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ORIGINAL ARTICLES

Acetaminophen at Different Doses Protects Brain Microsomal Ca^{2+}-ATPase and the Antioxidant Redox System in Rats

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Abstract Acetaminophen, an analgesic and antipyretic drug, rescues neuronal cells from mitochondrial redox impairment and reactive oxygen species (ROS). Excessive administration of acetaminophen above the recommended daily dose range has some negative effects on the brain. We investigated the effects of different doses of acetaminophen on Ca^{2+}-ATPase and the antioxidant redox system in rats. Seventy rats were randomly divided into seven equal groups. The first was used for the control. One dose of 5, 10, 20, 100, 200, and 500 mg/kg acetaminophen was intraperitoneally administered to rats constituting the second, third, fourth, fifth, sixth, and seventh groups, respectively. After 24 h, brain cortical samples were taken and brain microsomal samples were obtained by ultracentrifugation. Brain and microsomal lipid peroxidation (LP) and brain calcium levels in the sixth and seventh groups were increased compared to control. LP levels in the second, third, and forth groups; brain vitamin E levels; brain and microsomal glutathione peroxidase (GSH-Px); and Ca^{2+}-ATPase activity in the sixth and seventh groups were lower than in control, although brain vitamin E concentrations in the second, third, fourth, and fifth groups and microsomal GSH-Px activity in the third and fourth groups were higher than in control. Brain cortical β-carotene and vitamin A concentrations did not differ in the seven groups. In conclusion, 5–100 mg/kg acetaminophen seems to have protective effects on oxidative stress-induced brain toxicity by inhibiting free radicals and supporting the antioxidant redox system.

Keywords Acetaminophen · Antioxidant · Oxidative stress · Brain · Apoptosis · Ca^{2+}-ATPase · Rat

Introduction

Oxidative stress is defined as an imbalance between higher cellular levels and reactive oxygen species (ROS), e.g., superoxide and hydroxyl radicals (Yatin et al. 2000) and cellular antioxidant defense (Halliwell 2006). Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism, i.e., mitochondrial oxidation and phagocytosis. In order to scavenge ROS, various defense systems exist in the brain. Glutathione peroxidase (GSH-Px), a selenium-containing enzyme, is responsible for the reduction of hydro- and organic peroxides in the presence of reduced glutathione (GSH) (Whanger 2001). GSH is a hydroxyl radical and singlet oxygen scavenger, and it participates in a wide range of cellular functions (Whanger 2001; Nazıroğlu 2007a, 2007b). Vitamin E (α-

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tocopherol) is the most important antioxidant in the lipid phase of cells (Nazıroğlu 2007a). Vitamin A (retinol) serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (Halliwell 2006).

If ROS are not controlled by enzymatic and nonenzymatic antioxidants, they can cause oxidative injury, i.e., peroxidation of cell membrane phospholipids, proteins (receptor and enzymes) and DNA. The brain is extremely susceptible to oxidative damage induced by these ROS because it generates very high levels of ROS due to its very high aerobic metabolism and blood perfusion and it has relatively poor enzymatic antioxidant defense (Nazıroğlu 2007a). The brain contains polyunsaturated fatty acids (PUFAs), which can readily be peroxidized (Özmen et al. 2007). Lipid peroxidation (LP) causes injury to cell and intracellular membranes and may lead to cell destruction and subsequently cell death (Yatin et al. 2000; Eren et al. 2007). Brains are protected by antioxidants from peroxidative damage (Halliwell 2006; Nazıroğlu et al. 2009).

Acetaminophen (*N*-acetyl-*p*-aminophenol) is a widely used over-the-counter antipyretic and analgesic drug with unappreciated antioxidant and anti-inflammatory properties. The molecular mechanisms of action of acetaminophen are controversial and still poorly understood (Tripathy and Grammas 2009a). The possible pro- and anti-inflammatory actions of acetaminophen are complex because high acetaminophen administration initiates a series of both pro- and anti-inflammatory cascades (Yee et al. 2007). It is likely that there are other, as yet unidentified physiological targets for both the toxic and therapeutic actions of acetaminophen.

There is considerable interest in drugs that affect oxidative homeostasis in the brain because oxidative stress is thought to contribute to the pathogenesis of many neurodegenerative diseases. Increasing evidence suggests that acetaminophen has unappreciated antioxidant properties. For example, acetaminophen can protect dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP^+)–induced toxicity in mitochondria by scavenging ROS (Maharaj et al. 2004). Also, administration of acetaminophen to rats significantly attenuates quinolinic acid-induced superoxide generation (Maharaj et al. 2006). Acetaminophen has been shown to be a potent scavenger of ROS (Tripathy and Grammas 2009b). However, the antioxidant effects of different doses of acetaminophen on the brain have not been explored.

We evaluated whether there would be a protective effect of acetaminophen at different therapeutic doses on oxidative stress, enzymatic antioxidants, plasma membrane Ca^{2+}-ATPase (PMCA), calcium values in rat brain cortex and microsome.

Materials and Methods

Animals

Seventy male Wistar albino rats weighing 180 ± 10 g were used for the experimental procedures. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments, unless otherwise noted. The temperature was maintained at 22 ± 2 °C. A 12/12 h light/dark cycle was maintained, unless otherwise noted. The experimental protocol was approved by the ethical committee of the Medical Faculty of Suleyman Demirel University (SDU). Animals were maintained and used in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* prepared by the SDU.

Experimental Design

Acetaminophen at different doses was dissolved in physiological saline (0.9%, w/v). Animals were equally divided into seven groups according to the injected acetaminophen dose (Li et al. 2004; Lu et al. 2004) as follows: group I was the control group (n = 10), to which placebo (physiological saline) was intraperitoneally (i.p.) given; groups II, III, IV, V, VI and VII received i.p. 5, 10, 20, 100, 200 and 500 mg/kg body weight acetaminophen, respectively. Twenty-four hours after the injection of acetaminophen, the animals were killed under anesthesia and brain cortical samples were taken.

Anesthesia and Preparation of Brain Samples

Rats were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) administered i.p. before death and removal of the cortical samples. The brain was also taken as follows: The cortex was dissected out after the brain was split in the mid-sagittal plane. Following removal, the cortex was dissected from the total brain as described in our previous study (Eren et al. 2007).

Cortical tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (–30 °C) until processing (maximum 10 h). After weighing, half of the cortex was placed on ice, cut into small pieces using scissors and homogenized (2 min at 5,000 rpm) in five volumes (1:5, w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4), using a glass Teflon homogenizer (Caliskan Cam Teknik, Ankara, Turkey).

All preparation procedures were performed on ice. The homogenate was used for determination of LP and antioxidant levels. The remaining cortical samples were used for the Ca^{2+}-ATPase assay and isolation of microsomes by ultracentrifugation.

After addition of butylhydroxytoluol (4 µl/ml), brain homogenate and microsomal samples were analyzed immediately for LP levels and enzyme activities. Antioxidant vitamin analyses were performed within 3 months.

Isolation of Brain Microsome

Tissues were cleaned, minced and then homogenized in six volumes of freshly prepared buffer A containing 0.3 mol/l sucrose, 10 mmol/l HEPES HCl (pH 7.4) and 2 mmol/l dithiothreitol. The material was homogenized with the glass Teflon homogenizer. The homogenate was centrifuged (MS 80; Sanyo, Tokyo, Japan) at 85,000×*g* in a rotor (Sorvall; Teknolab, Ankara, Turkey) for 75 min. The supernatant was discarded, and the pellet was resuspended in the original volume of buffer A containing 0.6 mol/l KCl using four strokes of the pestle and centrifuged again at 85,000×*g* for 75 min. The pellet was resuspended in the original volume of buffer A. After centrifugation at 85,000×*g* for 75 min, the pellet was suspended in buffer A using four strokes of the pestle at a protein concentration of 2–7 mg/ml. The procedures took 10–12 h for eight samples. The samples were frozen and stored at –30 °C until assayed. The isolation procedure was carried out at +4 °C (Dogru Pekiner et al. 2005; Nazıroğlu et al. 2009).

Measurement of Microsomal Ca^{2+}-ATPase Activity

Ca^{2+}-ATPase activity was measured spectrophotometrically by the method of Niggli et al. (1981). The assay medium contained 120 mmol/l KCl, 60 mmol/l HEPES (pH 7, at 37 °C), 1 mmol/l $MgCl_2$, 0.5 mmol/l K_2-ATP, 0.2 mmol/l NADH, 0.5 mmol/l PEPA, 1 IU/l pyruvate kinase, 1 IU/l LDH and 500 mmol/l EGTA. After preincubation of the assay medium (total volume of 1 ml) for 4 min at 37 °C, 50 mg of the microsomal homogenate were added to the medium. After 2 min, the reaction was started by addition of 600 mmol/l $CaCl_2$. The ATPase activity as oxidation of NADH was followed by continuously measuring the absorbance at 340 nm.

Based on the extinction coefficient for NADH, $\varepsilon = 6.2 \times 10^6$ m^{-1}, the amount of NADH oxidized is equivalent to the hydrolyzed amount of ATP. Values were expressed as international units per milligram protein.

Determination of Total Brain Calcium Levels

Microsomal calcium levels were analyzed with a plasma optic emission atomic absorption spectrophotometer (ICP-OES, Optima 4300 DV; Perkin-Elmer Life and Analytical Sciences, Waltham, MA) by following the wet ashing procedure with nitric acid. Calcium values were measured at 422.7 nm in the atomic absorption spectrophotometer (Nazıroğlu et al. 2009).

LP Level Determinations

LP levels in the brain homogenate and microsomal samples were measured with the thiobarbituric acid reaction by the method of Placer et al. (1966). The values of LP in the brain and microsome were expressed as micromoles per gram protein. Although the method is not specific for LP, measurement of the thiobarbituric acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

Brain Cortical GSH, GSH-Px and Protein Assay

The GSH content of the brain homogenate and microsome was measured at 412 nm using the method of Sedlak and Lindsay (1968) as described in our previous study (Eren et al. 2007). GSH-Px activities of the brain homogenate and microsome were measured spectrophotometrically at 37 °C and 412 nm according to Lawrence and Burk (1976). The protein content in the brain cortex and microsome was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Analyses of Brain Cortical β-Carotene and Vitamins A and E

Vitamins A (retinol) and E (α-tocopherol) were determined in brain cortical samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). The vitamin A and E concentrations were measured in a spectrophotometer (model 1800; Shimadzu, Kyoto, Japan) at 325 and 535 nm, respectively. Calibration was performed using standard solutions of all-*trans*-retinol and α-tocopherol in hexane.

Statistical Analyses

All results are expressed as means ± SD. To determine the effect of treatment, data were analyzed using ANOVA. $P < 0.05$ was regarded as significant. Significant values were assessed with the LSD test. Data were analyzed using the SPSS statistical program (version 9.05; SPSS, Inc., Chicago, IL).

Results

LP Results

The mean brain cortical and microsomal LP levels in the seven groups are shown in Tables 1 and 2. The results showed that LP levels in the brain cortex and microsome in

the sixth and seventh groups were significantly higher than those in the control group, whereas brain cortical and microsomal LP levels in the third and fourth groups were lower than those in the control. Hence, high doses (200 and 500 mg) of acetaminophen caused an increase in oxidative stress of the brain, although moderate doses (10 and 20 mg) induced an antioxidant effect in the rat brain.

GSH-Px Activities and GSH Levels

The mean brain cortical and microsomal GSH-Px activities and GSH levels in the seven groups are shown in Tables 1 and 2. The results showed that the brain cortical and microsomal GSH-Px activities were significantly lower in the sixth and seventh groups than in the control group, although microsomal GSH-Px activity was significantly higher in the third and fourth groups than in control. Hence, GSH-Px activity in the brain cortex and microsome was decreased by the high-dose acetaminophen administration, although it was increased by moderate doses of acetaminophen. However, brain cortical and microsomal GSH levels did not significantly change in the seven groups with different amounts of acetaminophen.

Results of Antioxidant Vitamin Concentrations

The mean brain cortical β-carotene and vitamin A and E concentrations in the seven groups are shown in Table 1, and the β-carotene and vitamin A concentrations were not affected by the acetaminophen administrations. The brain cortical vitamin E concentrations were significantly lower in the sixth and seventh groups compared to control, whereas brain cortical vitamin E concentrations were significantly ($P < 0.05$) higher in the third, fourth and fifth groups than in control.

Results of Brain Cortical Calcium Level and Microsomal PMCA Activity

The mean microsomal PMCA activities in the seven groups are shown in Fig. 1. PMCA activities were significantly lower in the sixth and seventh groups than in control, although in the second, third and fourth groups they were significantly higher than in control.

The mean brain cortical calcium levels in the seven groups are shown in Fig. 2. The calcium levels were significantly higher in the sixth and seventh groups than in control, although levels in the second, third, fourth and fifth groups were not significantly changed by administration of different concentrations of acetaminophen.

Discussion

We found that the brain cortical and microsomal LP and microsomal calcium levels were increased by high doses (200 and 500 mg) of acetaminophen, although levels were decreased by moderate doses (10 and 20) of acetaminophen. The brain and microsomal GSH-Px and brain vitamin E levels as well as the PMCA activities increased in the moderate-dose acetaminophen groups, whereas their values were decreased by moderate doses of acetaminophen. Hence, high-dose acetaminophen administrations in the animals are characterized by increased LP and decreased PMCA, GSH-Px and vitamin E antioxidant values, although moderate doses of acetaminophen induced an antioxidant effect. A limited number of in vivo or in vitro studies in tissues except the brain microsome of experimental animals has been reported regarding the effects of acetaminophen on the antioxidant enzymatic system and on LP and PMCA values (Maharaj et al. 2004, 2006; Tripathy and Grammas 2009a). To the best of our knowledge, the

Table 1 Effects of different doses of acetaminophen on cortical GSH-Px activity and GSH, LP, calcium, vitamin E, vitamin A, and β-carotene levels in rats (mean ± SD)

Parameters	Control (n = 10)	5 mg (n = 10)	10 mg (n = 10)	20 mg (n = 10)	100 mg (n = 10)	200 mg (n = 10)	500 mg (n = 10)
GSH-Px(IU/g prot)	53.4 ± 9.2	50.1 ± 5.9	51.6 ± 6.0	51.6 ± 6.1	49.1 ± 7.4	42.7 ± 6.2*	38.7 ± 4.7*
GSH (μmol/g prot)	9.3 ± 1.2	9.4 ± 2.7	10.7 ± 2.5	10.2 ± 1.0	10.0 ± 2.4	9.5 ± 1.4	9.1 ± 1.5
LP (μmol/g prot)	795 ± 39	683 ± 57	615 ± 49*	607 ± 43*	756 ± 40	1222 ± 96**	1403 ± 91**
Vitamin E (μmol/g tissue)	17.4 ± 1.1	18.1 ± 2.2	21.3 ± 5.3*	28.6 ± 2.9*	22.9 ± 3.5*	14.6 ± 5.9*	12.5 ± 2.6**
β-carotene (μmol/g tissue)	1.4 ± 0.2	1.7 ± 0.5	1.6 ± 0.1	1.7 ± 0.3	1.7 ± 0.3	1.5 ± 0.2	1.3 ± 0.1
Vitamin A (nmol/g tissue)	2.4 ± 0.3	2.8 ± 0.5	2.8 ± 0.4	2.9 ± 0.3	2.8 ± 0.5	2.7 ± 0.4	2.8 ± 0.5

* $P < 0.05$ and ** $P < 0.01$ vs. control group

Table 2 Effects of different doses of acetaminophen on cortical microsomal GSH-Px activity and GSH and LP levels in rats (mean ± SD)

Parameters	Control (n = 10)	5 mg (n = 10)	10 mg (n = 10)	20 mg (n = 10)	100 mg (n = 10)	200 mg (n = 10)	500 mg (n = 10)
GSH-Px (IU/g prot)	8.4 ± 0.7	8.5 ± 1.5	9.8 ± 1.2*	9.6 ± 1.7*	7.4 ± 0.5	6.9 ± 0.8*	5.9 ± 1.5*
GSH (μmol/g prot)	5.4 ± 0.3	5.4 ± 0.6	5.6 ± 0.8	5.9 ± 0.6	5.9 ± 0.7	5.7 ± 0.6	6.7 ± 0.9
LP (μmol/g prot)	46.3 ± 4.4	41.3 ± 7.3	36.3 ± 5.2*	37.2 ± 2.9*	48.3 ± 5.6	56.3 ± 5.8*	62.1 ± 1.9**

* $P < 0.05$ and ** $P < 0.01$ vs. control group

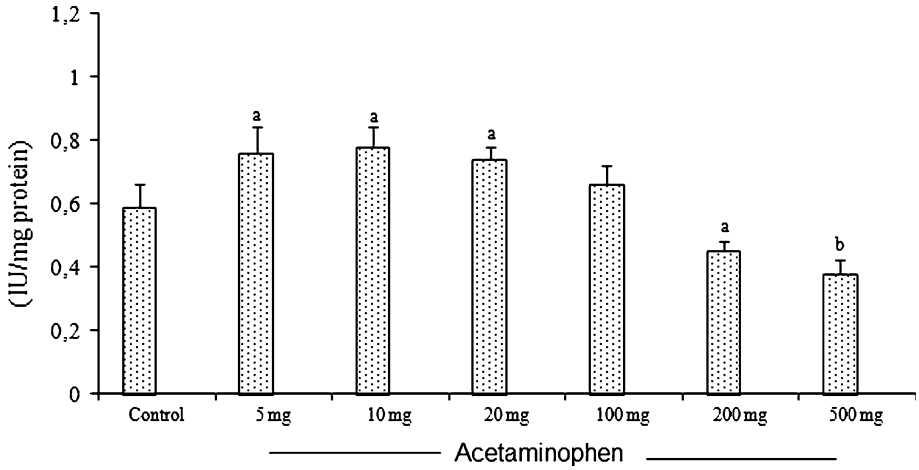

Fig. 1 Effects of different doses of acetaminophen on microsomal Ca^{2+}-ATPase activity in rats (mean ± SD and n = 10). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control

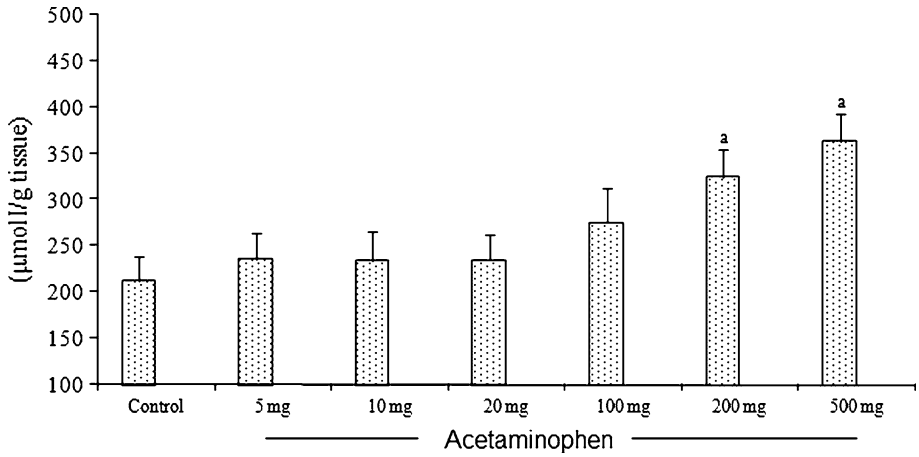

Fig. 2 Effects of different doses of acetaminophen on brain cortical calcium levels in rats (mean ± SD and n = 10). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control

current study is the first to compare the medicine with particular reference to its effects on oxidative stress and the antioxidant redox system in brain injury of rats.

The current study indicated that acetaminophen administration at high doses produced a significant increase in LP levels of the brain cortex and microsome, although moderate doses induced an antioxidant effect. Our results are in accordance with previous reports of an LP increase in brain endothelial cells (Maharaj et al. 2004, 2006; Tripathy and Grammas 2009a, 2009b). On the other hand, the current study is the first report regarding brain microsomal LP in acetaminophen-administered rats. High doses of acetaminophen may trigger a variety of biochemical process including activation of membrane phospholipases, proteases and nucleases (Tripathy and Grammas 2009a). Marked alterations in membrane phospholipid metabolism result in the liberation of LP and ROS. Hence, the involvement of LP as malondialdehyde in high-dose acetaminophen administration was attributed to the activation of membrane phospholipases. In the current study, GSH-Px and vitamin E in the brain cortex and microsome were decreased by high-dose acetaminophen. If GSH-Px levels decrease, superoxide radical production may increase and eventually lead to oxidative stress and LP (Nazıroğlu 2007a, 2007b).

Intake of large doses of acetaminophen results in severe hepatic necrosis (James et al. 2003). Oxidative stress mediated by the metabolite *N*-acetyl-*p*-benzoquinone-imine is considered the main cause of acetaminophen-

induced toxicity (Olaleye and Rocha 2008). Mice deficient in SOD are resistant to acetaminophen toxicity due to a reduction in activity of a key acetaminophen-metabolizing enzyme (cytochrome p450 2E1) (Lei et al. 2006). In the brain, overdose of acetaminophen (3 g/kg) caused a dramatic decrease of glutathione levels, ascorbic acid concentrations and SOD activity (Nencini et al. 2007). Pretreatment of endothelial cell culture with acetaminophen (25–100 μM) increased cell survival and inhibited superoxide-generating compound menadione (25 μM)-induced expression of inflammatory proteins and SOD activity (Tripathy and Grammas 2009a).

The above-documented toxic effects associated with oxidative stress are elicited in response to high doses of acetaminophen. However, several studies have directly demonstrated acetaminophen's capacity to function as an antioxidant (Tripathy and Grammas 2009a). During post-ischemia reperfusion in the heart, acetaminophen attenuates the damaging effects of peroxynitrite and hydrogen peroxide and limits protein oxidation (Jaques-Robinson et al. 2008). Acetaminophen is a phenolic compound which produces a clear inhibitory dose–response curve with peroxynitrite in its range of clinical effectiveness (Van Dyke et al. 1998). Acetaminophen protected hippocampal neurons and PC12 cultures from amyloid-beta peptide-induced oxidative stress through reduction of LP and by lowering cytoplasmic levels of peroxides (Bisaglia et al. 2002). In accordance with the reports, we observed in the current study that moderate doses of acetaminophen induced antioxidant effects due to its phenolic structure.

Inactivation of ROS can be carried out by antioxidant vitamins (Nazıroğlu 2007b). Vitamin E, α-tocopherol, is the most important antioxidant in the lipid phase of cells. Vitamin E acts to protect cells against the effects of free radicals, which are potentially damaging by-products of the body's metabolism (Yatin et al. 2000). Therefore, low antioxidant levels and high content of PUFA results in limited antioxidant defense in the brain. Vitamin E concentrations in the brain cortex were decreased in the high-dose acetaminophen group, although brain cortical vitamin E concentration was increased by moderate doses of acetaminophen. The decreased concentration of the antioxidant vitamins at high doses of acetaminophen could be due to its stimulation as a result of the increased production of free radicals.

The mechanism of action of acetaminophen is still unclear (Tripathy and Grammas 2009a). The hepatotoxicity of high doses of acetaminophen has traditionally been associated with necrosis (James et al. 2003). However, high-dose acetaminophen can also evoke apoptosis. The C-jun N-terminal kinase is thought to play a central role in acetaminophen-induced liver injury and in injury of glioma cells (Nakagawa et al. 2008). In contrast, low dose (350 μM) of acetaminophen appears to be protective in cardiac myocytes exposed to reperfusion injury via inhibition of the mitochondrial permeability transition pore and subsequent apoptotic pathway (Hadzimichalis et al. 2007). Similarly, Tripathy and Grammas (2009a) reported that acetaminophen at a very low dose (50 μM or 8 μg/ml) protected neurons exposed to oxidative stress. Acetaminophen inhibits the formation of the Parkinson disease toxin 1-methyl-4-phenylpridinium in mitochondria (Maharaj et al. 2004). Acetaminophen has also been shown to be a potent scavenger of peroxynitrite (Schildknecht et al. 2008). Taken together, these data are consistent with the results of the current study showing that acetaminophen at moderate doses significantly protects brain exposed to oxidative stress.

PMCA activity was decreased by administration of high doses of acetaminophen, whereas it was increased by moderate doses of acetaminophen. Brain cortical total calcium levels were higher in the high-dose acetaminophen groups than in control. In physiologically healthy cells, calcium concentrations outside of the cells were 12,000- to 20,000-fold higher than inside of the cells. It was reported that if the cell membrane calcium channels degenerate by high levels of oxidative stress, calcium enters the cells from extracellular fluid, although PMCA activity decreases due to toxic effects of acetaminophen on the cell membranes (Gutiérrez-Martín et al. 2005; Zaidi et al. 2009). It has also been suggested that phenolic compound–containing drugs such as acetaminophen are associated with detrimental effects on brain antioxidant defense systems (Tripathy and Grammas 2009a, 2009b). In addition, calcium and calcium-dependent processes have been hypothesized to be involved in the induction of apoptosis (Nazıroğlu 2009). It has been shown that acetaminophen rectifies Ca^{2+} homeostasis and therefore decreases ROS production in the rat hippocampus (Maharaj et al. 2006). The alterations in PMCA activity and calcium levels by high doses of acetaminophen, which result in the generation of ROS, could therefore be attenuated by moderate doses of acetaminophen, which would also explain the potent action exhibited by moderate doses of this drug.

If mitochondrial cytosolic Ca^{2+} increases over 400 nm, it leads to depolarization of mitochondrial membranes (Colegrove et al. 2000; González et al. 2007). Uptake of Ca^{2+} into mitochondria stimulates the tricarboxylate cycle, resulting in augmented reduction of pyridine nucleotides, which may be one of the mechanisms of the coupling of neuronal and metabolic activity (Duchen 2000). On the other hand, exposure of mitochondria to high cytosolic free Ca^{2+} was shown to increase formation of ROS (Patel 2004). It has been reported that moderate doses of acetaminophen in cardiomyocytes modulated cytosolic Ca^{2+} levels by regulation of mitochondrial pore and Ca^{2+} influx (Hadzimichalis et al. 2007). In the current study the brain

cortical and microsomal LP levels were lower in the acetaminophen groups with moderate doses than in the control group. Modulation of microsomal pores and Ca^{2+} influx in brain cells by treatment with acetaminophen might cause a decrease in mitochondrial ROS production.

In conclusion, 10- and 20-mg acetaminophen administrations have a protective effect on oxidative stress and the antioxidant redox system. The beneficial effects of acetaminophen on glutathione and nonenzymatic antioxidant systems include regulation of GSH-Px enzyme activities and vitamin E levels in the cortex of the brain. The results in the brain may be of help to physicians in the treatment of oxidative stress–dependent brain degeneration with the drug.

References

Bisaglia M, Venezia V, Piccioli P, Stanzione S, Porcile C, Russo C, Mancini F, Milanese C, Schettini G (2002) Acetaminophen protects hippocampal neurons and PC12 cultures from amyloid beta-peptides induced oxidative stress and reduces NF-kappaB activation. Neurochem Int 41:43–54

Colegrove SL, Albrecht MA, Friel DD (2000) Dissection of mitochondrial Ca^{2+} uptake and release fluxes in situ after depolarization-evoked $[Ca^{2+}]_i$ elevations in sympathetic neurons. J Gen Physiol 115:351–370

Desai ID (1984) Vitamin E analysis methods for animal tissues. Methods Enzymol 105:138–147

Doğru Pekiner B, Daş Evcimen N, Nebioğlu S (2005) Diabetes-induced decrease in rat brain microsomal Ca^{2+}-ATPase activity. Cell Biochem Funct 23:239–243

Duchen MR (2000) Mitochondria and calcium: from cell signalling to cell death. J Physiol 529:57–68

Eren I, Nazıroğlu M, Demirdaş A (2007) Protective effects of lamotrigine, aripirazole and escitalopram on depression-induced oxidative stress in rat brain. Neurochem Res 32:1188–1195

González A, Pariente JA, Salido GM (2007) Ethanol stimulates ROS generation by mitochondria through Ca^{2+} mobilization and increases GFAP content in rat hippocampal astrocytes. Brain Res 1178:28–37

Gutiérrez-Martín Y, Martín-Romero FJ, Henao F, Gutiérrez-Merino C (2005) Alteration of cytosolic free calcium homeostasis by SIN-1: high sensitivity of L-type Ca^{2+} channels to extracellular oxidative/nitrosative stress in cerebellar granule cells. J Neurochem 92:973–989

Hadzimichalis NM, Baliga SS, Golfetti R, Jaques KM, Firestein BL, Merrill GF (2007) Acetaminophen-mediated cardioprotection via inhibition of the mitochondrial permeability transition pore-induced apoptotic pathway. Am J Physiol 293:H3348–H3355

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658

James LP, Mayeux PR, Hinson JA (2003) Acetaminophen-induced hepatotoxicity. Drug Metab Dispos 31:1499–1506

Jaques-Robinson KM, Golfetti R, Baliga SS, Hadzimichalis NM, Merrill GF (2008) Acetaminophen is cardioprotective against H_2O_2-induced injury in vivo. Exp Biol Med (Maywood) 233:1315–1322

Lawrence RA, Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 71:952–958

Lei XG, Zhu JH, McClung JP, Aregullin M, Roneker CA (2006) Mice deficient in Cu, Zn-superoxide dismutase are resistant to acetaminophen toxicity. Biochem J 399:455–461

Li XD, Xia YLv, He P, Han J, Wu C (2004) Conjugation metabolism of acetaminophen and bilirubin in extrahepatic tissues of rats. Life Sci 74:1307–1315

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin- phenol reagent. J Biol Chem 193:265–275

Lu ZB, Li Q, Ye BP, Bian S, Wang Y, Ruan QP, Wu WT (2004) Protective effects of shark hepatic stimulator substance against acute hepatic injury induced by acetaminophen in mice. Acta Pharmacol Sin 39:17–21

Maharaj H, Maharaj DS, Saravanan KS, Mohanakumar KP, Daya S (2004) Aspirin curtails the acetaminophen-induced rise in brain norepinephrine levels. Metab Brain Dis 19:71–77

Maharaj H, Maharaj DS, Daya S (2006) Acetylsalicylic acid and acetaminophen protect against MPP^+-induced mitochondrial damage and superoxide anion generation. Life Sci 78:2438–2443

Nakagawa H, Maeda S, Hikiba Y, Ohmae T, Shibata W, Yanai A, Sakamoto K, Ogura K, Noguchi T, Karin M, Ichijo H, Omata M (2008) Deletion of apoptosis signal-regulating kinase 1 attenuates acetaminophen-induced liver injury by inhibiting c-Jun N-terminal kinase activation. Gastroenterology 135:1311–1321

Nazıroğlu M (2007a) Molecular mechanisms of vitamin E on intracellular signaling pathways in brain. In: Goth L (ed) Reactive oxygen species and diseases. Research Signpost Press, Kerala, India, pp 239–256

Nazıroğlu M (2007b) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res 32:1990–2001

Nazıroğlu M (2009) Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. Neurochem Res. doi:10.1007/s11064-009-0015-8

Nazıroğlu M, Kutluhan S, Uğuz AC, Çelik Ö, Bal R, Butterworth PJ (2009) Topiramate and vitamin E modulates the electroencephalography records, brain microsomal and blood antioxidant redox system in pentylentetrazol-induced seizure of rats. J Membr Biol 229:131–140

Nencini C, Giorgi G, Micheli L (2007) Protective effect of silymarin on oxidative stress in brain. Phytomedicine 14:129–135

Niggli V, Adunyah ES, Penniston JT, Carafoli E (1981) Purified (Ca^{2+}-Mg^{2+})-ATPase of the erythrocyte membrane. Reconstitution and effect of calmodulin and phospholipids. J Biol Chem 256:395–401

Olaleye MT, Rocha BT (2008) Acetaminophen-induced liver damage in mice; effects of some medicinal plants on the oxidative defense system. Exp Toxicol Pathol 59:319–327

Özmen I, Nazıroğlu M, Alıcı HA, Sahin F, Cengiz M, Eren İ (2007) Spinal morphine administration reduces the fatty acid contents in spinal cord and brain in rabbits due to oxidative stress. Neurochem Res 32:19–25

Patel M (2004) Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med 37:1951–1962

Placer ZA, Cushman L, Johnson BC (1966) Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. Anal Biochem 16:359–364

Schildknecht S, Daiber A, Ghisla S, Cohen RA, Bachschmid MM (2008) Acetaminophen inhibits prostanoid synthesis by scavenging the PGHS-activator peroxynitrite. FASEB J 22: 215–224

Sedlak J, Lindsay RHC (1968) Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellmann' s reagent. Anal Biochem 25:192–205

Suzuki J, Katoh N (1990) A simple and cheap method for measuring vitamin A in cattle using only a spectrophotometer. Jpn J Vet Sci 52:1282–1284

Tripathy D, Grammas P (2009a) Acetaminophen inhibits neuronal inflammation and protects neurons from oxidative stress. J Neuroinflamm 6:10

Tripathy D, Grammas P (2009b) Acetaminophen protects brain endothelial cells against oxidative stress. Microvasc Res 77:289–296

Van Dyke K, Sacks M, Qazi N (1998) A new screening method to detect water-soluble antioxidants: acetaminophen (Tylenol) and other phenols react as antioxidants and destroy peroxynitrite-based luminol-dependent chemiluminescence. J Biolumin Chemilumin 13:339–348

Whanger PD (2001) Selenium and the brain: a review. Nutr Neurosci 4:81–97

Yatin SM, Varadarajan S, Butterfield DA (2000) Vitamin E prevents Alzheimer's amyloid beta-peptide (1–42)-induced neuronal protein oxidation and reactive oxygen species production. J Alzheimers Dis 2:123–131

Yee SB, Bourdi M, Masson MJ, Pohl LR (2007) Hepatoprotective role of endogenous interleukin-13 in a murine model of acetaminophen-induced liver disease. Chem Res Toxicol 20:734–744

Zaidi A, Fernandes D, Bean JL, Michaelis ML (2009) Effects of paraquat-induced oxidative stress on the neuronal plasma membrane Ca^{2+}-ATPase. Free Radic Biol Med. doi: 10.1016/j.freeradbiomed.2009.08.018