Food and Chemical Toxicology 123 (2019) 453–458

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

The effects of sulfite on cPLA$_2$, caspase-3, oxidative stress and locomotor activity in rats

Goksun Basaranlar[a], Narin Derin[a,*], Ceren Kencebay Manas[a], Gamze Tanriover[b], Mutay Aslan[c]

[a] Akdeniz University, Medical School, Department of Biophysics, Antalya, Turkey
[b] Akdeniz University, Medical School, Department of Histology and Embryology, Antalya, Turkey
[c] Akdeniz University, Medical School, Department of Biochemistry, Antalya, Turkey

ARTICLE INFO

Keywords:
Sulfite
Phospholipase A2
Hippocampus
Locomotor activity
Caspase-3
Antioxidant capacity

ABSTRACT

Sulfite is a commonly used preservative in food products, alcoholic beverages and pharmaceutical products. We investigated the effect of sulfite, on locomotor activity as well as the relationship of these effects with oxidant and antioxidant capacities, cPLA2 enzyme activity. Thirty male Wistar albino rats were randomly divided into two groups as control(C) and sulfite(S). Animals in the S group were given freshly prepared sulfite for 35 days via gastric gavage (100 mg/kg/day) while the C group received equal volumes of distilled water via gavage for the same period. Open-field tests were performed to all groups and animals were sacrificed. Total antioxidant capacity(TAC), TBARS levels, cPLA2 activity as well as amount of caspase-3 positive cells were analyzed on the hippocampi.

In the open field test, distance and velocity values of the S group increased with respect to controls. TBARS and cPLA2 activity were also increased in the S group, while levels of TAC decreased compared to controls. Immunohistochemical analysis showed that sulfite ingestion caused an increase in the amount of hippocampal caspase-3 positive cells.

In conclusion, sulfite seemed to increase locomotor activity. cPLA2 might play a role in ingested sulfite-induced oxidative stress and apoptotic cell death in the hippocampus.

1. Introduction

Sulfite compounds such as sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3), and sodium sulfite (Na_2SO_3) are added to food, beverages and drugs as preservative (E220 – E228) for their antioxidant and antimicrobial properties (Ough, 1986). Since all chemicals are potentially toxic, the minimum level of exposure above which unfavorable health effects may occur is determined primarily using animal-based studies in which the application of safety factors such as acceptable daily intake (ADI) value for the chemical is obtained (Martyn et al., 2013). In the case of sulfites (expressed as SO_2), the Joint Expert Committee on Food Additives (JECFA) has set the group ADI-value to 0.7 mg/kg/bw/day (Til et al., 1972). However, safety of the use of sulfites has raised concerns as sulfite intake is shown to exceed ADI levels in many countries. The neurotoxic effects of sulfites have been demonstrated by recent research (Kencebay et al., 2013; Noorafshan et al., 2013; Ozturk et al., 2011; Vandevijvere et al., 2010).

Sulfite is oxidized to sulfate by the mitochondrial enzyme sulfite oxidase (Yoshida et al., 2015). A deficiency of sulfite oxidase in humans is associated with severe neurological dysfunction suggesting that neuronal cells are highly susceptible to sulfite toxicity (Ahmad et al., 2008). Although the mechanism of sulfite toxicity has not been fully elucidated, free radicals were implicated in the increased toxicity of sulfite observed when intracellular reduced glutathione was compromised (Grings et al., 2017). Additionally, one electron oxidation of sulfite produces a sulfite radical (SO_3^-) which is suggested to induce DNA (deoxyribonucleic acid) damage (Ozturk et al., 2011; Shi, 1994; Shi and Mao, 1994), and mitochondrial injury through lipid peroxidation (Aydin et al., 2005; Derin et al., 2009).

Oxidative metabolic processes such as oxidative phosphorylation by the mitochondrial respiratory chain produce reactive oxygen species (ROS) and when ROS production exceeds the antioxidant defense capacity of an organism, oxidative stress occurs (Amin and Bano, 2018). Oxidative stress may cause structural damage to nucleotides, lipids and proteins. The neuronal plasma membrane is particularly prone to oxidative insult due to its high poly-unsaturated fatty acid content and various processes participate in the repair of oxidative damage to

* Corresponding author. Akdeniz University, Medical School, Department of Biophysics, 07070, Antalya, Turkey.
E-mail address: narinderin@akdeniz.edu.tr (N. Derin).

https://doi.org/10.1016/j.fct.2018.11.021
Received 28 August 2018; Received in revised form 6 November 2018; Accepted 8 November 2018
Available online 15 November 2018
0278-6915/

Abbrevations

ADI	acceptable daily intake
$cPLA_2$	calcium dependent cytoplasmic phospholipase A_2
$iPLA_2$	calcium independent phospholipase A_2
$sPLA_2$	calcium dependent secretory PLA_2
DNA	deoxyribonucleic acid
FAO/WHO	Food and Agriculture Organization/World Health Organization
HCl	hydrochloric acid
JECFA	Joint Expert Committee on Food Additives
PBS	phosphate buffered saline
PLA_2	phospholipase A_2
$K_2S_2O_5$	potassium metabisulfite
K_2SO_3	potassium sulfite
ROS	reactive oxygen species
$NaHSO_3$	sodium bisulfite
$Na_2S_2O_5$	sodium metabisulfite
Na_2SO_3	sodium sulfite
SCF	Scientific Committee for Food
SO_2	sulfite
SO_3^-	sulfite radical
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TAC	total antioxidant capacity

cellular components (Catala, 2010; Spiteller, 2010). In the case of membrane phospholipids, members of the phospholipase A_2 (PLA_2) family of fatty acid acylases catalyze hydrolysis of sn-2 lipid bonds in glycerol phospholipids (Adibhatla and Hatcher, 2008; Chuang et al., 2015). PLA_2 promotes the formation of lysophopholipids and free fatty acids such as arachidonic acid and docosahexaenoic acid (Farooqui and Horrocks, 2006; Murakami et al., 2011). About 20 isoforms of this enzyme were identified and divided into three categories: calcium independent PLA_2 ($iPLA_2$), calcium dependent secretory PLA2 ($sPLA_2$), and calcium dependent cytoplasmic PLA_2 ($cPLA_2$). The $cPLA_2$, generally localized to the cytosol, specifically cleaves arachidonic acid from phospholipid molecules and plays a key role in the release of this fatty acid in prostanoid synthesis (Boyanovsky and Webb, 2009). An important feature of $cPLA_2$ is its link to cell surface receptors that stimulate signaling pathways associated with activation of protein kinases and production of ROS. Furthermore, $cPLA_2$ activation has been implicated in apoptosis, the programmed cell death (Wang et al., 2014).

Apoptosis is morphologically characterized by cell shrinkage, membrane blebbing, and chromatin condensation, internucleosomal DNA fragmentation, and the formation of apoptotic bodies (Streck et al., 2014). The signaling pathways occurring during apoptosis involve the activation of cysteine proteases that are part of a large family of proteins known as caspases (Yagami et al., 2018). Caspases are a class of proteases instrumental in carrying out many cellular functions including cell differentiation, remodeling and death. The general mechanism of caspase-mediated cell death is highly conserved in different cell types, including neurons from brain structures such as cortex and hippocampus. Caspases are synthesized in cells as inactive proenzymes. Upon proteolysis, initiator caspases such as caspase-8, -9, -10, and −2 cleave executioner caspases-3, -6, and-7. These effector or executioner proteases then degrade structural proteins, signaling molecules, and DNA repair enzymes (Kumar, 2007). Among the executioner caspases, activation of caspase-3 plays an extremely important role in neuronal apoptosis and is considered the terminal event preceding cell death (Snigdha et al., 2012).

Apoptosis is suggested to underlie several mood disorders, one of which is anxiety (Krolow et al., 2014). Comprehensive study has been conducted to discover neural substrates and mechanisms that contribute to the etiology of anxiety, among which the imbalance between ROS generation and antioxidant defense system, as well as apoptosis have gained attention (Ding et al., 2014). Additionally, there is great evidence suggesting hippocampus mediate anxiety disorders (Adhikari et al., 2010; Zhu et al., 2016).

Considering the fact that hippocampal oxidative stress and apoptotic events, also demonstrated to play role in anxiety disorders (Ding et al., 2014), are induced by high sulfite intake (Akdogan et al., 2011; Chiarani et al., 2008), this study was designed to investigate whether high sulfite intake contributes to the development of anxiety by causing $cPLA_2$ mediated oxidative stress and apoptotic events in the rat hippocampus.

2. Materials and methods

2.1. Animals

Thirty adult male Wistar albino rats, weighing 300–350 g, were used in the present study. Animals were provided from Akdeniz University Animal Care Unit. All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University Medical School. Rats were kept in stainless steel cages in groups of 5 rats per cage and given food and water ad libitum. Animals were maintained at 12 h light–dark cycles and a constant temperature of 23 ± 1 °C at all times. Rats were divided into two experimental groups

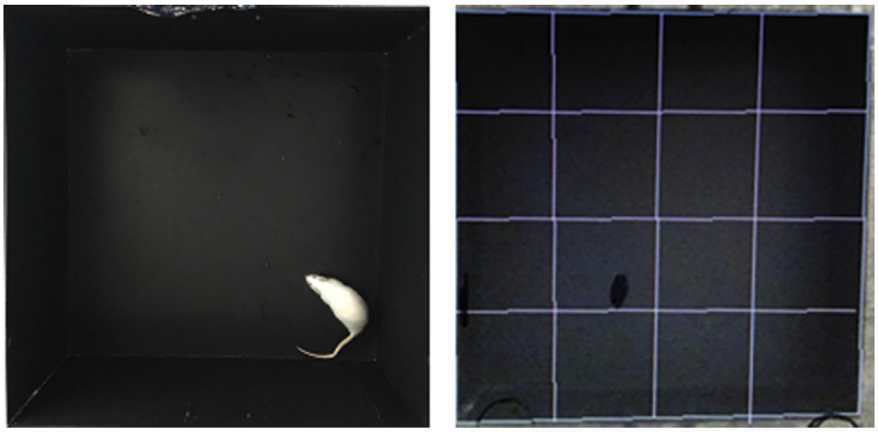

Fig. 1. Demonstrative photograph of open field experiment.

each consisting of 15 animals: Group 1: control group (C) and Group 2: rats administered $Na_2S_2O_5$ (S). Animals in S group were given freshly prepared $Na_2S_2O_5$ for 35 days by gastric gavage (100 mg/kg/day) (Hui et al., 1989; Ozturk et al., 2011) while the control group received equal volumes of distilled water via gavage for the same time period. On the 36th day, open-field tests were performed and rats were sacrificed on day 37.

2.2. Open field test

Behavior and locomotor activity were tested in an open field constructed of black Plexiglas (100 cm × 100 cm × 45 cm) arena (Fig. 1). Each rat was placed in the center of the open field and allowed a single exploration trial of 3 min. The floor and walls were cleaned thoroughly with 70% ethanol and allowed to air-dry after each trial to remove olfactory cues. Activity was monitored by a video camera and recorded to allow for later analysis (Noldus Ethovision XT System, The Netherlands). The field was virtually divided into 16 equally sized squares. Entry into a square was scored when the rat crossed the borders of a designated square with all four paws. Anxiety-like behavior and locomotor activity were evaluated by calculating the total number of squares entered and total distance moved, respectively (Hawley et al., 2011).

2.3. Biochemical analysis

Brain tissues of 10 rats were used for biochemical analysis. Following 5 weeks of drug administration, brains were perfused transcardially with heparinized saline and removed immediately. Then, hippocampi were dissected and stored at −80 °C until biochemical analysis. Brain tissues of 5 rats were perfused with 10% formalin and embedded in paraffin for immunohistochemical studies.

2.3.1. Hippocampal thiobarbituric acid reactive substances (TBARS) level determination

Levels of TBARS were measured by a fluorimetric method described by Wasowicz et al. (1993), using 1,1,3,3-tetraethoxypropane as standard. Harvested hippocampi were homogenized (Bio-Gen Pro-200) in ice cold 50 mM potassium phosphate buffer (pH:7.0). Homogenates were centrifuged (10.000 g, 15 min, 4 °C) (Sigma 3–18 K centrifuge) and supernatants were collected for lipid peroxidation analysis. Subsequently, supernatants (50 µl) were poured into a tube containing 29 mmol/l thiobarbituricacid (TBA) in acetic acid (8.75 mol/l), samples were placed in a water bath and heated for 1 h at 95–100 °C. Following the cooling period of the samples, 25 µl of 5M HCl was added and the reaction mixture was extracted by agitation for 5 min with 3.5 ml n-butanol. After centrifugation, the butanol phase was separated and the fluorescence of the butanol extract was measured in a fluorescence plate reader (Biotek-synergy Mx) using wavelengths of 525 nm for excitation, and 547 nm for emission. The results are presented as mmol/g protein.

2.3.2. Hippocampal total antioxidant capacity (TAC) measurement

Total antioxidant capacity was measured by a commercially available TAC assay kit (Cat. #RL0017, Rel Assay Diagnostics, Gaziantep, Turkey). The assay is calibrated with a stable antioxidant standard solution. The results are expressed as millimolarTrolox Equivalent per g protein (mmolTrolox Equiv./g protein).

2.3.3. $cPLA_2$ enzyme activity

$cPLA_2$ Enzyme activity in the hippocampus was measured using an assay kit (Cat. #765021 Cayman Chemical, Ann Arbor, MI, USA), according to manufacturer's instructions. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per g protein (pmol/min/mg protein).

2.4. Immunohistochemistry

Under deep urethane anesthesia, animals were perfused transcardially with 200 ml of saline followed by 300 ml of 10% paraformaldehyde solution. Brains were harvested and postfixed in the same paraformaldehyde solution for 1 day and embedded in paraffin. Five µm coronal sections were cut, collected on poly-L-lysine coated slides (Sigma–Aldrich, St. Louis, MO, USA) and incubated overnight at 56 °C. For cleaved caspase-3 assay, brain sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Sections were then treated with 10 mM citrate buffer, pH 6.0, for 5 min twice in a microwave oven, and left to cool down for 20 min. Following three washes in phosphate buffered saline (PBS), sections were incubated in a universal blocking reagent (BioGenex, San Ramon, CA, USA) for 7 min at room temperature in order to prevent nonspecific binding. Subsequently, sections were incubated overnight at 4 °C with cleaved caspase-3 (1:100, Cell Signaling #9661) primary antibody. After several washes in PBS, sections were incubated with goat-anti-rabbit-biotin (1:400; Vector Lab. Burlingame, CA, USA) secondary antibody for 60 min at room temperature and rinsed with PBS. Visualization was provided via Streptavidin-peroxidase complex (Dako, Carpinteria, CA, USA) and diaminobenzidine (BioGenex). Sections were counterstained with Mayer's hematoxylin (Dako) and mounted with Kaisers Glycerin Gelatine (Merck Kaisers Glycerin Gelatine, Cat No: 1.09242.0100). Slides were examined using Zeiss-Axioplan microscope (Oberkochen, Germany).

2.5. Protein determination

Protein concentrations of brain tissues were spectrophotometrically measured (Shimadzu RF-5500, Kyoto, Japan) by a protein assay reagent kit (Pierce, Rockford, IL) using a modified Bradford method (Bradford, 1976). Bovine serum albumin was used as the standard.

2.6. Statistical analysis

The differences in biochemical and open field data parameters were analyzed by Student's T-test. P values less than 0.05 were considered significant.

3. Results

3.1. Open field test

Velocity and total distance moved in the open field test from all experimental groups are shown in Table 1. The velocity and total distance moved in the open field test were significantly higher in the S group compared to controls.

3.2. TBARS and TAC levels

TBARS level was increased in the S group compared with the C group. TAC level of the S group (0.17 ± 0.04 mmol Trolox Equiv./g protein) was found to be significantly lower than controls (0.32 ± 0.07 mmol Trolox Equiv./g protein) (Table 2).

Table 1
Values of open-field locomotor activity.

Groups	Total distance (cm)	Velocity (cm/min)
C	812.1 ± 66.6	289.4 ± 23.7
S	840.5 ± 70.8*	301.4 ± 31.5*

The results are presented as mean ± SEM, n = 15 for each group. C: control, S: Sulfite.
*Statistically different versus the control group, $p < 0.05$.

Table 2
The effects of sodium metabisulfite on TBARS and TAC on hippocampus.

Groups	TBARS (mmol/g protein)	Total antioxidant capacity (mmolTrolox Equiv./g protein)
C	1.128 ± 0.038	0.32 ± 0.07
S	1.810 ± 0.133*	0.17 ± 0.04*

The results are presented as mean ± SEM, n = 10 for each group. C: control, S: Sulfite.
*Statistically different versus the control group, $p < 0.05$.

3.3. $cPLA_2$ enzyme activity

Data analysis showed that $cPLA_2$ activity was increased in the S group (121.56 ± 12.08 pmol/min/mg protein) compared with C group (72.97 ± 12.53 pmol/min/mg protein) (Fig. 2).

3.4. Caspase-3 immunohistochemistry

Immunohistochemistry results demonstrated caspase-3 positive neurons in hippocampal CA1, CA2 and CA3 regions (Fig. 3). Caspase-3 expressions were higher in the S group compared to the C group in the hippocampal area CA1, CA2, and CA3 (Fig. 3). Immunohistochemical staining of cleaved caspase-3 was localized in the nuclei of hippocampal areas in the S group. No immunoreactivity was observed on the slides where primary antibody was replaced with normal rabbit IgG in the hippocampi of all groups (Fig. 3, Negative control).

4. Discussion

Sulfites or sulfiting agents such as sodium metabisulfite, are additives widely used in the food industry predominantly as anti-browning agents, antioxidants and preservatives (Noorafshan et al., 2013). Acceptable Daily Intake (ADI) for sulfites was established by the Joint FAO/WHO (Food and Agriculture Organization/World Health Organization) Expert Committee on Food Additives (JECFA) in 1974 (0.7 mg/kg body weight i.e. equivalent to 42 mg for a 60 kg adult) and was also adopted by the Scientific Committee for Food (SCF) in 1994 (Commission of the European Communities, 1994) (Leclercq et al., 2000). Since then, dose-dependent effects of ingested sulfites have been investigated and daily doses higher than 25 mg/kg is found to be toxic for rats in various studies (Kencebay et al., 2013; Noorafshan et al., 2013; Ozturk et al., 2011; Til et al., 1972). Of note, in recent years sulfite consumption even at safe levels was suggested to show toxic effects by some researchers (Akdoğan et al., 2011; Ozturk et al., 2006; Noorafshan et al., 2013). In our previous studies we already demonstrated the toxic effects of sulfite at a dose of 100 mg/kg for 35 days in rat total brain tissues (Kencebay et al., 2013; Ozturk et al., 2011). While there is no data for sulfite dosage on hippocampus toxicity specifically, we used the above mentioned dose for the same period.

As sulfiting agents are known to have neurotoxic effects, hippocampal changes and related behavioral outcomes associated with sulfite consumption has also gained attention in the last decade (Akdogan et al., 2011; Kocamaz et al., 2012; Noorafshan et al., 2013). Recently Noorafshan et al. surprisingly suggested that long-term sulfite intake at safe level resulted in learning and memory impairment in rats in a radial arm maze test (Noorafshan et al., 2013). However, in studies conducted by Ozsoy et al. (2017) and Kucukatay et al. (2005), long-term acceptable daily sulfite intake failed to affect active avoidance performances of aged and adult rats, respectively. Open field test was used to evaluate the locomotor activity of the animals and the potential effects of excessive sulfite consumption on locomotor activity have been investigated for the very first time in the present study. Interestingly, sulfite has been shown to result in a significant increase in the locomotor activity, implying hyperactivity. That is, long-term sulfite overconsumption might contribute to the hyperactivity development in behavioral aspect. However, further investigations are needed to clarify the potential molecular pathways underlying this outcome and the accompanying behavioral alterations.

Oxidant properties of sulfites have been reported both in vitro and in vivo in numerous tissues such as liver, plasma, kidney, and brain tissues in the literature (Herken et al., 2009; Kencebay et al., 2013; Kucukatay et al., 2005; Niknahad and O'Brien, 2008; Ozturk et al., 2011; Vincent et al., 2004). In our study, sulfite consumption above safe level has been shown to prominently increase lipid peroxidation and decrease total antioxidant capacity in the hippocampus for the first time. Interestingly, two separate studies conducted by Kucukatay et al. (2005) reported that 6 weeks of 25 mg/kg daily sulfite ingestion did not affect TBARS levels and increased antioxidant capacity in the rat hippocampus (Kucukatay et al., 2007). The increment in hippocampal antioxidant capacity was speculated to be an adaptive response to sulfite dependent oxidative stress (Kucukatay et al., 2007). Based on our TAC data, here we might suggest that high amounts of sulfite could decrease total antioxidant capacity without leaving a chance of adaptation in the hippocampus. For sure, antioxidant system parameters should be investigated to support that opinion. Similar to our findings regarding TBARS levels, Kencebay et al. (2013) and Ozturk et al. (2011) also demonstrated long-term sulfite intake at doses above safe level produces oxidative stress in the brain. Furthermore, evidences such as the increase of ROS in hepatocyte cell lines (Niknahad and O'Brien, 2008) and canine kidney cells (Vincent et al., 2004) and the decrease of TAC in rat plasma (Herken et al., 2009) associated with sulfite exposure strengthen the hypothesis that sulfite toxicity is primarily mediated by oxidative stress. Hence it can be concluded that excessive sulfite consumption might activate various hazardous signaling pathways, such as arachidonic acid-PLA_2 pathway, and even cause apoptosis through oxidative stress (Korbecki et al., 2013).

PLA_2 Enzymes catalyzing the cleavage of fatty acids from sn-2 position of membrane phospholipids to release free fatty acids have been previously suggested to play role in sulfite neurotoxicity (Beck-Speier et al., 2003; Kencebay et al., 2013). Although Beck-Speier was the first author to claim the PLA_2 activation by sulfite exposure, a more specific information was provided in our previous study which reported daily toxic dose of sulfite administration to increase secretory PLA_2 ($sPLA_2$) level in the rat brain (Kencebay et al., 2013). In the present study, we aimed to focus on the $cPLA_2$ response to sulfite overconsumption in the rat hippocampus. Our findings pointed out that sulfite caused a significant increase of $cPLA_2$ activity in the hippocampus. Since $cPLA_2$ enzymes are implicated in ROS generation and oxidative stress development (Wang et al., 2014), one contributor mechanism mediating toxic actions of sulfites and sulfiting agents might involve $cPLA_2$ activity increase.

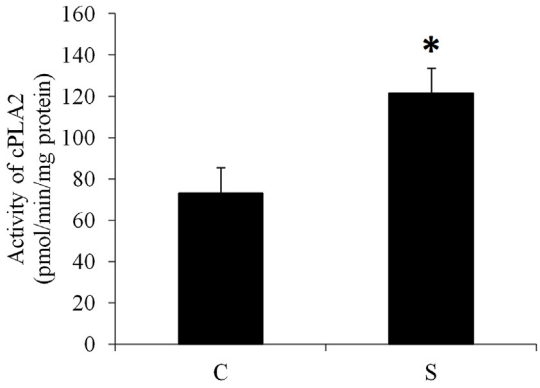

Fig. 2. $cPLA_2$ activity in the hippocampus. The results are presented as mean ± SEM, n = 10 for each group. C: control, S: Sulfite *Statistically different versus the control group, $p < 0.05$.

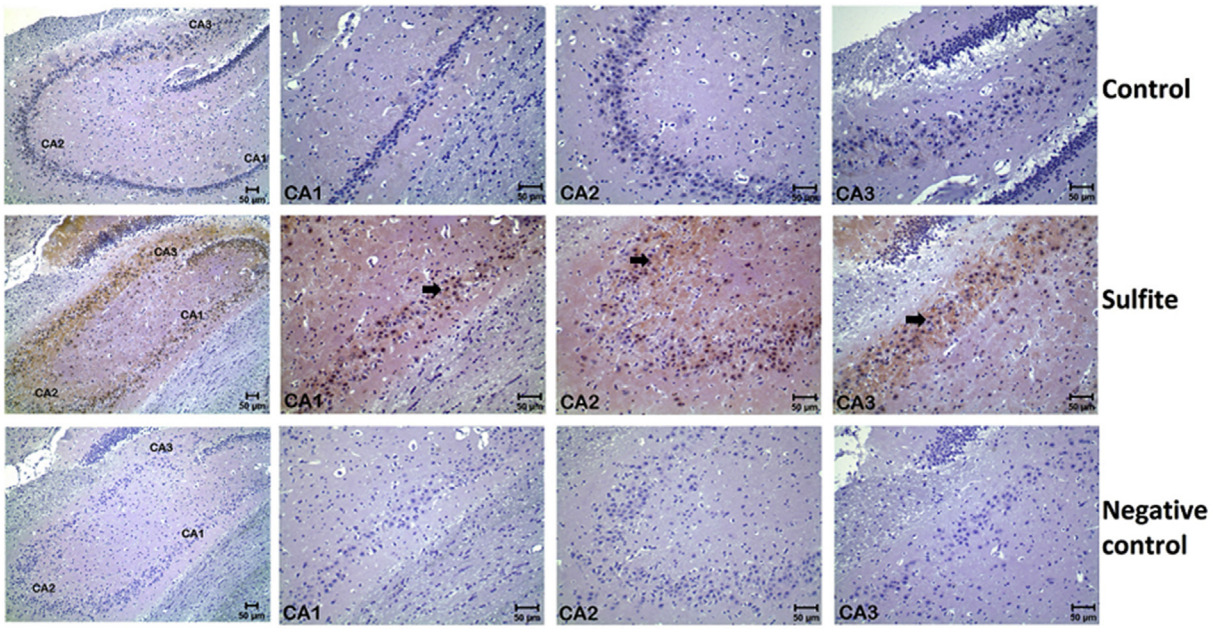

Fig. 3. Immunohistochemical staining of caspase-3 in hippocampus. Expression of caspase-3 in the hippocampal area CA1, CA2, and CA3. Arrows indicate cleaved caspase-3 immunopositive cells.

Another phenomenon that $cPLA_2$ enzymes take part in is apoptosis (Wang et al., 2014). Apoptotic pathways are known to include the activation caspase signaling (Yagami et al., 2018). Because executioner caspase-3 plays pivotal role in neuronal apoptosis and we wanted to see whether long-term sulfite overconsumption results in apoptotic events in rat hippocampus, cleaved caspase-3 immunreactivity was measured in the present study. Immunohistochemical analysis showed that toxic dose of sulfite intake increased the number of apoptotic cells in the hippocampus. Apoptotic actions of sulfites in the brain have been demonstrated by numerous studies, some of which reported sulfite intake, even at lower doses, to cause neuronal loss in the hippocampus (Akdogan et al., 2011; Kencebay et al., 2013; Kocamaz et al., 2012; Ozturk et al., 2011). Kocamaz et al. (2012) demonstrated that 6 weeks of 70 mg/kg daily sulfite consumption resulted in pyramidal neuron loss in the rat hippocampus by optical fractionator method. Likewise by using the same method, Akdogan et al. (2011) showed CA1, CA2, CA3 pyramidal neuron loss in the rat hippocampus at the end of a 6-week sulfite intake period with a daily dose of 25 mg/kg. On the other hand, a recent study with the same exposure protocol (25 mg/kg of daily sulfite intake for 6 weeks) by Ozsoy et al. (2017) failed to demonstrate cleaved caspase-3 immunreactivity increase in the hippocampi of aged rats. The difference between outcomes of sulfite intake in young and old animals gives rise to new questions that need to be answered by future studies.

5. Conclusion

Daily sulfite intake above safe levels might give rise to both behavioral and molecular alterations which in turn result in harmful outcomes. Locomotor activity increase was accompanied by the increases of lipid peroxidation, $cPLA_2$ activity, caspase-3 immunreactivity and the decrease of total antioxidant capacity in the hippocampus as a result of long-term sulfite overconsumption in the present study. Our findings suggest a role for $cPLA_2$ in the sulfite neurotoxicity in rat hippocampus.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the Research Foundation of Akdeniz University, Turkey (Project number: 2008.01.0103.009).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.11.021.

References

Adhikari, A., Topiwala, M.A., Gordon, J.A., 2010. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron 65, 257–269.
Adibhatla, R.M., Hatcher, J.F., 2008. Phospholipase A(2), reactive oxygen species, and lipid peroxidation in CNS pathologies. BMB reports 41, 560–567.
Ahmad, A., Ahmad, S., Baig, M.A., 2008. Purification and characterization of sulfite oxidase from goat liver. Indian J. Biochem. Biophys. 45 (6), 379–386.
Akdogan, I., Kocamaz, E., Kucukatay, V., Yonguc, N.G., Ozdemir, M.B., Murk, W., 2011. Hippocampal neuron number loss in rats exposed to ingested sulfite. Toxicol. Ind. Health 27, 771–778.
Amin, F., Bano, B., 2018. Damage of cystatin due to ROS-generation and radical-scavenging activity of antioxidants and associated compounds. Int. J. Biol. Macromol. 119, 369–379.
Aydin, S., Yargicoglu, P., Derin, N., Aliciguzel, Y., Abidin, I., Agar, A., 2005. The effect of chronic restraint stress and sulfite on visual evoked potentials (VEPs): relation to lipid peroxidation. Food Chem. Toxicol. 43, 1093–1101.
Beck-Speier, I., Dayal, N., Denzlinger, C., Haberl, C., Maier, K.L., Ziesenis, A., Heyder, J., 2003. Sulfur-related air pollutants induce the generation of platelet-activating factor, 5-lipoxygenase- and cyclooxygenase-products in canine alveolar macrophages via activation of phospholipases A2. Prostag. Other Lipid Mediat. 71, 217–234.
Boyanovsky, B.B., Webb, N.R., 2009. Biology of secretory phospholipase A2. Cardiovasc. Drugs Ther. 23, 61–72.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Catala, A., 2010. A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochem. Biophys. Res. Commun. 399, 318–323.
Chiarani, F., Bavaresco, C.S., Dutra-Filho, C.S., Netto, C.A., Wyse, A.T., 2008. Sulfite increases lipoperoxidation and decreases the activity of catalase in brain of rats. Metab. Brain Dis. 23, 123–132.
Chuang, D.Y., Simonyi, A., Kotzbauer, P.T., Gu, Z., Sun, G.Y., 2015. Cytosolic phospholipase A2 plays a crucial role in ROS/NO signaling during microglial activation through the lipoxygenase pathway. J. Neuroinflammation 12, 199.
Derin, N., Akpinar, D., Yargicoglu, P., Agar, A., Aslan, M., 2009. Effect of alpha-lipoic acid on visual evoked potentials in rats exposed to sulfite. Neurotoxicol. Teratol. 31,

34–39.
Ding, L., Zhang, C., Masood, A., Li, J., Sun, J., Nadeem, A., Zhang, H.T., O'Donnell, J.M., Xu, Y., 2014. Protective effects of phosphodiesterase 2 inhibitor on depression- and anxiety-like behaviors: involvement of antioxidant and anti-apoptotic mechanisms. Behav. Brain Res. 268, 150–158.
Farooqui, A.A., Horrocks, L.A., 2006. Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12, 245–260.
Grings, M., Moura, A.P., Parmeggiani, B., Pletsch, J.T., Cardoso, G.M.F., August, P.M., Matté, C., Wyse, A.T.S., Wajner, M., Leipnitz, G., 2017. Bezafibrate prevents mitochondrial dysfunction, antioxidant system disturbance, glial reactivity and neuronal damage induced by sulfite administration in striatum of rats: implications for a possible therapeutic strategy for sulfite oxidase deficiency. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1863 (9), 2135–2148.
Hawley, W.R., Grissom, E.M., Dohanich, G.P., 2011. The relationships between trait anxiety, place recognition memory, and learning strategy. Behav. Brain Res. 216, 525–530.
Herken, E.N., Kocamaz, E., Erel, O., Celik, H., Kucukatay, V., 2009. Effect of sulfite treatment on total antioxidant capacity, total oxidant status, lipid hydroperoxide, and total free sulfydryl groups contents in normal and sulfite oxidase-deficient rat plasma. Cell Biol. Toxicol. 25, 355–362.
Hui, J.Y., Beery, J.T., Higley, N.A., Taylor, S.L., 1989. Comparative subchronic oral toxicity of sulphite and acetaldehyde hydroxysulphonate in rats. Food Chem. Toxicol. 27, 349–359.
Kencebay, C., Derin, N., Ozsoy, O., Kipmen-Korgun, D., Tanriover, G., Ozturk, N., Basaranlar, G., Yargicoglu-Akkiraz, P., Sozen, B., Agar, A., 2013. Merit of quinacrine in the decrease of ingested sulfite-induced toxic action in rat brain. Food Chem. Toxicol. 52, 129–136.
Kocamaz, E., Adiguzel, E., Er, B., Gundogdu, G., Kucukatay, V., 2012. Sulfite leads to neuron loss in the hippocampus of both normal and SOX-deficient rats. Neurochem. Int. 61, 341–346.
Korbecki, J., Baranowska-Bosiacka, I., Gutowska, I., Chlubek, D., 2013. The effect of reactive oxygen species on the synthesis of prostanoids from arachidonic acid. J. Physiol. Pharmacol. 64, 409–421.
Krolow, R., Arcego, D.M., Noschang, C., Weis, S.N., Dalmaz, C., 2014. Oxidative imbalance and anxiety disorders. Curr. Neuropharmacol. 12, 193–204.
Kucukatay, V., Savcioglu, F., Hacioglu, G., Yargicoglu, P., Agar, A., 2005. Effect of sulfite on cognitive function in normal and sulfite oxidase deficient rats. Neurotoxicol. Teratol. 27, 47–54.
Kucukatay, V., Bor-Kucukatay, M., Atsak, P., Agar, A., 2007. Effect of ingested sulfite on hippocampus antioxidant enzyme activities in sulfite oxidase competent and deficient rats. Int. J. Neurosci. 117, 971–983.
Kumar, S., 2007. Caspase function in programmed cell death. Cell Death Differ. 14, 32–43.
Leclercq, C., Molinaro, M.G., Piccinelli, R., Baldini, M., Arcella, D., Stacchini, P., 2000. Dietary intake exposure to sulphites in Italy–analytical determination of sulphite-containing foods and their combination into standard meals for adults and children. Food Addit. Contam. 17, 979–989.
Martyn, D.M., McNulty, B.A., Nugent, A.P., Gibney, M.J., 2013. Food additives and preschool children. Proc. Nutr. Soc. 72, 109–116.
Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T., Yamamoto, K., 2011. Recent progress in phospholipase A(2) research: from cells to animals to humans. Prog. Lipid Res. 50, 152–192.
Niknahad, H., O'Brien, P.J., 2008. Mechanism of sulfite cytotoxicity in isolated rat hepatocytes. Chem. Biol. Interact. 174, 147–154.
Noorafshan, A., Asadi-Golshan, R., Karbalay-Doust, S., Abdollahifar, M.A., Rashidiani-Rashidabadi, A., 2013. Curcumin, the main part of turmeric, prevents learning and memory changes induced by sodium metabisulfite, a preservative agent, in rats. Exp. Neurobiol. 22, 23–30.
Ough, C.S., 1986. Determination of sulfur dioxide in grapes and wines. J. AOAC Int. 69, 5–7.
Ozsoy, O., Aras, S., Ozkan, A., Parlak, H., Gemici, B., Uysal, N., Aslan, M., Yargicoglu, P., Agar, A., 2017. The effect of ingested sulfite on active avoidance in normal and sulfite oxidase-deficient aged rats. Toxicol. Mech. Methods 27, 81–87.
Ozturk, O.H., Kucukatay, V., Yonden, Z., Agar, A., Bagcı, H., Delibas, N., 2006. Expressions of N-methyl-D-aspartate receptors NR2A and NR2B subunit proteins in normal and sulfite-oxidase deficient rat's hippocampus: effect of exogenous sulfite ingestion. Arch. Toxicol. 80, 671–679.
Ozturk, N., Yargicoglu, P., Derin, N., Akpinar, D., Agar, A., Aslan, M., 2011. Dose-dependent effect of nutritional sulfite intake on visual evoked potentials and lipid peroxidation. Neurotoxicol. Teratol. 33, 244–254.
Shi, X., 1994. Generation of $SO_3^{\cdot-}$ and OH radicals in $SO_3^{(2-)}$ reactions with inorganic environmental pollutants and its implications to $SO_3^{(2-)}$ toxicity. J. Inorg. Biochem. 56, 155–165.
Shi, X., Mao, Y., 1994. 8-Hydroxy-2'-deoxyguanosine formation and DNA damage induced by sulfur trioxide anion radicals. Biochem. Biophys. Res. Commun. 205, 141–147.
Snigdha, S., Smith, E.D., Prieto, G.A., Cotman, C.W., 2012. Caspase-3 activation as a bifurcation point between plasticity and cell death. Neurosci. Bull. 28, 14–24.
Spiteller, G., 2010. Is lipid peroxidation of polyunsaturated acids the only source of free radicals that induce aging and age-related diseases? Rejuvenation Res. 13, 91–103.
Streck, E.L., Goncalves, C.L., Furlanetto, C.B., Scaini, G., Dal-Pizzol, F., Quevedo, J., 2014. Mitochondria and the central nervous system: searching for a pathophysiological basis of psychiatric disorders. Rev. Bras. Psiquiatr. 36, 156–167.
Til, H.P., Feron, V.J., De Groot, A.P., 1972. The toxicity of sulphite. I. Long-term feeding and multigeneration studies in rats. Food Chem. Toxicol. 10, 291–310.
Vandevijvere, S., Temme, E., Andjelkovic, M., De Wil, M., Vinkx, C., Goeyens, L., Van Loco, J., 2010. Estimate of intake of sulfites in the Belgian adult population. Food Addit. Contam. 27, 1072–1083.
Vincent, A.S., Lim, B.G., Tan, J., Whiteman, M., Cheung, N.S., Halliwell, B., Wong, K.P., 2004. Sulfite-mediated oxidative stress in kidney cells. Kidney Int. 65, 393–402.
Wang, W., Wolff, M.W., Reichl, U., Sundmacher, K., 2014. Avidity of influenza virus: model-based identification of adsorption kinetics from surface plasmon resonance experiments. J. Chromatogr. A 1326, 125–129.
Wasowicz, W., Neve, J., Peretz, A., 1993. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. Clin. Chem. 39, 2522–2526.
Yagami, T., Yamamoto, Y., Koma, H., 2018. Pathophysiological roles of intracellular proteases in neuronal development and neurological diseases. Mol. Neurobiol. Aug 10. https://doi.org/10.1007/s12035-018-1277-4.
Yoshida, M., Nakagawa, M., Hosomi, R., Nishiyama, T., Fukunaga, K., 2015. Low molybdenum state induced by tungsten as a model of molybdenum deficiency in rats. Biol. Trace Elem. Res. 165 (1), 75–80.
Zhu, W., Mao, Z., Zhu, C., Li, M., Cao, C., Guan, Y., Yuan, J., Xie, G., Guan, X., 2016. Adolescent exposure to cocaine increases anxiety-like behavior and induces morphologic and neurochemical changes in the hippocampus of adult rats. Neuroscience 313, 174–183.