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# Fish Oil and Antipsychotic Drug Risperidone Modulate Oxidative Stress in PC12 Cell Membranes Through Regulation of Cytosolic Calcium Ion Release and Antioxidant System

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**Abstract** Oxidative stress is a critical route of damage in various psychological disorders such as schizophrenia, although fish oil and risperidone (RISP) induce antioxidant effects in the human body. However, the mechanisms behind these effects remain elusive. We investigated the effects of fish oil and RISP in the PC12 cell line by evaluating $Ca^{2+}$ mobilization, lipid peroxidation (LP) and antioxidant levels. PC12 cells were divided into eight flasks: control, fish oil, RISP, $H_2O_2$, fish oil + $H_2O_2$, RISP + $H_2O_2$, fish oil + RISP and fish oil + RISP + $H_2O_2$. Cells were incubated with fish oil and RISP for 24 and 48 h, respectively. Then, cells were exposed to $H_2O_2$ for 15 min before analysis. $Ca^{2+}$ release and LP levels were higher in the $H_2O_2$ group than in the control, RISP and fish oil groups, although their levels were decreased by incubation of cells in fish oil and RISP. Glutathione peroxidase activity, reduced glutathione and vitamin C levels in the cells were lower in the $H_2O_2$ group than in the control, RISP and fish oil groups, although levels were higher in cells incubated with fish oil and RISP than in those in the $H_2O_2$ groups. In conclusion, these results indicate that RISP and fish oil induced protective effects on oxidative stress in PC12 cells by modulating cytosolic $Ca^{2+}$ release and antioxidant levels.

**Keywords** Schizophrenia · Fish oil · $Ca^{2+}$ release · Oxidative stress · PC12 cell

**Abbreviations**

| | |
|---|---|
| EPUFA | Essential polyunsaturated fatty acid |
| GSH | Reduced glutathione |
| GSH-Px | Glutathione peroxidase |
| LP | Lipid peroxidation |
| RISP | Risperidone |
| $PLA_2$ | Phopspholipase A2 |
| PC12 | Rat pheochromocytoma-derived cell line |

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## Introduction

Reactive oxygen substances (ROS) and lipid peroxides are produced by a free radical chain reaction, which can also be initiated by ROS (Halliwell 2006). The ROS, i.e., singlet oxygen, superoxide anion, perhydroxyl and hydroxyl radicals, contribute to tissue damage (Nazıroğlu 2009). ROS also cause injury by reacting with biomolecules such as lipids, proteins and nucleic acids as well as by depleting enzymatic and/or nonenzymatic antioxidants in the brain (Whanger 2001). There is also evidence that ROS play an important role in the pathogenesis of many diseases, particularly in neurological and psychiatric diseases due to the central nervous system vulnerability to oxidative stress (Bilici et al. 2001; Mahadik et al. 2001). Glutathione peroxidase (GSH-Px) detoxifies hydrogen peroxide to water (Whanger 2001), although vitamin C has an inhibitory role on superoxide and hydroxyl radicals in the cytosol of cells (Halliwell 2006; Nazıroğlu 2007a). Therefore, ROS can be

indirectly evaluated by measurement of some antioxidants such as GSH-Px, reduced glutathione (GSH) and vitamin C (Halliwell 2006; Nazıroğlu 2007b).

Oxidative stress is primarily or secondarily involved in the pathogenesis of psychiatric diseases such as schizophrenia. The coexistence of increased oxidative stress with symptoms of depression and schizophrenia in patients has been reported, as evidenced by defective plasma antioxidant defenses in association with enhanced susceptibility to lipid peroxidation (LP) (Sivrioğlu et al. 2007; Dietrich-Muszalska et al. 2010). Significant correlations were also found between the severity of schizophrenia, as well as the length of index episode and duration of illness, and alterations in GSH-Px activity, GSH and LP levels (Zhang et al. 2006; Sarandol et al. 2007). As a potent antioxidant, vitamin C and its combination with antipsychotics in the treatment of schizophrenia have been suggested (Mahadik et al. 2001). Essential polyunsaturated fatty acids (EPUFAs) have been shown to improve some of the psychopathological symptoms of schizophrenia (Mahadik and Evans 2003). Hence, antidepressant therapeutic intervention may be associated with normalization of the critical oxidative redox system along with alleviating depressive and schizophrenic symptoms.

Some studies have reported an increase of LP levels in the plasma of first-episode schizophrenic patients who had not taken any antipsychotic drugs (Mahadik et al. 1998). EPUFAs are critical for brain development as well as function (Whanger 2001) and are affected in schizophrenia. Decreased levels of membrane EPUFAs observed in schizophrenia may be associated partly with an increase of phospholipase $A_2$ ($PLA_2$) activity (Arvindakshan et al. 2003). $PLA_2$ catalyzes the removal of PUFA from phospholipid molecules, which then take part in signaling reactions or are recycled back into the phospholipid molecules. Essentially fatty acid reduction in the membrane of patients with schizophrenia and, consequently, cell injury might result from excessive ROS generation or an impaired antioxidant defense redox system (Mahadik and Evans 2003). Ultimately, this process could lead to neuronal membrane stability and perhaps cell death (Halliwell 2006). However, Ranjekar et al. (2003) did not find an increase in plasma LP levels in schizophrenia. Thus, the role of LP in the pathophysiology merits further research in cases of schizophrenia.

Antipsychotics are the drugs of choice for the long-term management of schizophrenia. However, antipsychotic drugs may affect lipid metabolism (Zhang et al. 2006). Classic antipsychotic drugs are suggested to increase oxidative stress and oxidative cell injury in the brain (Mahadik et al. 1998), whereas the second-generation antipsychotic drugs do not exhibit such effects or they may have antioxidant effects (Kropp et al. 2005; Dietrich-Muszalska et al. 2010). Risperidon (RISP) could have different effects on oxidative stress and antioxidant systems in the cells. However, the mechanisms of these effects are not fully understood.

Evidence is increasing to indicate that mitochondrial pathophysiology and oxidative stress may also be the most critical components in the pathophysiology and outcome of schizophrenia (Ben-Shachar and Laifenfeld 2004; Bubber et al. 2004). $Ca^{2+}$ is a key regulator of cell survival since cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) is a major regulatory factor for a large number of cellular processes, such as muscle contraction, metabolism, secretion and even cell differentiation and apoptosis. However, the sustained elevation of intracellular $Ca^{2+}$ plays a role in cell death (Nazıroğlu 2009). The proapoptotic effects of $Ca^{2+}$ are mediated by a diverse range of $Ca^{2+}$-sensitive factors that are compartmentalized in various intracellular organelles, including endoplasmic reticulum and mitochondria (Putney and McKay 1999). If the free intracellular $Ca^{2+}$ concentration increases due to degeneration of cation channels, this causes loss of physiologic cell functions (Halliwell 2006; Nazıroğlu 2007a, b). Excessive $Ca^{2+}$ load to the mitochondria may induce apoptosis by both stimulating the release of apoptosis-promoting factors such as ROS from the mitochondrial intermembrane space to the cytoplasm and impairing mitochondrial function and cation channels (Wang 2001; Nazıroğlu 2007a).

PC12 cells have been widely used in studies of oxidative stress-induced protection models (Wang et al. 2005; Akan et al. 2009). Since the modulatory effects of fish oil and RISP on cellular survival and death in molecular pathways have not yet been clarified, we focused on the dual effect of fish oil + RISP in undifferentiated PC12 cell lines by checking its effect on LP and antioxidant levels and $Ca^{2+}$ release from intracellular stores evoked by $H_2O_2$.

## Materials and Methods

### Chemicals

Cell culture flasks were bought from TPP Company (Trasadingen, Switzerland). All chemicals (fish oil, cumene hydroperoxide, KOH, NaOH, thiobarbutiric acid, 1,1,3,3 tetraethoxypropane, 5,5-dithiobis-2 nitrobenzoic acid, tris-hydroxymethyl-aminomethane, cumene-hydroperoxide, glutathione, butylhydroxytoluol) were obtained from Sigma-Aldrich (St. Louis, MO), and all organic solvents (*n*-hexane, ethyl alcohol) and RPMI 1640 medium were purchased from Merck (Darmstadt, Germany). Fura-2 was purchased from Promega (Madison, WI). All reagents were analytical grade. All reagents except the phosphate buffers were prepared daily and stored at +4°C. Reagents were equilibrated at room

temperature for 30 min before an analysis was initiated or reagent containers were refilled. Phosphate buffers were stable at +4°C for 1 month.

## Cell Culture

PC12, a rat pheochromocytoma-derived cell line, was selected as the neuronal model, which was originally described by Greene and Tischer (1976). The cell line was obtained from DSMZ Cell Lines Bank (Braunschweig, Germany). Before treatment, PC12 cells were placed in a poly-d-lysine-coated cell culture flask at a density of $1 \times 10^5/cm^2$ and allowed to attach for 24 h in RPMI 1640 medium supplemented with 10% donor horse serum, 5% fetal bovine serum and a mixture of 1% of penicillin/streptomycin/L-glutamine. Cells were incubated at 37°C in a humid 5% $CO_2$ and 95% air environment (Akan et al. 2009).

## Study Groups

PC12 cells were divided into eight groups as follows:

- Group 1: Control group. Cells were incubated with only RPMI medium for 24 h.
- Group 2: Fish oil group. A therapeutic dose of fish oil was calculated according to the content of decosahexaenoic acid (DHA, 60 μM) and eicosapentaenoic acid (EPA, 75 μM) in the fish oil. DHA and EPA were dissolved in 0.1 ml DMSO stock solution, which was diluted with medium (Arab and Rossary 2006). Cells were incubated with 60 μM DHA and 75 μM EPA for 24 h.
- Group 3: Risperidone group. Cells were incubated with RISP (40 μg/ml) for 48 h (Bai et al. 2002).
- Group 4: $H_2O_2$ group. Cells were exposed to $H_2O_2$ (100 μM) (Uğuz et al. 2009).
- Group 5: Fish oil + $H_2O_2$ group. Cells were incubated fish oil (DHA, 60 μM and EPA, 75 μM) for 24 h before $H_2O_2$ incubation. Cells were exposed to $H_2O_2$ (100 μM) for 15 min.
- Group 6: RISP + $H_2O_2$ group. Cells were exposed to $H_2O_2$ (100 μM) after incubation with RISP (40 μg/ml) for 48 h.
- Group 7: Fish oil + RISP group. Cells were incubated in fish oil (60 μM DHA) and RISP (40 μg/ml) for 24 and 48 h, respectively.
- Group 8: Fish oil + RISP + $H_2O_2$ group. Cells were incubated in fish oil (60 μM DHA) and RISP (40 μg/ml) for 24 and 48 h, respectively. Then, cells were exposed to $H_2O_2$ (100 μM) for 15 min.

At the end of the treatments, half of the cells were washed in the medium and stored at −33°C. LP and antioxidant analyses were performed within 1 week. The remaining cells were immediately used for cytosolic $[Ca^{2+}]_i$ analysis. During the $[Ca^{2+}]_i$ analysis, the eight groups were exposed to $H_2O_2$ for stimulating $[Ca^{2+}]_i$ release.

## Measurement of Cytosolic $[Ca^{2+}]_i$

Cells were loaded with fura-2 by incubation with 4 μM fura-2 acetoxymethyl ester (fura-2/AM) for 30 min at room temperature according to a procedure published elsewhere (Uğuz et al. 2009). Once loaded, cells were washed and gently resuspended in Na-HEPES solution containing (in mM) NaCl, 140; KCl, 4.7; $CaCl_2$, 1.2; $MgCl_2$, 1.1; glucose, 10; and HEPES, 10 (pH 7.4). The eight groups were exposed to $H_2O_2$ to stimulate $[Ca^{2+}]_i$ release. Fluorescence was recorded from 2-ml aliquots of a magnetically stirred cellular suspension ($2 \times 10^6$ cells/ml) at 37°C using a spectrofluorometer (Carry Eclipsys; Varian, Sydney, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored using the fura-2 340/380 nm fluorescence ratio and calibrated according to the method of Grynkiewicz et al. (1985). In experiments where calcium-free medium was indicated, $Ca^{2+}$ was omitted and 2 mM ethylene glycol-bis (2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) was added.

$Ca^{2+}$ release was estimated using the integral of the rise in $[Ca^{2+}]_i$ for 150 s after addition of $H_2O_2$ (Uğuz et al. 2009; Espino et al. 2009). $Ca^{2+}$ release is expressed in nanomolar concentrations, taking a sample every second (nM s), as previously described (Heemskerk et al. 1997).

## LP Determinations

LP levels in PC12 cells were measured with the thiobarbituric acid reaction by the method of Placer et al. (1966). Thiobarbituric acid-reactive substances were quantified by comparing the absorption to the standard curve of malondialdehyde (MDA) equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of LP in PC12 cells were expressed as nanomoles per gram protein. Although the method is not specific for LP, measurement of the thiobarbituric acid reaction is an easy and reliable method (Halliwell 2006), which is used as an indicator of LP and ROS activity in biological samples.

## GSH, GSH-Px and Protein Assay

The GSH content of PC12 cells was measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of PC12 cells were measured spectrophotometrically at 37°C and 412 nm according to the method of

Lawrence and Burk (1976). The protein content in PC12 cells was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

### Vitamin C Analyses

Vitamin C (ascorbic acid) in PC12 cell samples was quantified according to the method of Jagota and Dani (1982). The absorbance of the samples was measured spectrophotometrically at 760 nm.

### Statistical Analysis

Data are expressed as means ± SEM of the numbers of determinations. Statistical significance was analyzed using the SPSS program (9.05; SPSS, Inc., Chicago, IL). To compare the different treatments, statistical significance was calculated by the Mann–Whitney *U* test analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

## Results

### Effects of Fish Oil and RISP on Cytosolic $[Ca^{2+}]_i$ Release

The effects of fish oil and RISP on cytosolic $[Ca^{2+}]_i$ release in PC12 cells are shown in Fig. 1. Cells were incubated with RISP and fish oil with or without $H_2O_2$ (100 μM) for 15 min. Cytosolic $[Ca^{2+}]_i$ release in PC12 cells were significantly ($P < 0.001$) higher in the $H_2O_2$ group than in the control, fish oil and RISP groups. Cytosolic $[Ca^{2+}]_i$ release was significantly ($P < 0.01$) lower in the fish oil + $H_2O_2$ and RISP + $H_2O_2$ groups than in the $H_2O_2$ group. Cytosolic $[Ca^{2+}]_i$ release was significantly ($P < 0.05$) lower in the fish oil + RISP + $H_2O_2$ group than in the control, fish oil and RISP groups. Hence, we found that RISP and fish oil induced protective effects against oxidative stress-induced $[Ca^{2+}]_i$ release. Cytosolic $[Ca^{2+}]_i$ release was also significantly ($P < 0.001$) lower in the fish oil + RISP and fish oil + RISP + $H_2O_2$ groups than in the fish oil + $H_2O_2$ and RISP + $H_2O_2$ groups. Hence, the protective effect of the fish oil and RISP combination was higher ($P < 0.001$) than that of RISP or fish oil alone against oxidative stress-induced $[Ca^{2+}]_i$ release.

### Effects of Fish Oil and RISP on LP Levels

The effects of fish oil and RISP on LP levels in PC12 cells are shown in Fig. 2. LP levels were significantly ($P < 0.01$) higher in the $H_2O_2$ group than in the control, fish oil and RISP groups. LP levels were significantly ($P < 0.01$) lower in the fish oil + $H_2O_2$ ($P < 0.05$), RISP + $H_2O_2$ ($P < 0.01$), fish oil + RISP ($P < 0.01$) and fish oil + RISP + $H_2O_2$ ($P < 0.05$) groups than in the $H_2O_2$ group only. Hence, we found that RISP and fish oil induced protective effects against oxidative stress-induced LP levels. However, the

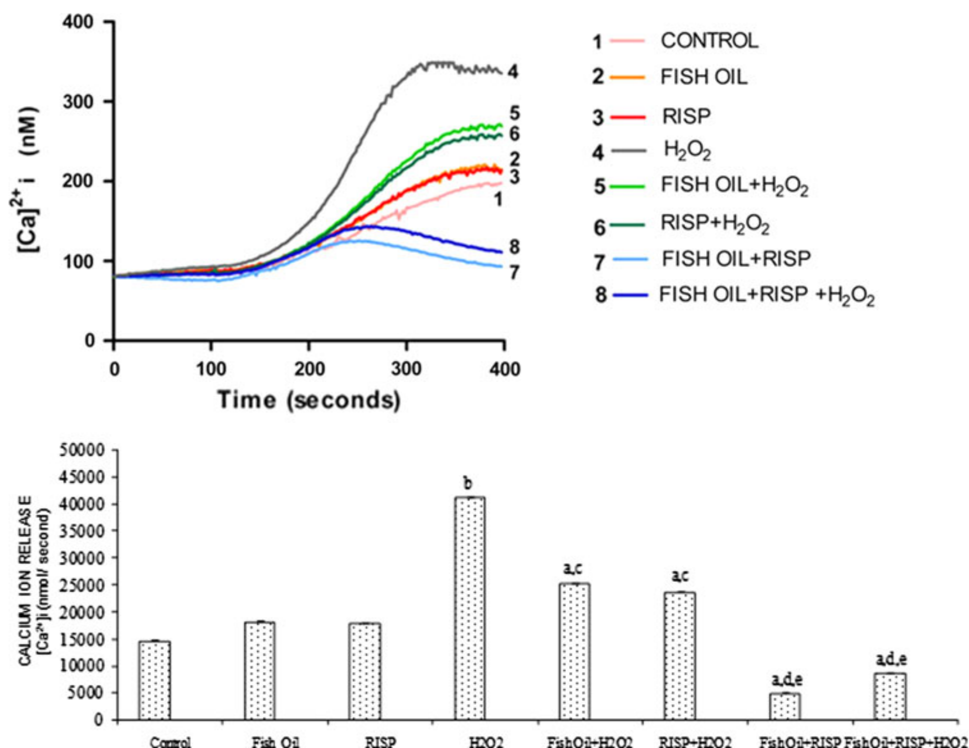


**Fig. 1** Effects of fish oil and RISP on cytosolic $[Ca^{2+}]_i$ release in PC12 cells ($n = 8$, mean ± SD). [a] $P < 0.05$ and [b] $P < 0.001$ vs. control, fish oil and RIS; [c] $P < 0.01$ and [d] $P < 0.01$ vs. $H_2O_2$; [e] $P < 0.001$ vs. fish oil + $H_2O_2$ and RIS + $H_2O_2$

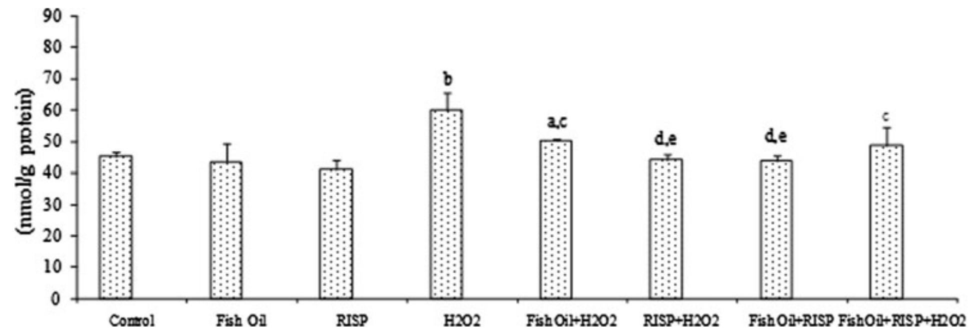


**Fig. 2** Effects of fish oil and RISP on LP levels in PC12 cells ($n = 8$, mean ± SD). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control, fish oil and RIS; [c] $P < 0.05$ and [d] $P < 0.01$ vs. $H_2O_2$; [e] $P < 0.05$ vs. fish oil + $H_2O_2$

protective effect of RISP on LP levels was higher ($P < 0.05$) than in the fish oil group.

## Effects of Fish Oil and RISP on GSH-Px, GSH and Vitamin C

GSH-Px ($P < 0.05$), GSH ($P < 0.05$) and vitamin C ($P < 0.01$) levels in PC12 cells were significantly lower in the $H_2O_2$ group than in the control, fish oil and RISP groups (Figs. 3, 4, and 5). GSH-Px, GSH and vitamin C levels were significantly higher in the fish oil + $H_2O_2$ and RISP + $H_2O_2$ groups than in the $H_2O_2$ ($P < 0.01$) and control ($P < 0.05$) groups. GSH-Px, GSH and vitamin C levels were also significantly higher in the fish oil + $H_2O_2$ and fish oil + RISP groups than in the $H_2O_2$ ($P < 0.01$) and control, fish oil, and RISP ($P < 0.05$) groups. Hence, we found that RISP and fish oil induced protective effects on GSH, vitamin C and GSH-Px values against oxidative stress-induced decrease. GSH-Px, GSH and vitamin C levels were also significantly ($P < 0.001$) higher in the fish oil + RISP group than in the control ($P < 0.01$), $H_2O_2$ ($P < 0.001$), fish oil + $H_2O_2$ and RISP + $H_2O_2$ ($P < 0.05$) groups. GSH-Px, GSH and vitamin C levels were significantly ($P < 0.05$) lower in the fish oil + RISP + $H_2O_2$ group than in the fish oil + RISP group.

## Discussion

The most important finding of this study is that membrane LP levels as MDA and $[Ca^{2+}]_i$ values were elevated significantly in PC12 cells exposed to oxidative stress com-

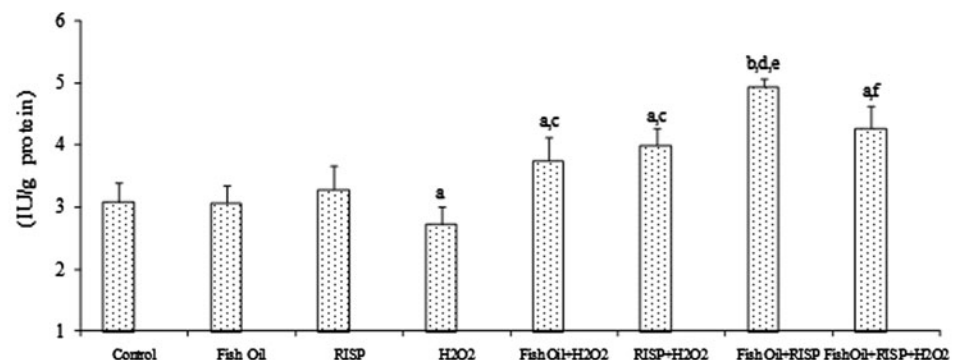


**Fig. 3** Effects of fish oil and RISP on GSH-Px activity in PC12 cells ($n = 8$, mean ± SD). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control, fish oil and RIS; [c] $P < 0.01$ and [d] $P < 0.001$ vs. $H_2O_2$; [e] $P < 0.05$ vs. fish oil + $H_2O_2$ and RISP + $H_2O_2$; [f] $P < 0.05$ vs. fish oil + RISP

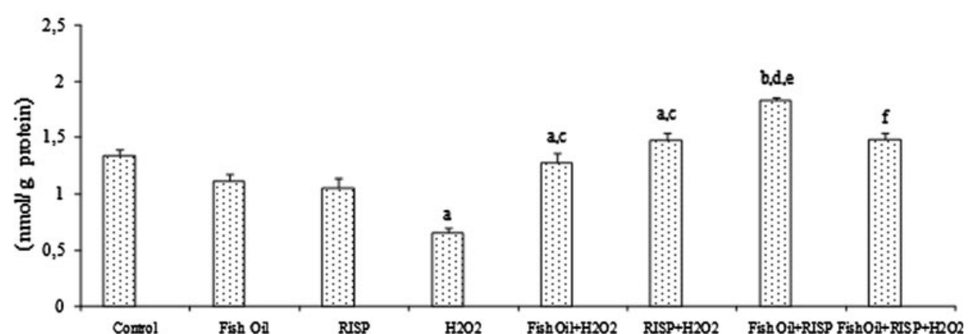


**Fig. 4** Effects of fish oil and RISP on GSH levels in PC12 cells ($n = 8$, mean ± SD). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control, fish oil and RIS; [c] $P < 0.01$ and [d] $P < 0.001$ vs. $H_2O_2$; [e] $P < 0.05$ vs. fish oil + $H_2O_2$ and RISP + $H_2O_2$; [f] $P < 0.05$ vs. fish oil + RISP

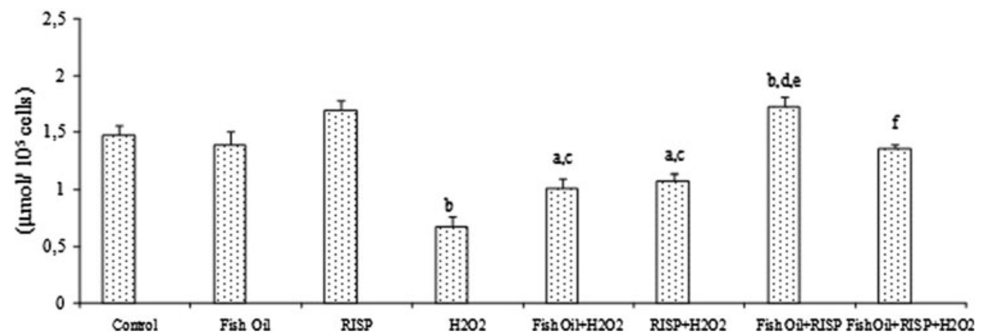


**Fig. 5** Effects of fish oil and RISP on vitamin C levels in PC12 cells ($n = 8$, mean ± SD). [a] $P < 0.05$ and [b] $P < 0.01$ vs. control, fish oil and RIS; [c] $P < 0.01$ and [d] $P < 0.001$ vs. $H_2O_2$; [e] $P < 0.05$ vs. fish oil + $H_2O_2$ and RISP + $H_2O_2$; [f] $P < 0.05$ vs. fish oil + RISP

pared to control PC12 cells, whereas GSH, GSH-Px and vitamin C values were decreased by incubation with oxidative stress. LP and $[Ca^{2+}]_i$ values were reduced to control levels by fish oil and RISP supplementation, whereas GSH, GSH-Px and vitamin C values were increased by the supplementations. To our knowledge, this is the first comparative study of RISP and fish oil on $[Ca^{2+}]_i$ release and the oxidative and antioxidant system in cell culture medium. There is evidence that psychotic disorders might impair antioxidant defense and increase LP as antipsychotic treatment itself increases oxidative stress and induces irreversible neuropathological changes in animal and human models (Mahadik et al. 1998; Akyol et al. 2002).

LP levels were found to be elevated significantly in PC12 cells exposed to oxidative stress compared to control PC12 cells. However, LP levels were decreased by fish oil supplementation, whereas GSH, GSH-Px and vitamin C values were increased by the supplementation. The elevation of LP as an indicator of ROS production and one of the key indices of membrane pathology may be involved in the pathophysiology of schizophrenia (Akyol et al. 2002). It has been suggested that LP may be one of the major mechanisms of the consistently observed reduction of EPUFAs in schizophrenia (Mahadik et al. 1998). Reduced levels of phospholipids and EPUFAs in erythrocyte membranes (Keshavan et al. 1993) and brain homogenates (Horrobin et al. 1991) from patients with schizophrenia have been reported. Recent studies have reported that EPUFAs were reduced in postmortem brain tissue from schizophrenic patients (Zhang et al. 2006). In contrast to our results, in the study of Pillai et al. (2007) GSH-Px activity in rats did not change with 90-day RISP treatment.

LP levels in the current study were decreased by RISP supplementation. Oxidative stress induced by antipsychotic treatment is a hypothesis that should be taken into account concerning schizophrenia. There is also evidence of an impaired antioxidant defense and increased ROS-mediated cellular injury in animal models and patients with schizophrenia (Mahadik et al. 1998, 2001, 2003). For a long time, the theory that striatal postsynaptic dopamine receptor supersensitivity causes schizophrenia has been widely accepted, but new evidence may change this model. A new hypothesis combining the facts that antipsychotics enhance striatal glutamatergic neurotransmission by blocking presynaptic dopamine receptors and cause neuronal damage by oxidative stress was presented (Tsai et al. 1998). Using the model of glutaminergic neurotransmission and the evidence of long-lasting persistence of antipsychotics in the human brain and the "fast disassociation hypothesis" of antipsychotics as an explanation for atypical antipsychotics (Kapur and Seeman 2001; Kropp et al. 2005), our results may explain why RISP showed lower levels of oxidative stress in the current study. Similarly, Kropp et al. (2005) reported that plasma LP levels in patients with schizophrenia significantly decreased with RISP treatment.

ROS act as subcellular messengers in such complex processes as mitogenic signal transduction, gene expression and regulation of cell proliferation when they are generated excessively or when enzymatic and nonenzymatic defense systems are impaired (Nazıroğlu 2007a, b). Superoxide dismutase converts superoxide radical to $H_2O_2$. The major intracellular antioxidant enzyme GSH-Px detoxifies $H_2O_2$ to water and removes organic hydroperoxides (Halliwell 2006). Neuronal cells are very vulnerable to oxidative stress because of their elevated consumption of oxygen and consequent generation of large amounts of ROS (Whanger 2001; Nazıroğlu 2007b). Results of the current study demonstrate an increase in GSH-Px activity and vitamin C levels in response to RISP and fish oil treatment of oxidative stress-exposed PC12 cells. Enhancement of their levels may provide an effective defense from the damaging effects of not only superoxide anion and $H_2O_2$ but also the highly reactive and damaging hydroxyl radical (Halliwell 2006). Moreover, the observed restraint stress-induced reductions in GSH levels and activities of GSH-Px scavenging damage the second line of antioxidant defense. GSH is a multifunctional intracellular nonenzymatic thiol antioxidant, and the GSH system is very important in cellular defenses against oxidative stress. A similar depletion of brain GSH has previously been reported in rats under stress-induced depression (Eren et al. 2007; Zafir et al. 2009). RISP is found to reverse effectively this coordinate oxidative stress-induced decline in GSH and GSH-Px by restoring disrupted GSH pathways; it is plausible that antipsychotic supplementation may exert

the observed antioxidant effects via restoration of critical GSH-related processes such as ROS scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulfide status of proteins and regulation of cell signaling and repair pathways (Whanger 2001; Zhang et al. 2006). Similarly, Zhang et al. (2006) reported decreased activity of GSH-Px in patients with schizophrenia. In contrast, some other studies (Reddy et al. 1991; Yao et al. 1999) failed to find any differences in GSH-Px activity between schizophrenic patients and normal controls. Numerous research groups also have reported elevated GSH-Px activity in schizophrenia (Herken et al. 2001).

It has been suggested that EPA inhibits $PLA_2$, which is elevated in schizophrenia, and that this may prevent the reduction of membrane EPUFAs. However, the findings of elevated levels of $PLA_2$ are consistent (Arvindakshan et al. 2003). Also, the increased $PLA_2$ seems to be associated with the increased peroxidation and worsening of clinical symptoms (Ross 2003). Several studies have indicated that elevation of $PLA_2$ is associated with increased peroxidation and is thus a repair response to replace peroxidated EPUFAs (Arvindakshan et al. 2003). It is likely that, in the current study, use of PUFAs may have reduced LP levels and subsequently reduced levels of $PLA_2$ and, thus, may have contributed to the increase in membrane PUFAs. This may also support the advantage of fish oil supplementation in oxidative stress-induced schizophrenia. However, this needs to be systemically investigated by simultaneous analysis of oxidative stress and membrane EPUFAs in cell culture medium.

Cytosolic $Ca^{2+}$ has been presented as a key regulator of cell survival, but this ion can also induce apoptosis in response to a number of pathological conditions (Nazıroğlu 2009). In addition, the mitochondria act as $Ca^{2+}$ buffers by sequestering excess $Ca^{2+}$ from the cytosol (Hajnóczky et al. 2006). $Ca^{2+}$-mobilizing agonists can effectively produce a rapid, simultaneous and reversible cessation of the movements of both the endoplasmic reticulum (ER) and mitochondria, which is strictly dependent on a rise in $[Ca^{2+}]_i$. This inhibition in mitochondrial motility reflects an increased mitochondrial $Ca^{2+}$ uptake and, thus, enhances the local $Ca^{2+}$-buffering capacities of mitochondria, with important consequences for signal transduction (Bubber et al. 2004). $Ca^{2+}$ overloading in mitochondria can induce an apoptotic program by stimulating the release of apoptosis promoting factors like cytochrome *c* and by generating ROS due to respiratory chain damage (Hajnóczky et al. 2006; Nazıroğlu 2007a). Furthermore, mitochondria have been found to play a pivotal role in $Ca^{2+}$ signaling (Hajnóczky et al. 2006). In fact, the release of $Ca^{2+}$ from ER stores by IP3 receptors has been implicated in multiple models of apoptosis as being directly responsible for mitochondrial $Ca^{2+}$ overload (Putney and McKay 1999; Bubber et al. 2004). Stored $Ca^{2+}$ is crucial for a number of cellular functions, including signal transduction cascades that respond to stress conditions (Nazıroğlu, 2007a). We provided compelling evidence that mitochondrial $Ca^{2+}$ uptake evoked by rises in $[Ca^{2+}]_i$ induces mitochondrial membrane depolarization. Our results indicate that the blockade of both $Ca^{2+}$ uptake into mitochondria with fish oil and RISP and rises in $[Ca^{2+}]_i$ was able to decrease LP mediated by $H_2O_2$, which releases $Ca^{2+}$ from intracellular stores.

In conclusion, fish oil and RISP have protective effects on oxidative stress, the antioxidant redox system and $Ca^{2+}$ release in $H_2O_2$-exposed PC12 cells. Our current study on PC12 cells taken together with in vitro studies on improved cognition with fish oil and RISP in schizophrenia suggest an unappreciated therapeutic potential for this drug in psychiatric diseases such as schizophrenia and depression, which are characterized by oxidative stress.

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