Trichloroethylene-induced Cellular Damage was Associated with Significant changes in the Concentrations of Caspase-3 and Glutathione in human lymphocytes

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Abstract

Trichloroethylene (TCE) is a volatile organic compound that is widely used in industries and potential source of environmental contamination. The aims of this study were focused on the effects of TCE on cellular viability and evaluation of critical markers like caspase-3 and glutathione (GSH) in human lymphocytes. The experiments were studied in thirty volunteers. The TCE concentrations of 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L were treated in human lymphocytes for 48 h. Lymphocytes were cultured and effect of TCE on viability of lymphocytes was studied by MTT assay. The expression of caspase-3 in TCE treated lymphocytes was studied by ELISA and the concentration of glutathione in TCE treated lymphocytes was studied by colorimetric technique. The results showed that, in the experimental groups at various TCE concentrations had lower cellular viability than the control group ($p<0.05$). The caspase-3 concentration was increased when TCE concentration increased ($p<0.05$). The glutathione concentration was decreased when TCE concentration increased ($p<0.05$). It is indicated that TCE effected on cellular viability. The expression of caspase-3 enzyme and glutathione concentrations were changed by TCE toxicity in lymphocytes.

Keywords: Trichloroethylene, Lymphocyte, Cellular viability, Caspase-3 enzyme, Glutathione.

Introduction

Trichloroethylene (TCE) is a volatile and colorless liquid that is miscible with many non-polar organic solvents. It has been found in underground water and many surface waters as a result of the manufacture, use, and disposal of the chemical.It is used mainly as a degreaser for metal parts. It is also an ingredient in adhesives, paint removers, typewriter correction fluids. Trichloroethylene is readily absorbed in

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the gastrointestinal tract and the lungs. In humans and animals, initial uptake following inhalation is rapid. The major metabolic route is oxidation (by cytochrome P-450 mixed-function oxygenase) giving trichloroethanol,tricholoroethanol-glucuronide and trichloroacetic acid as the major metabolites (identified in both humans and animals). Chloral hydrate is an intermediate in this oxidative biotransformation and its formation is probably preceded by the conversion of trichloroethylene to its epoxide. Several minor metabolites of trichloroethylene have also been identified, including the mercapturic acid N-acetyl-S-(dichlorovinyl)-L-cysteine (DCVC), which is formed in the kidneys from the glutathione conjugate of trichloroethylene. Occupational exposure to trichloroethylene is associated with excess incidences of liver cancer, kidney cancer and non-Hodgkin lymphoma.[1,2,3]

Caspases are a family of cysteinyl aspartate-specific proteases that are highly conserved in multicellular organisms and function as central regulators of apoptosis. Caspase-3 is one of the key mediators in apoptosis. This protein cleaves and activates caspases 6 and 7 and the protein itself is processed and activated by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease. Alternative splicing of this gene results in two transcript variants that encode the same protein. Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways. Activated Caspase-3 is responsible for the cleavage of poly ADP-ribose polymerase, actin and sterol regulatory element binding protein, which are associated with apoptosis. The zymogen feature of caspase-3 is necessary because if unregulated, caspase activity would kill cells indiscriminately.[4] Caspase-3 has been found to be necessary for normal brain development as well as its typical role in apoptosis, where it is responsible for chromatin condensation and DNA fragmentation. It is now being shown that caspase-3 may play a role in embryonic and hematopoietic stem cell differentiation.[5]

Glutathione (GSH) is a tripeptide formed by glutamic acid, cysteine, and glycine. The glutamic acid forms a particular gamma-peptic bond with cysteine by its gamma glutamyl group. Two forms of GSH are possible: the reduced form (GSH) which represents the majority of GSH, reaching millimolar concentration in the intracellular compartment, and the oxidized form (GSSG) that is estimated to be less than 1% of the total GSH. Intracellularly, the majority of GSH is found in the cytosol (about 90%), while mitochondria contain nearly 10% and the endoplasmic reticulum contains a very small percentage conjugates with GSH, either spontaneously or enzymatically, in reactions catalysed by GSH-S-transferases (GST). Human GSTs are divided into two distinct family members: the membrane-bound microsomal and cytosolic family members. The conjugates formed are usually excreted in the bile. It can undergo modification to mercapturic acid. Another important GSH function is the maintenance of the intracellular redox balance and the essential thiol status of proteins. Glutathione plays important roles in antioxidant defence nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation).[6] Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes).[7]

A major function of glutathione (GSH) is the detoxification of xenobiotics and some endogenous compounds. Caspase-3 is one of the key mediator in apoptosis. TCE can produce oxidative stress in human.Oxidative stress can disturb GSH homeostasis and affect apoptosis mechanism. Cellular damage and its potential role in TCE-mediated pathogenesis is not clearly understood. The relationship between the depletion of cellular glutathione, generation of ROS, and activation of caspase-3 is not known. In this work, we aim to understand the relationship between these events as they relate to oxidative stress. Therefore, to assess the effect of TCE exposure on lymphocyte was studied. This study aims to investigate the effects of TCE on the cellular viability, the concentration changes of caspase-3 enzyme and glutathione in

human lymphocytes. Glutathione and caspase-3 concentration changes due to trichloroethylene toxicity may be associated with oxidative stress and lead to increase the progression of cancer.

Materials and Methods

Chemicals

Trichloroethylene (CAS No. 79-01-6) was purchased from Lobachemie (Mumbai, India). MTT (3-[4.5-dimethy-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) was procured from Bio Basic Canada (Ontario, Canada). Dimethylsulphoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, USA.). RPMI medium and chemical for cell culture were purchased from Thermo Fisher Scientific (Massachusetts, USA).

Specimen collection

Heparinized blood was collected from 30 healthy volunteers (age 20-25 years and no history of hematologic diseases and genetic disorders) after getting their informed consent. Approximately 5 mL of blood was collected from each healthy volunteer. The peripheral blood mononuclear cell (PBMC) isolation was done following Debey's study[8]. Lymphocytes were cultured for a study on the effects of TCE on lymphocyte viability, the concentration changes of caspase-3 enzyme and glutathione in human lymphocytes.

Study on the effect of TCE on lymphocyte viability

Human lymphocyte cultures from the same subject were separated to be both control (n = 30) and experimental (n = 30) groups. The study about lymphocyte viability was performed by added TCE conc. 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L to the experimental groups, respectively. The $1x10^5$ cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 24 h. After 24 h, the concentrations of TCE 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L were added in lymphocyte cultures and incubated for 48 h. After 48 h incubation time, MTT assay was done to analyse the viability of lymphocytes. For MTT assay, the content was centrifuged at 600 g for 10 min and 0.5 mL of 300 µg/mL MTT in phosphate buffer saline solution (PBS) was added to each well and incubated for 4 h at 37°C. The medium was removed and formazan was dissolved in DMSO and the optical density was measured at 570 nm. using a Bio-assay reader.

Study on the effect of TCE on the caspase-3

The $1x10^5$ cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 24 h. After 24 h, the concentrations of TCE 0.002, 0.004, 0.008, 0.016 mM/L and 0.032 mM/L were added in lymphocyte cultures and incubated for 48 h. After 48 h incubation time, TCE treated lymphocytes were studied about the concentration change of caspase-3 by Human Caspase-3 Instant ELISA Kit (Invitrogen, USA).

Study on the effect of TCE on the glutathione

The $1x10^5$ cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 24 h. After 24 h, the concentrations of TCE 0.002, 0.004, 0.008, 0.016 mM/L and 0.032 mM/L were added in lymphocyte cultures and incubated for 48 h. After 48 h incubation time, TCE treated lymphocytes were studied about the concentration change of glutathione by Glutathione Colorimetric Assay Kit (Biovision, USA).

Statistics analysis

The toxicity of TCE on lymphocyte viability was tested by ANOVA. The effects of TCE on the concentration changes of caspase-3 and glutathione were analysed byANOVA and p values of less than 0.05 were considered statistically significant.

Results

Effect of TCE on viability of lymphocytes

The study about TCE-induced cytotoxicity at concentrations of 0.002, 0.004, 0.008, 0.016 and 0.032 mM/L for 48 h. were done. The results showed that the percentage of viable lymphocyte was decreased when the concentrations of TCE increased (Figure 1) ($p<0.05$). The 50% inhibitory concentration (IC_{50}) was 0.029 mM/L which effected growth or depletion of human lymphocytes.

Effect of TCE on the caspase-3 and glutathione

The results showed that the expression of caspase-3 reduced progressively in a dose dependent manner in relation to treatment of lymphocytes with TCE (*p*<0.05) (Figure 2). The results showed that the concentration of glutathione (reduced form) was changed when lymphocytes exposed with TCE as shown in Table 1. TCE reduced the concentration of glutathione (reduced form) in a dose dependent manner (*p*<0.05).

Table 1. The concentrations of glutathione in TCE exposed lymphocytes at the various concentrations of TCE.

TCE concentration (mM/L)	Reduced glutathione concentration (µg/ml)±SE
0	0.126±0.08
0.002	0.094±0.04*
0.004	0.067±0.01*
0.008	0.063±0.01*
0.016	0.040±0.02*
0.032	0.030±0.01*

*TCE significantly reduced reduced glutathione concentration at 48 h incubation time compared with the control group (*p*<0.05).

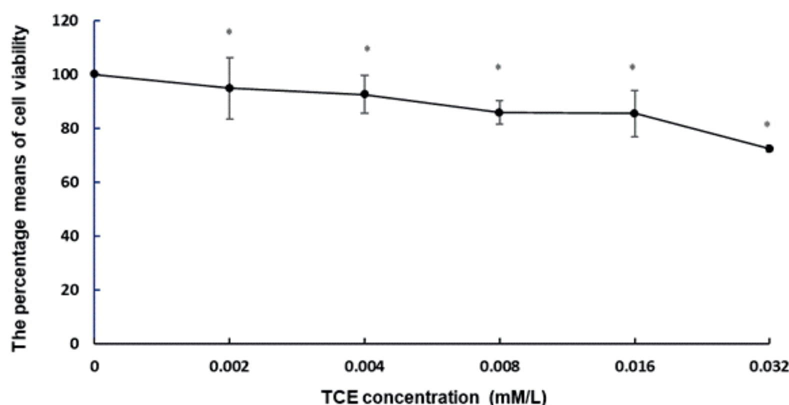

Figure 1. The percentage means of lymphocyte viability at the various concentrations of TCE.

*TCE significantly reduced lymphocyte viability at 48 h incubation time compared with the control group (*p*<0.05).

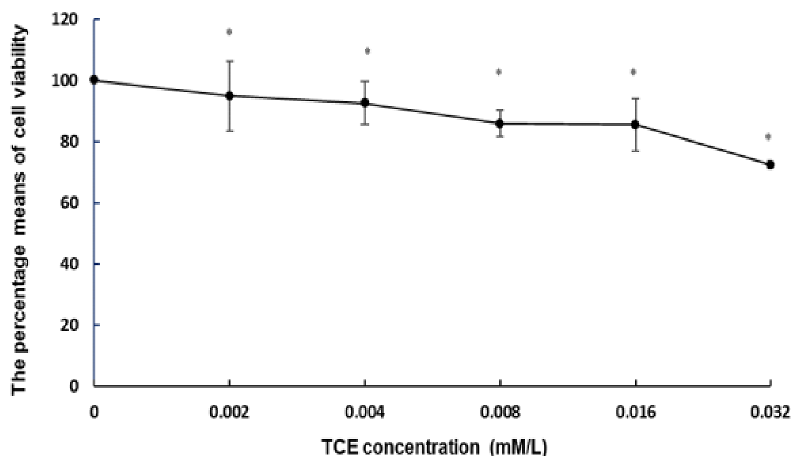

Figure 2. The percentage means of lymphocyte viability at the various concentrations of TCE..

*TCE significantly reduced lymphocyte viability at 48 h incubation time compared with the control group (p<0.05).

Discussion

Many studies have established that the direct treatment of cells with oxidants like hydrogen peroxide induces apoptosis and several non-oxidant apoptogenic agents such as tumor necrosis factor and cycloheximide also elicit oxidative stress. It is indicated that Reactive Oxygen Species (ROS) generation may be a conserved apoptotic event. Several studies suggest that ROS generation and antioxidant depletion mediate the events leading to apoptosis. However, the presence of an oxidative mechanism in apoptosis is unclear.[9]

TCE metabolism plays a critical role in eliciting its mutagenicity, carcinogenicity and other adverse health effects. Trichloroethanol, tricholoroethanol-glucuronide and trichloroacetic acid are the major metabolites and the mercapturic acid N-acetyl-S-(dichlorovinyl)-L-cysteine (DCVC) is a minor biotransformation product of TCE. TCE or its metabolite DCVC can stimulate the increasing ROS generation, increasing pro-inflammatory response, increasing apoptosis, and decreasing supply of energy metabolites. TCE can induce oxidative stress in cells. [10] Huang et al, 2015 observed that TCE was associated with increased variability of DNA methylation. DNA methylation may play a role in the pathogenesis of TCE exposure-related diseases and lymphocytes decreased with increased exposure to TCE. [11] Our study observed that TCE could decrease lymphocyte viability.

Apoptosis involves the activation of caspases. Caspases (cysteinyl aspartate proteases) are expressed as zymogens and become activated by an apoptotic signal. Once a caspase is activated, it may initiate an amplified apoptotic pathway by activating other caspases, leading to rapid cell death. 14 mammalian caspases have been identified and categorized into initiator, effector, and inflammatory caspases. Caspase-3 is the major effector caspase activated by initiator caspases in the execution phase of apoptosis. Once activated, the protease cleaves proteins involved in DNA and cytoskeleton structure, leading to irreversible self-destruction. The activation of caspase-3 may depend on redox status. Activated caspase-3 is responsible for the cleavage of poly ADP-ribose polymerase, actin and sterol regulatory element binding protein, which are associated with apoptosis. TCE-induced DNA damage was associated with significant activation of PARP-1 and increases in caspase-3, cleaved caspase-8 and -9 in the liver cells.[12] The effects of TCE toxicity were studied on the normal human liver cells (L02 cells) and hepatocytes with CYP2E1 gene overexpression. It showed that caspase-3, caspase-8 and caspase-9 mRNA expression increased by 30% - 600% in CYP2E1-overexpressing cells by TCE.[13] Oral administration of TCE in rats induced DNA strand breaks and induced the expression of caspase-3,-7 and -9.[14] TCE could induce oxidative stress, reduced cellular viability in lymphocytes and it also increased caspase-3 activation in this study. The concentration of caspase-3 was increased when TCE concentration increased.

Glutathione (GSH) is an important regulator of the cellular redox condition. Glutathione exists in both the cytosol and the mitochondria. It is the most abundant, non-protein physiological antioxidant and involved in regulation of the cell cycle. The sulfhydryl group (−SH) of the cysteine is involved in reduction and conjugation reactions that are usually considered as the most important functions of GSH. Sources of oxidants GSH plays a major role in removal of many reactive species. Glutathione regulates the action of glutathione-peroxidases and glutathione-transferases.[6] A decrease in the cellular antioxidant concentration is associated with the generation of an excess of ROS. Xenobiotics and some endogenous compounds are electrophiles and form conjugates with GSH, either spontaneously or enzymatically, in reactions catalysed by GSH-S-transferases (GST). Human GSTs are divided into two distinct family members: the membrane-bound microsomal and cytosolic family members. The conjugates formed are usually excreted in the bile, but can also undergo modification to mercapturic acid. Another important GSH function is the maintenance of the intracellular redox balance and the essential thiol status of proteins. Glutathione exists in reduced (GSH) and oxidized (GSSG) states. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of cellular oxidative stress where increased GSSG-to-GSH ratio is indicative of greater oxidative stress. In healthy cells and tissue, more than 90% of the total

glutathione pool is in the reduced form (GSH), with the remainder in the disulfide form (GSSG). In the reduced state, the thiol group of cysteinyl residue is a source of one reducing equivalent. Glutathione disulfide (GSSG) is thereby generated. The oxidized state is converted to the reduced state by NADPH. This conversion is catalysed by glutathione reductase. [15]TCE could alter glutathione redox homeostasis and glutathione precursors. [16] GSH plays a vital role in the protection of TCE-induced oxidative stress and apoptosis, which may be mediated through a p53-dependent pathway in human lung cancer cells. [17]TCE induced deactivation of cytochrome P-450 and loss of liver glutathione in male rat. [18]Its depletion could induce apoptosis.[19]

Experimental animal and human data indicate that TCE metabolism occurs through two major pathways: cytochrome P-450 (CYP)-dependent oxidation and glutathione (GSH) conjugation catalyzed by GSH S-transferases (GSTs).GSH depletion can induce apoptosis. The disturbances in GSH homeostasis are involved in the etiology and progression of many human diseases including cancer. While GSH deficiency, or a decrease in the GSH/glutathione disulphide (GSSG) ratio, leads to an increased susceptibility to oxidative stress implicated in the progression of cancer, elevated GSH levels increase the antioxidant capacity and the resistance to oxidative stress as observed in many cancer cells.

TCE-induced DNA damage was associated with increases in the levels of caspase-3 in liver cells and glutathione plays a vital role in the protection of TCE-induced oxidative stress and apoptosis, which may be mediated through a p53-dependent pathway.[12,17] The concurrent depletion of glutathione and generation of ROS may be required for the activation of caspase-3. Our study observed that TCE treated lymphocytes reduced glutathione concentration in cells. The concentration of glutathione was decreased when TCE concentration increased. It is concluded that TCE caused oxidative stress in cells. Oxidative stress caused by TCE treated lymphocyte could reduce lymphocyte viability and it changed the concentrations of caspase-3 and glutathione in human lymphocytes.

Conclusion

TCE could reduce viability of lymphocytes and significantly changed the expression of caspase-3 and glutathione concentrations in human lymphocytes.

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Ethical Clearance: This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Thammasat University ethical committee. The project identification code was EC 175/2020 and date of approval was Jan 20, 2021.

Conflict of Interest: The authors declare no conflicts of interest.

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