Turk J Vet Anim Sci
24 (2000) 109–112

Acute Phase Effect of Trichloroethylene Ingestion on Some Biological Markers in Dogs

Recep ASLAN
AKÜ, Veterinary Faculty, Department of Physiology, Afyon-TURKEY
Mehmet TÜTÜNCÜ
YYÜ, Veterinary Faculty, Department of Internal Medicine, Van-TURKEY
S. Oktay ASLAN
HÜ, Medical Faculty, Department of Toxicology, Şanlıurfa-TURKEY
Yılmaz DÜNDAR
AKÜ, Veterinary Faculty, Department of Biochemistry, Afyon-TURKEY

Received: 19.06.1998

Abstract: Trichloroethylene (TE) is an environmental toxic solvent hazardous to human and domestic animals and well known in the industrial sector. The purpose of this study was to determine whether oral TE plays a role in lipid oxidation and tissue damage. Fourteen dogs were treated with an oral toxic dose of 0.5 ml/kg T.E.

The acute changes that occurred in creatine kimase (CK), malondialdehyte (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were investigated in blood samples taken before application and 8, 24, 48, 72 and 168 hours after treatment. SOD and GSH-Px activities decreased, protein, albumin and alcalen phosphate did not change and the others increased 8 and 24 h after the TE treatment. Forty-eight hours after the treatment, MDA, CK, albumin and AST increased, while SOD and GSH-Px decreased. All markers returned to their normal levels after 72 h. It was concluded that TE plays a role in oxidative stress and tissue damage in the acute phase. Repeated intake of oral TE can reach serious toxicity in domcstic animals living around industial zones polluted with chemicals.

Key Words: trichloroethylene, peroxidation, oxidative stress, environmental, toxic, damage.

Köpeklerde Oral Trikloretilen Alımının Bazı Biyomarkerlere Akut Dönem Etkisinin Araştırılması

Özet: Triklor etilen (TE), endüstriyel olarak pek çok alanda kullanılması nedeniyle insan ve evcil hayvan sağlığını olumsuz yönde etkilemektedir. Bir solvent olan TE'nin inhalasyonunda etkili olabileceği ortaya konmuş olmakla birlikte, oral alımına bağlı etkileri üzerinde yeterli sayılabilecek tartışmanın olmadığı da dikkat çekicidir. Oysa TE içeren ürün ve ambalaj malzemeleri bir kimyasal çevre kirliliğine yol açarak çöplük ve fabrika çevrelerindeki canlıların, özellikle evcil hayvanların, oral olarak TE içeren ürünler almasına yol açabilmektedir. Bu çalışma ile önemli bir kimyasal çevre kirliliği faktörü olan TE'nin oral alımındaki etkilerinin araştırılması amaçlanmıştır. TE için toksik doz olarak bilinen 0.5 ml/kg düzeyinin bazı serum yaşamsal parametrelerini akut fazda ne şekilde etkilediği köpekler üzerinde deneysel bir çalışma ile araştırıldı. Çalışmada deney amacıyla 14 köpek kullanıldı. Toksik dozda TE hayvanlara susam yağı içinde orogastrik sonda yardımı ile sabah açlığında uygulandı. Deney hayvanlarından kan numuneleri uygulama öncesinde ve TE verildikten sonraki 8., 24., 72., 168. saatlerde alınarak lipid peroksidasyonu ürünlerinden malondialdehit (MDA), albumin, protein, alkalen, antioksidan hücresel enzimlerden süpeoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px), karaciğer katabolik enzimlerinden AST, GGT ve ALT ile doku hasarı göstergelerinden kreatin kinaz (CK) düzeyleri araştırıldı. TE uygulamasını izleyen 8. ve 24. saatte SOD ve GSH-Px düzeylerinin azaldığı protein albumin ve alkalen konsantrasyonunun anlamlı bir değişme göstermediği; MDA, CK, AST, GGT ve ALT'nın ise serum düzeylerinin istatistiksel önemde arttığı görüldü. Uygulama sonrası 48. saate ilişkin analizlerde MDA, CK, AST ve albumin düzeylerinin kontrol verilerine göre yine yüksek, SOD ve GSH-Px seviyelerinin düşük olduğu; 72. ve 168. saat ölçümlerinde elde edilen verilerin ise kontrollere göre istatistiksel öneme haiz bir farklılık göstermediği izlendi. TE'nin toksik dozda oral alımının akut dönemde lipitlerin peroksidasyonunu artırıp antioksidan enzimleri azalttığı; organizmada oksidan-antioksdan dengenin bozulduğu ve karaciğer enzimlerinden AST, GGT, ALT ile CK'nın konsantrasyonunu yükselterek bir toksikasyona yol açtığı, ancak bu toksikasyonun ve değişen göstergelerin medikal girişime gerek kalmaksızın spontan olarak gerilediği ve 3. günden itibaren normala döndüğü belirlendi. Kimyasal bir çevre kirliliği oluşturan TE kaynaklarının tekrarlayan etkileri göz önüne alındığında, TE'nin kirlilik kaynakları ile temas kuran canlıların sağlığını olumsuz etkileyen bir tehdit olabileceği görülmektedir.

Anahtar Sözcükler: trikloretilen, peroksidasyon, oksidatif stres, çevre, toksik, yıkım.

Introduction

In nature, animals are exposed to a number of chemicals that have hazardous effects on living cells. Interaction between chemicals and bodies may involve pharmacokinetic or pharmacodynamic effects resulting in modulation of oxidative stress, toxicity and tissue damage (1). An unsaturated hydrocarbon solvent, TE has been in use as a raw material in industrial plants, as an anesthesic in surgical medicine, and as an insecticide in agriculture for more than 50 years (1, 2). Hydrocarbons are known to be life-threatening agents. Some experimental and clinical studies have reported that TE has caused adverse health, having toxic effects on the central and peripheral nervous system, the skin, liver, kidney and heart (1, 2, 3). It has been reported that trichloroacetic acid, an end product of TE biotransformation, is mainly effective metabolite in TE toxicity as follows.

$$Cl_2C = CHCl \rightarrow HCCl$$
$$\downarrow$$
(monochloroacetic acid) ClH_2CCOOH Cl_3CCH_2OH (trichloroethanol)
$$\downarrow$$
$Cl_3C\ COOH$ (trichloroacetic acid)

The mechanistic explanation of this toxicity is the increased free radical production and antioxidant depletion during the metabolism of various xenobiotics (4). These effects of TE on the oxidative metabolism of the body have been reported by various authors (5, 6, 7). There are no satisfactory data in the literature concerning environmental pollution, tissue damage and oxidative stress related to the effects of oral TE administration on domestic animals. It is known that dogs are used in industrial areas for security, and stray dogs roam freely. The aim of the present study was to investigate whether oral TE administration plays a role in blood cell peroxidation, antioxidant agent concentration and tissue damage in dogs. The acute phase changes of protein, albumin, alcalen phosphatase, ALT, AST, creatine kinase, MDA (an end product of lipid peroxidation) and the antioxidant enzymes SOD and GSH-Px were studied in dogs.

Material and Method

Fourteen dogs of both sexes were used in the study. They were 1-2 years old and weighed 9-12 kgs. After an 8h starvation period, 0.5 ml/kg TE in sesame oil was introduced into the stomach by an orogastric tube.

A total of 5 ml of blood was drawn after overnight fasting to control before, at 8, 24, 48, 72 h and 7 days after TE administration. Oxidative stress was estimated by the method of Jain et al. (8) based on TBARS reactivity. MDA, an end product of fatty acid oxidation, reacts with TBA to form a colored complex that has maximum absorbance at 532 nm. For this purpose, 0.2 ml of serum was suspended in 0.8 ml phosphate-buffered saline (pH 7.4), and 0.025 ml BHT and 0.5 ml 30% TCA was added. Tubes were vortexed and allowed to stand in ice for at least 2 h and centrifuged at 2000 rpm for 15 minutes. One ml of each supernatant was transferred to another tube, and 0.075 ml 0.1mol/l EDTA and 0.25 ml 1% TBA were added. Tubes were mixed and kept in a boiling water bath for 15 min and cooled. Absorbance of the sample was read at 532 nm.

MDA status was measured by colorimetric method by spectrophotometer (Shimadzu UV 1201V), CK, LDH, SOD, GSH-Px and other concentrations were measured by the enzymatic method with an autoanalyzer (Technicon RA-XT).

The Shapiro-Willks test was used to evaluate the results. Paired t-test was used for statistical comparisons among means. Data was expressed as mean±SD.

Results

The parameters mentioned above and the values are shown in the Table. Twenty-four hours after TE administration, SOD and GSH-Px activities decreased, and protein, albumin and alkaline phosphate did not change significantly, whereas all other markers increased. At 48 hours MDA, CK and ASt were elevated, while SOD and GSH-Px were lowered. After 72 hours, all parameters had returned to their normal levels.

Discussion

High levels of lipid peroxidation markers as MDA, increased tissue damage products and elevated liver enzyme concentrations are the best-known signs of toxic and oxidative reactions in different biological cases of humans and animals. TE is widely used in all regions of the world, and it can also enter human and domestic animal tissues by a variety of routes, including inhalation (2, 9) and ingestion (10). This may result in acute and oxidative stress and poisoning.

There are few reports on the blood lipid peroxidation, antioxidant enzymes and tissue damage markers associated with acute effects of oral TE administration in domestic animals. In the majority of studies done on TE and other toxic agents, only liver enzymes have been studied (9, 12).

Table. Profile of the measured markers in the study. Data are given as mean±SD and those with the sign (*) are statistically significant ($p<0.05$) in comparison to the control values.

	Control	8th h	24th h	48th h	72th h	7th day
MDA nm/ml	1.19±0.1	2.27±0.31*	2.09±0.57*	1.64±0.42*	1.21±0.32	1.23±0.27
CK U/lt	119.2±13	141.4±24*	138.9±12*	141.1±19*	126.6±17	116.3±21
SOD U/gHb	0.987±0.1	0.832±0.2*	0.786±0.3*	0.823±0.4*	0.928±0.2	0.963±0.3
GSPx U/gHb	67.29±13	48.57±9.5*	51.17±9.3*	50.9±12.6*	68.2±15.3	69.37±12
Protein U/gHb	6.51±0.4	6.22±0.4	6.27±0.32	6.09±0.64	6.18±0.25	6.38±0.36
Albmin U/gHb	3.3±0.58	3.7±0.83	3.2±0.69	3.3±0.64	3.3±0.37	3.2±0.41
AST U/gHb	23.6±5.7	39.8±7.27*	35.6±8.44*	30.2±8.9*	25.7±6.5	25.1±7.3
GGT U/gHb	6.7±1.39	9.1±2.15*	9.4±2.25*	7.6±1.07	7.3±1.2	7.3±1.7
ALT U/gHb	24.8±7.9	36.4±10.6*	33.2±11.3*	28.6±10.43	23.5±5.9	23.6±6.3
Alcalen U/gHb	42.6±14	45.7±22.2	43.2±16.7	40.4±16.7	40.1±13.6	41.3±14.9

Nelson et al. (3), Kavwamoto et al. (5), Gonthier et al. (4), Davidson et al. (13) and other researchers have determined that the effects of TE inhalation on the microsome and mitochondrial mixed function of oxidative mechanism included the inducement of free radical production in liver and brain during metabolism in some experimental animals. Atkinson et al. (7) showed that the MDA levels of the liver of mice given TE were significantly higher than those of controls. A maximum increase in the level of oxygen consumption in liver microsomes was observed in these rats. It was reported that all these toxic and environmental polluting chemicals increased the microsome's NADPH level, and that oxidative stress and its markers, such as MDA, elevated approximately 200%. Although they studied the liver and brain tissue of rats, their findings are similar to the plasma MDA level results of our study.

In contrast, Kefalas et al. (14) reported that lipid peroxidation was not responsible for TE-induced enhancement of toxicity in rat hepatocytes.

Increase in oxygen consumption, NADPH disappearance and MDA production in microsomes and tissues are all indicative of elevated oxidative stress (4). Increased oxidative stress may be involved in the induction of TE-associated hepatotoxicity (7), when oxidative stress increases or antioxidants fail, a condition of oxidative stress related to excessive molecular and tissue damage (15).

In conclusion, the CK, LDH and MDA results of this study confirm the validity of the hypothesis of interaction between free radical production and tissue damage. Thus the results of the present study suggest that oral TE administration plays an oxidative and damaging role in the blood plasma and red blood cells of dogs in the acute phase. With repetition, oral TE administrating may cause serious damage. It is concluded that further studies are needed to clarify the cause of increased lipid peroxidation products, tissue damage markers and insufficient antioxidant activity in domestic animals exposed to TE.

References

1. Barton H.A., Reech J.R., Godin C.S., Randal GM., Chloroethylene mixtures: pharmacokinetic modeling and in vitro metabolism of vinylchloryde, trichloroethylene in rat. Toxicol Appl Pharmacol. 1995, 130 (2): 237.
2. McCunney JR. Diverse manifestations of Trichloroethylene. Br J Ind Med. 1988, 45: 122.
3. Nelson MA., Bull JR. Induction of strand break in DNA by trichloroethylene metabolites in rat and mouse liver in vivo. Toxicol Appl Pharmacol. 1988, 94: 45.
4. Gonthier BP., Barret LG. in vivo spin-trapping of free radicals produced during trichloroethylene and the ethylether metabolism. Toxicol Lett. 1989, 47: 225.
5. Kawamoto T., Hobara T., Nakamura K. et al. Induction of cytochoromo P-450 isozymes with long term trichloroethylene treatment. Toxicology. 1988, 53: 239.
6. Cojocel C., Beuter W., Müller W., Mayer D. Lipid peroxidation: a possible mechanism of trichloroethylene induced nephrotoxicity. Toxicology. 1989 55: 131.

7. Atkinson A., Meeks RG., Roy D. Increased oxidative stress in the liver of mice treated with trichloroethylene trans-1, 2-dichloroethylene in rat. Biochem Mol Biol Int. 1993, 31 (2): 297.

8. Jain SK, McVie R., Duett J. Erythrocyte membrane lipid peroxidation and glucolisated hemoglobin in diatebes. Diabetes. 1989, 38: 1539.

9. Kimmerle G., Eben A. Metabolism, excretion and toxicology of trichloroethylene after inhalation: experimental exposure on rats. Archive fur Toxikol. 1983, 30: 115.

10. Steup DR., Hall P., McMillan DA., Time course of hepatic injury and recovery following coadministration of carbontetrachloride and TE in rats. Toxicol Pathol. 1993, 21 (3): 327.

11. Steinberg AD., DeSesso Jm. Have animal data been used inappropriately to estimate risk to humans form environmental trichloroethylene? Regul Toxicol Pharmacol. 1993, 18: 137.

12. Wang GZ., Takano T., Tomita K. et al. Interaction of trichloroethylene and isopropyl alcohol in the perfused rat liver. Nippon Eiseigaku Zasshi. 1993, 48 (5): 1000.

13. Davidson IW., Beliles RP. Consideration of the target organ toxicity of trichloroethylene in terms of metabolic toxicity and pharmacokinetics. Drug Metab Rev. 1991, 23: 493.

14. Kefalas V., Stacey NH. Potentiation of carbontetrachloride-induced lipid peroxidation by trichloroethylene in isolated rat hepatocytes: no role in enhanced toxicity. Toxicol Appl Pharmacol. 1989, 101: 158.

15. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem. 1995, 41: 1819.