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# Merit of quinacrine in the decrease of ingested sulfite-induced toxic action in rat brain

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ABSTRACT

We aimed at investigating the effects of sulfite-induced lipid peroxidation and apoptosis mediated by secretory phospholipase A2 (sPLA2) on somatosensory evoked potentials (SEP) alterations in rats. Thirty male albino Wistar rats were randomized into three experimental groups as follows; control (C), sodium metabisulfite treated (S), sodium metabisulfite + quinacrine treated (SQ). Sodium metabisulfite (100 mg/kg/day) was given by gastric gavage for 5 weeks and 10 mg/kg/day quinacrine was applied as a single dose of intraperitoneal injection for the same period. The latencies of SEP components were significantly prolonged in the S group and returned to control levels following quinacrine administration. Plasma-S-sulfonate level was increased in S and SQ groups. TBARS levels in the S group were significantly higher than those detected in controls. Quinacrine significantly decreased brain TBARS levels in the SQ group compared with the S group. Quinacrine treatment did not have an effect on the increased sPLA2 level of the sulfite administered group. Immunohistochemistry showed that sulfite caused an increase in caspase-3 and TUNEL positive cells, restored to control levels via quinacrine administration. This study showed that sPLA2 might play a role in ingested sulfite-induced SEP alterations, oxidative stress, apoptotic cell death and DNA damage in the brain.



## 1. Introduction

Since 1959, FDA (Food and Drug Administration) has accepted sulfite compounds as safe and therefore these chemical agents are used as a food additive agent (Schroeter, 1966). Five sulfite salts including 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 commonly used as antioxidants in food and pharmaceutical preparations (Gunnison and Jacobsen, 1987). Once ingested, sulfite salts react with water leading to the generation of bisulfite ($HSO^{-3}$), sulfite ($SO_3^{-2}$), and sulfurdioxide ($SO_2$) (Gunnison, 1981; Mottley and Mason, 1988). Previous studies have shown that ingested sulfite enters the systemic circulation by gastrointestinal absorption and distributed essentially to all body tissues including the brain (Gunnison and Jacobsen, 1987; Gunnison and Benton, 1971).

Sulfite can react with a variety of humoral and cellular components and can cause toxicity (Gunnison and Palmes, 1976; Hayatsu and Miller, 1972; Rencuzogullari et al., 2001). Hence, sulfite is detoxified by sulfite oxidase in the mammalian tissues. Sulfite oxidase, a molybdenum-containing enzyme located in the intremembranous space of the mitochondria, oxidizes sulfite to sulfate in a two-electron oxidation step and protects cells from sulfite toxicity (Cohen and Fridovich, 1971; Feng et al., 2007). If there is deficiency of sulfite oxidase or exposure to excessive sulfite, the sulfite undergoes one electron oxidation reactions, catalyzed by peroxidases to form sulfur trioxide anion radical ($SO_3^-$) (Mottley and Mason,

*Abbreviations:* ADI, acceptable daily intake; $HSO^{-3}$, bisulfite; cPLA2, cytosolic calcium dependent phospholipase A2; iPLA2, cytosolic calcium independent phospholipase A2; DNA, deoxyribonucleic acid; HCl, hydrochloric acid; PRA, pararosaline hydrochloride; PBS, phosphate buffered saline; KCN, potassium cyanide; $K_2S_2O_5$, potassium metabisulfite; $K_2SO_3$, potassium sulfite; PGH, prostaglandin H synthase; sPLA2, secretory phospholipase A2; $NaHSO_3$, sodium bisulfite; NaCl, sodium chloride; NaOH, sodium hydroxide; $Na_2S_2O_5$, sodium metabisulfite; $Na_2SO_3$, sodium sulfite; SEP, somatosensory evoked potential; $SO_4^-$, sulfate radical; $SO_3^{-2}$, sulfite; $SO_3OO$, sulfite peroxyl radical; $SO_3^+$, sulfite radical; $SO_3^-$, sulfur trioxide anion radical; $SO_2$, sulfurdioxide; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TBS, tris buffered saline; WHO, World Health Organization.

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1988). The sulfite radicals can react with oxygen molecules forming sulfite peroxyl radical ($SO_3OO^{\cdot}$) and sulfate radical ($SO_4^{-}$) (Mottley and Mason, 1988). There are in vitro studies suggesting that sulfite radicals induce DNA injury and (Niknahad and O'Brien, 2008; Ozturk et al., 2011; Rencuzogullari et al., 2001) mitochondrial damage causing excessive reactive oxygen species generation, mediated by lipid peroxidation (Aydin et al., 2005; Derin et al., 2009; Green and Reed, 1998). Reactive oxygen species are produced by a number of cellular oxidative metabolic processes, monoamine oxidases, mitochondrial respiratory chain and PLA2 pathway (Adibhatla et al., 2003).

Phospholipase A2 is a family of enzymes that catalyze the cleavage of fatty acids from sn-2 position of membrane phospholipids to release free fatty acids (Farooqui and Horrocks, 2006). The PLA2 family is classified into three main groups in the brain tissue: cytosolic calcium dependent PLA2 (cPLA2), cytosolic calcium independent PLA2 (iPLA2) and secretory PLA2 (sPLA2) (Farooqui et al., 1997). sPLA2 is synthesized in intracellular compartments and secreted into extracellular space where it binds to cell surface receptors, identified N type, in neurons (Matsuzawa et al., 1996). The highest activity of sPLA2 is established in hippocampus, medulla oblongata and cerebral cortex (Thwin et al., 2003). sPLA2 causes lipid peroxidation and apoptosis through its products such as arachidonic acid and lysophosphatidylcholine (Toborek et al., 1999). It has been reported that AA produces ROS by regulation of the activity and the expression of NADPH oxidase and by dysfunction of mitochondria (Pompeia et al., 2003; Balboa and Balsinde, 2006; Farooqui et al. 2007). Arachidonic acid-induced apoptosis has been characterized in various cell models by several assays that have shown leakage of cytochrome c from mitochondria, mitochondrial depolarization, phosphatidylserine externalization, caspase activation, poly(ADP)-ribose polymerase cleavage, DNA fragmentation, chromatin condensation, nuclear breakdown and loss of membrane integrity. (Köller et al., 1997; Vento et al., 2000; Garrido et al., 2000; Scorrano et al., 2001). Lysophosphatidylcholine-induced apoptosis is dependent on activation of Bax and caspase (Kakisaka et al., 2012). On the other hand, lysophosphatidylcholine stimulated production of superoxide anion partly through membrane-associated NADH-dependent superoxide anion production systems (Kugiyama et al., 1999).

Quinacrine, a non selective inhibitor of PLA2, was used to elucidate the effects of sulfite ingestion on regulation of enzyme protein levels. Quinacrine was selected because it is has been shown to cross the blood–brain barrier (Dubin et al., 1982).

Apoptosis, also known as programmed cell death, is a biological process that plays an important role in the development of the nervous system (Sastry and Rao, 2000). The specific morphological changes associated with apoptosis are cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation, and the formation of apoptotic bodies (Chandra et al., 2000; Jang et al., 2002). The signaling pathways occurring during apoptosis involve the activation of cysteine proteases that are part of a large family of proteins known as caspases (Aggarwal, 2000). There are two types of caspases: initiator caspases (caspase 8, 10, 9, 2) and effector caspases (caspase 3, 7, 6). The effector caspases are activated by the active initiator caspases. In particular, the most widely studied member of the caspase family is caspase-3. Caspase-3 is an effector caspase, partially or totally responsible for the proteolytic cleavage of several proteins (Cohen, 1997; Ko et al., 2009).

Somatosensory evoked potentials (SEPs) are often used to evaluate the function of afferent pathways from the sensory receptors up to the somatosensory cortex (Desmedt and Cheron, 1980; Canu et al., 2003). The components of SEP waves are characterized by the latency, reflecting the conduction velocity of different stages of afferent pathway and, the amplitude, corresponding to the postsynaptic response to the quantity of sensory input (Canu et al., 2003; Herr et al., 2007; Shapiro, 2002). These potentials have become an important diagnostic method when evaluating complications caused by neurotoxic agents (Lukács et al., 2007; Herr et al., 2007; Lebrun et al., 2000). Our group previously showed that sulfur dioxide, one of the most common air pollutants, affects SEP latencies and amplitudes in rats (Kucukatay et al., 2003). However, the effect of ingested sulfite-induced SEP alterations has not been reported in literature.

This study aimed to investigate whether sPLA2 plays a role in sulfite mediated SEP alterations through apoptosis and lipid peroxidation in rats. In this context, thiobarbituric acid reactive substances (TBARS), sPLA2 protein levels, TUNEL and cleaved caspase-3 levels were determined in the brain. Additionally, plasma-S sulfonate levels were also measured as a biomarker of ingested sulfite accumulation in the plasma.

## 2. Materials and methods

### 2.1. Preparation of animals

Healthy male Wistar albino rats, aged three months, weighing 300–350 g were used in this 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 housed 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 three experimental groups consisting of 10 rats each: Group 1: control (C); Group 2: rats treated with $Na_2S_2O_5$ (S); Group 3: rats treated with $Na_2S_2O_5$ + quinacrine (SQ). Animals in S and SQ groups were given by gastric gavage (100 mg/kg/day) of freshly prepared $Na_2S_2O_5$ for 5 weeks (Hui et al., 1989; Ozturk et al., 2011). Quinacrine, non-spesific PLA2 inhibitor, was applied as a single dose (10 mg/kg/day) of intraperitoneal injection in the SQ group for 5 weeks (Hirose et al., 2007) while the control group received distilled water by gavage and saline by intraperitoneal injection for the same period.

### 2.2. SEP recordings

At the end of 5 weeks, rats were deprived of food for 24 h and then SEPs were recorded. SEPs were recorded with stainless steel subdermal electrodes (Medelec 017K024, Medelec Manor Way, Old Woking Surrey, United Kingdom) under ether anesthesia. The active electrode was placed over the left somatosensory area of the cerebral cortex (0.4 cm to the right of bregma); the reference was 1.0 cm anterior to bregma on the midline. A ground electrode was placed on the animal's tail (Kanda et al., 1989; Kucukatay et al., 2003).

SEPs were recorded using Biopac MP100 data acquisition equipment (Biopac System, Inc.) The electrical stimulus was a square-wave, constant-voltage impulse delivered at a rate of 1/s transcutaneously to the right posterior tibial nerve at the ankle. The stimulus duration was 0.5 ms, at an intensity sufficient to the produce a definite twitch of the big toe. Analysis time was set to 150 ms, the sampling rate was 1000 Hz, and the frequency bandwidth of the amplifier was 1–3000 Hz. The gain was selected as 20 μV/div. The body temperature of rats was maintained between 37 °C and 37.5 °C using a heating pad during the SEP recording (Panjwani et al., 1991). Two hundred responses were averaged. Sweeps contaminated with large artifacts were rejected by the computer. To ensure the response reproducibility at least two averages were obtained.

### 2.3. Biochemical investigations

After SEP recordings, heparinized blood was collected from the abdominal aorta of rats under urethane anesthesia and used for the determination of plasma-S-sulfonate levels. For other biochemical analysis, brains of rats were perfused transcardially with heparinized saline, removed immediately and stored at −80 °C. On the other hand, for immunohistochemical studies brains were perfused with by 10% formalin and embedded in paraffin.

### 2.4. Plasma-S-sulfonate analysis

Plasma-S-sulfonate levels were measured by the method of Gunnison and Palmes (Gunnison and Palmes, 1973). One milliliter of plasma was mixed with 0.2 ml of a solution containing 0.027 mM NaOH and 0,125 mM KCN. The mixture was incubated at 35 ± 1 °C under nitrogen for 1 h. Following incubation, the mixture was cooled in ice and transferred to a cellulose dialysis bag and dialyzed at 4 °C against 5 ml of dialysate containing 10 mM glycine buffers at pH 10.2 for 4 h. After the dialysis, 200 μl of each reagent given below was added to 1.4 ml of dialysate in the following order. 0.15 M HCl, sodium tetrachloromercurate solution [0.18 M