Research Article

Manual Acupuncture Attenuates iNOS and TNF-Alpha Production and Modulates Stress Oxidative after Acute Seizures in Mice

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ABSTRACT

The use of acupuncture in the treatment of Central Nervous System (CNS) disorders is an age-old practice. Although only a few studies have proven its efficacy, evidence has indicated the use of acupuncture to treat different types of seizures. Therefore, the present study aimed to evaluate the effect of Manual Acupuncture (MAC) using a chemical acute model. Also the effect of MAC on modulation of oxidative stress parameters, nitric oxide (NO) and TNF-α production after Pentylenetetrazole (PTZ)-induced acute seizure was evaluated. Mice received PTZ (88 mg/kg s.c.) once on the eleventh day, after MAC treatment. MAC was applied at acupoint GV20 received the application daily during ten consecutive days. Diazepam (DZP) (2 mg/kg) was used as positive control. Also, we evaluated the MAC effect associated with DZP (MAC/DZP) at a low dose (0.15 mg/kg). The results demonstrated that MAC or MAC/DZP were not able to reduce seizure occurrence or to increase the latency to the first seizure. PTZ-induced seizure caused significant neuronal injury, oxidative stress, and expression of the pro-inflammatory Tumor Necrosis Factor-α (TNF-α) and Inducible Nitric Oxide Synthase (iNOS). These effects were reversed by treatment with MAC or MAC/DZP. These results indicated that the stimulation of acupoint GV20 by MAC showed no potential antiepileptogenic effect in the model used, although it greatly promoted neuronal protection, which may result from antioxidant and anti-inflammatory effects observed here.

INTRODUCTION

Complementary and alternative medicine such as Manual Acupuncture (MAC) and electro acupuncture are practiced as a form of therapy for more than 3,000 years in Asia [1,2]. Medical doctors practice acupuncture under the guidance of meridian theory to achieve de qi status [3]. To perform acupuncture, thin and sterile metal needles are used to penetrate specific stimulation points termed acupoints [1].

Neurologic disorders have been shown to benefit from acupuncture, especially neurodegenerative diseases, including Alzheimer's Disease (AD) and Parkinson's Disease (PD), once the acupuncture can inhibit the accumulation of toxic proteins in neurological diseases, modulate energy supply based on glucose metabolism, depress neuronal apoptosis [2]. Some clinical studies have demonstrated that acupuncture also produces favorable effects on varied types of epilepsy, such as an absence seizure,

febrile convulsion, generalized clonic-tonic seizure, and even status epilepticus, improving the patient's quality of life [4].

Epilepsy is a common neurological disorder that affects approximately 0.5%–1.0% of the general population [5] and is characterized by recurrent and excessive neuronal discharges that result in detrimental effects on patients and relatives' health and quality of life, generating important psychological and economic burdens to them [6,7]. Antiepileptic Drugs (AEDs) are usually the first-line treatment for epilepsy. However, approximately 30% of patients continue to have seizures [4,8]. This has encouraged a considerable research for new and complementary treated with less or no adverse effects like MAC, which is safely used in children and pregnant women [1,9].

Experimental data have demonstrated the intrinsic relationship between epilepsy, oxidative stress, and inflammation, and showed that specific inflammatory molecules such as free radicals generated by oxidative stress are involved in a significant loss of neuronal cells after cerebral insults from seizures [10-12]. Khalil et al. [13] found that the electric acupuncture suppressed tumor necrosis factor-α (TNF-α) and interleukin-1 b (IL-1b) in the striatum and midbrain, similarly, bee venom acupuncture therapy suppressed TNF-α and IL-1b in PD animal. In previous studies, we have demonstrated that MAC has antioxidant and anti-inflammatory beneficial effects on chronic seizure in mice [6]. Therefore, the aim of this study was to determine the effect of MAC alone or associated with diazepam on seizures using a Pentylenetetrazole (PTZ)-induced acute model in mice. In addition, the effect of MAC on modulation of oxidative stress parameters, Nitric Oxide (NO) and TNF-α production was evaluated.

MATERIAL AND METHODS

Animals

Seventy two male CF-1 mice (2-3 months of age, 30-40 g) obtained from the biotery of the Federal University of Rio Grande do Sul (UFRGS) were used in this work. Animals were housed in plastic cages (5 per cage), with water and food ad libitum, under a 12 h light/dark cycle (lights on at 7:00 AM), and at a constant temperature of 23 ± 2°C. All experimental procedures were carried out in accordance to the national and international legislation (Guidelines of Brazilian Council of Animal Experimentation – CONCEA – and EU Directive 2010/63/EU for animal experiments), with the approval of the Committee on the Ethical Use of Animals of UFRGS (authorization number 28514). All protocols were designed aiming to reduce the number of animals used to a minimum, as well as to minimize their suffering. Mice were assigned randomly into groups according to the experimental model.

Drugs and pharmacological procedures

Pentylenetetrazole (PTZ) was purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Diazepam (DZP) (Compaz®) was purchased from Cristália Produtos Químicos e Farmacêuticos Ltda and was used as the reference anticonvulsant drug for comparison (positive control). PTZ and DZP were dissolved in saline solution/NaCl 0.9% (SAL) and administrated subcutaneously (s.c.) and intraperitoneally (i.p.) respectively at a volume of 10 mL/kg body weight. Primary antibodies (anti-TNF-α [Sc52746], anti-iNOS [Sc7271], and anti-β-actin [Sc69879]), and secondary antibodies were obtained from Santa Cruz Biotechnology (California, USA).

PTZ-induced acute seizures and MAC treatment

Seizure induction was carried out as described by Coelho et al. [14] with minor modifications. The PTZ dose used was 88 mg/kg (s.c.), obtained through a pilot test. This convulsant agent was administered once on the eleventh day, after MAC treatment. Mice were allocated into eight groups that received the application of MAC or not. Animals that received MAC treatment were divided into three groups and called MAC Sal/Sal, MAC Sal/PTZ, MAC DZP 0.15 mg/kg/PTZ. Animals that did not receive a MAC application were divided into four groups and received Sal/Sal, Sal/PTZ, DZP 2mg/kg/PTZ or DZP 0.15 mg/kg/PTZ. One group was called Sham Sal/PTZ (Figure 1).

Animals treated with MAC received the application daily during ten consecutive days. Acupuncture needles (Dong Bang Acupuncture Inc., Republic of Korea) were inserted to a depth of 3mm at GV 20 acupoint (Baihui) [15], located at the midmost point of the parietal bone, and turned at a rate of two spins per second for 15s, during 10 minutes. Right after ten days of MAC treatment, on the eleventh day animals were given intraperitoneal injections of DZP (0.15 mg/kg) or saline, 30 min prior the PTZ administration. It was also used the "nonacu" to control the non-specific effects of acupuncture

stimulation (sham group). This point was set at a point that was approximately 3mm to the lateral side of the tail on the gluteus muscle, and was simulated at the same time and duration of application on the acupoint. Injections were given between 7 and 11 a.m.

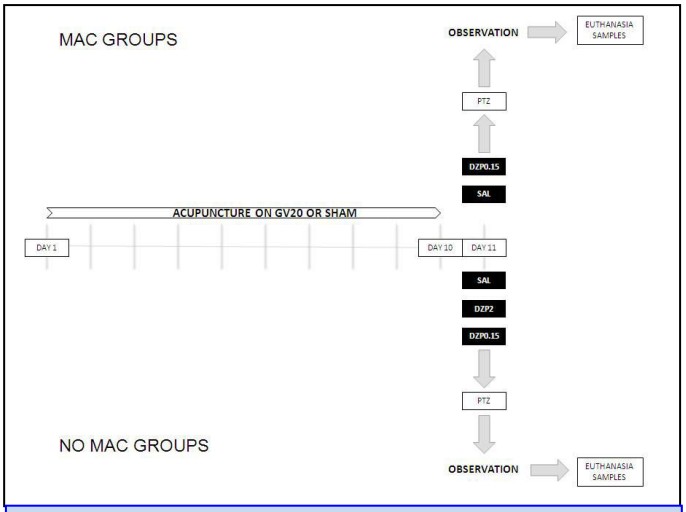

Figure 1: The timeline of PTZ-induced acute seizures in mice.

DZP, a GABAA receptor agonist, was used in this study as a positive control (2 mg/kg) or it was used in low dose (0.15 mg/kg) to assessment of its association with MAC in the PTZ-induced acute seizures.

In groups not submitted to the MAC or sham treatment, animals were given intraperitoneal injections of DZP (0.15 mg/kg), DZP (2 mg/kg) or saline, 30 min before the convulsive stimuli (PTZ 88 mg/kg). Immediately after PTZ injection, mice were placed individually in acrylic observation chambers for 30 min. and behavioral seizure was observed. The occurrence of clonic forelimb seizures as long as or lasting longer than 3 s and the latency to the first seizure was also observed at each PTZ administration day.

Tissue homogenization

Frozen cortex from each treated mice was homogenized in ice-cold phosphate buffer (KCl 140 mmol/L, phosphate (20 mmol/L, pH 7.4) and centrifuged at 12,000 rpm for 10 min, the supernatant was used. Protein concentration was measured according to the method of Lowry et al. [16] using serum bovine albumin as standard.

Free radical levels

Homogenates were overlaid with 100 µL of 25 µM Dichlorofluorescein Diacetate (DCFDA) and were placed back in the incubator for an additional 30 min at 37°C. At the end of the incubation period, plates were removed, and fluorescence of the homogenates was measured on a SpectraMax M2e Microplate Reader (Molecular Devices, MDS Analytical Technologies, Sunnyvale, California). The excitation/emission wavelengths for DCFDA were 480/520 nm. Values of Relative Fluorescence (RFU) were expressed as RFU mg−1 protein [17].

Superoxide dismutase (SOD) activity

SOD activity was evaluated by quantifying the inhibition of superoxide-dependent autoxidation of epinephrine, verifying the absorbance of the samples at 480 nm [18]. Briefly, to 20 µL of the homogenate was added 170 µL of a mixture containing 50 mM Glycine buffer pH 10.2 and 10 mM Catalase. After that, 10 µL of epinephrine were added and the absorbance was immediately recorded every 30 s for 12 min at 480 nm in SpectraMax M2e Microplate Reader (Molecular Devices, MDS Analytical Technologies, Sunnyvale, California). The inhibition of epinephrine autoxidation occurs in the presence of SOD, whose activity can be then indirectly assayed spectrophotometrically. One SOD unit is defined as the amount of SOD necessary to inhibit 50% of epinephrine autoxidation and the specific activity is reported as SOD Units/mg protein.

Catalase (CAT) activity

CAT activity was assayed according to the method described by Chance and Machley [19], based on the disappearance of H_2O_2 at 240 nm. Briefly, 10 µL of the homogenate was added to 180 µL of 20 mM Potassium phosphate buffer pH 7.2. Subsequently, 10 µL of 5 mM H_2O_2 were added and the absorbance was immediately recorded every 30 s for 10 min, using SpectraMax M2e Microplate Reader (Molecular Devices, MDS Analytical Technologies, Sunnyvale, California). One CAT unit was defined as one µmol of hydrogen peroxide consumed per minute and the specific activity was calculated as CAT Units/mg protein.

Western blotting

Frozen cortex from each treated mice was homogenized in ice-cold (Tris/acetate 20 mM, pH 7.5, sucrose, EDTA 1 mM, EGTA 1 mM, Triton X-100 1%, ortovanadate 1 mM, sodium glycerophosphate 1 mM, sodium fluoride 5 mM, sodium pyrophosphate 1 mM, β-mercaptoethanol 5 mM, bezamidine 1

mM, PMSF 35µg/mL, leupeptine 5µg/mL). The samples were homogenized and incubated on ice for 20 min before being centrifuged at 12,000 g for 15 min. The supernatants were assayed for protein concentration.

For the western-blot analysis, a 30µg sample of total proteins was separated on an SDS-polyacrylamide gel 15% and transferred to a polyvinylidene difluoride membrane. The membranes were blocked by incubation in TBS buffer containing Tween-20 0.1%, and dried milk 5% for 1 h at room temperature, and then washed with TBS buffer containing Tween-20 0.1%. Next, membranes were blotted overnight at 4°C with the primary antibodies (1/1,000) mentioned above. The blots were washed three times with TBS 0.1% Tween and developed with peroxidase-linked secondary antibodies (1/3,000). All blots were developed by ECL Western Blotting Detection Kit Reagent and detected using a ChemiDoc (Bio-Rad) imaging system [10].

STATISTICAL ANALYSIS

The Fisher Exact Test was applied to analyze the percentages of seizures. The latency data were analyzed by the Kruskal-Wallis test followed by Dunn's test ($p = 0.05$). Results were expressed as means ± Standard Error of the Mean (SEM). Experiments with homogenates were independently repeated at least three times, with triplicate samples for each treatment. Data were analyzed by one-way analysis of variance (ANOVA), and means were compared using Tukey's test, and the P value of ≤ 0.05 considered as statistically significant. Values represent the mean ± Standard Deviation (SD). Data were analyzed using GraphPad Prism v.5 program (Intuitive Software for Science, San Diego, CA, U.S.A.).

Results

This work aimed to evaluate the effect of MAC alone or associated to DZP on PTZ-induced acute seizures (MAC/DZP/PTZ). After the last treatment, 100% of the animals in the SAL/PTZ group (negative control) showed seizure behavior lasting at least 3 s. These results confirm that the convulsive PTZ dose used in this study (88 mg/kg) was able to induce a generalized tonic–clonic seizure (Figure 2A).

The development of PTZ-induced seizure was only prevented by pretreatment with DZP 2 mg/kg ($P \leq 0.001$). MAC and its association to DZP were not able to change the convulsive behavior of the animals (occurrence of seizure or latency to the first bilateral forelimb clonus lasting more than 3 sec; (Figure 2A and B)).

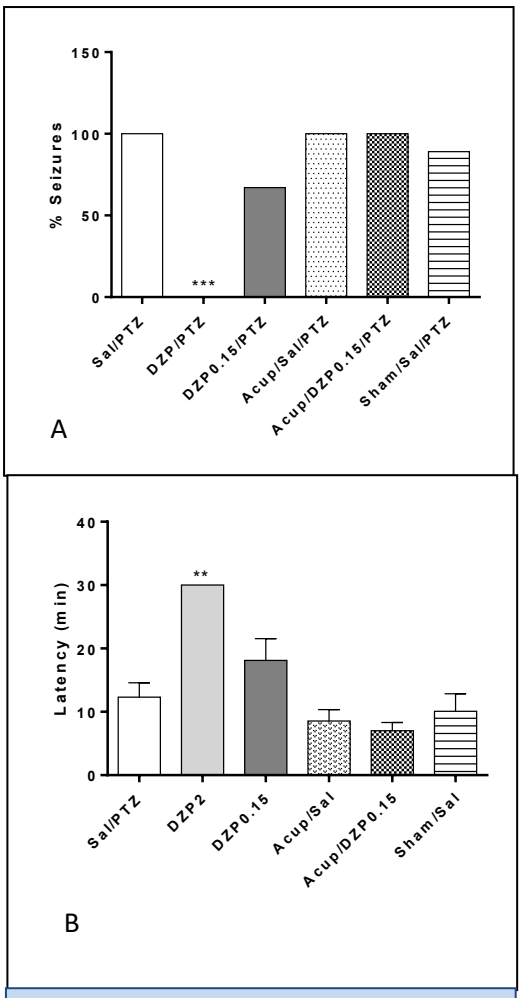

Figure 2: MAC effect on PTZ-induced acute seizures in mice. (A) Percentage of animals presenting seizures (clonic forelimb convulsions lasting more than 3 s) in the Sal/PTZ, DZP 2.0 mg/Kg/PTZ, DZP 0.15 mg/Kg/PTZ, MAC sal/PTZ, MAC DZP 0.15 mg/Kg/PTZ, Sham sal/PTZ groups after PTZ administration (88 mg/kg, s.c.). n = 09 animals per group (*** $P \leq 0.001$; Fisher's Exact Test, compared to Sal/PTZ group). (B) Latency for the 1st clonic forelimb convulsion as long as or longer than 3 s in mice submitted to PTZ-induced acute seizures. Diazepam (DZP) was used as positive control. n = 09 animals per group (** $P \leq 0.01$ compared to the Sal/PTZ group). The comparison between the six groups, according to the number of treatments were analyzed by using one-way analysis of variance (ANOVA). Kruskal-Wallis test followed by Dunn's test was used to complete the evaluation statistical calculations.

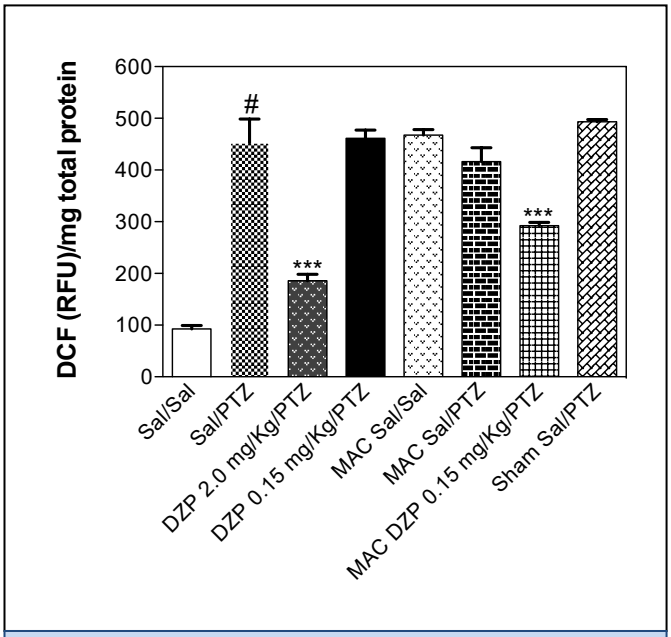

Figure 3: MAC effect on oxidative stress promoted by Pentylenetetrazole (PTZ) induced acute seizures in the cerebral cortex of mice. DCF was used to determine ROS generation in cells homogenates. More oxidizing extracellular conditions demonstrated an increase in DCF fluorescence and are indicative of an increase in intracellular ROS generation. Values represent the mean ± SD of N = 3 animals per group. *** P ≤ 0.001 compared to the saline/PTZ group; # P ≤ 0.001 compared to the saline/saline group.

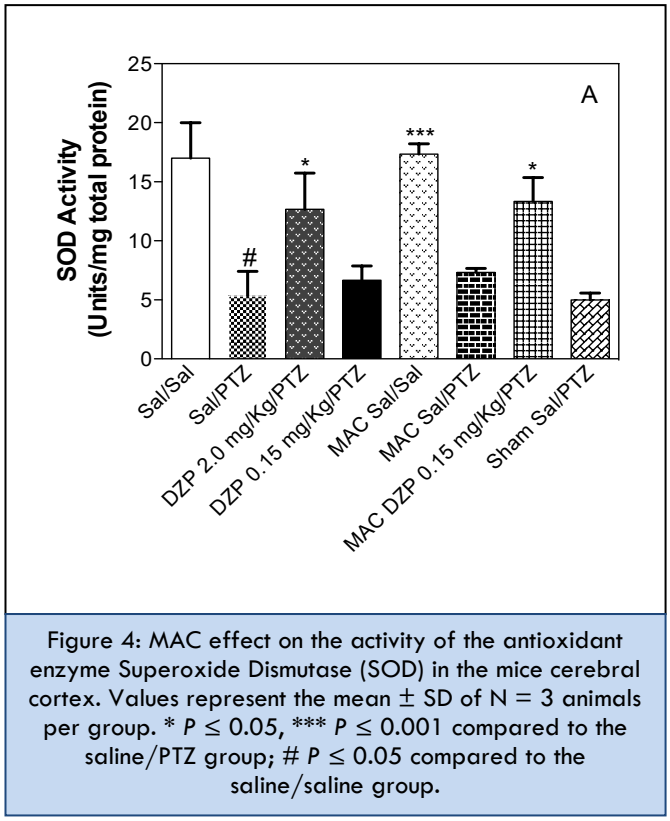

Figure 4: MAC effect on the activity of the antioxidant enzyme Superoxide Dismutase (SOD) in the mice cerebral cortex. Values represent the mean ± SD of N = 3 animals per group. * $P \leq 0.05$, *** $P \leq 0.001$ compared to the saline/PTZ group; # $P \leq 0.05$ compared to the saline/saline group.

Studies have shown that higher levels of ROS can be generated in PTZ-induced seizures. Thus, we monitored changes in DCFDA fluorescence intensity to assess intracellular ROS induced by the seizure of PTZ. The results indicated the MAC/DZP0.15/PTZ suppressive effect on ROS production, showing a significant difference compared to the SAL/PTZ control group ($P \leq 0.001$; Figure 3), just as DZP 2mg/kg was able to significantly decrease ROS production (DZP2/PTZ). Conversely, DZP0.15/PTZ administered alone did not change the DCFDA level.

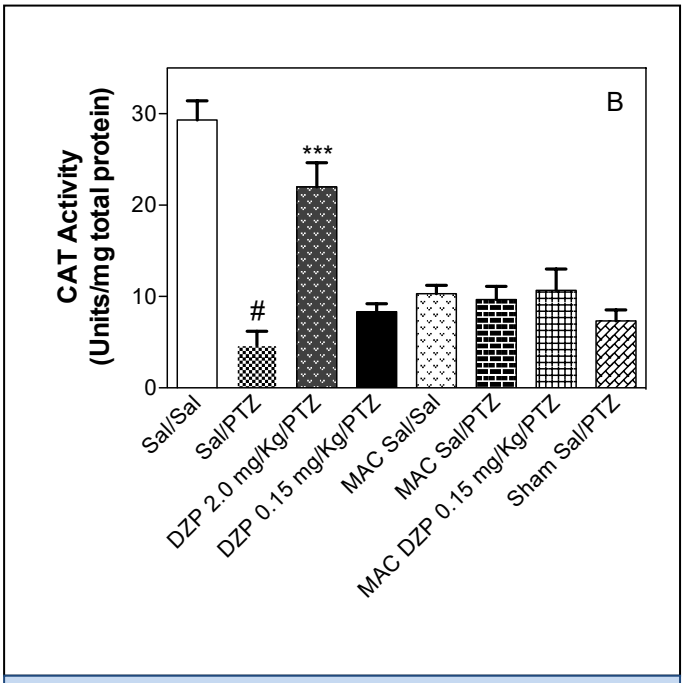

Figure 5: MAC effect on the activity of the antioxidant enzyme Catalase (CAT) in the mice cerebral cortex. Values represent the mean ± SD of N = 3 animals per group. *** $P \leq 0.001$ compared to the saline/PTZ group; # $P \leq 0.01$ compared to the saline/saline group.

In an attempt to elucidate whether the antioxidant properties of the MAC were mediated by an increase in antioxidant enzymes, Superoxide Dismutase (SOD) and Catalase (CAT) activities were measured (Figures 4 and 5). MAC/DZP0.15/PTZ treatment increased SOD activity after PTZ-induced seizure in the cerebral cortex when compared to the SAL/PTZ group ($P \leq 0.05$). CAT activity was not altered by MAC alone or combined with a low dose of DZP. Interesting to note that even in the absence of seizures (MAC/SAL/SAL) acupuncture increased the SOD activity. SOD and CAT activities were higher in DZP2/PTZ compared to the negative control. DZP0.15/PTZ was not able to induce any change.

iNOS plays an important role in generating the production of NO in cells. The expression of iNOS is usually high in neuron cells induced by inflammatory factors. TNF-α is one of the main neurotoxic agents secreted in the CNS, which can also produce neuronal death, directly and indirectly, by induction of NO and reactive oxygen species. The increased level of the iNOS and TNF-α proteins developed in PTZ-induced seizure was reduced by MAC alone or associated to DZP (Figure 6). Our results demonstrate that the increasing expression of iNOS and TNF-α after PTZ-induced seizure in the cerebral cortex was inhibited by MAC (MAC/SAL/PTZ) and its association to DZP (MAC/DZP0.15/PTZ).

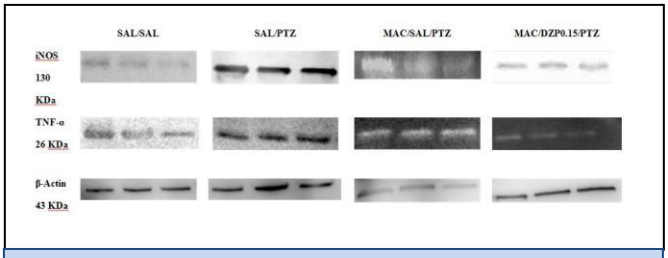

Figure 6: MAC effect on the expression of iNOS and TNF-α protein in the cerebral cortex of mice. The iNOS and TNF-α protein were examined by western blot analysis.

DISCUSSION

The ability of MAC to modulate seizures in a PTZ-induced inflammation model was evaluated based on the beneficial effects of AC on oxidative stress and inflammatory parameters, as reported in previous studies [6,20-22]. Although MAC alone did not produce a less convulsive behavior effect, it was able to reduce ROS production, increase SOD activity and improve the inflammatory parameter measured after PTZ-induced inflammation. Similarly, studies have provided evidence that MAC reduces oxidative stress and apoptosis in hippocampal neurons and improves behavioral performance in a rat model of Alzheimer's disease [23], and significantly reduces the ROS production and improve depressive behavior in psychological stress-induced depression rats [24]. Reinforcing this evidence, previous studies have shown that MAC increases SOD activity in spinal cord tissues in traumatic spinal cord injury in rats [21], also increasing catalase, superoxide dismutase and glutathione peroxidase activities in the hippocampus of alcoholic rats [25].

This is the first attempt to evaluate the protective effect of MAC in the acute PTZ-induced model in mice associated with oxidative stress and neuroinflammation parameters. Expressed in response to external stimuli, inducible NOS (iNOS) produces high NO concentrations [26] after stressful conditions such as ischemia or convulsions [27]. NO may play a proepileptogenic role in the genesis and development of some types of epilepsy. This is in line with other reports that indicate a proconvulsive role of NO in the development of various types of kindling. In this study, increased level of iNOS developed in the PTZ-induced acute seizure was observed. It may be possible for iNOS expression to somehow regulate synaptic activity by facilitating increased glutamate content and reducing GABA levels, NO is known to be able to modulate the presynaptic release of excitatory amino acids [28].

De Luca et al. [29] found that iNOS-Deficient Mice reach the kindled status more slowly than control (mice expressing the enzyme), suggesting that in basal condition the GABA-benzodiazepine inhibitory inputs are unaltered by iNOS mutation. Since there is no correlation between excitatory and inhibitory neurotransmitter imbalance and kindling development, it is possible that this difference between knockout and control mice is due to the presence of iNOS, which may be involved in long-term brain plasticity. In this sense, our results demonstrate that increasing expression of iNOS after PTZ-induced seizure in the cerebral cortex was inhibited by MAC (MAC/SAL/PTZ) and its association with DZP (MAC/DZP0.15/PTZ).

Inflammatory expressions of cytokines have been distinctly induced under various harmful conditions, according to recently published studies [30]. Microglial cells are specialized macrophages with a role in the immune system, while astrocytes, the most numerous glial cells, induce inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) when activated, contributing to neuronal death through a sustained inflammatory response [31]. In this study, PTZ administration increased the level of pro-inflammatory cortical TNF-α, known to participate in the induction and maintenance of epilepsy, as revealed using other epileptic models. Other studies have shown that TNF-α overexpression, among other inflammatory cytokines in the mouse brain, is linked to spontaneous seizures, highlighting its

crucial role in epilepsy. Therefore, high levels of brain TNF-α in PTZ-treated mice may be related to a proepileptogenic potential, possibly lowering the seizure induction threshold - this is linked to a higher seizure susceptibility - and thus laying the basis for the onset of epilepsy [32,33]. In this context, our results showed that the MAC (MAC/SAL/ PTZ) and MAC associated with a low dose of DZP (MAC / DZP0.15 / PTZ) significantly inhibited this proinflammatory factor. This result corroborates the findings of a previous study showing that MAC applied to rats submitted to spinal cord injury was able to reduce the expression of inflammatory cytokines, including IL-1β, IL-6 and TNF-α [21].

Most antiepileptics have the main effect of improving GABAA response by facilitating the opening of gamma-aminobutyric acid (GABA)-activated chloride channels [34]. The mechanism of action of benzodiazepines consists in increasing the frequency of channel opening events, enhancing the conductance of chloride ions, thus inhibiting the action potential [35]. However, its clinical use is limited by its side effects, such as sedation, psychomotor impairment, ataxia, myorelaxation, amnesia, drug interactions, tolerance, withdrawal symptoms and dependence [36,37].

Therefore, an alternative is needed that can improve its effectiveness, reducing undesirable effects and thus reducing tolerance and dependence. Unlike DZP, whose anticonvulsant mechanisms are well known, several studies have proposed different mechanisms to explain the effects of AC, among them raising cell proliferation and neuroblast differentiation by increasing the levels of brain-derived neurotrophic factor (BDNF) and phosphorylated cyclic AMP response element-binding (CREB) protein, and it was reported to exert a neuroprotective effect on dopaminergic neurons through anti-inflammatory and neurotrophic effects [1,38,39].

Several clinical evaluations have shown that AC has positive effects on various types of epilepsy. Benefits include absence of seizure as well as febrile seizures, generalized clonic-tonic seizure, and even Status Epilepticus (SE). In general, therapeutic benefits include improved electroencephalogram (e.g., reduction of peak wave and desynchronization), epileptic symptoms (e.g. decreased seizure frequency and lack of seizures), severity of SE, functional recovery and quality of life [1,6,40-43].

Preclinical studies have demonstrated the antiepileptic effects of AC in several experimental models of epilepsy. Yang et al. [44] observed that acupuncture treatment may contribute to its protective effect on seizure-induced brain injury, through up-regulating GRP 78 protein expression and down-regulating CHOP protein expression level in epilepsy rats. Chen et al. [4] showed that Electroacupuncture (EA) can reach the 27% reduction in PTZ-induced epileptiform neuronal activities of the ventroposterior lateral thalamus of rats. The antiepileptic effect of EA is related to its role in down-regulating hippocampal Ca^{2+} level increase in PTZ-like epilepsy in rats [45]. Guo et al. [46] realized that EA at ST36 acupoint reduced the times of spontaneous recurrent seizure and elevated the expression of GAD(67) mRNA in the dentate gyrus, suggesting EA at ST36 may have a healing effect on epileptic rats. AC treatment has a protective effect on pyramidal cells of the hippocampal CA1 and CA3 regions in epileptic rats, which is related to the normal function of the intracellular PI(3)K/Akt signaling pathway [15]. EA, when applied to head acupuncture points (e.g. GV20), improves cognitive deficits and prevents atrophy of some limbic structures in pilocarpine-epileptic rats, which has been shown to depend on the serotonergic system [47].

In addition, AC facilitates the release of neuropeptides by modulating gene expression in neuronal cells in the CNS, inducing deep physiological effects and activating self-healing mechanisms. Besides, AC promotes neurogenesis in various diseases through concomitant stimulation in ST36 and GV20 acupoints, which ameliorates hippocampal-related neuropathologies, such as epilepsy and ischemia, inducing neuroblast differentiation and cell proliferation [48].

Looking at the findings, we can suppose that MAC has an anti-inflammatory effect as it reduces TNF-α levels, which may play an important role in epileptogenesis, being responsible for increasing microglial glutamate release, positively regulating AMPA receptors - increasing glutamine transmission - allowing excessive calcium uptake - causing neurotoxicity - and inducing GABA receptor endocytosis, which reduces the inhibitory unit and causes relevant changes in excitability [49]. In addition to inflammation, Nitric Oxide (NO) plays an essential role in epileptogenesis and excitotoxicity in the brain [50], so by evaluating the results, we can presume that MAC has an antioxidant effect, perhaps not by sequestering free radicals,

but through increased SOD activity, as well as of reduction of the enzyme inducible Nitric Oxide Synthase (iNOS) which is expressed essentially in all cell types in the nervous system, particularly in conditions such as epilepsy, both in humans and in spontaneously epileptic mice [50] and can have neurotoxic effects, especially on glial cells [51].

In this work, combined treatment of MAC with a low dose of DZP decreases ROS levels, as well as increases SOD activity. DZP has distinct actions as a positive allosteric modulator of GABAA receptors, however the mechanism underlying the effects of MAC should be enlightened. This association attenuates neuroinflammation after PTZ-induced seizure, as demonstrated by inhibition of TNF-α and iNOS expression, maybe without the adverse effects associated with high doses of benzodiazepines. Considering the findings of this wok, we suppose that reduction in TNF-α and iNOS levels, as well as an increase in SOD activity, were not strong enough to translate into a decrease in convulsive behavior of animals, as previously observed in work published by our group [6]. Thereafter, in an attempt to mitigate neurological symptoms of epileptogenesis, an association therapy, including MAC and benzodiazepine may become a new therapeutic strategy.

CONCLUSIONS

Our findings reveal that the stimulation of acupoint GV20 by MAC did not decrease PTZ- induced acute seizures, but MAC alone or MAC in association with a low DZP dose demonstrated neuroprotective effects, improving brain antioxidant defenses, and decreasing the expression of the pro-inflammatory TNF-α and iNOS. Thus, MAC should be more thoroughly investigated in further studies focused on its potential use in epilepsy and other disorders associated with the CNS, or as a possible adjuvant in conventional pharmacotherapy.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this publication and there has been no significant financial support for this study that could have influenced its outcome.

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