TOLUENE AND P-XYLENE MIXTURE EXERTS ANTAGONISTIC EFFECT ON LIPID PEROXIDATION IN VITRO

EWA SAWICKA and ANNA DŁUGOSZ

Wrocław Medical University, Wrocław, Poland
Department of Toxicology

Abstract

Objectives: Previous research on a group of workers occupationally exposed to styrene, ethylene glycol, toluene, p-xylene and their mixture showed elevated levels of the main products of lipid peroxidation: malondialdehyde and 4-hydroxynonenal (MDA+4-HNE) in plasma [1]. Moreover, an earlier in vitro study indicated a synergistic interaction between styrene and ethylene glycol on lipid peroxidation [2]. Therefore, it seemed interesting to investigate the effect of combined exposure to toluene and p-xylene on lipid peroxidation and define the type of the interaction.

Materials and Methods: An in vitro model of human placenta mitochondria was used in the study. The concentration of TBARS (thiobarbituric active reagent species) was measured by spectrophotometry, and of hydroxyl radical (•OH) by assessment of deoxyribose degradation. It was investigated whether the administration of coenzyme Q10 (CoQ10) could have a protective function (if given before solvent exposure) or a reparatory function (if given after exposure) in solvent-induced oxidative stress.

Results: Exposure to p-xylene at concentrations ranging from 5.3 to 265 μg/ml produced an increase in TBARS concentration. The results showed that p-xylene had a stronger influence on lipid peroxidation than toluene. The mixture of toluene and p-xylene induced an antagonistic effect on lipid peroxidation, measured as TBARS concentration. The mechanism connected with •OH generation was found to play an important role in the oxidative damage to lipids resulting from p-xylene exposure. Administration of coenzyme Q10 at the doses of 3.0 and 12.0 μg/ml successfully decreased the TBARS level that was elevated after solvent exposure.

Conclusions: In contrast to the synergistic effect that the mixture of styrene and ethylene glycol had on lipid peroxidation (previous study), an antagonism between toluene and p-xylene could be observed. The coenzyme Q10 can be considered a protective agent against lipid peroxidation.

Key words: P-xylene, Toluene, Antagonism, Lipid peroxidation, Coenzyme Q10, In vitro

INTRODUCTION

The generation of free radicals is a constant process due to physiological metabolic transformations or pathological changes. Free radicals are formed by gaining or losing an electron or by homolytic fission of the covalent bond. This way a hydroxyl radical (•OH) can be produced from H2O2. The hydroxyl radical is the most reactive species. It binds and oxidizes DNA, proteins and lipids, which leads to lipid peroxidation. The toxic effect of some organic solvents is connected with the formation of free radicals (e.g. carbon tetrachloride) [3]. A preliminary study conducted on a group of workers occupationally exposed to styrene, ethylene glycol, toluene, p-xylene and their mixture in the paint and lacquer industry showed a statistically elevated concentration of the two main products of lipid peroxidation: malondialdehyde and 4-hydroxynonenal (MDA+4-HNE) in plasma, compared to the values found for controls [1].

In order to evaluate which of the solvents present in work environment could be most responsible for enhanced lipid peroxidation, their influence on lipid peroxidation (TBARS level) was investigated in an in vitro model of human placenta mitochondria. The model was previously employed to yield information on cell membrane processes [2,4].
The former in vitro study showed that exposure to ethylene glycol or styrene had no influence on TBARS concentration in mitochondria, but the combined exposure produced a synergistic interaction resulting in an increased TBARS level at all the concentrations examined (p = 0.000–0.0017). A similar effect referred also to 'OH generation.

The purpose of the present study was to evaluate in vitro the effect that exposure to toluene or p-xylene or their mixture has on TBARS concentration and 'OH production. It seemed interesting to investigate what type of interaction may result from the combined exposure. To this end it was necessary to assess exposure to a mixture of solvents that is common in industrial settings where the workers can be simultaneously exposed to a number of chemicals. Interaction may refer to the metabolism of the chemicals, their absorption or the effect on receptors, especially when the chemicals have a similar chemical structure [5]. Workers’ protection against adverse effects of exposure is very important. An earlier study concerning a group of workers in the paint and lacquer industry showed a statistically significant decrease in plasma concentration of MDA+4-HNE after four weeks of supplementing coenzyme Q₁₀ (ubiquinone, CoQ₁₀) [1]. Moreover, CoQ₁₀ protected against lipid peroxidation caused by ethylene glycol and styrene mixture in vitro, by significantly decreasing TBARS level in human placenta mitochondria. Coenzyme Q₁₀, a lipophilic electron carrier in the mitochondrial respiratory chain, is synthesized in all the tissues and cells of the organism to serve as an antioxidant in homeostasis. Under exposure conditions, the local biosynthesis of CoQ₁₀ can be scarce [6]. Coenzyme Q₁₀, in its reduced form acts as a free radical scavenger. It contributes to the regeneration of vitamin E from its oxidized form [7,8]. In the present project, the role of CoQ₁₀ in exposure to toluene or p-xylene and their mixture was evaluated.

### MATERIALS AND METHODS

Mitochondria isolated from human placenta from natural deliveries were used in the present study. The mitochondria were isolated after Radi and Lass, by homogenizing the material in 5 mM Tris-HCl buffer, pH 7.4, containing 0.23 M mannitol, 0.07 M sucrose, 1 mM EDTA and 0.2% bovine serum albumin (BSA). The homogenate was centrifuged at 1000×g (4°C) and then recentrifuged under the same conditions at 12,000×g. The mitochondrial supernatant was collected and washed (3×) in 50 mM Tris-HCl buffer, pH 7.4, containing 0.575% KCl at 4°C, and centrifuged for 10 min at 13,000–14,000 rpm. The resulting mitochondria were suspended in the above buffer and stored at –80°C (no longer than 3 months) until use [9,10]. The model was adapted for hydrocarbons and antioxidants by Długosz and Piotrowska [4].

Coenzyme Q₁₀ (Q₁₀ 904944, JEMO PHARM) was added to the mitochondrial suspension before and after the treatment with toluene or p-xylene and their mixture. CoQ₁₀ was administered at three doses: 1.5; 3.0 and 12.0 μg/ml as a 30 μl solution in pentane. Solvents: (a) toluene pure p.a., d = 0.866 g/cm³ (PPH POCh S.A., Gliwice, Poland), (b) p-xylene pure p.a., d = 0.880 g/cm³ (PPH POCh S.A., Gliwice, Poland), and their mixture used in the paint and lacquer industry were evaluated. The solvents were added to the mitochondrial suspension in 30 μl at the doses of 2.5–265.0 μg/ml, as shown in Table 1. The results were compared with the control K, without the solvents. TBARS content was measured using thiobarbituric acid [11]. Hydroxyl radical concentration was assessed using the method of deoxyribose degradation according to Rice-Evans [12]. Mitochondrial proteins were measured by the Lowry method [13]. For each dose of toluene or p-xylene, 10–12 samples were analyzed.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Density (g/ml)</th>
<th>10 000×</th>
<th>5 000×</th>
<th>2 000×</th>
<th>1 000×</th>
<th>500×</th>
<th>200×</th>
<th>100×</th>
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<tbody>
<tr>
<td>P-xylene</td>
<td>0.880</td>
<td>2.6</td>
<td>5.3</td>
<td>13.2</td>
<td>26.0</td>
<td>53.0</td>
<td>132.0</td>
<td>265.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.866</td>
<td>2.5</td>
<td>5.1</td>
<td>12.9</td>
<td>25.9</td>
<td>51.8</td>
<td>129.5</td>
<td>259.0</td>
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</table>
Evaluation of the effects of solvent exposure on lipid peroxidation

Mitochondrial suspension (1 ml) was incubated at 37°C for 30 min with 30 μl of a given solvent solution: toluene or p-xylene, at doses specified in Table 1. Then 0.5 ml of 20% trichloroacetic acid (TCA), 1.5 ml of 0.67% thiobarbituric acid (TBA), 30 μl of 1% buthylhydroxytoluene (BHT) were added and the samples were incubated at 85°C for 15 min. TBARS concentration was measured by spectrophotometry at 535 nm and expressed as nmol per mg mitochondrial protein using the molar absorption coefficient of 1.56×10^5 M^-1 cm^-1 [4,11]. The results were compared with the control K1, without the solvents, prepared following the same procedure.

Evaluation of the effects of solvent exposure on hydroxyl radical formation

Mitochondrial suspension (0.5 ml) was incubated at 37°C for 15 min with 0.5 ml of 20 mmol/l deoxyribose and 15 μl of the examined solvent solution at doses as above and then centrifuged. To 0.8 ml of supernatant, 0.5 ml of 20% TCA, 1.5 ml of 0.67% TBA, 30 μl of 1% BHT were added and the samples were incubated at 85°C for 15 min. Hydroxyl radical concentration was measured by spectrophotometry under the same conditions as above [11–13]. The results were compared with the control K2, without the solvents, prepared following the same procedure.

Evaluation of the reparative effect of CoQ_{10} on human placental mitochondria stimulated with the solvents

The mitochondrial suspension was preincubated with 30 μl of the examined solvents at 37°C for 30 min (Table 1) and then 30 μl of CoQ_{10}(at doses of 1.5; 3.0 and 12 μg/ml), prepared as above, was added. The incubation was continued at 37°C for 30 min and then the TBARS analysis was performed.

Evaluation of the protective effect of CoQ_{10} on human placental mitochondria stimulated with the solvents

The mitochondrial suspension at doses as above was preincubated with CoQ_{10} at 37°C for 30 min. Then 30 μl of the examined solvents (Table 1) was added and the incubation was continued at 37°C for 30 min. The TBARS level was determined as described above.

Evaluation of toluene and p-xylene interaction

The mitochondrial suspension (1 ml) was preincubated at 37°C for 30 min with the mixture: 30 μl of toluene and 30 μl of p-xylene solutions diluted 10 000×, 5000×, 2000×, 1000× and 500× (Table 1). The TBARS level was determined using thiobarbituric acid. All the experiments conformed to relevant Polish regulations and ethical standards.

Statistics

The results of the study were analyzed using Student’s t-test. To define the correlation, Pearson correlation coefficient was used. Statistical evaluation of results was performed using Statistics 6.0. Differences at p < 0.05 were regarded as statistically significant.

RESULTS

The solvents considered in the present study were found to have influence on lipid peroxidation measured as TBARS level in mitochondria. Toluene at four concentrations: 2.6; 5.2; 12.9 and 25.9 μg/ml enhanced lipid peroxidation in mitochondria, but the effect was statistically significant only at the doses of 12.9 and 25.9 μg/ml. These two doses produced an increase in TBARS level to 2.2 and 3.64 nmol/mg protein, respectively, as compared to the findings for controls (2.06 nmol/mg protein).

Higher doses of toluene (51.8–259.0 μg/ml) inhibited lipid peroxidation (Fig. 1).
It seems that the effect differs depending on toluene concentration.
In p-xylene exposure, the effect was congeneric and strong. A significant increase in TBARS concentration could be noted for every dose of p-xylene, from 5.3 to 265 μg/ml. The most significant increase in TBARS level referred to the doses of 26.5; 132.5 and 265.0 μg/ml. Respective TBARS concentrations amounted to 3.16; 3.39 and 3.43 nmol/mg protein and differed significantly from the control value of 2.06 nmol/mg protein (p = 0.0018; p = 0.0001; p = 0.0004, respectively) (Fig. 1). This finding indicates a stimulating effect of p-xylene on lipid peroxidation.
Exposure to the mixture of p-xylene and toluene was found to inhibit lipid peroxidation. After combined exposure at doses ranging from 2.5–2.6 μg/ml to 51.8–53.8 μg/ml, the TBARS concentration was significantly lower than after exposure to toluene only or p-xylene only. This type of interaction between toluene and p-xylene is an antagonistic effect. The influence of combined exposure to toluene and p-xylene on TBARS level was weaker than the effect of particular solvents. Exposure to solvent mixture produced a decrease in TBARS level at all the doses. The antagonism is most clearly seen at solvent concentrations of 12.9–26.5 μg/ml. Both p-xylene and toluene administered separately at these doses produced a statistically significant increase in TBARS level but given as a mixture they had no influence on the level of thiobarbituric acid reactive substances (Fig. 2).
The second stage of our experiment was conducted to investigate the mechanism of the changes induced by toluene and p-xylene exposure. It was interesting to find out whether they were connected with hydroxyl radical generation.
Exposure to toluene enhanced •OH generation only at the highest dose of 259.0 μg/ml while to p-xylene at the two highest doses of 132.0 and 256.0 μg/ml (Fig. 3). A statistically significant linear correlation (r = 0.3817; p = 0.001) between •OH radicals and TBARS concentration was noted only for p-xylene (Fig. 4).
In general, toluene and p-xylene at medium concentrations had no influence on hydroxyl radical generation. However, the positive correlation for p-xylene indicates that exposure to this solvent may partially account for the stimulating effect on lipid peroxidation. Also the mechanism pertaining to the combined exposure is connected in part with the influence on •OH generation because both p-xylene and toluene at the lowest doses of 2.6 μg/ml and 2.5–5.1 μg/ml, respectively, induced a statistically significant increase in TBARS concentration (p = 0.0018; p = 0.0001; p = 0.0004, respectively) (Fig. 1). This finding indicates a stimulating effect of p-xylene on lipid peroxidation.
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significant decrease in \( \cdot \)OH level, as compared to control values (Fig. 3).

The next step in our research was to assess the function of coenzyme Q\(_{10}\) as an antioxidant in p-xylene- or toluene-enhanced lipid peroxidation. It was also worth examining the possible interaction between the solvents and coenzyme Q\(_{10}\). The protective role of CoQ\(_{10}\) (when administered before incubation with solvents) and reparative role (administered after incubation) was investigated. Coenzyme Q\(_{10}\) was used at the three following doses: 1.5; 3.0 and 12.0 \( \mu \)g/ml. Toluene and p-xylene were administered at the doses which had increased TBARS concentration in the previous study.

In exposure to toluene (29.5 \( \mu \)g/ml), the lowest dose of CoQ\(_{10}\) (1.5 \( \mu \)g/ml) was found to have a protective effect, decreasing TBARS concentration from 3.64 to 3.09 nmol/mg protein (\( p = 0.0002 \)). Also CoQ\(_{10}\) at 1.5 \( \mu \)g/ml in p-xylene exposure at 26.0 \( \mu \)g/ml had a protective effect and decreased TBARS concentration from 2.9 to 2.27 nmol/mg protein (\( p = 0.018 \)), a better result was obtained when a higher dose of CoQ\(_{10}\) (3.0 \( \mu \)g/ml) was used. Under conditions of toluene-only exposure, a protective effect was observed (decrease in TBARS level from 2.29 to 1.67 nmol/mg protein; \( p = 0.003 \)) or from 3.64 to 3.04 nmol/mg protein; \( p = 0.0005 \)) (Fig. 5). In exposure to p-xylene, CoQ\(_{10}\) at 3 \( \mu \)g/ml had both a protective and reparative effect on TBARS concentration. The protective function was reflected as TBARS decrease, e.g. from 2.45 to 1.68 nmol/mg protein (\( p = 0.00064 \)) or from 2.9 to 2.28 nmol/mg protein (\( p = 0.0075 \)) and the reparative one also as TBARS decrease, e.g. from 2.45 to 1.99 nmol/mg protein (\( p = 0.0035 \)) or from 2.9 to 2.2 nmol/mg protein (\( p = 0.0012 \)) (Fig. 6). CoQ\(_{10}\) at the highest dose (12.0 \( \mu \)g/ml), used in exposure to p-xylene, exhibited a very strong activity both as a protective and a reparative agent and produced a highly significant decrease in TBARS level (\( p = 0.0000 \)) (Fig. 7).

The results showed that CoQ\(_{10}\) could protect from and reduce (repair) lipids peroxidation caused by p-xylene, but the reparative effect requires higher doses than does the protective activity.

**DISCUSSION**

Lipid peroxidation is the most commonly measured effect of oxidative stress in humans. It can be inhibited by antioxidants. Increased lipid peroxidation after exposure to a toxic substance indicates the formation of free radicals. [15]. Among the xenobiotics which may have influence on biological membranes are the organic solvents, mostly aromatic hydrocarbons. There are numerous studies describing the effect of solvents on lipid peroxidation. Most of them are focused on the activity of ethanol...
or methanol in this process. The aim of the present study was to evaluate the effects of a single and combined exposure to p-xylene or toluene on TBARS concentration in the mitochondria.

Toluene after oxidation in vivo is conjugated with glycine and excreted as hippuric acid (HA) in urine. Xylenes (three isomers) are oxidized in vivo to toluic acid, conjugated with glycine and excreted as methylhippuric acid (MHA) in urine. HA and MHA are often used as biological markers in the studies on occupational exposure to these solvents [16]. The TBARS level can be used as an indicator of oxidative damage to lipids by toluene or xylene.

In our earlier in vivo studies, a statistically significant increase in MDA+4-HNE concentration in plasma was observed after workers’ exposure to a mixture of solvents. Our present findings indicate that p-xylene may play a role in this effect — we noted a statistically significant influence of p-xylene on TBARS concentration in mitochondria. However, the biological effect of exposure to a mixture of chemicals is much more complicated. As regards the influence of p-xylene on TBARS level, we presume that the solvent may stimulate lipid peroxidation. The stimulatory effect was less definite with respect to \( ^\text{•} \)OH generation in mitochondria for it was not noted for every p-xylene concentration under study. The significant linear correlation between the dose of p-xylene and TBARS concentration \((r = 0.446; p = 0.000)\) points out that p-xylene is involved in the mechanism connected with \( ^\text{•} \)OH formation. The stronger influence of p-xylene on TBARS level, compared to the effect of toluene, may be associated with a higher lipophilicity and a better penetration of p-xylene into the lipid layer. The in vitro studies on toluene and p-xylene acting on isolated liver mitochondria that were conducted by Revilla et al. indicate that xylene may have influence on oxidative stress. At the concentration 0.1–1.0 mM, xylene induced a significant increase in ROS (reactive oxygen species) generation. As for toluene, it did not produce such an effect even at the concentration of 5 mM [17].

Also the study by Costa et al. revealed an influence of exposure to toluene and xylene vapours on the viability and integrity of the barrier function of human skin. To evaluate the role of oxidative stress, some biomarkers of oxidative damage as the lipid peroxidation products were investigated. Reduced glutathione depletion, decreased activity of antioxidant enzymes and oxidative damage of biological macromolecules were observed. The results showed an enhancement of lipid peroxidation after dermal exposure to xylene and toluene. The amount of peroxidation product, measured as MDA concentration, was higher in the case of xylene exposure [18]. Moreover, the type of isomer, namely para-isomer for xylene, could have increased the oxidative potential [19]. The para-substituted aromatic hydrocarbons exhibit an increased ability to form pseudochinonic radicals. These radicals transfer the extra electron to molecular oxygen thus increasing the generation of superoxide anion radicals [20].

Toluene is often used in industry as a thinner component. Thinners generated reactive oxygen species and caused an increase in lipid peroxidation products (MDA+4-HNE) in all the brain regions in rats [15]. It was observed that toluene degrades faster than xylenes and benzenes. Toluene appears to be the most easily degradable aromatic hydrocarbon under anoxic condition [21]. It was demonstrated that intraperitoneal injection of toluene produced a significant increase in the rate of reactive oxygen species and a decrease in glutathione levels in the brain of rats [15]. The question is how a mixture of toluene and p-xylene can act on lipid peroxidation. A combined exposure to a variety of organic solvents frequently occurs in industrial settings [22,23].

Our previous in vitro research indicated a synergistic interaction between styrene and ethylene glycol. The present study revealed that the effect of exposure to toluene and p-xylene mixture on TBARS in vitro is quite different and can be classified as an antagonistic one. In literature, there are some examples of the antagonistic activity of toluene and p-xylene in animal or human studies. Tardif et al. demonstrated that in combined exposure of rats, the quantity of urinary metabolites decreased by 20–30% for hippurid acid [HA] and by 40% for methylhippurid acid [MHA]. The mechanism of this interaction may be connected with a competitive activity on cytochrome P-450.
Many solvents are metabolised by cytochrome CYP 2E1 into reactive intermediates including epoxides and ROS. The induction of CYP450 was found to increase liver toxicity after exposure to some chemicals such as carbon tetrachloride, acetone or acetaminophen [25]. It is well known that the metabolic interaction of organic solvents is dependent on the kind of solvent, the metabolic rate and the sequence of exposure. Also the induction or inhibition of enzymes has influence on the biotransformation and may cause interaction. Some studies demonstrated that the excretion of benzoic acid after exposure to toluene and xylene mixture was dependent on the dose of the solvents. Low doses of toluene in the mixture with xylene inhibited urinary excretion of benzoic acid in rats, but a higher dose increased the excretion [26]. The effects of experimental exposure to toluene (100 ppm), xylene (100 ppm) and their mixture (50 ppm of toluene and 50 ppm of xylene) on the CNS function were studied in 10 male volunteers aged 25–35 years. The effect of combined exposure appeared to be weaker than the effect of xylene-only exposure, but stronger than that of toluene-only exposure [27]. Tardif et al. showed that the pharmacokinetic interaction was not apparent at low levels of exposure, namely 50 ppm of toluene and 40 ppm of m-xylene. They concluded that the interaction of toluene and xylenes is likely when the concentration of each is higher than 50 ppm.

In a human volunteer study, metabolic interaction took place when humans were exposed to a combination of 95 ppm toluene and 80 ppm xylene (mostly m-isomer) [28,29]. The problem of combined exposure and interaction is very complicated. Sometimes it is difficult to define one type of interaction for a mixture of two solvents. For example, an antagonism seen in one part of the nervous system after exposure to a mixture of xylene and n-hexane or toluene and dichloromethane does not exclude a possibility of synergism in another part. Both the synergistic and antagonistic effect can occur within the same organ or system after combined exposure to the same chemicals [23]. In the present study, the antagonistic toluene/p-xylene effect on lipid peroxidation may be associated with a solvent-induced mutual deactivation of radicals and on the penetration of the mixture to the water and lipid phases. Toluene and p-xylene can probably decrease each other’s concentration in tissue fluids and this may partially explain the mechanism of the antagonistic effect. Tabatabaie et al. postulated that in exposure to toluene, it is benzaldehyde, toluene metabolite, that is responsible for ROS generation. The mechanism is connected with inactivation of glutathione peroxidase. The antagonistic effect of toluene and p-xylene mixture on lipid peroxidation may result from a mutual inhibition of toluene conversion to benzaldehyde by p-methybenzaldehyde deriving from p-xylene [30]. Some experiments on rats showed that the metabolism of m-xylene at the dose of 150 ppm was suppressed by toluene co-exposure at 150 ppm [5].

Our present in vitro studies partially explain how toluene and p-xylene exposure in the paint and lacquer industry may have influence on lipid peroxidation. In contrast to the synergistic effect of styrene combined with ethylene glycol, an antagonism between toluene and p-xylene is also possible.

The last stage of our experiment focused on evaluating the antioxidative effect of CoQ$_{10}$ on enhanced lipid peroxidation after p-xylene or toluene exposure. The protective or reparative effect of CoQ$_{10}$ was evaluated. Coenzyme Q$_{10}$ is a part of the antioxidant defence mechanism in the body. Coenzyme Q$_{10}$ is the only lipid-soluble antioxidant that is synthesized endogenously. In its reduced form (ubiquinol), CoQ$_{10}$ inhibits protein and DNA oxidation, but it is the effect on lipid peroxidation that has been studied most extensively. Ubiquinol inhibits peroxidation of cell membrane and also that of lipoprotein fractions in the circulatory system [31]. Oxidative stress, e.g. after occupational exposure to organic solvents, may enhance endogenous requirement of CoQ$_{10}$. Apart from numerous reports on the beneficial effects of CoQ$_{10}$ in various diseases [32,33], there are only a few publications on the role of CoQ$_{10}$ in occupational exposure. They concern CoQ$_{10}$ administration in exposure to carbon tetrachloride and the protection of the liver cells [34,35]. Our earlier in vitro studies demonstrated that CoQ$_{10}$ could be effective in protecting against lipid peroxidation induced by a mixture of styrene and ethylene glycol or Solvesso and Bawanol, the solvents used in the paint and lacquer industry [1,2].
In the present study, the lower dose of CoQ_{10}, 1.5 μg/ml, appeared to be ineffective. Higher doses of CoQ_{10} (3.0 and 12.0 μg/ml) had to be used to achieve a beneficial effect on lipid peroxidation. The reduced form of CoQ_{10} protects lipid membranes from lipid peroxidation both at the initiation and propagation phase. The antioxidant effect of CoQ_{10} may be associated with its ability to directly react with superoxide radicals and ADP-Fe^{3+}-O_2^{-} complex. In such cases, CoQ_{10} acts as a free radical scavenger and can inhibit the initiation of lipid peroxidation. Coenzyme Q_{10} can also neutralise lipid radicals (L^{-}) or superoxide lipid radicals (LOO^{-}), thus inhibiting the propagation phase. The higher efficiency of CoQ_{10} as a protective rather than reparative agent can be associated with a greater activity of the superoxide radicals than of the hydroxyl radicals in the processes under study. Moreover, the CoQ_{10} protection may be a consequence of the competitive inhibition of p-xylene transformation to p-chinone derivatives by semichinone, which results from the conversion of CoQ_{10}.

**CONCLUSIONS**

As evidenced by the study results, exposure to a mixture of toluene and p-xylene exerts an antagonistic effect on lipid peroxidation, which is reflected by a decreased level of TBARS in mitochondria. On the other hand, exposure to p-xylene was found to increase TBARS and hydroxyl radical concentrations in mitochondria, with a statistically significant dose-response correlation. Furthermore, a relationship could be noted between TBARS level and 'OH generation after exposure to p-xylene. Administration of coenzyme Q_{10} at the concentrations of 3.0 and 12.0 μg/ml has successfully decreased the formation of TBARS induced by p-xylene.

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