Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chemical Neuroanatomy 29 (2005) 173–178
Journal of CHEMICAL NEUROANATOMY
www.elsevier.com/locate/jchemneu

Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: Biochemical and histological studies

Ahmet Gurel [a,*], Omer Coskun [b], Ferah Armutcu [a], Mehmet Kanter [b], Oguz Aslan Ozen [c]

[a] *Department of Biochemistry, Zonguldak Karaelmas University, Faculty of Medicine, 67600 Kozlu, TR-44069 Zonguldak, Turkey*
[b] *Departments of Histology and Embryology, Zonguldak Karaelmas University, Faculty of Medicine, 67600 Kozlu, TR-44069 Zonguldak, Turkey*
[c] *Department of Anatomy, Zonguldak Karaelmas University, Faculty of Medicine, 67600 Kozlu, TR-44069 Zonguldak, Turkey*

Received 14 June 2004; received in revised form 7 January 2005; accepted 7 January 2005

Abstract

Formaldehyde (FA) can cause severe central nervous system impairment. But, there are only a few studies about biochemical and histopathological changes of frontal cortex and hippocampal tissue caused by FA toxicity. The aim of our study was to investigate these changes occurring after chronic formaldehyde toxicity in frontal cortex and hippocampal tissues, and protective effect of Vitamin E (vit E) against oxidative damage. Eighteen rats were divided into three groups: (1) control, (2) treated with FA (FAt), and (3) treated with FA and vit E (FAt + vit E) groups. After the treatment, the animals were sacrificed and frontal cortex and hippocampal tissues were removed for biochemical and histopathological investigation. FA significantly increased tissue malondialdehyde (MDA) and protein carbonyl (PC) levels and also decreased superoxide dismutase (SOD) and catalase (CAT) enzyme activities in frontal cortex and hippocampal tissue compared to control. Vit E treatment decreased MDA and PC levels and prevented inhibition of SOD and CAT enzymes in the tissues. In the FAt group, the neurons of both tissues became extensively dark and degenerated with picnotic nuclei. The morphology of neurons in FAt + vit E group was protected well, but not as neurons of the control group. The number of neurons in frontal cortex and hippocampal tissue of FAt group was significantly less than both control and FAt + vit E groups. It was concluded that vit E treatment might be beneficial in preventing FA-induced oxidative frontal cortex and hippocampal tissue damage, therefore, shows potential for clinical use.

Keywords: Formaldehyde; Lipid and protein oxidation; Antioxidant enzymes; Frontal cortex; Hippocampal tissue; Vitamin E

1. Introduction

Formaldehyde (FA), being a very reactive compound, can react with different macromolecules, such as proteins and nucleic acids or with low molecular weight substances as amino acids (Cheng et al., 2003; Metz et al., 2004). It is widely used in medicine and industry. Humans are exposed to FA from both direct environmental sources, as well as from the metabolism of xenobiotics. Everyday exposure to FA includes building materials (e.g. paint, plywood), cosmetics, cigarette smoke, photochemical smog and even various fruits (Conaway et al., 1996; Trezl et al., 1998). Physiological FA can be formed by the metabolism of L-methionine, histamine or methylamine, and can contribute to biological methylation by folic acid (Trezl et al., 1990).

FA causes central nervous system symptoms, which include headache, malasia, insomnia, anoreaxia and dizziness (Solomons and Cochrane, 1984). In addition, some studies have linked chronic FA exposure in humans to neurodegenerative disorders (Kilburn, 1994). Pitten et al. (2000) reported that the animals exposed to formaldehyde needed more time to find the food and made more mistakes.

FA, after administration, fastly diffuses to many tissues, including brain. In a postmortem study FA and its metabolites, methanol and formic acid, were found at the similar concentration in the brain (Nishi et al., 1988). When ingested, FA is rapidly taken up and metabolized as shown by the blood increase of formic acid. There are at least seven enzymes that catalyze the oxidation of FA in animal tissues, namely

* Corresponding author. Tel.: +90 372 261 0169; fax: +90 372 261 0155.
E-mail address: dragurel@yahoo.com (A. Gurel).

0891-0618/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchemneu.2005.01.001

aldehyde dehydrogenase, xanthine oxidase, catalase, peroxidase, aldehyde oxidase, glyceraldehyde-3-phosphate dehydrogenase, and a specific NAD-dependent formaldehyde dehydrogenase (Cooper and Kini, 1962).

Reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical, could be produced by endogenous sources, as cell aerobic metabolism and inflammation, or by exposure to a variety of chemical and physical agents. ROS are important mediators of cellular injury, and play a putative role in oxidative stress and can contribute to a variety of diseases, or be present in situations of toxicity (Halliwell, 1997), such as in the presence of a toxic dose of methanol (Kadiiska and Mason, 2000). ROS-initiated oxidative stress can be regulated by cell defense mechanisms, which include superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Halliwell and Gutteridge, 1989).

Vitamin E (vit E) is a term corresponding to a small group of tocopherols of which α-tocopherol is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability (Di Mascio et al., 1991). Vit E may also affect oxidative changes which occur in other cell organelles. Moreover, it is known that antioxidants, such as Vitamin C, GSH and selenium, may act synergistically, preventing lipid peroxidation and cell destruction (Ozdil et al., 2004).

The aim of our study was to investigate a possible protective effect of vit E treatment on lipid and protein oxidation and antioxidant enzyme activities and histopathological changes in the frontal cortex and hippocampal tissues of rats treated with FA.

2. Materials and methods

2.1. Animals

Three-month-old Wistar albino rats, weighing between 250 and 300 g, were obtained from Zonguldak Karaelmas University Medical Faculty Experimental Research Center and housed in separate cages in standard conditions, with a 12/12 h light–dark cycle. The animals were given standard rat chow and water ad libitum.

2.2. Experimental groups

2.2.1. Test drugs

FA treated (FAt), and FA + vit E treated (FAt + vit E) groups received intraperitoneal injection of 10 mg/kg FA (37% formaldehyde, Merck, Darmstadt, Germany) for 10 days at the same time in the morning. In addition, FAt + vit E group received intramuscular injection of 300 mg/kg vit E (Evigen, Turkey) for 10 days. Eighteen male rats were randomly allotted into one of three experimental groups: control, FAt, and FAt + vit E groups. Each group consisted of six animals.

After the treatment, the animals were sacrificed and frontal cortex and hippocampal tissues were removed for biochemical and histological investigation.

2.3. Biochemical analysis

2.3.1. Preparation of tissue samples

Tissues were homogenized in a four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using a homogenizer for 2 min at 5000 rpm, after cutting the organs into small pieces. Levels of malondialdehyde (MDA) and protein carbonyl (PC), and CAT activity were determined in this homogenate. Also some of the homogenate was taken, centrifuged and its supernatant was separated. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was taken and used in the SOD activity and protein assays. All preparation procedures were performed at +4 °C.

2.3.2. MDA determination

The tissue MDA levels were determined by the method of Draper and Hadley (1990) based on the reaction of MDA with thiobarbituric acid (TBA) at 95 °C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 95 °C for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3-tetramethoxypropane). Results were expressed as nmol/mg tissue.

2.3.3. PC determination

Oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as previously described (Levine et al., 1990). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH and the absorbance was read at 370 nm. The results were calculated using the extinction coefficient of 22,000 for aliphatic hydrazone. Results were expressed as nanomoles per milligram tissue (nmol/mg tissue).

2.3.4. SOD activity determination

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lyzate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was

defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per mg protein.

2.3.5. CAT activity determination

CAT (EC 1.11.1.6) activity was determined according to Aebi's method (1974). The principle of the method was based on the determination of the rate constant (s^{-1}, k) of the H_2O_2 decomposition rate at 240 nm. Results were expressed as k (rate constant) per mg protein.

2.3.6. Protein assays

Protein assays in the samples were made by the method of Lowry et al. (1951).

2.4. Histological examinations

Frontal cortex and hippocampal tissue were harvested from the sacrificed animals, and the tissues were fixed in 10% neutral buffered formaline, embedded in paraffin, sectioned at 5 μm thickness and then, stained with heamatoxyline and eosine. Histological specimens were examined in light microscopy (Nikon Optiphot II, Japan).

2.5. Microscopic examination

Microscopic examination was carried out at a magnification of 400 and the counts of neurons were determined per square millimeter by using a standardized ocular grid. Intact or partially degenerated neurouns were counted. The density and distribution of neurons were examined in the sections stained with heamatoxyline and eosine. Tissue sections were examined under light microscopy (×400) and the number of the neurons counted in random high-power fields using a Nikon Optiphot 2 light microscope incorporating a square graticule in the eyepiece (eyepiece ×10, objective ×40, a total side length of 0.225 mm). Neurons density was assessed by counting the number of cells in 200 high power fields in frontal cortex and hippocampal tissue preparations of each group. The neurons density in each site was calculated and recorded as number of NCs/mm^2. The tissue compartments were used to record the neurons distribution in the frontal cortex and hippocampal tissue.

2.6. Statistical analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for windows, version 11.0). All data were presented in mean (±) standard deviations (S.D.). Differences in measured parameters among three groups were analyzed by Kruskal–Wallis test. Dual comparisons between groups that present significant values were evaluated with Mann–Whitney U-test. The differences were considered to be significant when the probability was less than 0.05.

3. Results

3.1. Biochemical analysis

3.1.1. MDA levels of the tissues

MDA levels in the frontal cortex and hippocampal tissues were found to be significantly higher in the FAt group than the control group. Treatment with vit E prevented elevation of MDA levels significantly (Tables 1 and 2).

3.1.2. PC levels of the tissues

In the FAt group, there was a significant increase in protein oxidation levels in the frontal cortex and hippo-

Table 1
Frontal cortex tissue MDA and PC levels and SOD and CAT enzyme activities of all groups

Group	MDA (nmol/mg tissue)	PC (nmol/mg tissue)	SOD (U/mg protein)	CAT (k/mg protein)
Control	7.19 ± 1.31	0.166 ± 0.025	0.287 ± 0.029	1.356 ± 0.291
FAt	15.28 ± 3.95*	0.337 ± 0.060*	0.198 ± 0.024*	0.858 ± 0.150*
FAt + vit E	8.02 ± 1.67	0.173 ± 0.033	0.262 ± 0.046	1.204 ± 0.199

Values are expressed as mean ± S.D., and $n = 6$ for all groups. FAt: formaldehyde treated; FAt + vit E: formaldehyde and vit E treated; S.D.: standard deviation; MDA: malondialdehyde; PC: protein carbonly, SOD: süperoxide dismutase; CAT: catalase.
* $p < 0.05$ when compared to control and FAt + vit E groups.

Table 2
Hippocampal tissue MDA and PC levels and SOD and CAT enzyme activities of all groups

Group	MDA (nmol/mg tissue)	PC (nmol/mg tissue)	SOD (U/mg protein)	CAT (k/mg protein)
Control	9.74 ± 1.77	0.189 ± 0.031	0.271 ± 0.031	1.246 ± 0.152
FAt	16.45 ± 3.18*	0.375 ± 0.076*	0.187 ± 0.022*	0.889 ± 0.161*
FAt + vit E	10.65 ± 2.14	0.204 ± 0.031	0.261 ± 0.033	1.147 ± 0.198

Values are expressed as mean ± S.D., and $n = 6$ for all groups. FAt: formaldehyde treated; FAt + vit E: formaldehyde and vit E treated; S.D.: standard deviation; MDA: malondialdehyde; PC: protein carbonly; SOD: süperoxide dismutase; CAT: catalase.
* $p < 0.05$ when compared to control and FAt + vit E groups.

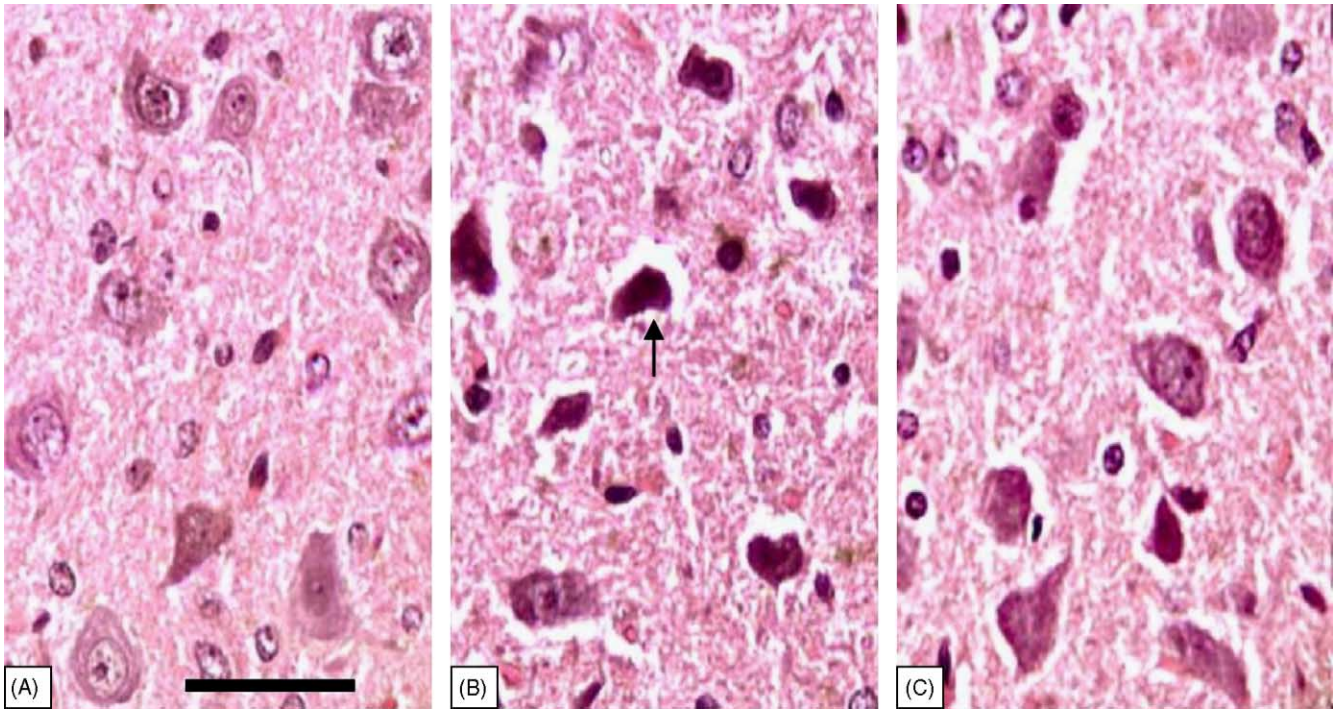

Fig. 1. In haematoxylin and eosin stained sections: (A) frontal cortex histology in control rats and (B) frontal cortex histology in formaldehyde treated rats. Severe degenerative changes and shrinkage cytoplasma and extensively dark picnotic nucleus are seen in neuronal cells (arrow). (C) Frontal cortex histology in formaldehyde and vit E treated rats. Less degenerative changes, and slight shrunken in cytoplasma and nucleus are seen in neuronal cells. Scale bar = 25 μm.

campal tissues, when compared with the control group. Treatment with vit E significantly prevented protein oxidation (Tables 1 and 2).

3.1.3. SOD and CAT activities of the tissues

SOD and CAT activities in the frontal cortex and hippocampal tissues were found to be significantly less in the FAt group than in the control group. The activities of both enzymes were kept at a level similar to the control group in FAt + vit E group (Tables 1 and 2).

3.2. Histological findings

In the control group, the morphology of neurons in frontal cortex (Fig. 1A) and CA_4 region of the hippocampal tissue was normal (Fig. 2A). The most consistent findings in histologic sections of both regions of neurons in FAt group stained with heamatoxyline and eosine were the severe degenerative changes, shrunken cytoplasma and extensively dark picnotic nuclei (Figs. 1B and 2B). In FAt + vit E group, the severity of degenerative changes in the cytoplasma and especially nucleus of frontal cortex and hippocampal tissues (Figs. 1C and 2C, respectively) were less than those in FAt group. The number of neurons in frontal cortex and hippocampal tissue of FAt group was also significantly less than control and FAt + vit E groups (Table 3).

4. Discussion

This experiment was carried out to evaluate the biochemical and histopathological changes in frontal cortex and hippocampal tissues due to oxidative damage by FA and protective effect of Vitamin E. FA has a neurotoxic and a slight carcinogenic effect. Although the neuron cytotoxic action of FA is not fully understood, it is thought to be mediated by the activation of free radical producing enzymes, and also, by the inhibition of free radical scavenger systems, thereby enhancing the production of the ROS. It has also been shown that FA and methanol are substrates for cytochrome P-450 monooxygenase system II E1 isozyme (CYP 2E1), which is important in the brain (Whelan et al., 1998) and can thus be oxidised by the endoplasmic reticulum by peroxidase, aldehyde oxidase and xanthine oxidase (Cooper and Kini, 1962). Activity of these enzymes have previously been shown to exhibit an unusually high rate of oxidase activity with the subsequent formation of ROS. The result of ROS formation is damage to an array of biomolecules found in tissues, including membrane lipids, proteins and nucleic acids. The brain and nervous system may be especially prone to radical damage, as brain has a high content of easily peroxidizable unsaturated fatty acids and as brain requires very high amounts of oxygen per unit weight. Membrane-associated polyunsaturated fatty-acids are readily attached by ROS in a process that results in

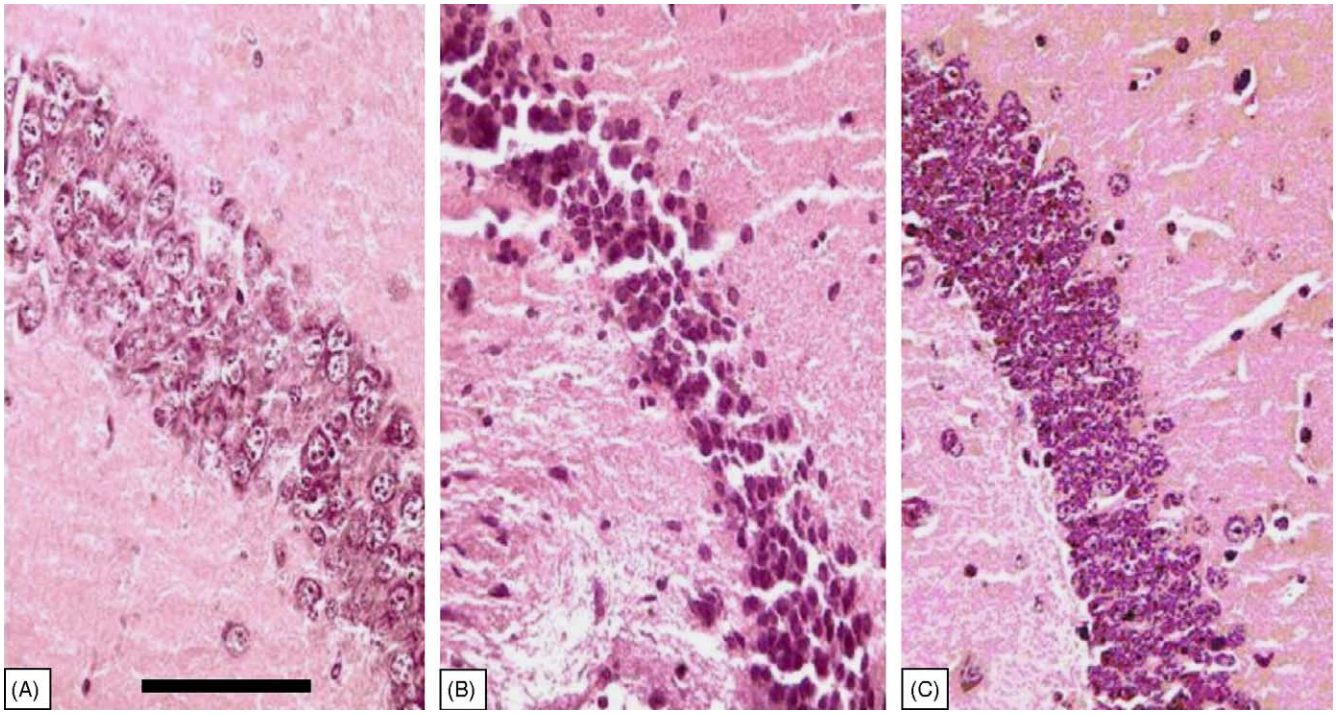

Fig. 2. In haematoxylin and eosin stained sections: (A) hippocampal tissue histology in control rats and (B) hippocampal tissue histology in formaldehyde treated rats. Severe degenerative changes and shrinkage cytoplasma and extensively dark picnotic nuclei are seen in neuronal cells. (C) Hippocampal tissue histology in formaldehyde and vit E treated rats. Less degenerative changes and slight shrunken in cytoplasma and nuclei are seen in neuronal cells. Scale bar = 25 μm.

the peroxidation of lipids. Peroxidation of membrane lipids can disrupt membrane fluidity and cell compartmentation, which can result in cell lysis. Thus oxygen radical-initiated lipid peroxidation and protein oxidation may contribute to the impaired cellular function and necrosis associated with toxicity of FA or its derivates (Datta and Namasivayam, 2003). Therefore, FA administration caused neurodegeneration, which is associated with the generation of ROS, resulting in oxidative damage. In this study, FA administration caused significant increases in MDA and PC, which are products of lipid and protein oxidation, respectively. This observation is in agreement with previous studies, where elevated levels of lipid peroxidation products were increased in different tissues (Teng et al., 2001; Tang et al., 2003). In this study, FA treatment caused severe degenerative changes in neurons of frontal cortex and hippocampal tissue and vit E treatment prevented this neuronal damage.

Table 3
The numbers (number/mm^2) of neurons in the frontal cortex and hippocampal tissue of control, FAt, and FAt + vit E groups

Groups	Frontal cortex tissue	Hippocampal tissue (CA_4)
Control	72.36 ± 5.34	102.63 ± 14.17
FAt	52.30 ± 3.83*	76.56 ± 4.27*
FAt + vit E	65.43 ± 3.98	91.52 ± 13.44

The data is expressed as mean ± standard deviation (S.D.), ($n = 6$). FAt: formaldehyde treated; FAt + vit E: formaldehyde and vit E treated.
* $p < 0.05$ when compared to FAt and FAt + vit E groups.

It has been suggested that there may be a relation between lipid and protein oxidation and tissue injury. In this experiment, it was found that FA significantly increased tissue MDA and PC levels. Teng et al. (2001) reported that the addition of FA to hepatocytes resulted in the generation of ROS and induction of lipid peroxidation in a dose- and time-dependent manner. Addition of the antioxidant vit E prevented cytotoxicity. We observed that the treatment of vit E showed an antiperoxidative effect in the rat frontal cortex and hippocampal tissues. By this way, MDA and PC levels in the tissues were also preserved near to the level of the control values. This anti-oxidative effect of vit E may be explained by its direct free radical scavenger property (Packer and Landvik, 1990; Sakamoto et al., 1990; Di Mascio et al., 1990).

The anti-oxidative defense system may scavenge ROS that has important role in initiation of lipid peroxidation process. The antioxidant defense system operates through enzymatic and nonenzymatic components. Antioxidant enzyme SOD is the first line of defense to scavenge superoxide anions generated in cytosolic and mitochondrial compartments of the cell. The function of this enzyme is to convert two superoxide radical molecules into oxygen and hydrogen peroxide. CAT causes direct breakdown of hydrogen peroxide to oxygen and water. In this present study, we examined the effect of FA intoxication in activities of SOD and CAT in hippocampus and frontal lobe tissues of the rats. The data of the present study show that activities of the antioxidant enzymes, such as SOD and CAT, significantly decreased in the brain tissues of

FA-injected rats after 10 days of intoxication. Our results are in agreement with reports of other workers, which suggest that FA exposure in experimental animals causes depression of their antioxidant system due to increased lipid peroxidation and formation of free radicals (Farooqui et al., 1986; Datta and Namasivayam, 2003). The impaired antioxidant enzyme activities in the tissues may cause an enhanced ROS-induced membrane lipid peroxidation and protein oxidation leading to delayed apoptotic/necrotic cell death in the frontal lobe and hippocampus. The drop in SOD and CAT activities showed that the activities of enzymes had been exhausted to scavenge the vast generation of ROS. Improvement of antioxidant enzyme activity in FAt + vit E group might be a result of free radical scavening effect of this vitamin. ROS might be removed from the environment by the help of free OH groups owned by vit E and enzyme consumption might be prevented by this way.

In conclusion, this study has shown that various biochemical abnormalities are produced in the frontal cortex and hippocampal tissues in response to the administration of FA. These effects include oxidative damage and impairment of structure and function of neurons and appear to be mediated through the production of free radicals. Pre-treatment with vit E conferred protection against such changes.

References

Aebi, H., 1974. Catalase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, pp. 673–677.

Cheng, G., Shi, Y., Sturla, S.J., Jalas, J.R., McIntee, E.J., Villalta, P.W., Wang, M., Hecht, S.S., 2003. Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem. Res. Toxicol. 16, 145–152.

Conaway, C.C., Whysner, J., Verna, L.K., Williams, G.M., 1996. Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation. Pharmacol. Ther. 71, 29–55.

Cooper, J.R., Kini, M.M., 1962. Biochemical aspects of methanol poisoning. Biochem. Pharmacol. 11, 405–416.

Datta, N.J., Namasivayam, A., 2003. In vitro effect of methanol on folate-deficient rat hepatocytes. Drug Alcohol Depend. 71, 87–91.

Di Mascio, P., Devasagayam, T.P., Kaiser, S., Sies, H., 1990. Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. Biochem. Soc. Trans. 18, 1054–1056.

Di Mascio, P., Murphy, M.E., Sies, H., 1991. Antioxidant defense systems: the role of carotenoids, tocopherols and thiols. Am. J. Clin. Nutr. 53, 194S–200S.

Draper, H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186, 421–431.

Farooqui, M.Y., Upreti, R.K., Ahmed, A.E., Ansari, G.A., 1986. Influence of intraperitoneally administered formaldehyde on bile production and tissue glutathione levels in rats. Res. Commun. Chem. Pathol. Pharmacol. 53, 233–236.

Halliwell, B., Gutteridge, J.M.C., 1989. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, pp. 186–187.

Halliwell, B., 1997. Antioxidants and human disease: a general introduction. Nutrition 55, S44–S52.

Kadiiska, M.B., Mason, R.P., 2000. Acute methanol intoxication generates free radicals in rats: an ESR spin trapping investigation. Free Radic. Biol. Med. 28, 1106–1114.

Kilburn, K.H., 1994. Neurobehavioural impairment and seizures from formaldehyde. Arch. Environ. Health. 49, 37–44.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186, 464–478.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 183, 265–275.

Metz, B., Kersten, G.F., Hoogerhout, P., Brugghe, H.F., Timmermans, H.A., de Jong, A., Meiring, H., ten Hove, J., Hennink, W.E., Crommelin, D.J., Jiskoot, W., 2004. Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J. Biol. Chem. 279, 6235–6243.

Nishi, K., Yamada, M., Wakasugi, C., 1988. Formaldehyde poisoning: report of an autopsy case. Nippon Hoigaku Zasshi 42, 85–89.

Ozdil, S., Bolkent, S., Yanardag, R., Arda-Pirincci, P., 2004. Protective effects of ascorbic acid, DL-alpha-tocopherol acetate, and sodium selenate on ethanol-induced liver damage of rats. Biol. Trace Elem. Res. 97, 149–162.

Packer, L., Landvik, S., 1990. Vitamin E in biological systems. Adv. Exp. Med. Biol. 264, 93–103.

Pitten, F.A., Kramer, A., Herrmann, K., Bremer, J., Koch, S., 2000. Formaldehyde neurotoxicity in animal experiments. Pathol. Res. Pract. 196, 193–198.

Sakamoto, W., Fujie, K., Handa, H., Ogihara, T., Mino, M., 1990. In vivo inhibition of superoxide production and protein kinase C activity in macrophages from vitamin E-treated rats. Int. J. Vitam. Nutr. Res. 60, 338–342.

Solomons, K., Cochrane, J.W., 1984. Formaldehyde toxicity. Part II. Review of acute and chronic effects on health. S. Afr. Med. J. 66, 103–106.

Sun, Y., Oberley, L.W., Li, Y.A., 1988. Simple method for clinical assay of superoxide dismutase. Clin. Chem. 34, 497–500.

Tang, M., Xie, Y., Yi, Y., Wang, W., 2003. Effects of formaldehyde on germ cells of male mice. Wei Sheng Yan Jiu 32, 544–548.

Teng, S., Beard, K., Pourahmad, J., Moridani, M., Easson, E., Poon, R., O'Brien, P.J., 2001. The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. Chem. Biol. Interact. 130–132, 285–296.

Trezl, L., Tyihak, E., Lotlikar, P.D., 1990. Nonenzymatic protein methylation and its biological significance in protein methylation. In: Paik, W.K., Kim, S. (Eds.), Protein Methylation. CRC Press, Boca Raton, FL.

Trezl, L., Hullan, L., Szarvas, T., Csiba, A., Szende, B., 1998. Determination of endogenous formaldehyde in plants (fruits) bound to L-arginine and its relation to the folate cycle, photosynthesis and apoptosis. Acta Biol. Hung. 49, 253–263.

Whelan, H.T., Bajic, D.M., Karlovits, S.M., Houle, J.M., Kindwall, E.P., 1998. Use of cytochrome-P450 mono-oxygenase 2 E1 isozyme inhibitors to delay seizures caused by central nervous system oxygen toxicity. Aviat. Space Environ. Med. 69, 480–485.