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The Effects of Sodium Arsenite on Glutathione and Malondialdehyde Levels in Mice Tissues

Sodyum Arsenitin Fare Dokularında Malondialdehit ve Glutation Düzeyine Etkisi

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Abstract
In this study, the oxidative damage of sodium arsenite ($NaAsO_2$) was investigated by measuring the malondialdehyde (MDA) and glutathione (GSH) levels. $NaAsO_2$ was administered Swiss Albino mice (35 mg/kg) and sacrificed after one hour. MDA and GSH levels were determined in liver, kidney, brain and heart tissues by UV-Spectrophotometric methods. After $NaAsO_2$ treatment MDA levels decreased in liver, brain and kidney ($p<0.001$), on the other hand, increased significantly in heart tissues ($p<0.01$). No alteration was observed in liver and kidney GSH levels, however, $NaAsO_2$ caused significant depletion in GSH in both heart and brain ($p<0.01$) tissues.

Key words: Sodium arsenite, lipid peroxidation, glutathione, malondialdehyde

Introduction
Inorganic arsenic, an important environmental toxicant, is a common constituent of the earth's crust and has both natural and industrial sources (Sakurai *et al.*, 2002, Couchane, 2001). Arsenicals have a widespread usage in paper, glass, dye, rubber, and leather industries. Exposure to inorganic arsenic compounds in drugs, drinking water and occupational environments (i.e. manufacturing process of pesticides, herbicides and other agricultural products) is correlated with a significantly elevated risk of skin, lung, liver and bladder cancer (Son *et al.*, 2001, Schinella *et al.*, 1996).

Acute arsenic poisoning is more common than chronic intoxication nowadays (Leikin *et al.*, 1991). Despite the efforts to ban the arsenicals from human environment, cases of acute intoxication, either by accidental selfadministration by children or by suicidal intention by adults, are still reported each year (Mückter *et al.*, 1993). Very low doses such as 20 mg of arsenic may cause symptoms of acute intoxication (Leikin *et al.*, 1991). This explains the increase in the amount of acute cases occuring.

Arsenic is very rarely found in its pure state. It rather exists in either trivalent [As (III)] or pentavalent [As (V)] oxidation state as an unstable sulfide or oxide, or as a salt of sodium, potassium or calcium (Aronson, 1994). Arsenic toxicity is postulated to be primarily due to the binding of trivalent form of arsenicals, including sodium arsenite and arsenic trioxide, to sulfydryl-containing enzymes. In addition, trivalent arsenicals have more inhibitory effect on glutathione peroxidase (GPx) activity than pentavalent compunds (Couchane, 2001). While pentavalent arsenicals which is readily found naturally in the earth and food, do not produce

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toxicity; trivalent forms of it that is used industrially and found as a food contaminate seem to be the problem (Miller *et al.*, 2002).

Oxidative damage plays an important role in the effects of arsenic, as it does for other metals, such as iron, copper, nickel, chromium, cadmium, lead and mercury (Buzard, 2000). Free radicals formed as a consequence of oxidative damage, have the ability to interact with lipids, proteins and DNA (Kehrer, 1993) leading to cell injury and cell death eventually (Figure 1).

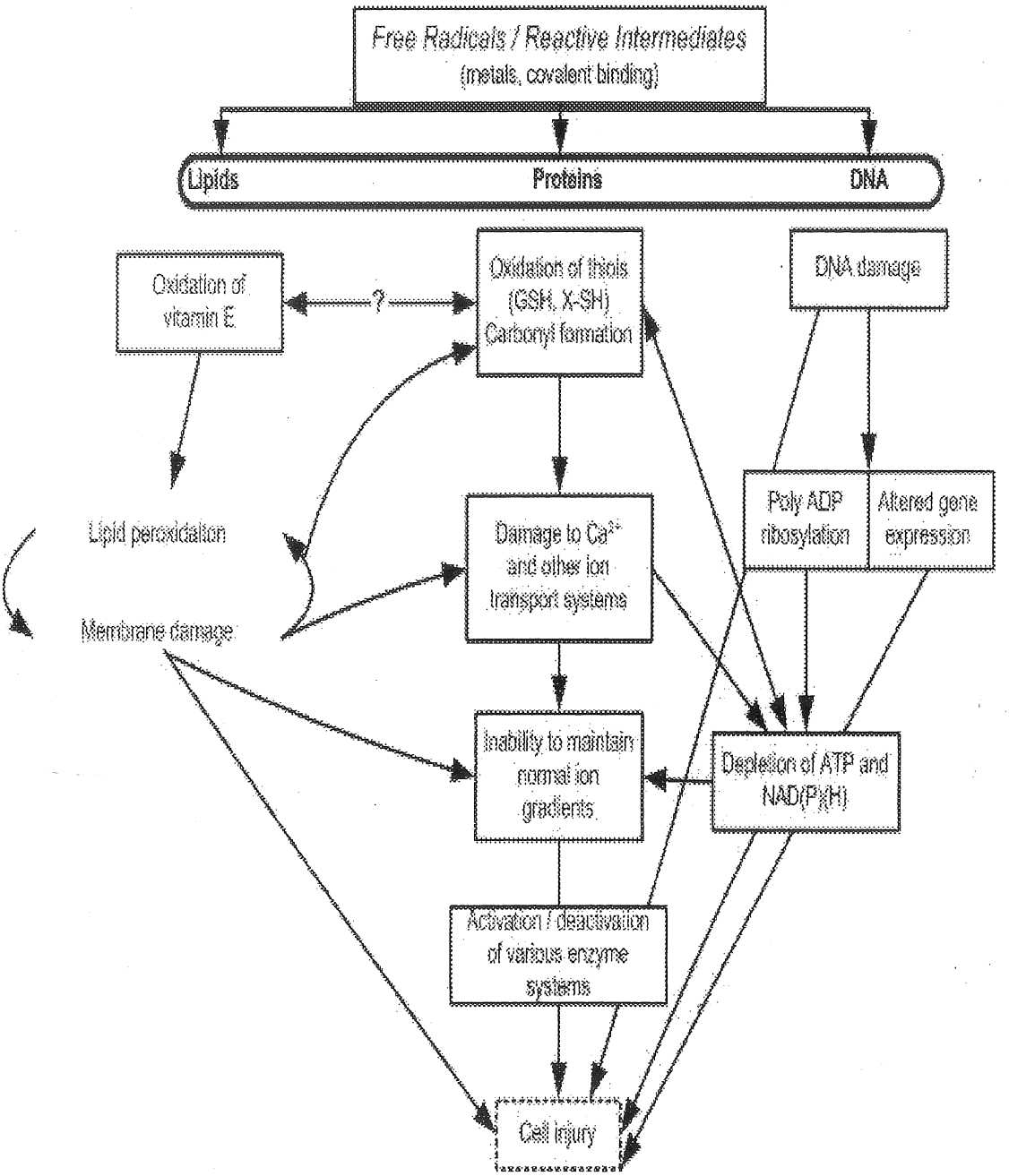

Figure 1. The ability of free radicals to interact with lipids, proteins and DNA provides a potential for numerous changes that could affect cell functioning (Kehrer, 1993).

Oxidative detorioration of membrane lipids caused by the attack of free radicals on polyunsaturated fatty acids (PUFA) is known as Lipid Peroxidation (LPO) (Horton, 1987). Reactive oxygen species generated in response to arsenic exposure lead to accumulation of intracellular hydrogen peroxide by activation of flavoprotein-dependent superoxide-producing enymes such as NADPH oxidase (Bernstam, 2000, Chen *et al.*, 1998).

Arsenic disturbs natural oxidation and reduction equilibria through various mechanisms involved in redox reactions with endogenous antioxidants and cellular antioxidant systems (Sen, 1998). Glutathione (GSH), an endogenous antioxidant system, is one of the most abundant cellular thiol and both GSH and GSH-related enzymes are crucial antioxidants in the detoxification of arsenic and other carcinogens. Glutathione-related enzymes are:

(a) Glutathione peroxidase (GPx): prevents the formation of reactive oxygen species (ROS) catalyses hydrogen peroxide.
(b) Glutathione reductase (GR): catalyses the reduction of oxidized glutathione (GSSG) by NADPH (Hazelton, 1980).

In mammalian cells, GPx and catalase are the key enzymes regulating the levels of ROS and protecting cells from arsenite damage (Chen *et al.*, 1998).

Although chronic toxicity of arsenic has been studied extensively by many researchers, there is not much evidence about acute toxicity mechanism of arsenic. The purpose of this work was to investigate the effect of arsenic on the activity of GSH and to measure an oxidative stress biomarker malondialdehyde (MDA), one of the end products of LPO.

Materials and Methods

Chemicals: The chemicals; sodium arsenite, chloroform, apirogen bidistilled water, NaCl, sucrose, Tris (aminomethanehydrochloride), Na_2EDTA (ethylenediaminetetraacetic acid), acetic acid, NaOH and sodium dodecyl sulfate (SDS) from MERCK; 1,1,3,3-tetraethoxypropane, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid:DTNB) from SIGMA were purchased.

Animals: Swiss Albino male mice (weighing 25-30 gr) were obtained from Refik Saydam Hıfzısıhha Institute, Animal Care Unit, Ankara. The mice were hold in normal laboratory conditions (20-22 °C, natural day-night cycle) and fed with commercial chow and water until 12 h before the injections.

Treatment: Animals were divided into two groups consisting 10 mice in each and treated as follows: (a) control group received apirogen bidistilled water; (b) received sodium arsenite 35 mg/kg, sodium arsenite was dissolved in apirogen bidistilled water and for each mice, 0.2 ml injected intraperitonally. One hour later from the last administrations, mice were sacrificed just after the chloroform exposure. Heart, lung, kidney and brain tissues were immediately excised and placed into polypropylene tubes after washing with 0.9 % NaCl and stored at –20 ^{0}C.

Homogenization: In experimental period, the stored animal tissues were taken and weighed exactly as 0.5 g and homogenized in 4.5 ml of 0.25 M sucrose to obtain a 10 % w/v suspension in order to measure lipid peroxidation in tissues. Tissue weighing 0.2 g was homogenized with 8 ml 0.02 M Na_2EDTA to measure GSH level.

Lipid peroxidation in tissues: The method of Jamall and Smith (Jamall, *et al.*, 1985) was used to determine lipid peroxidation in tissue homogenates (10% w/v). 1.1.3.3-Tetraethoxypropane was used as standard for the calibration curve in UV-Spectrophotometry. Homogenates were centrifuged for 45 min, at 4 ^{0}C and 3000 rpm. Homogenates (0.2 ml of each) was transferred to vials and mixed with 0.2 ml SDS solution (8.1 %), 1.5 ml acetic acid (20 % v/v, adjusted to pH 3.5 with NaOH), 1.5 ml of 0.8 % (w/v) solution of TBA, and distilled water in order to adjust the final volume to 4 ml. Tightly capped vials are heated in a boiling water bath for 60 min.the vials are then cooled under running water. Equal volumes of tissue blank or test sample and 10 % TCA were centrifuged at 1000 rpm for 10 min. The absorbance values of supernatants were

measured at 532 nm against tissue blank. The tissue TBA reactive products were expressed as nmoles of MDA/g wet weight.

Glutathione in tissues: GSH was measured by Sedlak and Lindsay's method (Sedlak, 1968). Method is based upon the reduction of Ellman's reagent by sulphydryl groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH.

Aliquots of 5 ml of the homogenates were mixed in 15 ml test tubes with 4 ml distilled water and 1 ml of 50% TCA. Solutions were centrifuged for 15 min at 3000 rpm. Four ml Tris buffer (0.4 M, pH:8.9) and 0.1 ml DTNB were added to 2 ml of supernatant and then shaken. The absorbances were measured at 412 nm. The nitromercaptobenzoic acid anion has an intense yellow color and can be used to measure SH groups. Absorbences should be taken in 5 min in order to prevent color fading. GSH levels are expressed as μmol/g wet weight.

Statistical analysis: The data obtained were analyzed by one-way analysis of variance (ANOVA) and Student-Newman-Keul's-Multiple comparison test for the possible significant interrelation between the various groups. The data were analyzed with the help of Instat computer software.

Results and Discussion

Arsenic compounds are widely distributed natural toxicants. Chronic exposure to arsenic results in liver injury, peripheral neuropathy, peripheral vascular and blackfoot diseases, and increased incidence of skin, lung, bladder and liver cancer. Chronic arsenic exposure increases the rate of formation of active oxygen species including superoxide anion radical (O_2^-) and hydroxyl radical (OH) through a chain reaction. These reactions bring about lipid peroxidation and glutathione depletion in chronic exposure (Figure 2).

Our investigation was established on these facts and we tried to find parallel results with these evidences. However, by an intraperitoneal *acute* exposure of arsenic did not create the same results. In the first part of this study, the effect of high dose of sodium arsenite (35 mg/kg, i.v.) on MDA levels for liver, kidney, brain and heart were observed by using Jamall and Smith's method.

Arsenite may exert its toxicity through reactions with thiols in cells especially dithiols (Aposhian, 1989). On the other hand, recent results suggest that arsenic may also exert its toxicity through the generation of reactive oxygen species. Arsenic compounds during their metabolism in cells may generate reactive oxygen species (Yamanaka *et al.*, 1991). These reactive oxygen species bring about oxidative stress that can result in;

(a) adaptation by up-regulation of defence systems

i. completely protect against damage

ii. protect against damage, but not completely

iii. "over protect" e.g. the cell is then resistant to higher levels of oxidative stress imposed subsequently

(b) cell death by two essential mechanism which are necrosis and apoptosis generation of reactive oxygen species (ROS) followed by apoptosis is a critical contributor to arsenic-induced cell death (Bernstam, 2000).

(c) tissue injury.

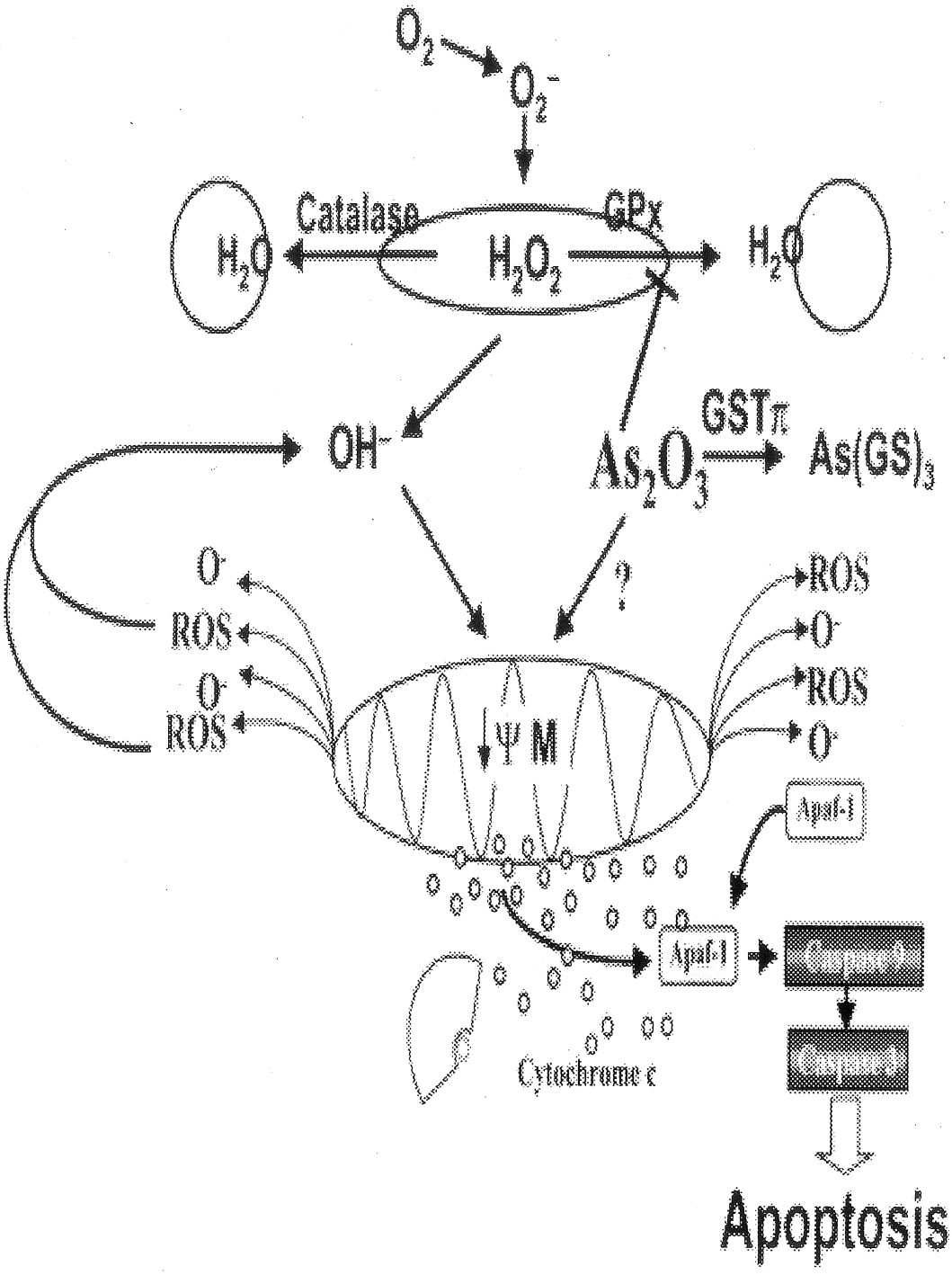

Figure 2. Arsenic trioxide induces apoptosis by an increase in intracellular levels of hydrogen peroxide, and other ROS, depleting GSH which lowers mitochondrial membrane potential and leads to cytochrome *c* release and subsequent activation of the caspase pathway (Chen *et al.*, 1998).

In tissue injury, oxidative stress can cause damage to all molecular targets: DNA, proteins, carbohydrates and lipids. Prospects for therapeutic intervention probably confounded because it

is often unclear which is the primary molecular target of oxidative stress. For example DNA is an important early target of damage when hydrogen peroxide is added to many mammalian cells; increase DNA strand breakage occurs before *detectable* lipid peroxidation or oxidative protein damage. The word "detectable" is emphasized because such conclusions obviously depend on on the assays used to measure such damage. For example measurement of protein carbonyls would not detect important early oxidative protein damage by oxidation of essential –SH groups on membrane ion transporters. However, if lipid peroxidation is a late stage in injury, then therapies directed against it might have little beneficial effect. Their failure may lead to the erroneous conclusion that oxidative stress is not important. Arsenite normally can increase the intracellular peroxide level. However, these peroxides did not cause the formation of malondialdehyde, which is the product of peroxidation of the lipids. Arsenite should follow a different pathway producing other aldehydes such as hydroxynonenal (Halliwell, 1999).

Following sodium arsenite administration, significant decrease in MDA level was observed in liver, kidney and brain ($p<0.001$) (Figure 3). However, this was an unexpected result for acute arsenic exposure because many heavy metals cause lipid peroxidation as an increase in MDA levels. Heart is the mostly affected organ with a significant increase in MDA level ($p<0.01$).

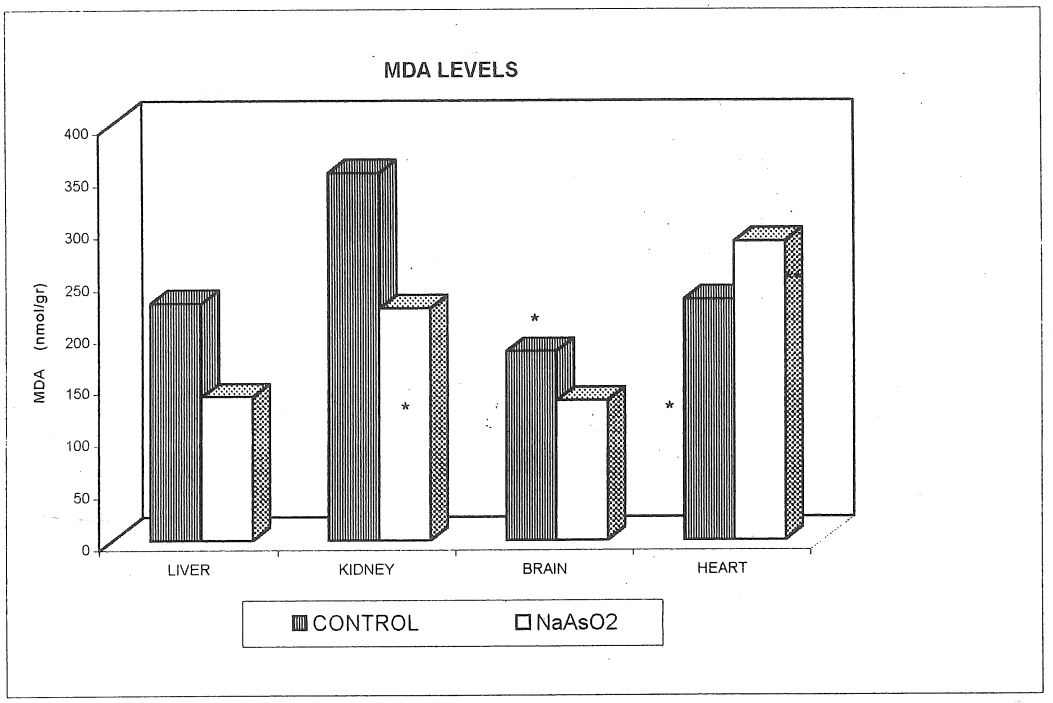

Figure 3. Malondialdehyde levels of mice tissues after acute arsenic exposure (*$p<0.001$, **$p<0.01$).

Arsenic alone is a poison and can cause cancer, but arsenic-containing compounds have been used medically for more than 2.000 years. The results for heart tissue in our study is parallel with the side effect of medicine used in APL (acute promyelocytic leukamia) treatment called "trisenox" which contains arsenic trioxide APL is a cancer where abnormal or immature white blood cells crowd out proper white cells and red blood cells in the bone marrow and blood. Arsenic trioxide apparently is used for the treatment of APL like retinoic acid: Instead of killing cells, it causes those immature white cells to mature into normal cells. Trisenox can cause very serious side effects. The sudden increase of working white blood cells results in

(a) inflammation

(b) fluid accumulation especially in the heart and lung tissue.

And these events may lead to sudden death. Trisenox also causes a heartbeat irregularity and this irregularity can lead to arrhythmias (Bachleither hofmann *et al.*, 2002). These evidences indicate that while arsenic trioxide may be used for the treatment of a hazardous cancer, APL; it however shows its toxicity by inducing tachicardia and this evidence may explain the results obtained for heart tissue in the current study.

Glutathione is a ubiquitous cellular constituent that is the most abundant thiol reducing agent in mammalian tissues. It is an important nonprotein source of -SH groups in living organisms and appears to

play a key role in detoxification of arsenic (Klaassen, 1985). GSH functions are:

(a) maintenance of thiol groups of proteins

(b) detoxification of foreign compounds and xenobiotics that react with sulphydryl groups

(c) act as an antioxidant defence mechanism.

However its physiological significance is still under elucidation (Hazelton, 1980).

Arsenic compounds bind to –SH groups and can inhibit several enzymes, including glutathione reductase. The interaction of arsenic with glutathione and its related enzymes may result in the inhibition or activation of the enzymes by changing their redox status, and this may lead to the alteration of their biological function (Barchowsky, 1999). Arsenic exposure increases the amount of oxidized glutahione (GSSG) and decreases cellular glutathione, which is also called reduced glutathione, GSH by inhibiting the glutathione reductase enzyme activity.

No significant change in liver and kidney GSH levels was observed for the sodium arsenite administered groups. However, the GSH level was significantly decreased in the brain and heart tissues ($p<0.01$) (Figure 4). An in vitro study by Li and Chou indicated that prolonged trivalent arsenic exposure can induce a dose dependent increase in total cellular GSH after the initial cellular GSH pool is depleted as part of a feedback mechanism (Li, 1999). This evidence may explain the results obtained in GSH levels.

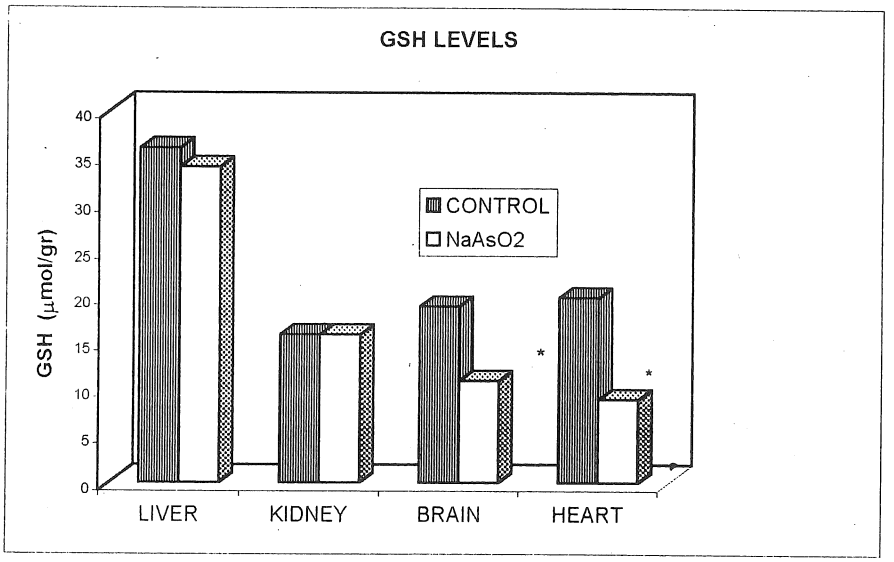

Figure 4. Glutathione levels of mice tissues after acute arsenic exposure (* $p<0.01$).

One reason for the low concentration of GSH in brain may be the high oxygen concentration. Another reason may be the insufficient amount of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in brain. This insufficiency brings about the lack of detoxicification of redox cycling xenobiotics and other foreign compounds (Pellegreni-Giampietro, 1994). Therefore GSH depletion is significant in this tissue.

According to the result obtained in this study, it is not convenient to measure MDA as a biomarker of LPO in mice tissues. However, that does not bring about the result that no LPO takes place. Moreover, tissue injury and glutathione depletion may be the cause of LPO not the consequence of it. Therefore, death may occur before LPO takes place. Another approach to this conflicting result may be related with the end product of LPO. Considering other end products of LPO in further investigations may lead us to observe the exact mechanism of sodium arsenite.

Özet

Bu çalışmada, sodyum arsenitin yaratmış olduğu oksidatif hasarın belirlenmesi amacı ile lipid peroksidasyon ürünü olan malondialdehit (MDA) düzeyindeki artış ve tüketilen glutation miktarı araştırıldı. Sodyum arsenit uygulanan Swiss Albino fareler enjeksiyondan bir saat sonra sakrifiye edildi. Alınan karaciğer, böbrek, beyin ve kalp dokularında UV-Spektrofotometre üzerine kurulu yöntemler ile MDA ve GSH düzeyi saptandı. Soyum arsenit, karaciğer, böbrek ve beyinde anlamlı bir MDA düzeyi düşüşüne neden olurken ($p<0.001$), kalpte artış gözlendi ($p<0.01$). Kalp dışındaki dokularda oksidatif hasara bağlı olarak malondialdehit oluşumu saptanmadı. Karaciğer ve böbrek dokularında glutation düzeyinde bir değişim gözlenmezken, kalp ve beyinde anlamlı glutation tüketimi saptandı ($p<0.01$).

References

Aposhian, H.V. (1989). Biochemical toxicology of arsenic. *Rev. Biochem. Toxicol.* 10:265-299.

Aronson, S. M. (1994). Arsenic and old myths. *R. I. Med.* 77: 233-234.

Bachleitner-Hofmann T., Kees M. and Gisslinger H. (2002). Arsenic trioxide: acute promyelocytic leukemia and beyond. *Leuk Lymphoma.* 43(8):1535-40.

Barchowsky, A., Roussel, R. R., Klei, L. R., James, P. E., Ganju, N., Smith, K. R. and Dudek, E. (1999). Low levels of arsenic trioxide stimulate proliferative signals in primary vascular cells without activating stress effector pathways. *Toxicol. Appl. Pharmacol.* 159 (1): 65-75.

Bernstam, L. and Nriagu, J. (2000). Molecular aspects of arsenic stress. *J. Toxicol. Environ. Health B Crit. Rev.* 3 : 293-322.

Buzard, G. S. and Kasprzak, K. S.C. (2000). Possible roles of nitric oxide and redox cell signaling in metal-induced toxicity and carcinogenesis: a review. *J Environ. Pathol. Toxicol. Oncol.* 19(3): 179-199.

Chen, Y. C., Lin-Shiau, S. Y. and Lin, J. K. (1998). Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J. Cell Physiol.* 177: 324-333.

Couchane, S. and Snow, E.T. (2001). In vitro effect of arsenicel compounds on glutathione-related enzymes. *Chem. Res. Toxicol.* 14: 517-522.

Halliwell B. and Gutteridge J.M.C. (1999). Free radicals in biology and medicine. Third Edition. Oxford University Press. pp.621-623.

Hazelton, G. A. and Lang, C. A. (1980). Glutathione contents of tissue in the aging mouse. *Biochem. J.* 188: 25-30.

Horton, A.A. and Fairhurst, S. (1987). Lipid peroxidation and mechanism of toxicity. *CRC Crit. Rev. Tox.,* 18:27-69.

Jamall, I. S. and Smith, J. C. (1985). Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation in the rat heart: A possible mechanism of cadmium cardiotoxicity. *Toxicol. Appl. Pharmacol.* 80: 33-42.

Kehrer P.J. (1993). Free radicals as mediators of tissue injury and disease. *Critical Rev. Toxicol.* 23 (1): 21-48.

Klaassen, C.D., Bracken, W.M., Dudley, R.E., Goering, P.L., Hazelton, G.A. and Hjelle, J.J. (1985) Role of sulfhydryls in the hepatotoxicity of organic and metallic compounds. *Fundam. Appl. Toxicol.* 5 : 806-815.

Leikin, J.B., Leikin, R.E.G., Evans, M.A., Wiener, S. and Hyrhorczuk, D.O. (1991). Immunotherapyin acute arsenic poisoning. *J. Toxicol. Clin. Toxicol.* 29(1):59-70.

Li, Y.M. and Broome J. D. (1999). Arsenic targets tubulin to induce apoptosis in myeloid leukemia cells. *Cancer Res.* 59: 776-780.

Miller, W. H., Schipper, H. M., Lee, J. S., Singer, J. and Waxman, S. (2002). Mechanisms of action of arsenic trioxide. *Cancer Res.* 62 (14) : 3893-3903.

Mückter, H., Liebl, B., Reichl, F.X., Hunder, G., Walther, U. and Ficht, B. (1997). Are we ready to replace dimercaprol (BAL) as an arsenic antidote?. *Hum. Exp. Tox.* 16:460-465.

Pellegreni-Giampietro, D. E. (1994). Free radicals in diagnostic medicine. Plenum Press, New York, pp:59-71.

Sakurai, T., Kaise, T. and Matsubara, C. (1998). Inorganic and methylated arsenic compounds induce cell death in murine macrophages via different mechanisms. *Chem. Res. Tox.* 11:273-283.

Schinella, G.R., Tournier, H.A., Buschiazzo, H.O. and DE Buschiazzo, P.M. (1996). Effect of arsenic (V) on the antioxidant defense system: in vitro oxidation of rat plazma protein. *Pharm. Toxicol.* 79 (6): 293-296

Sedlak, J. and Lindsay, R. H. (1968). Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* 25: 192-205.

Sen, C. K. (1998). Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem. Pharmacol.* 55: 1747-1758.

Son, M. H., Kang, K. W., Lee, C. H. and Kim, S.G. (2001). Potentiation of arsenic-induced cytotoxicity by sulphur amino acid deprivation (SAAD) through activation of ERK1/2, p38 kinase and JNK1: the distinct role of JNK1 in SAAD-potentiated mercury toxicity. *Toxicol. Lett.* 121: 45-55.

Yamanaka K., Hasegeva A., Sawamura R. and Okada S. (1991). Cellular response to oxidative damage in lung induced by the administration of dimethylarsenic acid, a major metabolise of inorganic arsenics, in mice. *Toxicol. Appl. Pharmacol.* 108: 205-213.

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