Can thiamine pyrophosphate prevent desflurane induced hepatotoxicity in rats?

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**ABSTRACT**

**PURPOSE:** To investigate the effects of thiamine pyrophosphate (TPP) against desflurane induced hepatotoxicity.

**METHODS:** Thirty experimental animals were divided into groups as healthy (HG), desflurane control (DCG), TPP and desflurane group (TDG). 20 mg/kg TPP was injected to intraperitoneally TDG. After one hour of TPP administration, desflurane was applied for two hours. After 24 hours, liver tissues of the animals killed with decapitation were removed. The oxidant/antioxidant levels and ALT, AST and LDH activities were measured. The histopathological examinations were performed in the liver tissues for all rats.

**RESULTS:** Notwithstanding the levels of oxidants and liver enzymes were significantly increased (p<0.0001), antioxidant levels were significantly decreased in DCG (p<0.0001). On contrary to the antioxidant parameters were increased (p<0.05) the oxidant parameters and liver enzymes were decreased in TDG (p<0.0001). Whereas multiple prominent, congestion, hemorrhage and dilatation were observed in sinusoids and lymphocyte-rich inflammation results in the centrilobular and portal areas of liver tissue in DCG, these findings were observed less frequently in TDG.

**CONCLUSION:** Thiamine pyrophosphate prevented liver oxidative damage induced with desflurane and may be useful in prophylaxis of desflurane induced hepatotoxicity.

**Key words:** Thiamine Pyrophosphate, Oxidative Stress. Rats.
Can thiamine pyrophosphate prevent desflurane-induced hepatotoxicity in rats?

**Introduction**

The desflurane is a halogenated ether anesthetic which has been synthesized as a result of the studies conducted by Terrel et al.\(^1\) in order to find an ideal inhalation agent. Desflurane is known to have important advantages such as ability to provide anaesthetic depth more easily and quicker recovery. Notwithstanding these important advantages, it has also serious disadvantages that may negatively affect human health; recent studies demonstrated that hepatotoxic effect emerges due to the use of halogenated anesthetics. Martin et al.\(^3\) reposted desflurane to have serious hepatotoxic effects. Even cases of hepatotoxicity related to desflurane and resulted in death are found in the literature\(^3\). In an experimental study by Arslan et al.\(^7\), desflurane was found to produce hepatic damage in aged female rats\(^8\). Again desflurane was argued to create oxidative stress in the liver of aged male rats by increasing oxidants and decreasing antioxidants\(^9\). As is known, hepatocellular integrity is indicated by the plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH) and gamma glutamyl transferase (GGT). Determination of transaminase activities is one of the most commonly used tests in the diagnosis of hepatic diseases. In a case report presenting hepatotoxicity of desflurane, Martin et al.\(^3\) demonstrated that, ALT, AST, alkaline phosphatase total bilirubine, direct bilirubine and gamma glutamyl transferase were rapidly elevated. This information indicates that, oxidative stress is an important factor in hepatotoxicity caused by desflurane and antioxidant therapy may be beneficial. Thiamine pyrophosphate (TPP) that we tested against hepatotoxicity of desflurane is an active metabolite of thiamine (TM). In their study, Yilmaz et al.\(^5\) stated that TPP prevented alcohol-induced oxidative stress in the rat liver. Ksiaoglu et al.\(^9\) argued that, TPP protected the liver tissue against oxidative damage by inhibiting increase of the oxidant and decrease of the antioxidant parameters in the liver tissue related to paracetamol. The significant antioxidant feature of TPP suggests that it may protect the liver tissue against desflurane induced damages. No information was found in the literature screening about the protective effects of TPP against hepatotoxicity with desflurane in rats. Therefore, objective of this study was to investigate biochemically and histopathologically effects of TPP on the desflurane induced hepatotoxicity in rats.

**Methods**

The animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the Local Animal Ethics Committee of Recep Tayyip Erdogan University, Rize, Turkey (Ethics Committee Number: 2015/28, Dated: 27.04.2015). The experimental animals were obtained from the Recep Tayyip Erdogan University, Medical Experimental Research and Application Center.

A total of 30 male albino Wistar rats weighed between 235 or 245g and aged between four or five months, were randomly selected for the experiment. The animals were housed and fed in the pharmacology laboratory at normal room temperature (22°C) for one week before the experiment in order to provide adaptation to their environment.

**Chemical substances**

Of the chemical substances used for the experiments, thiopental sodium was provided by IE Ulaga-Turkey. Thiamine and thiamine pyrophosphate were obtained from Biopharma-Russia. Desflurane were obtained from Eczacıbasi, Istanbul, Turkey.

**Experimental groups**

The experimental animals were composed from three group as healthy (HG; n:10), desflurane control (DCG; n:10) and TPP + desflurane administration (TDG; n:10) rat groups.

**Experimental procedure**

The rats had free access to food and water until 2 h before anaesthesia procedure and were kept in a room at 20-24°C with a rhythm of 12 h light and 12 h darkness. The anesthetic gas vaporizer was calibrated before beginning of the study. In performing the experiment, TDG group was intraperitoneally (ip) injected 20 mg/kg TPP\(^10,11\). The HG and DCG groups were administered distilled water through the same route. After one hour of TPP and distilled water administration, anesthesia procedure was applied on the TDG and DCG rats in transparent plastic box with sizes of 40x40x70 cm. The container that allowed observation of the rats, were connected to the anesthesia machine with half open inlet using fixed hoses. For the maintenance of the minimum alveolar concentration (MAC) 1, Desflurane (Suprane\(^5\)) was administered at 6% volume inspiratory concentration, in a flowrate of 6 L min\(^-1\) 100% O\(_2\) for 2 hours. Anesthetic gas was released in the cage containing 100% oxygen. The healthy group was not subjected to any procedures. The blood samples were collected 24 hours after anesthesia. Then the liver tissues of the animals killed with
decapitation were removed. ALT, AST and LDH activities were measured in the blood samples collected. In addition, oxidant/antioxidant levels were studied in the liver tissue and all livers tissues of histopathological examinations were performed.

**Biochemical analysis**

**Determination of malondialdehyde (MDA)**

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (Elisa) to assay the level of rat MDA in samples (Elisa kit, Eastbiopharm Co. Ltd, China). Add malondialdehyde (MDA) to monoclonal antibody enzyme well which is pre-coated with rat malondialdehyde (MDA) monoclonal antibody, incubation; then, add malondialdehyde (MDA) antibodies labeled with biotin, and combined with streptavidin-hrp to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution a, b, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the rat substance malondialdehyde (MDA) of sample were positively correlated.

**Determination of myeloperoxidase (MPO) activity**

Potassium phosphate buffer of pH=6 which contained 0.5% HDTMAB (0.5% hexadecyl trimethyl ammonium bromide) was prepared in order to determine MPO in the liver tissue homogenates. The mixture obtained then was centrifuged at 10,000 rpm for 15 minutes at +4 °C. Supernatant part was used as the analysis sample. Oxidation reaction with MPO mediated H2O2 which included 4-amino antipyrin/phenol solution as substrate was used in determination of the activity of MPO enzyme.

**Nitric oxide (NO) analysis**

Tissue NO levels were measured as total nitrite + nitrate levels with the use of the Griess reagent as previously described. The Griess reagent consists of sulfanilamide and N-(1-naphthyl)-ethylenediamine. The method is based on a two-step process. The first step is the conversion of nitrate into nitrite using a nitrate reductase. The second step is the addition of the Griess reagent, which converts nitrite into a deep purple azo compound; photometric measurement of absorbance at 540 nm is due to the fact that this azo chromophore accurately determines nitrite concentration. NO levels were expressed as μmol/g protein.

**Determination of total glutathione (tGSH) levels**

This kit uses enzyme-linked immune sorbent assay (Elisa) based on the biotin double antibody sandwich technology to assay the rat total glutathione (Elisa Kit, Eastbiopharm Co. Ltd, China). Add total glutathione to the wells, which are pre-coated with total glutathione monoclonal antibody and then incubate. After that, add anti glutathione antibodies labeled with biotin to unite with streptavidin-hrp, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate a and b. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of rat total glutathione are positively correlated.

**Determination of glutathione peroxidase (GSHPX) activity**

GSHPX was determined by monitoring NADPH production at 340 nm and 25 °C. The assay mixture contained 10 mm magnesium chloride, 0.2 mm NADPH, 0.1 u/ml gr and 0.1 m GSH in 100 mm tris-hydrochloride buffer solution at ph 8.0. Assays were carried out in triplicate and the activities were followed up for 60 s. One unit of activity (u) is defined as the amount of enzyme required to reduce 1 μmol/min of NADPH under the assay conditions. The activity of Gpx was calculated using the extinction coefficient of 6.22 mm⁻¹ cm⁻¹.

**Determination of glutathione reductase (GSHRD) activity**

GSHRD enzyme activity was measured by Beutler’s method. One enzyme unit is defined as the oxidation of 1 mmol NADPH per min under the assay condition (25 °C, ph 8.0).

**Determination of catalase (CAT) activity**

The CAT activity was measured by the Aebi method. In this method, 20 ml enzyme solution was added to the 1 ml 10mm H2O2 in 20 mm potassium phosphate buffer (ph 7.0) and incubated at 25 °C for 1 min. Initial reaction rate was measured from the decrease in absorbance at 240 nm.

**Determination of superoxide dismutase (SOD) activity**

SOD activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with nitro blue tetrazolium to form formazan dye. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction and is expressed as EU/ml.
Alanine transaminase (ALT), aspartate aminotransferase (AST) analysis

Venous blood samples were collected into tubes without anticoagulant. Serum was separated by centrifugation after clotting and stored at -80°C until assayed. Serum AST and ALT activities were measured spectrophotometrically as liver function tests, and LDH activity, as a marker of tissue injury, using a Cobas 8000 (Roche) autoanalyzer with commercially available kits (Roche Diagnostics, GmBH, Mannheim, Germany).

ALT analysis

Quantitative determination of the serum ALT (Alanine Aminotransferase) was studied by spectrophotometric method using Roche cobas 8000 autoanalyser. According to the International Federation of Clinical Chemistry (IFCC), pyridoxal 5'-phosphate method catalyzes the reaction between 3,4 ALT L-alanine and 2 - oxoglutarate. The pyruvate formed is reduced by NADH with a reaction catalyzed by lactate dehydrogenase (LDH) in which L-lactate and NAD⁺ are formed. Pyridoxal phosphate functions as a coenzyme in the amino transfer reaction. 

\[
\text{L-Alanine} + 2\text{-oxoglutarate} \rightarrow \text{pyruvate} + \text{L-glutamate.}\]

Rate of the NADH oxidization is proportional to the ALT activity.

AST analysis

Quantitative determination of the serum AST (Aspartate Aminotransferase) was studied by spectrophotometric method using Roche cobas 8000 autoanalyser. According to the International Federation of Clinical Chemistry (IFCC), pyridoxal 5'-phosphate method catalyzes the transfer of an amino acid group between L-aspartate and 2- oxoglutarate in order to form 3,4 AST oxaloacetate and L-glutamate. The oxaloacetate reacts with NADH in the presence of malate dehydrogenase (MDH) in order to provide formation of NAD⁺. Pyridoxal phosphate functions as a coenzyme in the amino transfer reaction.

\[
\text{L-Aspartate} + 2\text{-oxoglutarate} \rightarrow \text{oxaloacetate} + \text{L-glutamate.}\]

Rate of the NADH oxidization is proportional to the AST activity.

Lactate dehydrogenase (LDH) analysis

Quantitative determination of serum LDH (Lactate Dehydrogenase (P-L)) was studied with spectrophotometric method in Roche cobas 8000 autoanalyzer. This is a standard method optimized according to Deutsche Gesellschaft für Klinische Chemie (DGKC). LDH catalyzes the reaction between pyruvate and NADH for formation of NAD⁺ and L-lactate.

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{L-laktat} + \text{NAD}^+.\]

Initial rate of NADH oxidation is proportionate to the catalytical LDH activity. Reduction in the absorbance was determined by measurement at 340 nm.

Histopathological analysis

The liver tissues removed from rats were fixed in 10% buffered formalin solution. Sections of 5 µm thickness were obtained from the paraffin blocks that were obtained following routine tissue processes and stained with hematoxylin and eosin (H&E). All the sections were coded and examined under light microscope (Olympus CX 51, Tokyo, Japan) by the same pathologist who was blind to the applied protocol.

Statistical analysis

The results obtained from the experiments were expressed as “mean ± standard error of the mean” (x ± SEM). The statistical analysis was conducted using analysis of variance (ANOVA) followed by post-hoc comparisions (Tukey-B). All the statistical processes were carried out with “IBM SPSS Statistics Version 20” statistical software and p< 0.05 values were considered statistically significant.

Results

Biochemical results

As it is seen in Figure 1, levels of MDA, MPO and NO were respectively measured as 0.55 ± 0.04 nmol/ml, 2.5 ± 0.37 u/ml and 3.1 ± 0.34 µmol/l in the liver tissue of healthy animals. However, these values were increased as 1.85 ± 0.05 nmol/ml, 5.4 ± 0.39 u/ml and 13 ± 2 µmol/l in the animals exposed to desflurane inhalation for 2 hours (p<0.0001). In TDG these values were respectively found as 0.63 ± 0.03 nmol/ml, 2.9 ± 0.24 u/ml and 3.38 ± 0.32 µmol/l. Therefore when we compared HG to DCG and TDG the difference between HG and TDG was found statistically insignificant (p>0.05). However despite of the fact that the levels of tGSH, GSHPx and GSHRd were respectively found as 345 ± 7.8 nmol/ml, 0.667 ± 0.009 u/ml and 0.529 ± 0.007 µmol/l in the healthy group, these values decreased to respectively 147.83 ± 12.2 nmol/ml, 0.993 ± 0.004 u/ml and 0.204 ± 0.009 µmol/l levels in DCG. The differences were statistically significant (p<0.0001). However
TPP prevented the reduction of the tGSH, GSHPx and GSHRd levels with desflurane as the respective levels of $317 \pm 8.4$ nmol/ml, $0.690 \pm 0.006$ u/ml and $0.492 \pm 0.003$ µmol/l (Figure 2). Again the difference between HG and TDG was found statistically significant ($p<0.0001$).

**FIGURE 1** - The effects of thiamine pyrophosphate on MDA, MPO and NO levels in rats administered desflurane. *$p<0.0001$ according to HG group; **$p>0.05$ according to HG group.

CAT and SOD values were respectively found as $0.120 \pm 0.006$ u/ml and $8.8 \pm 0.21$ u/ml in the healthy group. While desflurane increased the activities of CAT to $0.51 \pm 0.05$ u/ml ($p<0.001$), SOD value decreased to $5.2 \pm 0.47$ u/ml ($p<0.0001$), the activity of CAT as $0.123 \pm 0.01$ u/ml and activity of SOD as $9.1 \pm 0.44$ u/ml were found in TDG (Figure 3). When we compared HG to TDG, these differences were statistically insignificant ($p>0.05$).

**FIGURE 2** - The effects of thiamine pyrophosphate on tGSH, GSHPx and GSHRd levels in rats administered desflurane. *$p<0.0001$ according to HG group.

**FIGURE 3** - The effects of thiamine pyrophosphate on CAT and SOD levels in rats administered desflurane. *$p<0.0001$ according to HG group; **$p>0.05$ according to HG group.
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The activities of AST, ALT and LDH that are used to evaluate the liver function were respectively found as 29 ± 1.25 u/ml, 20 ± 1.39 u/ml and 151 ± 2.96 u/ml in the blood samples of the healthy group, desflurane changed the activities of AST, ALT and LDH as respective 274 ± 8.20 u/ml, 157 ± 13.35 u/ml and 630 ± 12.71 u/ml. Whereas these activities were found as 194 ± 4.62 u/ml, 86 ± 4.22 u/ml and 333 ± 16.71 u/ml in the blood samples of the animals treated with TPP (Figure 4). The differences between compared groups were statistically significant (p<0.0001).

**Histopathological results**

The histopathological evaluations that were performed for all animals, were completely subjective up to the pathologist’s evaluation. In Figure 5a, normal histological appearance of the liver tissues of the HG is monitored (H&E, x200). In Figure 5b, limited mild congestion, hemorrhage (long star), dilatation (star) and lymphocytic inflammation areas were observed in the liver tissue of the animals treated with TPP. However, there are multiple marked congestion, hemorrhage (long arrow) and dilatation (star) areas in the sinusoids of the liver tissues of DCG group which received desflurane (H&E, x200). Lymphocyte-rich inflammation signs (short arrow) are observed in the centrilobular and portal areas in Figure 5c. (H&E, x200).

**Discussion**

In this study, the protective effect of TPP against hepatotoxicity induced with desflurane in rats, was biochemically and histopathologically investigated. The liver is the organ that is most affected by the toxic effects. The informations from the literature indicates that the incidence of desflurane induced hepatotoxicity appears to directly correlated with anesthetic metabolism catalysed by cytochrome P 450 2E1 to trifluoroacetylated proteins. Still desflurane also has been reported to produce hepatotoxic effect by decreasing the amounts of vitamins that have antioxidant activity. Eroglu et al. reported that, desflurane causes oxidative stress by decreasing the value of vitamin- E in blood plasma. Erbas et al. stated that, total oxidant capacity is elevated in the patients who received desflurane. Sivaci R et al. found that oxidant and antioxidant defense mechanisms were resulted in superiority of oxidants in desflurane induced anesthesia. Similar to these studies the biochemical experimental results of our study demonstrated that desflurane increased the values of oxidants such as MDA, MPO and NO whereas reduced
the values of antioxidants as tGSH, GSHPx, GSHRd, CAT and SOD in the liver tissues. These results showed that desflurane led to oxidative damage in the liver tissue.

Aslan et al. reported that desflurane leads to oxidative stress in the rat liver by increasing lipid peroxidation. As is known, MDA is the final product of lipid peroxidation and the MDA value increases when the liver is damaged by oxidative stress. Again in our study desflurane was found to be increased the MDA value similar to the studies mentioned above.

There was no information in the literature about whether desflurane changes the activity of MPO in the liver or not. However, there are studies indicating that the agents which produce liver damage increases the activity of MPO. Therefore we found that the desflurane increased the MPO activity in the liver tissue too.

Another parameter which increased in the liver tissue of the animals given desflurane is NO. NO reacts with ROS, producing peroxynitrite (ONOOH) which is a potent oxidant and this in turn lead to the formation of HO• radical through advanced decomposition. It has not been demonstrated whether NO has a role in the pathogenesis of the desflurane hepatotoxicity. However, it has been explained in the literature with experimental data that NO can cause hepatotoxicity. The NO has been reported to initiate lipid peroxidation in the environment in which superoxide takes place. We interpreted the increased NO value as an indication of oxidative stress in liver tissue like the other increased oxidants. Finally our experimental results and these informations from the literature demonstrate that, desflurane occured oxidative stress in the liver tissue. The levels of tGSH, GSHPx, GSHRd, CAT and SOD were found to be decreased in desflurane group and we reported that desflurane changed oxidative/antioxidant balance in the liver tissue in favour of oxidants. Consequently it is understood from the studies mentioned above that desflurane has negative effect on various antioxidant systems and these knowledges are consistent with our results.

No studies were found in the literature screening about the protective effect of TPP against desflurane hepatotoxicity. However, TPP has been emphasized to protect the liver against paracetamol induced oxidative damage by increasing the values of GSH, GPO, GRx, GST, SOD and CAT and by decreasing the amounts of MDA and NO in the liver tissue. Another study reported that TPP significantly decreased MPO value which was increased with cisplatin in the cerebral tissue, in a dose dependent manner. We found that the values of these antioxidant parameters increased in TPP administered group. Consequently, TTP whose protective effect was tested against desflurane hepatotoxicity, changed the oxidant/antioxidant balance in favour of antioxidants in the liver tissue.

According to the reducing of antioxidants in the desflurane group, activities of AST, ALT and LDH were found to be increased. These enzymes are the most recognized and used ones in order to evaluate the liver functions. However these values reduced in TPP administration group and we interpreted these result as an improvement of liver tissue by preventing oxidative damage.

Fisher et al. stated that, sinusoidal dilatation is a vascular lesion of the liver which is developed due to medicines. Arslan et al. found that, neutrophil and leucocyte infiltration was increased in the liver damage caused by desflurane. As mentioned above, no information was found in the literature demonstrating about the protective effect of TPP against oxidative damage by desflurane in the liver tissue. However, it has been stated that, TPP improved the histopathological damage in the rat liver such as dilatation, inflammation, necrosis and apoptosis caused by methotrexate. The intense congestion, hemorrhage and dilatation areas were observed in the liver sinusoids and lymphocyte-rich inflammation in the centrilobular and portal areas for desflurane group with changed oxidant/antioxidant balance in favour of oxidant. These histopathological findings indicate a vascular damage occurred in the liver tissue. Whereas the use of TPP has led to improvement in histopathological findings. This is understandable when demonstrated Figure 5b and those results are consistent with literature.

Conclusions

The desflurane leads to oxidative stress that causes liver damage of rats. Thiamine pyrophosphate can prevent desflurane induced oxidative stress and the liver dysfunction. Consequently TPP may be useful for the prophylaxis of hepatotoxicity induced with desflurane.

References

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