kg) was administered by subcutaneous (SC) injection, 30 min before the application of pilocarpine to reduce its cholinergic effects (salivation, diarrhea, and lacrimation) (Fujikawa, 1995; Curia et al., 2008). Ketamine group (K): Mice received 3 injections every 30 min of ketamine (10 mg/kg; IP; Sigma-Aldrich, Germany). Post-treatment with ketamine group (P-K): Mice received 3 injections of 100 mg/kg pilocarpine every 20 min. Atropine (1 mg/kg, by SC injection) was administered before 30 min of pilocarpine injection. After 30 min of pilocarpine treatment, animals received 3 injections of ketamine (10 mg/kg; IP) every 30 min (Dhote et al., 2012; Mc Girr et al., 2017).

Blood sampling method

Fifteen days after the last injection, blood samples were first collected by retro-orbital sinus puncture through the medial canthus of the eye using clean 45 µL heparinized microhematocrit tubes. No anesthesia was used at the time of the blood sampling, to avoid the effects of anesthesia on the blood glucose levels. Mice were fixed by a retainer during blood collection from tail-tip (Togashi et al., 2016). Blood samples were collected using the routine technique. The samples remaining after the glucometer measurements were immediately centrifuged at 1000g for 10 min at room temperature to separate plasma. Plasma was stored at -80°C to measure: NO levels, lipoperoxidation, cholesterol, antioxidant enzyme activities (Catalase (CAT), Peroxidase (POD), Superoxide dismutase (SOD)) and uric acid.

Blood glucose levels

The blood glucose levels were measured by glucometers (ACCU-CHECK Compact Plus®; Roche Diagnostics, Japan). The amount of 0.05 mL of blood was used for the measurement.

Determination of protein concentration

Total soluble proteins were determined according to the biuret method (Ohnishi and Barr 1978), kit from (Biomaghreb Tunisia, Ref.20161). BIURET method, whose principle, is the formation of a colored complex between copper sulphate and binding pepticid (Ohnishi and Barr 1978). The intensity of the staining is proportional to the number of peptide bonds. At acidic pH soluble proteins bound with copper a colorful complex measurable at 546 nm.

Malondialdehyde (MDA) levels in plasma

Lipoperoxidation was determined by MDA measurement according to the double heating method (Draper and Hadley 1990). Briefly, aliquots from plasma were mixed with 2,6-di-tert-butyl-4-hydroxy-toluene (BHT) –
trichloroacetic acid (TCA) solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000xg for 5 min at +4°C. The supernatant was blended with 0.5N HCl, 120 mM TBA in 26 mM Tris and then heated at -80°C for 10 min. After cooling, the absorbance of the resulting chromophore was determined at 532 nm using a Beckman DU 640B spectrophotometer. MDA levels were determined by using an extinction coefficient of the MDA-Thiobarbituric acid complex of 1.56 105 M⁻¹cm⁻¹.

**Cholesterol levels in plasma**

The cholesterol was determined according to Allain et al. (1974). The principle of this method is enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminantipyrine in the presence of phenol and peroxidase complex measurable at 505 nm.

**Nitric Oxide (NO) determination**

Plasma NO was measured by quantification of the NO metabolites nitrite and nitrate according to a commercial Nitrite/Nitrate Assay Kit, colorimetric, Product Number: 23479, from (Sigma-Aldrich, Germany). The total concentration of NO metabolites is determined using the Griess test. In the process, NO₃ is converted to NO₂ by the enzyme Nitrate Reductase. The mechanism of the Griess assay is summarized by the azo coupling between the diazonium species, which are produced from sulfanilamide with NO₂, and naphthylethylenediamine, resulting in a colorimetric product (540 or 570 nm) proportional to the NO metabolite present (Green, 1989).

**Antioxidant enzyme activity assays**

All spectrophotometric analyses of plasma antioxidant enzyme activities were performed with a Beckman DU 640B spectrophotometer.

Catalase (CAT) activity was assayed by measuring the initial rate of H₂O₂ disappearance at 240 nm (Aebi, 1984). The reaction mixture contained 33 mM H₂O₂ in 50 mM phosphate buffer (pH 7.0) and CAT activity was calculated using the extinction coefficient of 40 mM⁻¹cm⁻¹ for H₂O₂.

Peroxidase (POD) activity was measured at 25°C using guaiacol as a hydrogen donor. The reaction mixture contained 9 mM guaiacol (Sigma-Aldrich, Germany), 19 mM H₂O₂ in 50 mM phosphate buffer (pH7) and 50 μL of enzyme extract in 1 mL final volume. The reaction was initiated by the addition of H₂O₂ and monitored by measuring the increase in absorbance at 470 nm. Peroxidase activity was expressed in nmol of guaiacol oxidized per min with a molecular extinction coefficient of 26.2 mM⁻¹cm⁻¹ for calculation (Chance and Maehly 1955).

Superoxide dismutase (SOD) activity was determined by using a modified epinephrine assay (Misra and Fridovich, 1972). At alkaline pH, superoxide anion O₂⁻ causes the autoxidation of epinephrine to adenochrome; while competing with this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. Enzyme extract was added in a 2 mL reaction mixture containing 10μL bovine catalase (0.4 U/μL; Sigma-Aldrich, Germany), 20 μL epinephrine (5 mg/ml; Sigma-Aldrich, Germany) and 62.5 mM sodiumcarbonate/sodium bicarbonate buffer (pH10.2). Changes in absorbance were recorded at 480 nm. Characterization of SOD isofoms was performed using Potassium cyanide (KCN)(3 mM) which inhibited Cu/Zn-SOD or H₂O₂ (5 mM) affecting both Cu/Zn-SOD and Fe-SOD. Mn-SOD was insensitive to both inhibitors.

**Non-enzymatic antioxidant assay (uric acid)**

Estimation of uric acid was performed by enzymatic, colorimetric 4-aminophenazone (4-AP) method using a commercially available Uric Acid kit, from (Biomaghreb, Tunisia, Ref. 20092) according to Barham and Trinder (1972).

**Statistical analysis**

Data were analyzed by one-way analyses of variance (ANOVA) with post hoc Tukey tests and are expressed as means ± standard error of the mean (SEM). Results with a p value <0.05 were considered significant.

**RESULTS**

**Ketamine decreases blood glucose levels in plasma of mice submitted to pilocarpine-induced epilepsy**

As shown in Figure 1, pilocarpine increased blood glucose levels by almost 213% (p<0.01) compared to control groups. Ketamine alone did not have significant effect on glucose levels compared with the control group. However, the administration of ketamine, before the induction of epilepsy, decreased significantly (p<0.01) the blood glucose levels in plasma of mice submitted to pilocarpine injections.

**Ketamine decreases malondialdehyde (MDA) levels in plasma of mice submitted to pilocarpine-induced epilepsy**

The oxidative stress in mice plasma was first assessed by measuring malondialdehyde (MDA). MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress. According to our results, pilocarpine significantly increased the plasma MDA level by almost (+202%) (Figure 2, p<0.01). Interestingly, treatment of mice with ketamine significantly decreased the plasma MDA levels in mice submitted to epilepsy (Figure 2,
Figure 1: Effect of ketamine on glucose levels induced by pilocarpine-induced epilepsy in mice plasma. Mice were intraperitoneally administered with saline solution (C), ketamine (K), pilocarpine (P), or post-treatment with ketamine (P-K). Glucose was determined. Results are expressed as means ± SE (n=10). **, p < 0.01 vs. C; §§, p < 0.01 vs. P.

Figure 2: Effect of ketamine on malondialdehyde (MDA) levels induced by pilocarpine-induced epilepsy in mice plasma. Mice were intraperitoneally administered with saline solution (C), ketamine (K), pilocarpine (P) or post-treatment with ketamine (P-K). Plasma MDA were determined. Results are expressed as means ± SE (n=10). **, p < 0.01 vs. C; §§, p < 0.01 vs. P.

p=0.01). However, ketamine alone had no significant effect on plasma MDA compared with the saline control group.

Cholesterol

There is no significant difference in cholesterol plasma levels (Figure 3).

Ketamine decreases Nitric Oxide (NO) levels in plasma of mice submitted to pilocarpine-induced epilepsy

We measured the plasma NO level (Figure 4). Pilocarpine increased plasma NO by almost (+255%, p=0.01). However, ketamine administered 30 min after pilocarpine reversed the increase of NO levels induced by pilocarpine.
Figure 3: Effect of ketamine on cholesterol levels induced by pilocarpine-induced epilepsy in mice plasma. Mice were intraperitoneally administered with saline solution (C), ketamine (K), pilocarpine (P), or post-treatment with ketamine (P-K), and plasma cholesterol were determined. Results are expressed as means ± SE (n=10). **, p < 0.01 vs. C; §§, p < 0.01 vs. P.

Figure 4: Effect of ketamine on Nitric Oxide (NO) levels induced by pilocarpine-induced epilepsy in mice plasma. Mice were intraperitoneally administered with saline solution (C), ketamine (K), pilocarpine (P), or post-treatment with ketamine (P-K), NO was determined. Results are expressed as means ± SE (n=10). **, p < 0.01 vs. C; §§, p < 0.01 vs. P.
Ketamine alone had no significant effect on NO levels compared with the control group (Figure 4).

**Ketamine increases antioxidant enzyme activity in plasma of mice submitted to pilocarpine-induced epilepsy**

Pilocarpine decreased expressively the levels of CAT (Figure 5A, p<0.01), POD (Figure 5B, p<0.01) and SOD (Figure 5C, p=0.01) compared to control group. Whereas treatment with ketamine after pilocarpine abrogated the pilocarpine-induced depletion of antioxidant enzyme activities by increasing CAT (p<0.01), POD (p<0.01) and SOD (p<0.02). Ketamine alone had no significant effect on plasma antioxidant enzyme activity compared with the control group.

**Ketamine increases non-enzymatic antioxidant assay (uric acid) levels in plasma of mice submitted to pilocarpine-induced epilepsy**

As shown in Figure 6, pilocarpine decreased significantly plasma uric acid by almost (+232%, p=0.03) but ketamine had no significant difference in plasma uric acid level compared to the control group. However, post-treatment with ketamine increased significantly the plasma uric acid by almost (-41%, p=0.03).

**DISCUSSION**

The aim of this study is to evaluate the effects of repeated sub-anesthetic doses of ketamine on oxidative stress induced by pilocarpine-induced epilepsy in mice plasma. The brain is susceptible to oxidative stress because of the high lipid content and oxidative metabolism (Azam et al., 2012). This was proven in experimental animals which showed oxidative damage after pilocarpine-induced seizure model (Lee et al., 2018). Data from previous studies reported that recurrent seizures increase the reactive oxygen species (ROS) in the brain (Franseva et al., 2000; Freitas et al., 2005; Barros et al., 2007). Furthermore, studies on epilepsy with animal models and evaluation of human brain tissue have revealed the oxidative stress is considered one of the mechanisms that could independently contribute to the disease progression in addition to serving as processes that underlie neuronal injury (Huang et al., 2012; Menon et al., 2014). It seems that the antioxidant system prevents free radical damage and also plays an important part in disease progression in the body (Menon et al., 2014). However, the exact mechanism of oxidative stress in epilepsy and treatments are not yet identified.

The effect of ketamine administration before and after the onset of epilepsy has been previously studied (Loss et al., 2012, TANNICH et al., 2019). However, the post-treatment effect of ketamine on plasma oxidative stress has not been tested. There is evidence that repeated administration of sub-anesthetic doses of ketamine may have beneficial long-term effects (Zanos et al., 2018). Previous studies show that repeated sub-anesthetic ketamine has been shown to improve clinical outcomes for treatment-resistant depression (Zanos et al., 2016). Weckmann et al. (2017) showed that Ketamine’s antidepressant effect is mediated by energy metabolism and antioxidant defense system. Moreover, there is increasing evidence that ketamine injection after pilocarpine appears to be sufficient to reduce epileptic status (Dhote et al., 2012). We hypothesize that ketamine has a beneficial antioxidant capacity that reverses the epilepsy-associated with the increase in pro-oxidants generation.

Our investigations showed that pilocarpine administration increased the blood glucose level in mice. Altered glucose is related to the mechanisms involved in the initiation and propagation of seizures induced by pilocarpine. These results are in agreement with a previous study that showed altered blood glucose levels 15 min after pilocarpine treatment, suggesting glucose changes during the initiation of seizures (Hussain et al., 1995; Shin et al., 2011). Hyperglycemia plays a key role in cerebral hyperexcitability. Thus, glucose produces several changes in variables related to the generation and elimination of oxygen free radicals in adult rats. However, high glucose levels increased free radicals such as superoxide anion generation as byproducts of mitochondrial oxidative phosphorylation (Chen et al., 2015). González et al. (2015) found that high glucose levels induce NO synthesis via the L-arginine/NO pathway. However, endothelial nitric oxide synthase leading to vascular ROS generation (Sobrevia et al., 1996; González et al., 2004). Interestingly, our data showed a significant decrease in blood glucose levels in post-treated groups with ketamine compared to the epileptic group. The reduction of blood glucose levels might be a consequence of an interaction between ketamine and N-methyl-D-aspartate (NMDA) receptors in the brain. The enhancement of ketamine blockade NMDA receptors and an increase in dopamine protected the animals against convulsions and mortality, while depletion of ketamine aggravated irrespective of the grade of hypoglycemia. Besides, Suleiman et al. (2009) show that the hypoglycemic effect of ketamine is possibly mediated through opioid receptors with an involvement of β-adrenoceptors that only become evident after blockade of α 2-adrenoceptors. Thus, ketamine likely acts as an antioxidant under conditions of hyperglycemia, leading to the protection of the endothelium in diseases associated with endothelial dysfunction such as epilepsy (Kobylarek et al., 2019).

Importantly, the level of MDA in plasma increased in the epileptic group compared to the control group in the present study. MDA is one of the fairly reactive metabolic products resulting from the effect of free oxygen radicals on tissues and a series of reactions during lipid peroxidation. The plasma MDA level is a sensitive indicator of lipid peroxidation and thus of oxidative stress (Menon et al.,
Figure 5: Effect of ketamine on antioxidant enzyme activities induced by pilocarpine-induced epilepsy in mice plasma. Mice were intraperitoneally administered with saline solution (C), ketamine (K), pilocarpine (P), or post-treatment with ketamine (P-K), and plasma catalase (CAT) (A), peroxidase (POD) (B), and superoxide dismutase (SOD) (C) activities were determined. Results are expressed as means ± SE (n=10). **, p < 0.01 vs. C; §§, p < 0.01 vs. P.

Moreover, it is well known that MDA increase affected the fluidity of the lipid bilayer membrane which is correlated to pathological or stress conditions including epilepsy (Deng et al., 2019). Ketamine works as a protective agent against oxidative stress and ischemia/reperfusion injury of the brain, kidney, muscle, heart, and intestine. Nevertheless, in our experiment, MDA levels were lower in the post-treatment with ketamine group compared with the other groups. This confirms that ketamine is an agent that protects against oxidative stress. The protective effects of these drugs may belong to their antioxidant properties. These results may indicate that ketamine acts as a regulating agent of the anti- and pro-oxidant system balance. Ketamine could influence on the antioxidant defense system, while reducing lipid peroxidation.

We also showed that no difference in plasma cholesterol was observed during the acute phase compared to the control group. Hence, previous studies have shown that
patients with epilepsy had increased carotid artery intima-media thickness and increased vascular risk factors (Tan et al., 2009). This coincides with (Luoma et al., 1979) who reported that epileptic patients showed elevated plasma HDL cholesterol levels.

Our results demonstrate that pilocarpine also increased plasma NO. In epilepsy, the role of NO has been examined in both in vivo and in vitro studies (Herberg et al., 1995; Maggio et al., 1995; Milatovic et al., 2002). In normal conditions, there is a balance between the production of NO and other mediators and their destruction by antioxidant systems (Freitas et al., 2005). Subsequently, the overproduction of NO observed in the pilocarpine mouse group suggests a decrease in the antioxidant system. This hypothesis is supported by our data showing the decrease of antioxidant enzyme activities (CAT, POD, and SOD) in the pilocarpine group. Additionally, increased NO level within the plasma, induced by pilocarpine, is associated with Ca\(^{2+}\) elevation which could alter several pathways. The accumulation of Ca\(^{2+}\) could induce NADPH oxidase or nitric oxide synthetase activation, resulting in the production of superoxide anion and NO respectively (Lu and Thompson 2012). Nevertheless, our data revealed that NO levels were lower after the treatment with ketamine compared with the other groups. Nitric oxide (NO) is synthesized from l-arginine by the enzyme NO synthase (NOS). However, the reduction of NO might be explained by the effect of ketamine on NO synthase enzyme. Our data corroborate with results already mentioned in literature dealing with antidepressant and antioxidant effects of ketamine that have been observed either in vitro or in vivo and various experimental settings. Zhang et al. (2013) suggest that the L-arginine-nitric oxide pathway is involved in the antidepressant effects of ketamine observed in rats and this involvement is characterized by the inhibition of brain and plasma total nitric oxide synthases (T-NOS), inducible NO synthase (iNOS), and endothelial NO synthase enzymes (eNOS) activities.

Regarding the activity of antioxidant enzymes, we showed that the plasma oxidative stress induced by pilocarpine is also characterized by decreased antioxidant enzymes activities as CAT, POD, and SOD allowing a high amount of ROS. These results are in agreement with another study showing that GSH and catalase activity plays an antioxidant role in the hippocampus during status epilepticus (Freitas et al., 2005; Carvalho et al., 2017). Therefore, the injection of ketamine after induction of epilepsy has increased antioxidant enzymatic activity (CAT, SOD, and POD). The mechanisms of regulation of antioxidant and the pro-oxidant system of blood plasma by ketamine during the epileptogenesis phase remain unknown; while several data sources indicate the importance of Ca\(^{2+}\) in the production of NO and MDA because of the cerebral hyperexcitability and cause the production of ROS in the brain during epilepsy. Lu et al., (2002) reported that the phosphorylation-mediated modulation of Ca\(^{2+}\) channel activity in response to lipid peroxidation may play important roles in the response of neurons to oxidative stress. It is well known that ketamine induces a balance by decreasing ROS production and increasing antioxidants in the brain. So, ketamine exerts potent antioxidant properties by counteracting the Fenton reaction of H\(_2\)O\(_2\) with free iron, leading to intracellular calcium dysregulation in the brain. This potential antioxidant effect could be explained by the blockade of NMDA receptors, which decreases Ca\(^{2+}\)and cerebral hyperexcitability (Yuryev et al., 2016). Another interesting feature of ketamine is its ability to increase plasma CAT and SOD activities after epilepsy. Increased activity in antioxidant enzymes could correspond to post-translational modifications as phosphorylation inducing some gain of function as reported by Shin et al. (2011) on epileptic animal models about increased CAT and glutathione peroxidase (GPx) activities after tyrosine phosphorylation. Thus, Den Hertog et al. (2005) showed that ROS significantly inhibits the activity of tyrosine phosphatases, resulting in increased tyrosine phosphorylation. Thus pilocarpine-induced H\(_2\)O\(_2\) production, which mainly originates from plasma (Shin et al., 2011), activates mitogen-activated protein kinase (MAPK), which in turn induces the expression of antioxidant genes and up-regulates the activities of antioxidant enzymes. The activation of MAPK also enhances H\(_2\)O\(_2\) production, forming a positive amplification loop (Zhang et al., 2006). Ketamine’s effects on oxidative stress are mediated, at least in part, by a mechanism dependent of MAPK signaling inhibition (Rêus et al., 2016; Weckmann et al., 2017). Some treatments also improved neuronal injury after epilepsy by enhancing the Nuclear factor E2-related factor 2/antioxidant response element (Nrf2/ARE) pathway to increase the antioxidative activity of the body (Liu et al., 2018).

Uric acid is the non-enzymatic antioxidant present in the highest concentration in human blood. Uric acid functions as a paradox as it acts as a plasma antioxidant or pro-oxidant within the cell (Sautin and Johnson, 2008). The role of uric acid as a marker of oxidative stress and its role as an antioxidant are still under debate (Glantzounis et al., 2005). Indeed, our study showed that pilocarpine decreased plasma uric acid. But ketamine alone had no difference on plasma uric acid compared to the control group. However, post-treatment with ketamine increased plasma uric acid. Therefore, uric acid may play a role in the antioxidant defense systems after pilocarpine-induced status epilepticus in mice. In brief, the present study demonstrated that these findings may explain, at least in part, the mechanisms underlying ketamine on the dynamics of the pro-oxidant/antioxidant system of blood plasma in this disease.

Conclusion

The present study demonstrates that ketamine exerts a potent antioxidant action against pilocarpine-induced oxidative stress in mice. The protective effect of ketamine is attributable, at least in part, to activation of plasma...
antioxidant systems, uric acid and reduction of MDA and NO levels which preserve the balance between pro-oxidant and antioxidant systems. Thus the administration of low dose ketamine alone does not affect plasma antioxidant systems and pro-oxidant in mouse plasma. This fact suggests that ketamine activates indirectly plasma antioxidant systems plasma only in the pathological conditions. Biochemical (western blots, ELISA analysis) and immunohistological studies will be performed to explain the effects of exposure to ketamine in adult epileptic mice.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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