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Perfluorooctanoic acid affects mouse brain and liver tissue through oxidative stress

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The aim of this study was to investigate oxidative stress induced by perfluorooctanoic acid (PFOA) in the brain and liver tissues of Balb/c mice as well as protective effects of taurine and coenzyme Q_{10} (CoQ_{10}) in both organs. For this purpose, animals were treated with PFOA (15 and 30 mg/kg) orally and their lipid peroxidation, total glutathione levels (GSH), and antioxidant enzyme activities measured and both tissues analysed for histopathological changes. Our results showed a dose-dependent decrease in body weight and increase in relative brain and liver weights, PFOA-induced lipid peroxidation and reduced glutathione peroxidase (GPx) activity in the brain tissue, and changes in GSH levels, GPx, superoxide dismutase (Cu-Zn SOD), and catalase (CAT) activities in the liver tissue. Pre-treatment with taurine or CoQ_{10} provided protection against PFOA-induced Cu-Zn SOD reduction in the liver tissue. Our findings evidence the depleting effect of PFOA on antioxidative systems and confirm that PFOA exerts its (neuro)toxicity through oxidative stress, but further research is needed to identify the exact toxicity mechanisms, especially in the brain.

KEY WORDS: body weight; CAT; CoQ_{10}; GPx; GSH; hepatotoxicity; *in vivo*; neurotoxicity; oxidative damage; PFOA; relative organ weight; SOD; taurine

Perfluorooctanoic acid (PFOA), a member of polyfluorinated compounds, is an environmentally persistent chemical with a wide range of industrial use due to its oil and water-repellent properties and heat and chemical reaction resistance. The major human exposure pathways include consumption of contaminated food and water as well as inhalation of dust. Daily PFOA exposure for general population is estimated to be 0.3–150 ng/kg (1–5). PFOA and related substances have been included in the list of restricted substances in Annex XVII to the EU Registration, Evaluation, Authorization and Restriction of Chemicals regulation (6). PFOA is also classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) (7).

Animal studies show that PFOA is primarily distributed in the liver and plasma, with lower concentrations reaching other organs, including the brain (8–10). In humans, it is found in serum, breast milk, liver, kidneys, lungs, seminal plasma, thyroid gland, bones, and brain (11–15), confirming its ability to cross the blood-brain barrier. In addition, considerable levels of PFOA have been reported globally in human serum. In the United States, mean serum level reported in the general population is 3.7 ng/mL (16) and as high as 428–12000 ng/mL in occupationally exposed workers (17). Its estimated half-life in retired fluorochemical production workers is 3.8 years (18).

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Several epidemiological and animal studies have shown a relationship between exposure to PFOA and adverse health effects in many organs and systems (2, 3), liver in particular (19, 20). However, knowledge about the effects of PFOA in the brain tissue is relatively scarce, even though the nervous system seems to be one of the most sensitive targets for this compound (21). In addition, due to its long serum half-life and ability to cross the placenta, PFOA raises concern about normal brain development especially in foetuses and newborns (22, 23).

The underlying mechanism of adverse effects has not been entirely explained, but numerous *in vivo* (24, 25) and *in vitro* (26–28) studies point to oxidative stress. The aim of our study was to look further into oxidative stress induced by PFOA in mouse brain and liver tissue and to see whether known antioxidants, taurine and coenzyme Q_{10} (CoQ_{10}) can provide any protection.

MATERIALS AND METHODS

Chemicals

PFOA (ammonium salt of perfluoroctanoic acid, 96 %), taurine, and CoQ_{10} were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Haematoxylin and eosin yellowish were obtained from Merck

Co. (Darmstadt, Germany). All other chemicals were analytical grade and purchased from Sigma-Aldrich Co.

Animals

The study included 10-week-old male Balb/c mice weighing 30–35 g from the Animal Care Unit of Erciyes University. All mice had free access to standard laboratory chow (Optima Yem, Bolu, Turkey) and tap water. They were kept in polycarbonate cages at 20–24 °C and 40–50 % relative humidity with a 12 h-light/dark cycle. The protocol was approved by the Animal Ethics Committee of Erciyes University (Decision No. 13/33) and followed the Directive 2010/63/EU.

Experimental design

The animals were randomised into eight experimental groups. Control groups (water and corn oil) consisted of six mice each, while other groups had seven mice. PFOA and taurine were dissolved in Milli-Q water and CoQ_{10} in corn oil. All solutions were freshly prepared, protected from light, and administered to the animals by gavage in the volume of 10 mL/kg body weight (bw) at doses given below. Control animals received the vehicle (distilled water or corn oil) in the same volume and route for 10 days.

PFOA was administered to mice in two different doses (15 and 30 mg/kg bw) for 10 days. Both doses are much lower than oral LD_{50} for mice (457 mg/kg) (29), and their choice was based on considerations of similar studies published earlier (30–33). Antioxidant control groups were receiving 100 mg/kg bw of taurine or 50 mg/kg bw of CoQ_{10} for 5 days and dose selection was based on effective doses reported by previous studies investigating chemically induced oxidative stress in mice (34–37). Finally, two groups of animals first received taurine or CoQ_{10} for five days and then PFOA (30 mg/kg bw) for another 10 days.

All animals were sacrificed by cervical dislocation under ketamine/xylazine (90/10 mg/kg bw) anaesthesia 24 h after receiving the last treatment dose. Body weights were taken before sacrifice. Whole brain and liver were removed, washed with ice-cold 0.9 % NaCl, blotted, dried, and weighed. Small tissue sections were fixed in formalin for histopathological examination. The remaining parts of the tissues were stored at -80 °C until biochemical and flow cytometric analysis.

Histological examination

Fixed tissue samples with 10 % neutral formalin were dehydrated with gradient ethanol solution and embedded in paraffin. The samples were then sliced to 5-μm slices with an automatic microtome. After staining with haematoxylin and eosin, pathological changes were observed under a Nikon Optiphot 2 light microscope (200× magnification, Tokyo, Japan).

Sample collection

The tissues were homogenised in 1.15 % KCl using glass-glass Potter-Elvehjem-type homogeniser to obtain 10 % (w/v) whole homogenates. A part of a whole homogenate was used to determine malondialdehyde (MDA) level, and the rest was centrifuged at 1500 *g* and 4 °C for 10 min. Collected supernatant was used to measure total glutathione (GSH) and antioxidant enzyme activities. For total GSH analysis, a part of the obtained supernatant was diluted with metaphosphoric acid (6 %), re-centrifuged at 1500 *g* and 4 °C for 10 min for further use. The other part of the supernatant was re-centrifuged at 9500 *g* and 4 °C for 25 min for determination of enzyme activities.

Analytical procedures

Lipid peroxidation was determined spectrophotometrically as described by Ohkawa et al. (38). The method measures the absorbance of thiobarbituric acid-reactive substances (TBARS) from the reaction of thiobarbituric acid (TBA) with MDA. TBARS levels are expressed in nanomoles of MDA per mg of protein.

Total tissue GSH concentration was measured using the method described by Akerboom and Sies (39). This method is based on continuous reduction of 5,5'-dithiobis(2-nitrobenzoic acid) to 5'-thionitrobenzoate by NADPH in a reaction catalysed by glutathione reductase in the presence of GSH. Total GSH was determined by spectrophotometric monitoring of reaction kinetics at 412 nm. Results are expressed as μmol/mg protein.

Brain and liver copper-zinc superoxide dismutase (Cu-Zn SOD) activities were determined using the method described by Arthur and Boyne (40) with slight modifications. Glutathione peroxidase (GPx) and catalase (CAT) activities were measured as described by Pleban et al. (41) and Aebi (42), respectively. All enzyme activities are expressed as units per mg protein (U/mg protein). Protein content in brain and liver homogenates and supernatants was determined as described by Lowry et al. (43). This method is based on measuring the absorbance of blue-coloured complex formed following the reaction of tyrosine and tryptophan residues with alkaline copper and Folin-Ciocalteu reagent at 540 nm.

Cell cycle analysis

Liver tissue samples were minced with a blade, suspended in phosphate buffer saline (PBS), filtered through a 37 μm pore nylon mesh, and centrifuged at 400 *g*. Liver cells isolated from all the experimental groups were fixed in 80 % ethanol at 4 °C overnight and centrifuged, and washed with and resuspended in PBS. After adding RNase and propidium iodide, liver cells incubated at 37 °C for 1 h. Fluorescence was measured for 10,000 cells with flow cytometry (EPICS XLMCL, Beckman Coulter Inc., Brea, CA, USA), based on which we were able to determine DNA content histograms with MultiCycle software from Phoenix Flow Systems (San Diego,CA, USA) and calculate the percentage of cells in respective phases (G1, S, and G2/M) of the cell cycle.

Statistical analysis

All data are expressed as medians and interquartile ranges save for the results of cell-cycle analysis results, which were reported as

medians. Kruskal-Wallis test was followed by the Mann-Whitney *U* test for analysing the statistical difference between control and treated groups using Statistical Package for the Social Sciences for Windows (SPSS, version 18.0; Chicago, IL, USA). A value of $p<0.05$ was considered statistically significant.

RESULTS

Body and organ weights

Body weights of mice treated with both doses of PFOA significantly decreased in a dose-dependent manner (Table 1) compared to control (12 % and 21 %, respectively, $p<0.05$). Relative brain weight significantly increased only in the 30 mg/kg PFOA group (21 %, $p<0.05$), while relative liver weights were significantly higher in both PFOA groups (291 % and 293 %, respectively, $p<0.01$). Both antioxidants seemed ineffective against PFOA-induced weight changes.

Histopathological changes

Histology of brain sections appeared normal in all groups (Figure 1). No significant abnormalities were detected in the neuronal or glial cells of the brain tissue. Livers also had normal histological appearance in the control, taurine, corn oil, and CoQ_{10}-treated groups (Figure 2, panels A, D, F, and G) with no significant changes in portal tracts and central veins. However, all PFOA-treated (Figure 2, panels B and C) and antioxidant+PFOA-treated groups (Figure 2, panels E and H) showed hepatocellular hypertrophy, necrosis, balloon degeneration, and eosinophilic cytoplasmic changes, whereas the 30 mg/kg PFOA group also showed more prominent necrosis (Figure 2, panel C).

Table 1 Body weight and relative brain and liver weight changes in mice treated with oral PFOA, CoQ_{10}, and taurine

Groups	Body weight change from baseline (%)	Relative brain weight (g/100 g)	Relative liver weight (g/100 g)
Control (water)	3.03 (-6.43–8.14)	1.25 (1.19–1.36)	5.39 (5.00–5.82)
PFOA (15 mg/kg)	**-11.76**[a] (-14.71–8.33)	1.50 (1.30–1.62)	**15.71**[d] (14.67–15.91)
PFOA (30mg/kg)	**-21.21**[a] (-28.57–0)	**1.51**[a] (1.27–1.65)	**15.79**[d] (15.00–17.44)
Taurine	-3.23 (-10.81–2.56)	1.28 (1.18–1.45)	5.09 (5.03–5.59)
Taurine + PFOA	-13.33 (-16.67–3.23)	1.46 (1.32–1.54)	**16.00**[d,e] (15.71–16.40)
Corn oil	-4.37 (-12.42–2.03)	1.33 (1.19–1.48)	5.47 (4.58–6.23)
CoQ_{10}	-7.89 (-8.33–0)	1.30 (1.24–1.36)	5.15 (5.15–5.37)
CoQ_{10} + PFOA	**-17.65**[b,c] (-22.86–9.38)	1.37 (1.36–1.56)	**15.86**[f,g] (13.09–17.04)

Data are expressed as medians and interquartile ranges (n=6 for water and corn oil groups; n=7 for treated groups). [a]$p<0.05$ and [d]$p<0.01$ relative to control; [b]$p<0.05$ and [f]$p<0.01$ relative to corn oil; [c]$p<0.05$ and [g]$p<0.01$ relative to CoQ_{10}; [e]$p<0.01$ relative to taurine

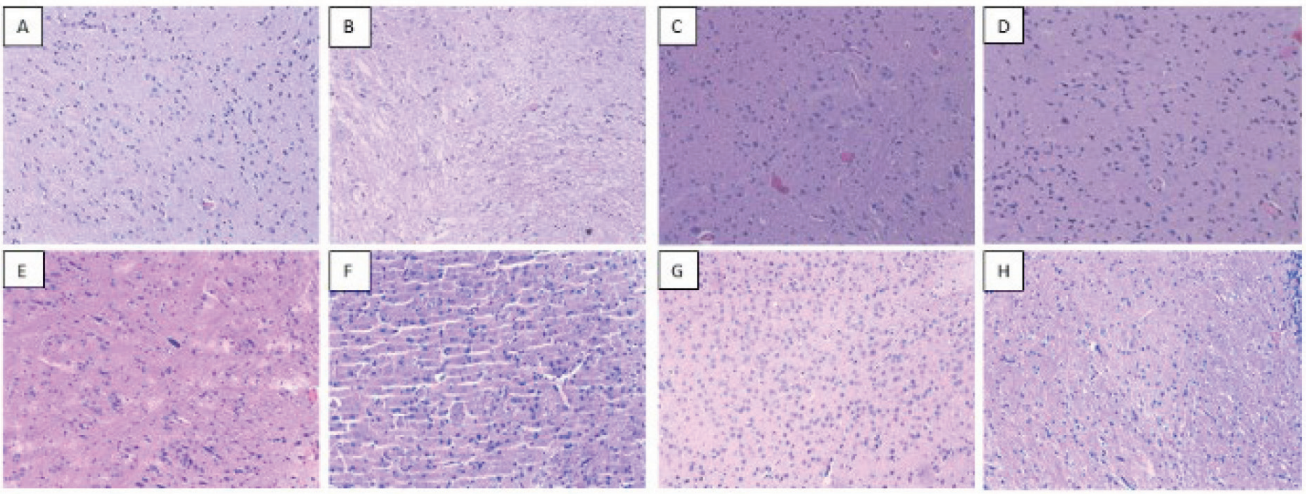

Figure 1 Histopathological changes in mouse brain tissue stained with hematoxylin and eosin (200× magnification). A – control; B – PFOA dose of 15 mg/kg bw; C – PFOA dose of 30 mg/kg bw; D – taurine dose of 100 mg/kg bw; E – taurine+PFOA dose of 30 mg/kg bw; F – corn oil; G – CoQ_{10} dose of 50 mg/kg bw; H – CoQ_{10}+PFOA dose of 30 mg/kg bw

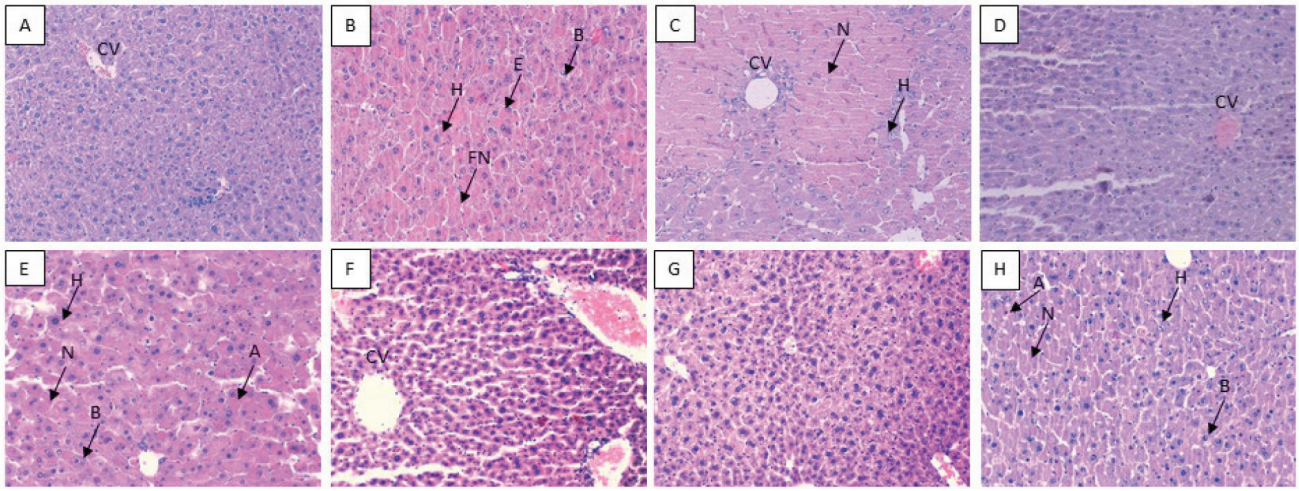

Figure 2 Histopathological changes in mouse liver tissue stained with hematoxylin and eosin (200× magnification). A – control; B – PFOA dose of 15 mg/kg bw; C – PFOA dose of 30 mg/kg bw; D – taurine dose of 100 mg/kg bw; E – taurine+PFOA dose of 30 mg/kg bw; F – corn oil; G – CoQ_{10} dose of 50 mg/kg bw; H – CoQ_{10}+PFOA dose of 30 mg/kg bw. Arrows: CV – central vein; H – hepatocellular hypertrophy; E – eosinophilic cytoplasm; B – balloon degeneration; FN – focal necrosis; N – necrosis; A – acidophilic cytoplasm

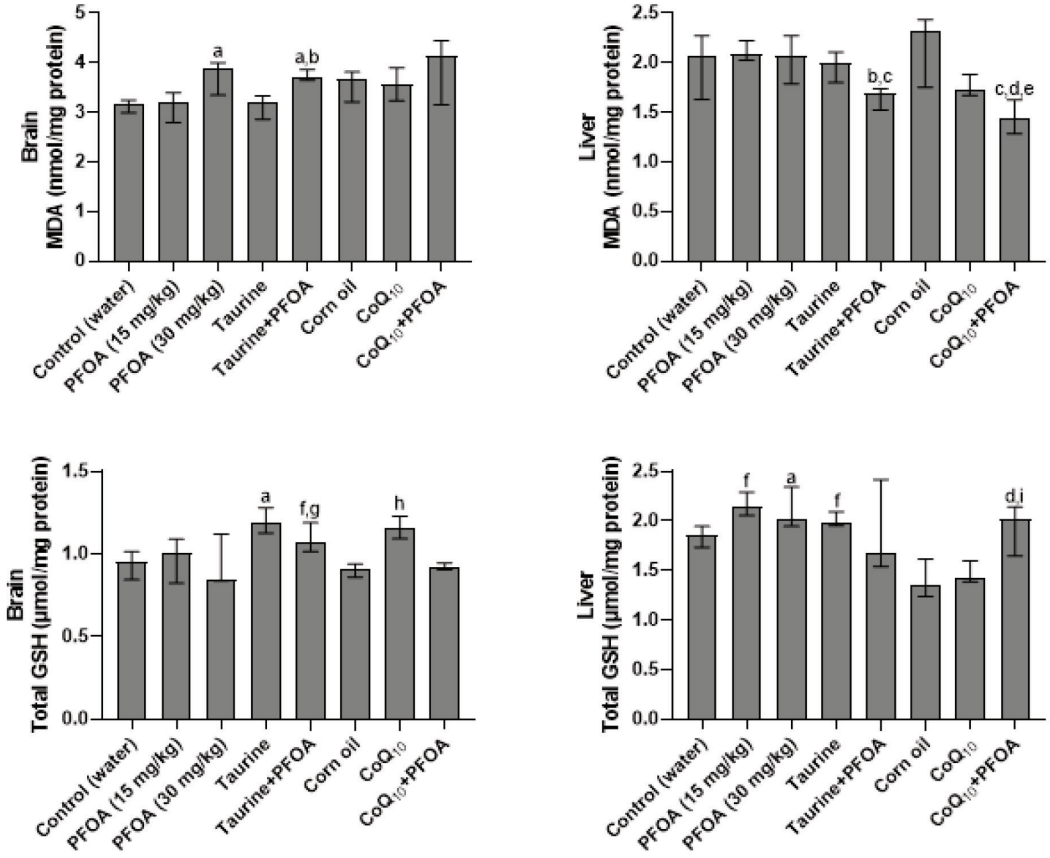

Figure 3 Total MDA and GSH levels in brain and liver tissues of mice treated with PFOA, taurine, and CoQ_{10} (data are expressed as medians and interquartile ranges, n=6 for water and corn oil groups; n=7 for treated groups). [a]$p<0.01$and [f]$p<0.05$ relative to control; [b]$p<0.01$ and [g]$p<0.05$ relative to taurine; [c]$p<0.01$ relative to PFOA (30 mg/kg); [d]$p<0.05$ and [h]$p<0.01$ relative to corn oil; [e]$p<0.05$ and [i]$p<0.01$ relative to CoQ_{10}

Lipid peroxidation and GSH levels

Significant changes were observed in brain MDA levels, which increased 1.22 times ($p<0.01$) in the 30 mg/kg PFOA group (Figure 3), and liver GSH, which increased 1.15 times in the 15 mg/kg PFOA group and 1.09 times in the 30 mg/kg PFOA group (Figure 3).

Neither antioxidant seemed to be effective against brain MDA or liver GSH increase. However, liver MDA levels decreased below control in animals pretreated with taurine or CoQ_{10} plus PFOA (Figure 3).

Antioxidant enzyme activities

Brain Cu-Zn SOD activities were not affected by PFOA treatment. However, both PFOA doses significantly decreased liver Cu-Zn SOD activities in a dose-dependent manner (Figure 4) relative to control (42 % with the lower and 53 % with the higher dose;

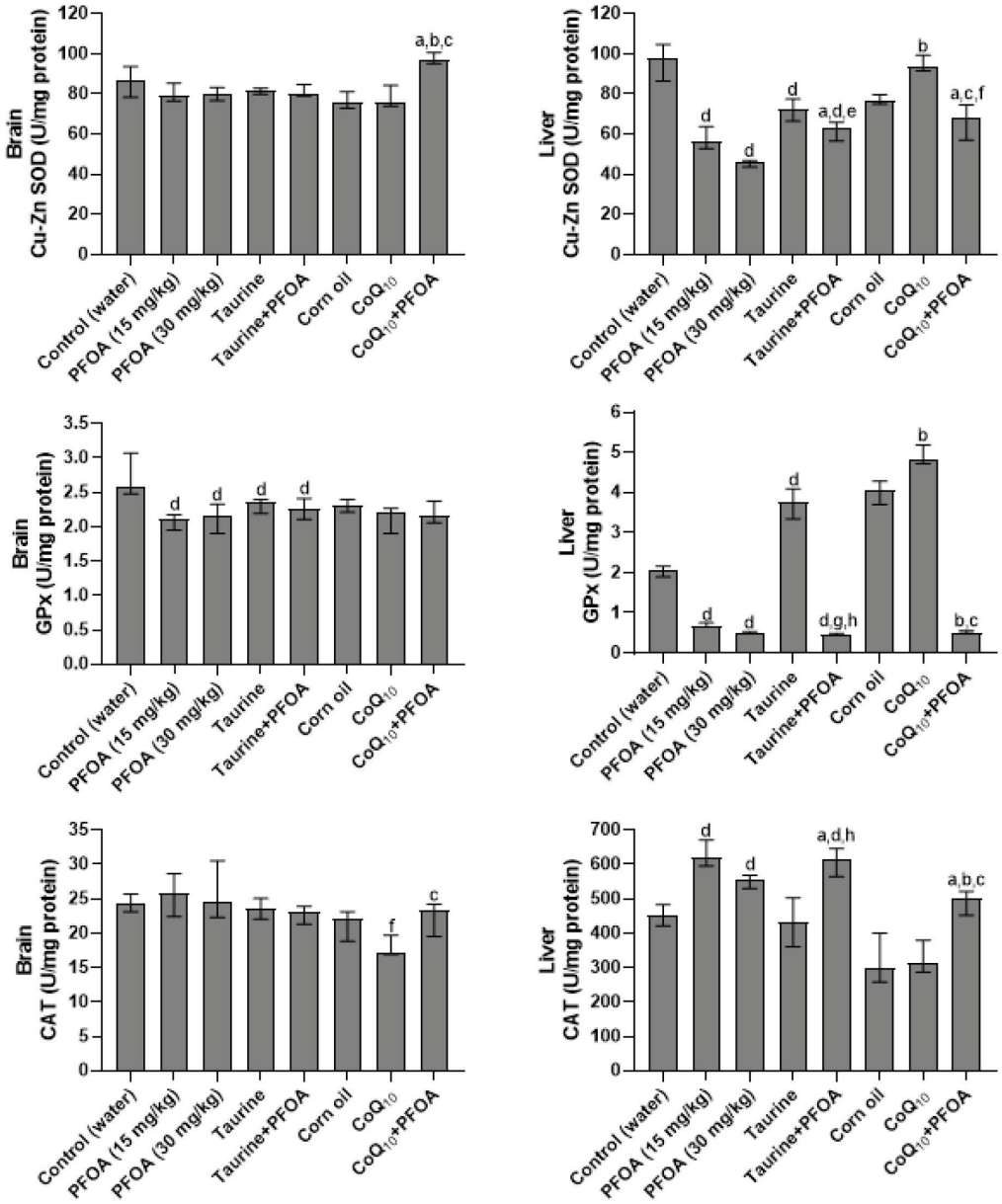

Figure 4 Antioxidant enzyme activity in brain and liver tissues of mice treated with PFOA, taurine, and CoQ_{10}. (data are expressed as medians and interquartile ranges, n=6 for water and corn oil groups; n=7 for treated groups). [a]$p<0.01$and [g]$p<0.05$ relative to PFOA (30 mg/kg); [b]$p<0.01$ and [f]$p<0.05$ relative to corn oil; [c]$p<0.01$ relative to CoQ_{10}; [d]$p<0.01$ relative to control; [e]$p<0.05$ and [h]$p<0.01$ relative to taurine

p<0.01). GPx activity in both tissues significantly dropped in both PFOA groups (18 % with the lower and 17 % with the higher dose in the brain and 67 % with the lower and 75 % with the higher dose in the liver; p<0.01 for all comparisons) (Figure 4). Lower and higher PFOA dose also significantly increased liver CAT activity (37 % and 24 %, respectively, p<0.01), whereas no significant change was observed in the brain tissue.

Taurine and CoQ_{10} were ineffective against PFOA-lowered GPx activity in the brain tissue, but significantly countered the effects of 30 mg/kg PFOA on SOD activity (p<0.01) in the liver. Furthermore, given alone, they significantly increased liver GPx activities relative to control (p<0.01). However, the two antioxidants differed in their effects against PFOA-induced CAT changes in the liver, as taurine pretreatment increased it significantly (p<0.01) and CoQ_{10} pretreatment lowered it significantly (p<0.01) relative to the 30 mg/kg PFOA group.

Liver cell cycle and apoptosis findings

Neither PFOA dose significantly affected liver cell cycle (Figure 5). With significantly more cells found in the G0/G1 phase than observed with the higher PFOA dose (p<0.05), taurine led to cell cycle arrest, whereas CoQ_{10} increased cell proportion in the G2/M phase.

DISCUSSION

Significant drops in body weight and increases in relative brain (with the higher PFOA dose) and liver weights (with both doses) in our study support earlier reports of PFOA effects (44–48). Unlike some reports (48, 49), however, we observed no significant histopathological changes in the brain at either PFOA dose, which may be owed to differences between animal species or in PFOA doses and treatment duration. On the other hand, in addition to hepatomegaly, we observed histopathological changes in the liver, including eosinophilic cytoplasm, ballooning degeneration, and necrosis, which is in line with earlier reports (20, 31, 46). Cui et al. (49) suggested that hepatomegaly might be attributed to the proliferation of peroxisome, smooth endoplasmic reticulum, and mitochondria. As a peroxisome proliferator, PFOA increases β-oxidation of peroxisomal fatty acids as well as the number and size of peroxisomes, which in turn leads to a series of morphological and biochemical changes, including hepatocellular hypertrophy (19).

As regards oxidative stress, our findings of significantly increased MDA levels in the brain tissue at the higher PFOA dose and lower GPx activity at both doses seem to corroborate earlier reports of its involvement in neurotoxic action of PFOA, including adverse developmental and behavioural effects in children (23, 27, 50–56).

As for the liver tissue, some studies suggest that PFOA induces lipid peroxidation (20, 24, 57), while others do not (32, 45, 51), and our findings are in line with the latter group. However, other oxidative stress parameters in the liver seem to have been affected by PFOA treatment, such as GSH as initial cell defence mechanism against oxidative stress or CAT, which increased significantly as reported elsewhere (32, 58–60) or Cu-Zn SOD and GPx activities, which dropped due to depletion or deactivation (24, 61–63). Increases in CAT activity, which is present in peroxisomes and

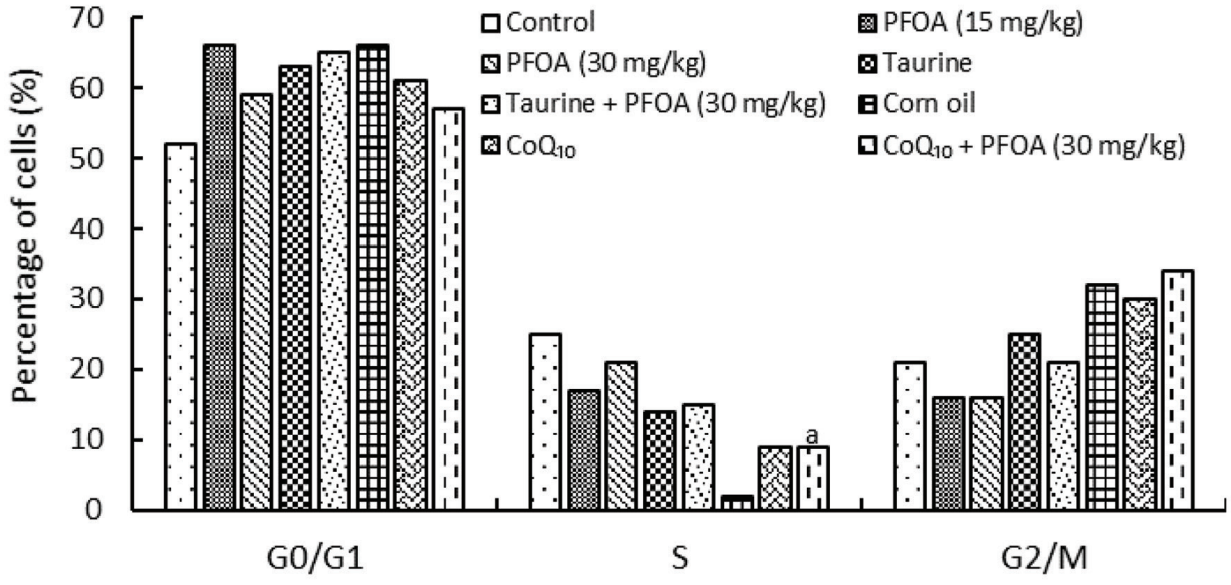

Figure 5 Distribution of liver cells by cycle in mice treated with, PFOA, taurine, and CoQ_{10} (data are expressed as medians, n=3). [a]p<0.05 relative to the PFOA (30 mg/kg) group

responsible for the reduction of H_2O_2, may be owed to reaction to higher prooxidant activity (58, 62), but when this prooxidant activity overcomes the protective capacity of CAT, its activity drops (64). The decline in SOD and GPx activity under PFOA exposure suggests that the effect of oxidative stress exceeds the antioxidant capacity of these enzymes, while increasing CAT levels might demonstrate counteraction of cells against oxidative damage.

Speaking of antioxidative action, both antioxidants in our study seem to be ineffective against PFOA-induced lipid peroxidation and GPx reduction in the brain. Other animal studies also reported inconsistent protective effects of taurine and CoQ_{10} against oxidative stress induced by a variety of agents (35, 65–67). Differences between our and other studies that found stronger antioxidant effects of taurine and CoQ_{10} may be owed to differences in protocol, as they used co-treatment instead of pretreatment. Nonetheless, our results confirm some antioxidative potential of taurine and CoQ_{10} against PFOA-induced oxidative stress in the liver, as both substances significantly increased SOD activity and lowered MDA levels in pretreatment with the lower PFOA dose, while maintaining GSH at control levels. However, only CoQ_{10} restored CAT activity to near control values.

As regards liver cell cycle distribution, we have shown that PFOA slightly increases the number of cells in the G0/G1 phase, which is in agreement with Shabalina et al. (68), and slightly decreases the number of cells in the G2/M phase. These results suggest that PFOA is capable of arresting cells in the G1 phase and of causing apoptosis and necrosis, probably by a pathway mediated by ROS and mitochondria reported elsewhere (45). Our study, however, has not detected apoptotic liver cells despite evidenced oxidative stress. This suggests that it takes higher PFOA doses or longer exposure for apoptosis to take place as reported in various studies mentioned above.

CONCLUSION

Our findings are important inasmuch as they evidence a depleting effect of PFOA on antioxidant systems, which corroborates earlier reports that PFOA induces oxidative stress and generates free radicals. They also show higher susceptibility of the brain tissue to PFOA because of its lower antioxidative capacity than liver. This points to a higher risk of neurodegenerative diseases and cancer and calls for more detailed investigation to understand the underlying oxidative stress mechanisms in different model systems.

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Conflict of interests

None to declare.

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Perfluorooktanoična kiselina oštećuje moždano i jetreno tkivo putem oksidacijskoga stresa

Cilj je ovog istraživanja bio utvrditi oksidacijski stres prouzročen perfluorooktanoičnom kiselinom (PFOA) u moždanom i jetrenom tkivu Balb/c miševa te zaštitno djelovanje taurina i koenzima Q_{10} (CoQ_{10}) u oba organa. U tu su svrhu životinje izložene PFOA-i u oralnoj dozi od 15 ili 30 mg/kg tjelesne težine te su u oba tkiva analizirane histopatološke promjene i izmjerene lipidna peroksidacija, ukupne razine glutationa (GSH) i aktivnosti antioksidacijskih enzima. Naši rezultati pokazuju smanjenje ukupne tjelesne težine i povećanje relativne težine organa ovisne o dozi, lipidnu peroksidaciju i smanjenu aktivnost glutation peroksidaze (GPx) u moždanom tkivu te promjene razina GSH i aktivnosti GPx, superoksid dismutaze (Cu-Zn SOD) i katalaze (CAT) u jetrenom tkivu. Prethodna primjena taurina ili CoQ_{10} zaštitila je životinje izložene PFOA-i od pada razina Cu-Zn SOD u jetrenom tkivu. Naši rezultati potvrđuju da PFOA iscrpljuje antioksidacijske sustave i djeluje (neuro)toksično putem oksidacijskoga stresa, no potrebna su daljnja istraživanja kako bi se precizno utvrdili njegovi mehanizmi toksičnosti, napose u mozgu.

KLJUČNE RIJEČI: CAT; CoQ_{10}; GPx; GSH; hepatotoksičnost; *in vivo;* neurotoksičnost; oksidacijsko oštećenje; PFOA; relativna masa organa; SOD; taurin; tjelesna masa