Effects of Exposure Duration to Liquefied Propane on Lipid Peroxidation and Antioxidant Enzymes in Gas Workers

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ABSTRACT:

BACKGROUND:

Toxic environmental agents include a host of chemicals and pollutants that may available in tobacco smoke and tar, the atmosphere, drugs, the work place, the food and water supply and from radiation and infectious organisms. These agents may exert multiple types of harmful effects on human body, including lipid peroxidation.

OBJECTIVES:

To assess the effects of duration of exposure on lipid peroxidation and antioxidant enzymes in liquefied propane gas workers.

METHODS:

Fifty five adult male gas workers (age mean 36. 6 ± 3.8 years) in liquefied propane gas stations in the District of Baghdad were enrolled in the present study. They were allocated into 3 groups according to the duration of exposure to LPG (1-9, 10-20 and more than 20 years). Twenty five healthy subjects, not exposed to LPG, with age mean comparable to that of workers (37.2 ± 4.0 years) were utilized as controls.

RESULTS:

The results showed significant differences in the levels of MetHb, lipid peroxidation parameters and antioxidant enzymes activities in LPG workers compared to controls. Meanwhile, workers with different duration of exposure to LPG demonstrated significant differences only in MetHb, MDA and catalase activity.

CONCLUSION:

Workers with different duration of exposure to LPG demonstrated significant differences only in MetHb, MDA and catalase activity. In conclusion, the changes in lipid peroxidation and antioxidant enzymes may be useful as indicator for the impact of duration of exposure in LPG workers

KEY WORDS: Liquefied propane gas; oxidative stress; lipid peroxidation; antioxidant enzymes

INTRODUCTION:

Air pollution is of major public and political concern, especially since the beginning of industrialization, and more recently because of automobile exhaust. However, the impact of air pollution has possibly been over estimated by the popular press and others [1]. Toxic environmental agents include a host of chemicals and pollutants that may available in tobacco smoke and tar, the atmosphere, drugs, the work place, the food and water supply and from radiation and infectious organisms. These agents may exert multiple types of harmful effects including: excessive production of reactive oxygen species (ROS) [2], enhanced plasma membrane lipid peroxidation [3], covalent binding of multiple reactive metabolites [4], depletion and/or alteration of natural antioxidants (glutathione and protein thiols) [5], Alterations of mitochondrial membrane potential [6] and alterations of intracellular calcium homeostasis [7].

The relationship between the environment and the concept of oxidative stress was based on the scientific evidence that free radicals and oxidative stress are of critical importance in the role that these agents play in various biochemical aspects during the life, like diseases, adaptive changes and physiological homeostatic and regulatory mechanisms [8]. In these regards, most pathological changes are resulted from the interaction between biomolecules and the environment. Biological macromolecules by themselves explain a very low percent of diseases, while the remainder can be attributed to external "environmental" factors that act in conjunction with both genetic and acquired susceptibility [9]. Abuse of gas fuel that contains liquid propane is spreading among people elsewhere. Abuse of liquefied petroleum gas (LPG) which contains propane as a major component is rare and usually not fatal, but the risk on the workers who deal with filling and packing of large quantities is still of significant importance[10].

Thus, two major approaches in prevention of environmental hazards of LPG are very well known: establishment of strategies to help people to modify hazardous life styles and/or use of chemoprevention (e.g. antioxidants), and reduction

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of involuntary exposure to the toxic agents in the work environment through improving conditions of work places and regular follow up for he general health status of the workers. This project was designed to asses the impact of the duration of exposure to LPG on the oxidative stress parameters and other health related biomarkers in LPG workers.

SUBJECTS AND METHODS:

This study was carried out on 55 healthy male adults with age mean (36. 6 ± 3.8 years), who work in liquefied propane gas (LPG) stations in different districts of Baghdad for the period from January 1st to December 31st 2004. Clinically, they did not present any pathological abnormalities and medically examined for this purpose, and the presence of any recognizable disorder was considered as exclusion criteria. They were allocated in to 3 groups according to the duration of exposure to LPG as follow: first group includes 18 male workers with age mean (35.3 ± 3.6 years) who have 1-9 years duration of exposure to LPG; second group includes 19 male workers with age mean (36.6 ± 3.8 years), who have 10-20 years duration of exposure to LPG and the third group includes 18 male workers with age mean (37 ± 3.4), who have more than 20 years duration of exposure to LPG. Twenty five healthy males, with age mean comparable to that of workers (37.2 ± 4.0 years), with no history of exposure to LPG were selected and served as controls. Blood samples (10 ml) were collected from all subjects by vein puncture and divided as follow: 8 ml of blood were taken into plain tube in order to obtain serum after clotting at room temperature.

Serum samples obtained after centrifugation at 3000 rpm for 10 minutes, and used for the estimation of malondialdehyde [11], glutathione [12], albumin [13], uric acid [14], total protein [15] and the activities of the antioxidant enzymes catalase [16] and glutathione-S-transferase [17]. The other fraction (2ml) was transferred to EDTA containing tube and used for the measurement of hemoglobin and methemoglobin [18,19]. Results obtained in the present study were presented as mean ± SE. Statistical analysis of data was performed utilizing unpaired Student's *t*-test, and ANOVA. Data with $P < 0.05$ were considered significantly different.

RESULTS:

Table 1 showed that blood levels of MetHb, MDA, GSH and the activities of the antioxidant enzymes CAT and GSH were significantly different (unpaired Student's *t*-test: P=0.001, 0.02, 0.04, 0.02, 0.002 respectively) in LPG workers compared to controls. Hemoglobin levels are not affected by the increase in the time of exposure to LPG, while MetHb was elevated with the increase in exposure time to LPG. Malondialdehyde level, the biomarker of lipid peroxidation, was found to be significantly elevated (ANOVA *test:* P=0.002) with increasing the time of exposure. Meanwhile, GSH levels showed to be significantly depleted with increasing exposure time to LPG. Both of the antioxidant enzymes, catalase and glutathione-S-transferase, were significantly affected (ANOVA *test:* P=0.001) by the increase in the time of exposure to LPG, where the activities of those enzymes strongly induced as a result of long-term exposure to LPG (Table1).

Table 1: Effect of duration of exposure to liquefied propane gas (LPG) on hemoglobin (Hb), methemoglobin (MetHb), malondialdehyde (MDA), glutathione (GSH), albumin, uric acid , total protein and the activities of catalase (CAT) and glutathione-S-transferase (GST) in the blood of LPG workers.

Parameters	Controls (n=25)	Duration of exposure (years)		
		1-9 (n=18)	10-20 (n=19)	> 20 (n=18)
Hb (gm/dL)	11.97 ± 0.32	14.6 ± 0.33* [a]	14.3 ± 0.51* [a]	13.0±0.32* [b]
MetHb % of Hb	0.17 ± 0.04	0.34 ± 0.06* [a]	1.60 ± 0.04* [b]	2.9 ± 0.90* [c]
MDA (mmol/L)	1.46 ± 0.21	1.51 ± 0.23 [a]	1.88 ± 0.34* [b]	2.37 ± 0.43* [c]
GSH (µmol/L)	358.0 ± 13.0	99.0 ± 6.0* [a]	89.0 ± 6.0* [a]	96.0 ± 4.0* [a]
Albumin (gm/dL)	4.32 ± 0.1	3.41 ± 0.23* [a]	3.6 ± 0.28* [a]	3.3 ± 0.13* [a]
Uric acid (gm/dL)	5.36 ± 0.24	5.43 ± 0.35 [a]	6.0 ± 0.45 [a]	5.54 ± 0.79 [a]
Total protein (gm/dL)	6.88 ± 0.15	7.57 ± 0.17 [a]	7.44 ± 0.26 [a]	7.35 ± 0.27 [a]
CAT (U/L)	1.94 ± 0.12	10.1 ± 0.2* [a]	9.1 ± 0.6* [b]	10.4 ± 0.13* [a]
GST (U/L)	16.9 ± 0.7	80.6±3.9* [a]	71.39 ± 5.3* [b]	80.8 ± 2.2* [a]

Values are expressed as mean ± SE; n= number of subjects; * significantly different compared to controls (P<0.05); values with non identical superscripts (a,b,c) are considered significantly different (P<0.05).

DISCUSSION:

Excessive formation of free radicals results in an increase in the process of lipid peroxidation, as evidenced by elevated levels of malondialdehyde (MDA), the end product of lipid peroxidation, in plasma and tissues of exposed subjects [20].

The results obtained in this study clearly showed that exposure to LPG resulted in an elevated levels of plasma MDA, and these varied according to duration of exposure, where longer duration of exposure to LPG associated with maximum values which are significantly different ($P<0.05$). Many authors reported the importance of exposure to many types of pollutants as a risk factor of oxidative stress; so elevated serum levels of MDA were reported in painters compared to controls [21]. Consequently, many parameters of oxidative stress like superoxide dismutase (SOD), MDA and others became objective indices for workers in health surveillance; and the role of these indices in the intoxication mechanism still need to be clarified [22]. It has been very well known that oxidative stress and lipid peroxidation nowadays suspected to be a common mechanism in many pathological changes; a fact which may shed a light on the mechanistic bases of different types of cellular or tissue damage produced by toxins and lead to a disease state; e.g. neurotoxicity, hepatic damage, nephrotoxicity and cancer [23,24]. In the data presented in table 1, the occupational hazard of exposure to LPG was found to be a potent depletor of GSH, where absorption through the skin and inhalation of this product provided a relatively free access to large quantities of this toxin. The increase in the level of CAT and CST activities in certain types of environmental conditions represents a compensatory mechanism to detoxify various types of toxins which can be effectively detoxified with GST. Such a type of enzyme induction is usually associated with increased levels of mRNA due to transcriptional activation of the gene encoding them. It is found that induction of GST by exposure to xenobiotics is responsible for protection against certain types of cancers [25]. In some cases, conjugation with glutathione enhances the toxicity of xenobiotics due to either formation of more potent toxins or the consequences of elimination of these metabolites may lead to specific target organ damage, like renal damage [26]. Moreover, induction of GST may lead to glutathione depletion due to extensive consumption by GST activity; and this predispose to increased susceptibility of cells and tissues to oxidative stress-induced damage. The results obtained in this study indicated that an induced oxidative stress is a common phenomenon during exposure to LPG, which could be related to the progressive nature of the exposure and/or to the development of secondary pathophysiological complications after a certain period of time. Methemoglobin (another index of lipid peroxidation) is a ferric-hemoglobin, where the heme-iron had been oxidized from ferrous to ferric. Under normal conditions the methemoglobin level is maintained less than 1% of total hemoglobin through its rapid reduction by the enzyme met-Hb reductase-NADH dependent enzyme [27]. Therefore, elevated levels of met-Hb, as observed in LPG workers, could result from either increased production or lowered ability to convert it back to normal hemoglobin. The elevated levels of met-Hb in those workers (table 1) could be related to the lowered NADPH pool in the erythrocytes and/or to an increased met-Hb production as a result of increased oxidative stress. The components of the LPG (97.8%propane, 1.5% isobutane, 0.1% n-butane, 0.2% propylene, and 0.4% other gases) are lipophilic so that after being taken up from the lungs into blood, they distributed at high concentrations in lipid-rich tissues such as brain and fat tissues, and also in liver, heart, and kidney [28,29]. Propane is less toxic than n-butane or isobutane having a weaker anesthetic effect and a negligible effect on heart. The LD50 value of propane is over 80% in the air whereas that of n-butane or isobutane is about 50% in experimental animal, also indicating propane is less lethal [28,30].The cause of death after propane gas inhalation is reported to be usually hypoxia [30,31],so long-term and continuous exposure may lead to fatal consequences.

CONCLUSION:

LPG contains isobutane in addition to propane, and breathing LPG for a long time may lead to accumulation of isobutane as well as propane in brain to a toxic level resulting in a possible anesthetic effect of isobutane rather than direct hypoxia.

REFERENCES:

1. Aust SD, Chignell CF, Bary TM. Free radicals in toxicology. Toxicol Appl Pharmacol 1993; 120: 168-178.
2. Ehlat DH. Radical ideas. Science 1998; 279: 1002-1003.
3. Forber JL, Kyle ME, Coleman JB. Mechanisms of cell injury by activated oxygen species. Lab Invest 1990; 62: 670-679.
4. Venitt S. Mechanisms of carcinogenesis and individual susceptibility to Cancer. Clin Chem 1994; 40: 1421-1425.

5. Comporti M. Glutathione depleting agents and lipid peroxidation. Chem Physics lipids 1987; 45: 143 – 169.
6. Horton AA, Fairhurst S. Lipid peroxidation and mechanisms of toxicity. Crit Rev Toxicol 1987; 18: 27-79.
7. Hippel, S, Elstner EF. Biological and Biochemical effects of air pollutants. In: Rice-Evans C, Halliwell B, Lunt GG. (Eds.), Free radicals and oxidative stress: environment, drugs and food additives. Portland Press, London, 1995; 153-161.
8. Ames BN, Shigenaga MK. oxidants are a major contributor of aging. Ann N Y Acad Sci 1992; 663: 85-96.
9. Bobak M, Leon DA. Air pollution and infant mortality. Lancet 1992; 340: 1010-1014.
10. Sugie H, Sasaki C, Hashimoto C, Takishita H, Nagai T, Nakamura S, et al. Three cases of sudden death due to butane or propane gas inhalation: analysis of tissues for gas components. Forensic Sci International 2004; 143: 211-214.
11. Buege JA, Aust SD. Microsomal Lipid Peroxidation. Method Enzymol 1978; 51: 302-307.
12. Godin DV, Wohaieb SA, Garnett ME. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol cell Biochem 1998; 84: 223-231.
13. Dinnel AE, Northam BE. New Automated dye-binding method for serum albumin determination with bromocresol purple. Clin Chem 1978; 24: 80-86.
14. Watts RW. Determination of uric acid in blood and urine. Ann Clin Biochem 1974; 11: 103-111.
15. Kingsley GR. The Direct Biuret method for the determination of serum proteins as applied to photometric and visual colorimetry. J Lab Clin Med 1942; 27: 840-845.
16. Aebi H. Erythrocyte Catalase. In: Berg-Meyer HV, (ed.), Methods in enzymatic analysis. Verlay Chemic, Weinheim, 1974; 673.
17. Habig WH, Pabst MJ, Jakoby WB. Colorimetric method for determination of glutathione-S- transferase in the serum. J Biol Chem 1974; 249: 7130-7136.
18. Drapkin DL, Austin J H. Spectrophotometric studies II, preparation from washed blood cells. Nitric oxide hemoglobin and sulfhemoglobin. J Biol Chem 1935; 112: 51-65.
19. Betke K, Steim H, Tonz O. A family with congenital methemoglobin due to reductase deficiency. Ger Med Month 1962; 7:217-219.
20. Baglia R. Ueda N, Shan SV, et al. Oxidant mechanisms in glomerular disease. Curr Nephrol 1997;20: 135-151.
21. Karagozler AA, Mehmet N, Lu KB, et al. Effects of long term solvent exposure on blood cytokine levels and antioxidant enzyme activities in house painters. J Toxicol Environ Health 2002; 65: 1273.
22. Jian L, Hu D. Antioxidative stress response in workers exposed to carbon disulfide. Int Arch Occup Environ Health 2000; 73: 503-506.
23. Kuloglu M, Atmaca M, Tezcan E, et al. Antioxidant enzyme activities and MDA levels in patients with obsessive-compulsive disorder. Neuropsychology 2002; 46: 27-32.
24. Bakan E, Tysi S, Polat MF, et al. Nitric oxide levels and lipid peroxidation in plasma of patients with gastric ulcer. Jpn J Clin Oncol 2002; 32: 162-166.
25. Zhang Y, Talalay P, Cho CG, Ponser G H. A major inducer of anticarcinogenic protective enzymes. Proc Natl Acad Sci USA 1992; 89: 2399-2403.
26. Dekant W, Vamvakas S. Glutathione-dependent bioactivation of xenobiotics. Xenobiotica 1993; 23: 873-887.
27. Firkin F, Chesterman C, Penngton D, et al. (Eds), de Gruchy's Clinical hematology in medical practice,(5th Ed.), Black Well Scientific Pub, 1989; 55-166.
28. Ago M, Ago K, M, Ogata A. fatal case of n-butane poisoning after inhaling anti-perspiration aerosol deodorant. Jpn J Legal Med 2002; 4:113–118.
29. Fuke C, Miyazaki T, Arao T, Morinaga Y, Takaesu H, Takeda T, et al. A fatal case considered to be due to cardiac arrhythmia associated with butane inhalation. Jpn J Legal Med 2002; 4: 134–138.
30. Rohrig TP. Sudden death due to butane inhalation. Am J Forensic Med Pathol 1997; 18: 299-302.
31. Kojima T, Takeoka T, Kobayashi H, Hara K, Kageura M, Nagata T. Determination of liquefied petroleum gas (mainly propane) in biological materials by gas chromatography and gas chromatography–mass spectrometry: a case report. Jpn J Legal Med 1979; 33: 80–85.