Original article

Effect of Alpha Lipoic Acid on hyperemia and trigeminovascular nociceptive activity induced by cortical spreading depression

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Objective Cortical spreading depression (CSD) is a phenomenon associated with migraine attack, which can cause cerebral hyperemia and activation of the trigeminovascular nociceptive system. Alpha-lipoic acid (ALA) was reported to have the potential of reducing attack frequency in migraine patients. However, the underlying mechanism was unclear. This study aimed to investigate the effects of ALA on cerebral hyperemia and activation of the trigeminovascular nociceptive system in a CSD migraine animal model.

Methods Wistar rats were divided into 7 groups: control group, CSD group, 4 CSD with ALA-pretreated groups, and a sumatriptan-pretreated group. pretreated groups received intravenous injection (i.v.) of saline or ALA at 10, 30, 100 or 300 mg/kg bodyweight 30 minutes before CSD induction or sumatriptan at 0.4 mg/kg bodyweight 5 minutes before CSD induction by placing 3 mg of solid potassium chloride (KCl) on the right parietal cortex. Cerebral blood flow was monitored using a laser Doppler flowmeter for 2 hours. After blood flow measurement, brain tissue was collected for c-Fos staining at the trigeminal nucleus caudalis (TNC).

Results Application of KCl produced a series of hyperemia peak characteristics of CSD. ALA pretreatment reduced amplitude and the number of hyperemic peaks, and increased the period (peak-to-peak duration), similar to sumatriptan pretreatment. Furthermore, 30, 100, and 300 mg/kg body weight of ALA showed a larger reduction of c-Fos positive cells than sumatriptan at the TNC.

Conclusion ALA pretreatment reduces CSD-induced cerebral hyperemia and activation of the trigeminovascular nociceptive system. These findings support the role of ALA as a prophylactic drug for migraine headache. **Chiang Mai Medical Journal 2015;54(4):185-96.**

Keywords: cortical spreading depression (CSD), hyperemia, trigeminovascular nociceptive system, alpha-lipoic acid (ALA), prophylactic migraine treatment

Introduction

Migraine headache is a significant source of both medical costs and lost productivity. Indirect costs are around 15 billion US $ worldwide, of which absence from work is the

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greatest component[1]. Those who do attend work with migraine are less effective by around one third[2]. Migraine attack is characterized by a severe, debilitating headache associated with nausea, vomiting, photophobia and/or phonophobia[3]. Management of migraine is more effective if medications are used early in an attack[4]. Medication such as sumatriptan is effective for both pain and nausea[4,5]. It is used for patients with moderate to severe pain or those with milder symptoms who do not respond to simple analgesics[6]. In general, all triptan medications appear to be equally effective with similar side effects. However, overuse of medications, including triptans, may cause headaches, which become more severe and frequent[7]. Thus, preventive measures, including prophylactic medications, are preferable to the frequent use of abortive drugs that might cause headaches from overuse. To prevent migraine effectively, medications should reduce the frequency or severity of migraine attacks by at least 50%, which would lead to improved quality of life[8].

Alpha-lipoic acid (ALA) is a natural metabolic antioxidant substance that provides neuroprotective effects in many conditions[9] including traumatic brain injury[10], cerebral ischemia[11], and neurodegenerative diseases[12]. Recently, a randomized controlled trial reported that the frequency of attack, headache days, and headache severity were reduced in patients receiving ALA for 3 months[13]. However, the trial was incomplete and the mechanism of ALA remained unknown. Therefore, this study aimed to determine the role and mechanism of ALA as a dietary supplement for migraine prevention by using cortical spreading depression (CSD) as a migraine model in rats. It has been demonstrated that CSD is not only a pathophysiologic mechanism of aura, but also of migraine headache via stimulation of the trigeminovascular nociceptive system[14]. Therefore, an animal CSD model has been well accepted and recommended for use as an investigative model for anti-migraine drug development. In addition, many drugs that can prevent migraine effectively have been shown to reduce CSD production[15].

CSD is depolarized waves moving across the surface of the cerebral cortex at the speed of 2-5 mm/minute[16]. Studies have shown that CSD occurs following several pathological conditions such as head trauma, stroke, exacerbated brain injuries and migraine. Several studies on migraine indicated strong association between CSD and the aura phase, which occurs prior to the headache phase in patients who have migraine with aura[17,18]. CSD can be induced in a rat migraine model by a variety of stimuli such as continuous cortical application of solid potassium chloride (KCl), infusion of a nitric oxide and mechanical stimulation of meninges[19,20]. It is accompanied by a series of changes in neurovascular responses, including marked alteration of ion homeostasis, release of several neurotransmitters and a short-lasting increase in regional cerebral blood flow or hyperemia[21-23]. CSD also could lead to migraine headache by stimulation of the pain pathway of the trigeminovascular nociceptive system. Increased pain signal transmission along the pain pathway of the trigeminovascular nociceptive system is indicated by an increased number of c-Fos positive cells at lamina I and II of the trigeminal nucleus caudalis (TNC)[14].

According to the CSD model, an effective anti-migraine drug should be able to reduce CSD, cerebral blood flow or hyperemia, and eventually the number of c-Fos positive cells at the TNC. An effective anti-migraine medication such as sumatriptan; a serotonin receptor agonist, was shown to reduce the release of nitric oxide responsible for vasodilation during CSD, and the number of c-Fos positive cells in rats and cats, when given before CSD induction[20]. Therefore, to determine the role of ALA in migraine prevention, it is essential that the effect of ALA on cerebral blood flow and number of c-Fos positive cells at the TNC in rats is comparable to that of sumatriptan under the induction of hyperemia and activation of the trigeminovascular nociceptive system by CSD, which is considered to be a prominent underlying mechanism of migraine headache.

Methods

Adult male Wistar rats (250–350 g) were purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. They were allowed to acclimatize to housing conditions for 1 week before the experiment. All experiments were conducted in accordance with the approved standard guidelines for animal experimentation of the Faculty of Medicine, Chulalongkorn University and the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes by the National Research Council of Thailand 1999.

Study design

Animals were divided into 7 groups (6 to 8 rats in each group) as follows: control, CSD, CSD with ALA pretreatment at the concentration of 10, 30, 100 or 300 mg/kg bodyweight and CSD with sumatriptan pretreatment. They were housed in a light/dark cycle with the light on from 6.00 to 18.00. Food and water was provided ad libitum.

Pretreated rats were given ALA or normal saline via intravenous (i.v.) injection 30 minutes before CSD induction or sumatriptan at 0.4 mg/kg bodyweight via i.v. 5 minutes before CSD induction. To induce the CSD, 3 mg of KCl was placed on the rats' cerebrocortical surface. Solid sodium chloride (NaCl) of the same weight was applied on the cortex of the control animals. The cerebral blood flow (CBF) was monitored continuously for 120 minutes using a Laser Doppler flowmeter. After completion of the CBF recording, all of the rats were sacrificed using an excessive dose of pentobarbital.

Surgical operation and CSD induction

The rats were anesthetized with pentobarbital sodium (50 mg/kg bodyweight, intraperitoneally) and mechanically ventilated through the tracheostomy opening with a positive pressure ventilator (Rodent ventilator model 683, Harvard Apparatus, South Natick, USA). Blood pressure was monitored continuously with an intra-arterial pressure transducer (Gould P23 Statham, USA) placed in a femoral artery. Data were recorded continuously and digitized using a data acquisition system for off-line analysis (PowerLab, ADInstruments, CO, USA). After tracheostomy and cannulation, the rats were placed on a surgical frame with their head fixed to a head holder. Craniotomy (2 mm in diameter) was performed on the parietal bone at 7 mm posterior and 1 mm lateral to the bregma. The dura was opened to expose the cortical surface. Three milligrams of solid KCl were placed directly on the surface of the parietal cortex. NaCl was placed instead of KCl in the control group.

CBF monitoring

To measure the CBF, an anterior craniotomy (2 mm in diameter) was performed in the parietal bone at 1 mm anterior and 1 mm lateral to the bregma. Hypothermia and drying of the cortical surface were prevented by superfusion with artificial cerebrospinal fluid (NaCl 118 mM, KCl 4 mM, $Na_2HPO_4.H_2O$ 1 mM, $NaHCO_3$ 25 mM, $CaCl_2.2H_2O$ 1.5 mM, $MgSO_4.7H_2O$ 1.2 mM, dextrose 5 mM in distilled water, pH 7.4, 37 ^oC). A fiber optic needle probe of the Laser Doppler flowmeter (MNP110XP; ADInstruments, CO, USA) was placed perpendicularly, at a distance of 2 mm above the cortical surface. The wavelength of the laser beam was 780 nm. Changes in CBF in response to CSD or non-CSD induction were recorded continuously for 2 hours.

Measurement of c-Fos expression

After taking CBF measurements, the rats were perfused transcardially with 250 ml of ice-cold phosphate buffered saline (PBS), pH 7.4. The cervical part of the spinal cord was removed and immersed in 4% paraformaldehyde in 0.1 M PBS, pH 7.4.

The caudal medulla (3 mm caudal to the obex) to the first cervical cord was fixed overnight in 4% paraformaldehyde in 0.1 M PBS, pH 7.4, and then placed in a cryoprotective solution consisting of 30% sucrose in 0.1 M PBS, pH 7.4. The tissue was cut into transverse serial sections at 30 µm thickness using a cryostat at -20 ^oC (Leica CM 1,580, Germany). One in every five sections was collected serially and then rinsed in 0.1 M PBS. All sections were prepared for c-Fos immunochemistry staining, as described by Supornsilchai W[24]. The number of c-Fos positive cells in lamina I and II of the TNC was determined using image analysis software (ImagePro® Plus; Media Cybernetics Inc., Bethesda, Maryland, USA).

Statistical analysis

All data were expressed as mean±standard error of mean (SEM), and analyzed for possible statistical significance using ANOVA for repeated measurements with the Student-Newman-Keuls Method. P-values of less than 0.05 were considered statistically significant.

Results

CSD-evoked changes in CBF

KCl application produced CSD that caused repeated cycles of cerebral hyperemia, while NaCl had no effect on CBF (Figure 1). Hyperemic peaks caused by CSD in each animal in the CSD groups had different amplitudes. Thus, amplitudes of all hyperemic peaks in

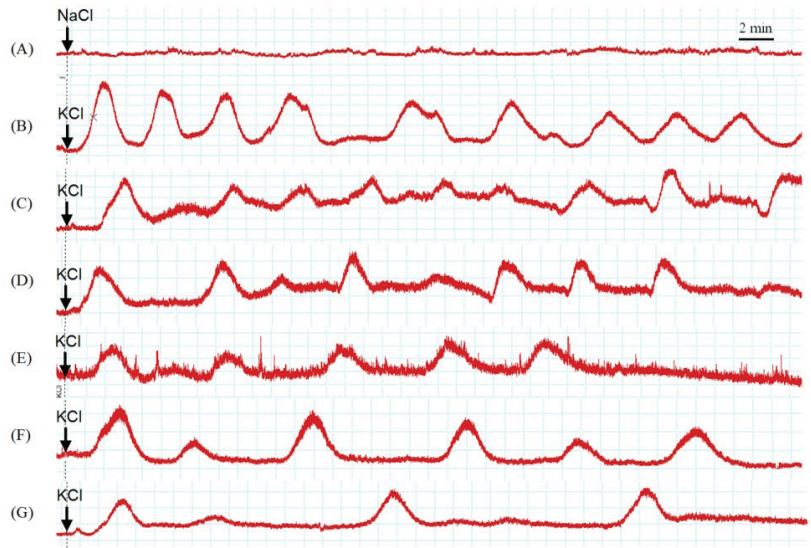

Figure 1. Changes of cerebral blood flow (CBF) with time after application of 3 mg of NaCl in the control group (A), 3 mg of KCl in the CSD group (B), and 3 mg of KCl in the CSD group with 30-minute pretreatment of ALA 10 (C), 30 (D), 100 (E), 300 (F) mg/kg bodyweight or 5-minute pretreatment with sumatriptan (G).

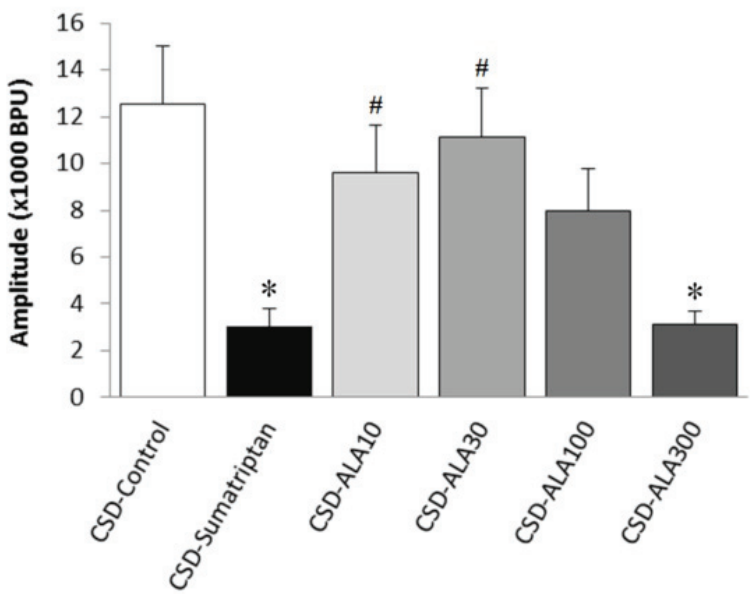

Figure 2. Average cumulative blood flow amplitude. Data are expressed as mean+SEM; n = 8 in the CSD-control group, n=7 in the CSD-ALA10 and CSD-ALA 30 group, n = 6 in other groups. All differences are significant at p <0.05. Asterisk (*) denotes significant difference between the CSD-control group and other groups; the hash mark (#) indicates significant difference between the sumatriptan-pretreated group and ALA-pretreated group.

each animal were added together during the recording period to produce a cumulative blood flow amplitude, which represented a total increase in blood flow caused by CSD waves. The average cumulative blood flow amplitude in the CSD control group was 12.54±2.50 x10^3 BPU (Figure 2). The average period (peak-to-peak duration) was 5.47±0.32 minutes

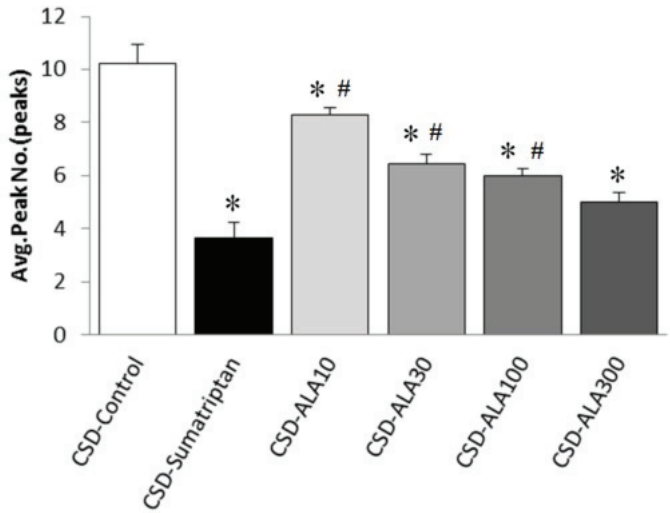

Figure 3. Average numbers of hyperemic peaks. Data are expressed as mean+SEM; n = 8 in the CSD-control group, n=7 in the CSD-ALA10 and CSD-ALA 30 group, n = 6 in the other groups. All differences are significant at p <0.05. Asterisk (*) denotes significant difference between the CSD-control group and other groups; the hash mark (#) indicates significant difference between the sumatriptan-pretreated group and ALA-pretreated group.

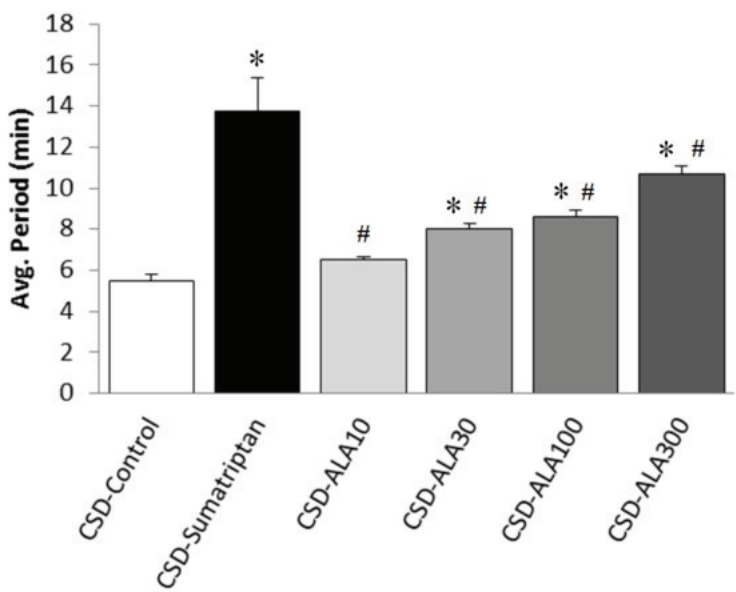

Figure 4. Average periods between cycles. Data are expressed as mean+SEM; n = 8 in the CSD-control group, n=7 in the CSD-ALA10 and CSD-ALA 30 group, n = 6 in other groups. All differences are significant at p <0.05. Asterisk (*) denotes significant difference between the CSD-control group and other groups; the hash mark (#) indicates significant difference between the sumatriptan-pretreated group and ALA-pretreated group.

(Figure 3). The average number of peaks per hour was 10.25±0.70 (ranging from 8 to 14) (Figure 1 and 4). The results confirmed that only KCl, and not NaCl, could induce CSD resulting in episodic increases of CBF.

Effect of sumatriptan on CSD-evoked changes in CBF

Pretreatment with sumatriptan 5 minutes before CSD induction by KCl reduced cerebral hyperemia significantly. The average cumu-

lative blood flow amplitude was reduced to 3.03±0.76x10^3 BPU, when compared with that of the CSD-control group (Figure 2). The average number of peaks per hour was 3.67±0.56, with that being a significant reduction when compared with CSD-control group (Figure 3). The average period was increased to 13.76±1.58 minutes (Figure 4). The results clearly show that sumatriptan reduced cerebral hyperemia by decreasing the amplitude of cumulative increase in CBF, decreasing the amount of episodic blood flow, and lengthening the period between individual blood flow events.

Effect of ALA on CSD-evoked changes in CBF

The average cumulative blood flow amplitude in the pretreated groups of ALA at 10, 30, 100 and 300 mg/kg bodyweight was 9.60±2.01, 11.15±2.07, 7.96±1.80 and 3.10±0.56x10^3 BPU, respectively (Figure 2). Only the pretreated groups of ALA at 300 mg/kg bodyweight could reduce cumulative blood flow amplitude significantly, when compared with the CSD-control group. The average number of peaks per hour in the pretreated groups of ALA at 10, 30, 100 and 300 mg/kg bodyweight was 8.29±0.29, 6.43±0.37, 6.0±0.26 and 5.00±0.38 peaks, respectively (Figure 3). The number of peaks in the pretreated groups of ALA at 30, 100 and 300 mg/kg bodyweight was reduced significantly when compared with the CSD-control group. The average cycle period was 6.49±0.17, 7.99±0.29, 8.63±0.32, and 10.67±0.42 minutes in the pretreated groups of ALA at 10, 30, 100 and 300 mg/kg bodyweight, respectively (Figure 4). A significant increase in cycle period was observed in only the pretreated groups of ALA at 100 and 300 mg/kg bodyweight when compared with the CSD-control group.

Comparing the effects of ALA and sumatriptan on CSD-evoked changes in CBF

ALA at 300 mg/kg bodyweight reduced local cerebral hyperemia by reducing cumulative blood flow amplitude (Figure 2) and average numbers of hyperemic peaks (Figure 3), as well as increasing the average period or duration between each peak (Figure 4). However, ALA at 300 mg/kg bodyweight did not lengthen the duration between each hyperemic peak as much as sumatriptan (Figure 4).

Although ALA at 30 and 100 mg/kg bodyweight could reduce the average peak number and prolong the duration between peaks, the effects were dissimilar to those of sumatriptan. ALA at 10 mg/kg bodyweight only affected the average number of peaks, and not the amplitude or period of hyperemic peaks, when compared with the CSD-control. However, the effect of ALA at 10 mg/kg bodyweight on the hyperemic peak number was less than that of sumatriptan (Figure 3). Thus, ALA at 300 mg/kg bodyweight had similar effects to sumatriptan regarding blood flow amplitude, number of peaks, and peak period.

Effect of CSD on the number of c-Fos positive cells at the trigeminal nucleus caudalis (TNC)

CSD increased the number of c-Fos positive cells at lamina I and II of the TNC (Figure 5). The average number of c-Fos positive cells in the CSD group (14.63±1.24 cells) was significantly higher than that of the non-CSD control group (1.42±0.35 cells). The result indicated that CSD activated the neurons at the TNC of the trigeminovascular nociceptive system.

Comparing the effects of ALA and sumatriptan on the number of c-Fos positive cells induced by CSD at the TNC

Sumatriptan reduced the number of c-Fos positive cells significantly at the TNC to 4.49±0.18 cells when compared to those in the CSD-control group (Figure 5). Similarly, ALA at 10, 30, 100, and 300 mg/kg bodyweight reduced the number of c-Fos positive cells to 4.04±0.22, 3.01±0.23, 2.74±0.16 and 2.32±0.27 cells, respectively (Figure 5). Interestingly, ALA at 30, 100, and 300 mg/kg bodyweight significantly reduced more c-Fos positive cells than sumatriptan.

Discussion

Patients with spontaneous migraine headaches have increased blood flow or hyperemia to the brain during an attack[25]. Sumatriptan is similar structurally to serotonin (5-HT), and it activates a specific receptor subtype in cranial and basilar arteries. Receptor activation causes constriction of the dilated arteries. The vasoconstrictive effect of sumatriptan is confirmed in this study, whereby cerebral hyperemia was reduced, as indicated by decreased cumulative blood flow and number of hyperemic peaks, as well as increased duration between hyperemic peaks.

As demonstrated by this study, 300 mg/kg bodyweight of ALA given 30 minutes prior to CSD induction mimicked the effects of sumatriptan. In Addition, ALA at every dosage was better than sumatriptan in reducing the number of c-Fos positive cells at the TNC. The results suggested that ALA is a better agent in reducing pain through activation of the trigeminovascular nociceptive system. It has been demonstrated that CSD induces oxidative stress in the trigeminal nociceptive system[26]. Oxidative stress produces neurogenic inflammation through the activation of TRPA1, leading to migraine headache[27]. Thus, ALA, with its anti-inflammatory and anti-oxidant properties[28], prevented or minimized activation of the trigeminovascular nociceptive system, as indicated by decreased c-Fos expression, and could reduce oxidative stress more than sumatriptan, which does not have anti-inflammatory activity. The data in this study also support the role of oxidative stress and inflammation as an important mechanism of migraine headache[29].

Additionally, the reduced number of hyperemic peaks signified the decrease in CSD production. Therefore, it is most likely that ALA is a better agent in preventing or minimizing activation of the trigeminovascular nociceptive system in response to CSD. Many studies have shown that the number of cortical hyperemic peaks corresponds with frequency of the CSD wave series, as detected by a direct current (DC) electrode[24,30]. The speed of depo-

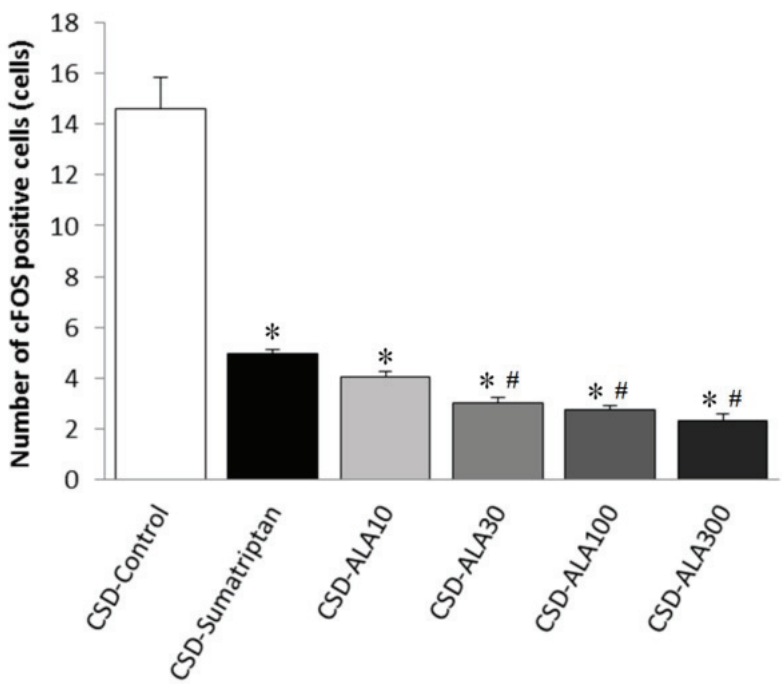

Figure 5. Numbers of c-Fos positive cells at the TNC. Data are expressed as mean+SEM; n = 6. All differences are significant at $p < 0.05$. Asterisk (*) denotes significant difference between the CSD-control group and other groups; the hash mark (#) indicates significant difference between the sumatriptan-pretreated group and ALA-pretreated group.

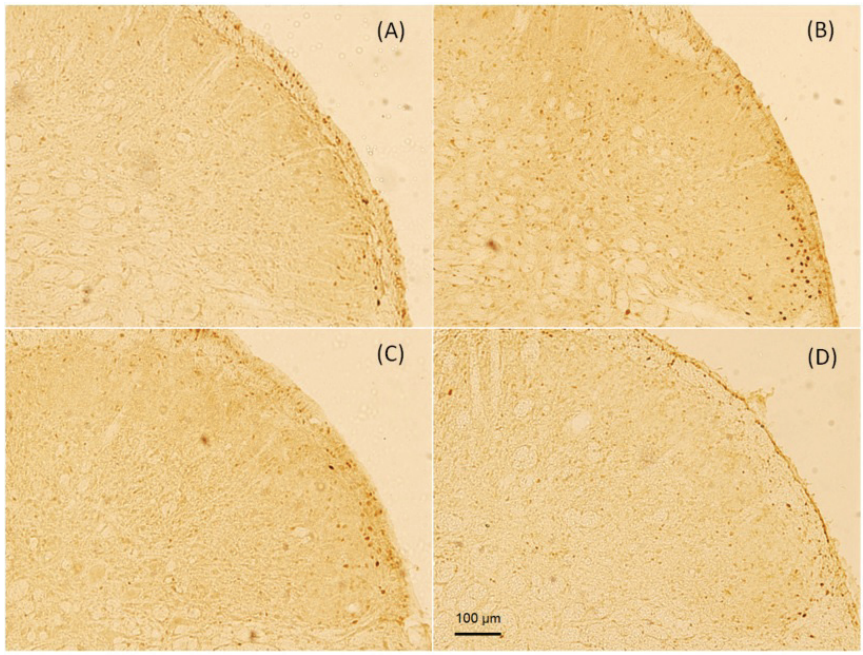

Figure 6. Effects of ALA or sumatriptan pre-treatment on CSD-evoked c-Fos expression at the trigeminal nucleus caudalis. Cells stained positive for c-Fos protein are indicated by a dark brown color. (A) non-CSD Control; (B) CSD control; (C) sumatriptan-pretreated; and (D) ALA 300-pretreated groups. Marker scale bar indicates a length of 100 µm.

larizing CSD waves traveling along the cortical surface of the patient's brain occurs during visual aura, prior to the development of migraine symptoms[17,18]. Besides migraine, CSD could occur spontaneously in hypoxic, ischemic, or hypoglycemic brain tissue, and could recover in a prolonged time course[31]. However, further investigation is required to determine the mechanism underlying CSD generation in humans. Attempts have been made to correlate CSD with migraine symptoms. Evidence in an animal model showed that CSD could activate the trigeminovascular nociceptive system[18]. Therefore, it is generally believed that CSD is an important pathophysiologic mechanism of migraine. For this reason, the reduction of CSD should minimize activation of the trigeminovascular nociceptive system, leading to a reduction in headache.

Sumatriptan has been shown to decrease activity of the trigeminal nerve[32], thereby reducing pain transmission to the central nervous system (CNS). Similar to sumatriptan, ALA at every dosage was shown to reduce activation of the trigeminovascular nociceptive system, as indicated by the decreased number of c-Fos positive cells at the TNC. Furthermore, ALA at 30, 100, and 300 mg/kg bodyweight showed a larger reduction of c-Fos positive cells than sumatriptan (Figure 5 and 6). Therefore, ALA is a good candidate agent for preventing migraine because of its ability to reduce both cerebral hyperemia and stimulation of the trigeminovascular nociceptive system, which together indicate suppression of CSD production.

Migraine was originally thought to be a systemic metabolic disorder, due to several lines of evidence[33]. During CSD initiation, the concentration of extracellular K+ ions increases, causing neurons to become excited and depolarized followed by a period of electrical silence on the brain cortical surface. Additional negative ion species move outwards to maintain electrical balance[34]. In response to CSD, the brain increases oxygen demand in order to generate energy[35-37] and maintain electrical balance. It has been estimated that when mitochondria generate energy, approximately 2% of the oxygen used is converted to oxygen

free radicals[38]. These free radicals are produced normally during oxidative metabolism and they can damage mitochondrial calcium. Not only does CSD activate trigeminovascular afferents[14], but also neurons causing increased intracellular Ca^{2+} ions that are taken up by mitochondria. Elevation of mitochondrial calcium levels leads to a production of free radicals that damage mitochondria further[39].

ALA is essential for normal oxidative metabolism, as a cofactor in mitochondrial α-keto acid dehydrogenase complexes[40]. Exogenously supplied ALA is taken up rapidly by cells and reduced to dihydrolipoate (DHLA). The reducing power of this reaction produces both Nicotinamide adenine dinucleotide (NADH) and Nicotinamide adenine dinucleotide plus hydrogen (NADPH)[41]. DHLA is one of the most potent naturally occurring antioxidants[42]. In fact, there is evidence that both ALA and DHLA are capable of scavenging a variety of free radicals[43-44]. Furthermore, DHLA appears to regenerate other endogenous antioxidants (e.g. vitamins C and E) and has a salubrious property for neutralizing free radicals without becoming one in the process itself[45,46]. Another way in which mitochondria may be important in neurodegeneration is through alterations in their effects on calcium homeostasis. Christoph Richter[47] reported that ALA also inhibits mitochondrial calcium transport, suggesting that ALA has specific effects on intracellular calcium homeostasis. Therefore, exogenously administered ALA has been considered a mitochondrial nutrient. It supports mitochondria to generate energy (NADH) for maintaining electrical balance, resulting in not only reduction of oxygen demand, but also reduction of hyperemia and elimination of oxygen radicals that damage mitochondria.

Therefore, evidence suggests that through improvement of metabolism, including mitochondrial oxygen free radicals and calcium balance, ALA can decrease the number and frequency of CSD waves, leading to concomitant changes in CBF and decreased activity of the trigeminovascular nociceptive system.

The results from this study clearly support the role of ALA as a prophylactic intervention for migraine, as demonstrated by the ability of ALA to reduce activation of the trigeminovascular nociceptive system that occurs in response to CSD; a hallmark of migraine headache.

Conflicts of interest

Authors declare no conflict of interest.

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ผลของกรดแอลฟา ลิโปอิกต่อเลือดมากเฉพาะที่ และกิจกรรมของการรับความเจ็บปวดจากหลอดเลือดตามประสาทไทรเจมินัล ที่ชักนำโดยปรากฏการณ์คอร์ติคัล สเปรดดิ้ง ดีเพรสชั่น

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วัตถุประสงค์ ปรากฏการณ์คอร์ติคัล สเปรดดิ้ง ดีเพรสชั่น (cortical spreading depression: CSD) เป็นหนึ่งในปรากฏการณ์ที่เชื่อมโยงกับอาการปวดศีรษะไมเกรน และก่อให้เกิดการไหลของเลือดไปยังสมองเพิ่มมากขึ้นร่วมกับการกระตุ้นการทำงานของระบบรับความเจ็บปวดตามประสาทไทรเจมินัลที่มาจากหลอดเลือดสมอง การศึกษาก่อนหน้านี้มีการรายงานว่ากรด แอลฟา ลิโปอิก (alpha lipoic acid: ALA) มีศักยภาพในการลดความถี่ในการปวดศีรษะไมเกรนของผู้ป่วย อย่างไรก็ตามยังไม่มีรายงานผลการศึกษากลไกการทำงานของ ALA แต่อย่างใด จึงเป็นที่มาของการศึกษานี้โดยมีเป้าหมายที่จะศึกษาผลของ ALA ต่อเลือดมากเฉพาะที่ และกิจกรรมของการรับความเจ็บปวดจากหลอดเลือดตามประสาทไทรเจมินัล ที่ชักนำโดย CSD โดยใช้หนูเป็นแบบจำลอง

วิธีการศึกษา หนูวิสตา (Wistar rats) จะถูกแบ่งออกเป็นกลุ่มควบคุม กลุ่มที่ได้รับ ALA มาก่อน และกลุ่มที่ได้รับยา sumatriptan มาก่อน หนูกลุ่มควบคุมได้รับน้ำเกลือเข้าทางหลอดเลือดดำ กลุ่ม ALA ได้รับสารขนาด 10, 30, 100 or 300 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม ทางหลอดเลือดดำ 30 นาทีล่วงหน้า ส่วนกลุ่ม sumatiptan จะได้รับสารขนาด 0.4 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม ทางหลอดเลือดดำ 5 นาที ก่อนการเหนี่ยวนำให้เกิด CSD โดยการวางผลึก potassium chloride (KCl) จำนวน 3 มิลลิกรัม ลงบนเปลือกสมองบริเวณ parietal cortex ข้างขวา จากนั้นจะมีการติดตามวัดการเปลี่ยนแปลงของปริมาณเลือดที่ไหลไปยังสมองเฉพาะที่เป็นเวลา 2 ชั่วโมง โดยใช้อุปกรณ์ laser Doppler flowmeter ภายหลังการวัดการไหลของเลือด เนื้อสมองจะถูกเก็บเพื่อนำมาย้อมสีหาปริมาณ c-Fos โปรตีนที่บริเวณ trigeminal nucleus caudalis (TNC) ของก้านสมอง

ผลการศึกษา การวางผลึก KCl ก่อให้เกิดการเพิ่มขึ้นของเลือดไปยังสมองเป็นช่วง ๆ ซึ่งเป็นลักษณะเฉพาะของการเกิดปรากฏการ์ CSD เช่นเดียวกับการให้ sumatriptan มาก่อน การให้ ALA มาก่อน สามารถลดขนาดและจำนวนของเลือดมากเฉพาะที่ และสามารถยืดช่วงระยะเวลาระหว่างการเกิดเลือดมากเฉพาะที่ ยิ่งไปกว่านั้น ALA ที่ขนาด 30, 100, และ 300 มิลลิกรัมต่อกิโลกรัม ยังสามารถลดจำนวนเซลล์ซึ่งสร้าง c-Fos ที่ TNC ได้มากกว่า sumatriptan

สรุปผลการศึกษา การให้ ALA ก่อนการเหนี่ยวนำให้เกิด CSD สามารถลดการเพิ่มของเลือดที่ไปยังสมอง และความเจ็บปวดที่เกิดขึ้นจากการกระตุ้นระบบประสาทไทร เจมินัล ผลการศึกษาที่ได้สนับสนุนการใช้ ALA เป็นยาที่มีบทบาทในการป้องกัน การปวดศีรษะไมเกรน **เชียงใหม่เวชสาร 2558;54(4):185-96.**

คำสำคัญ: ปรากฏการณ์คอร์ติคัล สเปรดดิ้ง ดีเพรสชั่น เลือดมากเฉพาะที่ กิจกรรมของการรับความเจ็บปวดจากหลอดเลือดตามประสาทไทรเจมินัล กรดแอลฟา ลิโปอิก การรักษาในเชิงป้องกัน การปวดศีรษะไมเกรน