Original Article

Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice

Takashi Matsuoka^{1,2}, Atsushi Takaki³, Hirokazu Ohtaki¹ and Seiji Shioda¹

¹Department of Anatomy, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-Ku, Tokyo 142-8555, Japan

²Department of Pediatrics, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-Ku, Tokyo 142-8555, Japan

³Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan

(Received May 11, 2010; Accepted July 5, 2010)

ABSTRACT — Formaldehyde (FA) is a commonly used chemical in everyday life and can react with many molecules in the human body. Although toxicity has been reported, exposure to FA has also been shown to have beneficial effects or no effect at all. In the present study, we examined the effect of FA inhalation on oxidative stress and inflammation in mice. Male adult ICR mice were exposed FA in gaseous form (0.1 ppm), and blood, urine, brain, lung and liver were obtained for 24 hr. Levels of 8-hydroxy-2'-deoxyguanosine (8OHdG) and NO₃⁻ were then determined by HPLC. A second group of mice were injected with 5 mg/kg lipopolysaccharide (LPS) after 24 hr of FA (3 ppm) inhalation and blood and organs were assayed for NO₃⁻ level and SOD activity. After exposure to a low dose of FA (0.1 ppm), the 8OHdG/dG ratio significantly increased in plasma. However, the ratio in urine and organs significantly decreased during 24 hr of FA exposure. The NO₃⁻ levels mirrored the 8OHdG/dG ratio. After 24 hr exposure to a high dose of FA (3 ppm), NO₃⁻ levels in plasma and liver were significantly lower than in control mice exposed to air only. The SOD activity of blood and urine were conversely increased in FA exposed animals. In the present study, we suggest that inhalation of FA at low doses influences the oxidative stress response in a tissue-specific manner. The FA may partially alleviate in some tissues like preconditioning in oxidative stress.

Key words: 8-hydroxy-2'-deoxyguanosine (8OHdG), Formaldehyde, Lipopolysaccharide (LPS), Nitric oxide (NO), Toxicity

INTRODUCTION

Formaldehyde (FA) is a colorless, highly flammable gas, and a very reactive compound. FA can react with different macromolecules, such as proteins, nucleic acids and low molecular weight substances such as amino acids (Cheng *et al.*, 2003; Metz *et al.*, 2004). It is widely used in the building, textile, paper product, resin, wood composite, insulating material, paint, plastic, fabric, adhesive and cosmetic industries, and is also found in photochemical smog and even in various fruits (Conaway *et al.*, 1996; Trézl *et al.*, 1998). In animal physiology, FA can be formed by the metabolism of L-methionine, histamine or methylamine, and can react with folic acid to contribute to biological methylation (Trézl *et al.*, 1998).

In 1995, the International Agency for Research on Cancer (IARC) concluded that FA is a probable human carcinogen (Cogliano *et al.*, 2005; IARC, 1995). Therefore, the toxicity of FA has attracted extensive studies in recent years. It is suspected that exposure to low levels of FA induces or aggravates airway inflammation (Fujimaki *et al.*, 2004). Moreover, inhalation of FA has been shown to inhibit learning and memory performance in mice (Lu *et al.*, 2008) and to cause increased oxidative stress (Zhou *et al.*, 2006) in rat testis and the developing rat liver (Kum *et al.*, 2007a). Conversely, other studies have suggested that FA inhalation does not influence systemic genotoxicity (Speit *et al.*, 2009), renal oxidative stress (Kum *et al.*, 2007b), asthmatic response (Ezratty *et al.*, 2007) or cerebellum development (Songur *et al.*, 2008). In con-

Correspondence: Seiji Shioda (E-mail: shioda@med.showa-u.ac.jp)

trast, inhalation of FA at low doses was shown to have a protective effect against allergic reactions and cell death induced by ovalbumin immunization (Fujimaki *et al.*, 2004; Lino *et al.*, 2009; Tsukahara *et al.*, 2006).

Reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical can be produced by endogenous sources, as well as through cellular aerobic metabolism and inflammation, or through exposure to a variety of chemical and physical agents. ROS are important mediators of cellular injury, and play a putative causing in oxidative stress. ROS can contribute to a variety of diseases and may be present in situations of toxicity (Halliwell, 1997; Kadiiska and Mason, 2000). ROS-initiated oxidative stress can be regulated by cell defense mechanisms, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). One major pathway of ROS-induced DNA damage involves a reaction at the C-8 position of 2'-deoxyguanosine to form 8-hydroxy-2'-deoxyguanosine (8OHdG). 8OHdG has been shown to cause mispairing during DNA replication, giving rise to G to T conversions, and consequently a G:C → T:A mutation occurs (Halliwell, 1997; Pilger and Rüdiger, 2006). Under normal conditions or less stressful conditions, oxidized DNA can be repaired *in vivo* by endonucleases, or by a base-specific glycosylase. In such cases, 8OHdG is transported through the blood and excreted into the urine without further modification. However, in pathological conditions such as cancer, inflammation, diabetes and neurodegeneration, 8OHdG levels increase in tissues, blood or urine in a cellular environment of oxidative stress. As such, 8OHdG is considered to be a marker of DNA oxidative stress (Loft *et al.*, 1993; Pilger and Rüdiger, 2006). Nitric oxide (NO) and the oxidative metabolite, peroxynitrite (ONOO⁻) are referred to as reactive nitrogen species (RNS). NO is synthesized from L-Arginine by three kinds of NO synthase (NOS) and can cause modification of neuronal function, dilation of blood vessels, disruption of host defenses, and inflammatory responses (Brüne *et al.*, 1998; Gordge, 1998).

In the present study, we exposed ICR mice to low amounts of FA gas and investigated the resultant levels of oxidative stress in plasma, urine and tissues. Moreover, we tested the effects of FA inhalation on sensitivity to lipopolysaccharide (LPS) challenge.

MATERIALS AND METHODS

FA exposure chamber

The experimental chamber [38 (W) x 52 (L) x 32 (H) cm] was made from glass and consisted of two compart-

ments: the experimental chamber and the FA vaporizing chamber, respectively (Fig. 1A). The two compartments were connected via a hole (5 cm diameter) with ventilator fan operating at 0.3 m³/min to diffuse the FA into the experimental chamber. Another hole (5 cm diameter) was located in the experimental chamber for exhaust. A beaker containing an appropriate concentration of formalin (Wako, Osaka, Japan) was set over a stirrer in the FA vaporizing compartment to produce gaseous FA. The concentration of FA in the experimental chamber was measured before and during each experimental phase by a smell sensor (Smell sensor portable model XG100V, New Cosmos Electric, Osaka, Japan) set adjacent to the exhaust. In accordance with previous reports (Naya and Nakanishi, 2005), we used FA concentrations of 0.1 ppm (low dose) and 3 ppm (high dose).

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Showa University. Male ICR mice (8 weeks of age) weighing 30-40 g were obtained from Japan Crea (Tokyo, Japan). All mice were housed in a temperature- and humidity-controlled environment for at least 2 weeks prior to the experiment.

Low dose FA exposure

A total of 43 male ICR mice were divided into two experimental groups; a non-FA inhalation control (n = 7) and four FA inhalation groups (n = 9 in each group). After 0 (non-FA inhalation control), 2, 4, 8, or 24 hr FA exposure, body weight was measured, and then mice were sacrificed by cervical dislocation. Urine (50 µl) and heparinized blood from the right ventricle were collected and the animals were perfused with PBS through the left ventricle to remove the blood. The urine samples (50 µl) were immediately added to 350 µl of preserving solution (0.5 mM EDTA, 5% methanol, 20% glycerol) to avoid further oxidation. Tissue samples (lung, liver, and brain) of approximately 150 mg were collected and stored in 250 µl of tissue preserving solution (0.5 mM EDTA, 5% methanol, 40% glycerol). Plasma (200 µl) was mixed with an equal volume of preserving solution. All samples were stored at 4°C. An overview of the experiment is shown in Fig. 1B.

High dose FA exposure

We analyzed the effect of FA exposure on the inflammatory response induced by LPS (*E.coli* O111:B4, Seikagaku Corp., Tokyo, Japan). The mice were divided into two groups: treated with either only LPS injected intraperito-

Oxidative stress by formaldehyde exposure

Fig. 1. Diagram showing the experimental chamber system for FA exposure (A) and experimental overview (B and C). A) FA vapor is released through constant stirring of FA solution within a vaporizing chamber. The gas is dispersed through the experimental chamber using an electric fan. A screen was set up between the electric fan and vents to intercept gas exhaust. FA concentration was measured near vents using an odor meter. B) Time schedule of low dose FA exposure. Mice were exposed to a low dose of FA vapor (0.1 ppm) in the experimental chamber over 24 hr. At time points of 0 (control), 2, 4, 8 and 24 hr, samples of tissues, blood and urine were collected. C) Time schedule of high dose FA (3 ppm) exposure. Mice were exposed to a high dose of FA (or room air as a control) in the experimental chamber for 24 hr. Following this, the mice were injected with LPS 5 mg/kg (i. p.), and samples were collected 4 hr later.

neally at 5 mg/kg ($n = 12$) was defined "LPS" group or was it LPS injection followed by FA (3 ppm) exposure at 5 mg/kg ($n = 12$) was defined "FA + LPS" group. The mice in the latter group were exposed to high dose FA for 24 hr, injected intraperitoneally with LPS and further

exposed to FA for 4 hr. At the end of the experiment, the mice were sacrificed as previously described. An overview of the experiment is shown in Fig. 1C.

Another set of animals ($n = 12$) were examined SOD activity. After 24 hr high dose FA or room air exposure,

plasma, urine and liver were collected as described above and were measured SOD activity.

Measurement of 8OHdG and dG

Tissue samples were prepared with a homogenizer (FastPrep FP120, BIO 101, La Jolla, CA, USA) and were centrifuged at 15,000 rpm for 10 min at 4°C. The supernatants were then treated on ice by a micro-dialysis system with a molecular weight cut-off of 50 kDa (Eicom, Kyoto, Japan). Efficiency of recovery of dG and 8OHdG were measured using the standard solutions to enable accurate correction for differences in individual membranes. dG and 8OHdG in the samples were then separated out with a column (CA5-ODS, Eicom) maintained at 2°C and quantified using an electrochemical detector (ECD) system (HITEC, Eicom). The electrode potential was set to 550 mV and flow rate at 230 µl/min with a mobile phase consisting of 0.1 M sodium phosphate buffer (pH 7.4), 2.5% methanol, and 90 mg/l sodium 1-octanesulfate. 8OHdG was detected at 3.32 nA and dG was detected by UV at 254 nm.

Measurement of NO metabolites

NO is highly unstable in solution and cannot be readily assayed. However, NO is metabolized to stable nitrite (NO_2^-) and nitrate (NO_3^-) ions and were thus measured as NO_3^- after conversion of NO_3^- to NO_2^- using nitrate reductase with HPLC (Yamada and Nabeshima, 1997). In brief, NO_2^- and NO_3^- concentrations in plasma, urine or supernatant of tissue extractions were measured using the HPLC NO detector system (ENO-10, Eicom). The injected sample was mixed with an equivalent volume of methanol and was subsequently separated using a reverse-phase separation column packed with polystyrene polymer (NO-PAK, 4.6 × 50 mm, Eicom). NO_3^- was reduced to NO_2^- in a reduction column packed with copper-plated cadmium filings (NO-RED, Eicom). The NO_3^- concentration was measured by addition of Griess reagent (Archer, 1993; Guevera *et al.*, 1998) to form a purple azo dye, and the resulting peak at 540 nm was quantified. In the present study, we measured the peak of NO_3^- because the majority of NO metabolite was NO_3^- and the peak of NO_2^- was not suitable for quantitative analysis.

Measurement of plasma IL-6 levels

Plasma IL-6 levels, which are an early inflammatory marker, were measured in a bioassay using an IL-6 dependent murine hybridoma cell line, B9. B9 cells were maintained in RPMI 1640 with 25 mM HEPES containing 10% heat inactivated (56°C, 30 min.) fetal bovine serum and a 1% antibiotic-antimycotic solution in the presence

of hybridoma growth factor (20 units/ml) and recombinant murine IL-6 (Boehringer Mannheim Biochemica, Mannheim, Germany). In 96-well plates, plasma samples (2 µl) were added to 198 µl of culture medium without IL-6. The 200 µl samples were then serially diluted 8-fold. Each plate included a standard row containing a dilution series of the recombinant murine IL-6, in which the concentration of the first well was 5 pg/ml. B9 cells (100 µl) ($2-4 \times 10^4/\text{ml}$) washed in culture medium were then added to each well and incubated in 5% CO_2 at 37°C for 72 hr. Bioactivity of IL-6 in the samples was measured fluorometrically following addition of Alamar Blue (20 µl), a fluorometric growth indicator (Alamar Biosciences Inc., Sacramento, CA, USA), for the final 8 hr of the incubation process. Fluorescence was measured using a Fluoroskan Ascent FL (LABOSYSTEMOY, Helsinki, Finland). The specificity of the B9 cell bioassay for measuring the bioactivity of IL-6 is well-established for *in vitro* and *in vivo* experiments (Yagi *et al.*, 2005).

Measurement of SOD activity levels

SOD activity in plasma, urine and supernatant of liver homogenates were measured by nitrite methods (Oyanagui, 1984) according to instruction manual (SRL, Tokyo, Japan). In brief, hydroxylamine, xanthine oxidase, hypoxanthine, EDTA-2Na, and the sample were incubated with or without KCN at pH 8.2, 37°C for 30 min. Then, Diazo dye-forming reagent formed by sulfanilic acid, N-1-naphthylethylenediamine and acetic acid was added and the absorption was measured at 550 nm.

Statistics

Data were analyzed using the Kruskal Wallis non-parametric analysis of variance test followed by Dunnett's multiple comparison test using JMP® software (Cary, NC, USA). A $p < 0.05$ was considered statistically significant.

RESULTS

To determine effects of FA inhalation on ROS, the 8OHdG/dG ratios were measured in urine, plasma, and tissues (lung, liver, and brain) for prior exposure to FA of up to 24 hr (Figs. 2 and 3). The urine dG level was 1.6 ± 0.2 µg/ml at 0 hr and increased significantly to 2.5 ± 0.2 µg/ml by 24 hr ($p < 0.05$). In contrast, plasma dG decreased significantly with time. There was no significant change over time in 8OHdG concentration in urine or plasma. Accordingly, due to the changes in dG concentration, the 8OHdG/dG ratio significantly decreased in urine over 24 hr (from 3.0 ± 0.4 ; $p < 0.05$ to 1.2 ± 0.2), and increased in plasma (from 9.0 ± 1.4 ; $p < 0.05$ to $25.8 \pm$

Oxidative stress by formaldehyde exposure

Fig. 2. Effect of low dose FA exposure on levels of dG (A, D), 8OHdG (B, E) and ratio of 8OHdG/dG (C, F) in urine (A, B, C) and plasma (D, E, F) of mice. Data was shown as mean $\pm$ S.E. (n = 7-9). * : $p < 0.05$ compared with control.

4.6). Tissue levels of dG, 8OHdG and the corresponding ratios are shown in Fig. 3. Levels of dG in lung and liver increased after low dose FA exposure, whilst levels of 8OHdG did not change significantly. Thus, the 8OHdG/dG ratios in lung and liver significantly decreased after 24 hr (lung, from $12.1 \pm 1.4/10^3$ to $5.0 \pm 1.6/10^3$ dG, $p < 0.05$; liver, from $4.5 \pm 1.1/10^3$ to $1.1 \pm 0.2/10^3$ dG, $p < 0.05$). However, levels of dG and 8OHdG in the brain did not show any significant changes over time. These results suggest that FA exposure influences oxidative stress even at low doses.

NO_3^- levels were also affected by FA exposure (Fig.

4). NO_3^- levels in the urine, liver and brain decreased by approximately 50% after 24 hr of FA exposure (urine, from 1.4 ± 0.2 to 0.8 ± 0.1 mM, $p < 0.05$; liver, from 19.2 ± 2.1 to 8.0 ± 1.2 μM , $p < 0.05$; brain, from 17.9 ± 3.1 to 5.8 ± 0.7 μM , $p < 0.05$). However, NO_3^- levels in plasma increased approximately 2-fold after 24 hr. No significant changes in NO_3^- levels were observed in the lung during this experiment. These results confirmed the 8OHdG/dG data in showing that FA exposure impacts oxidative stress levels, with increasing stress in plasma and decreasing stress in urine and tissues.

Levels of IL-6 were determined in plasma to analyze

Fig. 3. Effect of low dose FA exposure on levels of dG (A, D, G), 8OHdG (B, E, H) and ratio of 8OHdG/dG (C, F, I) in lung (A, B, C), liver (D, E, F) and brain (G, H, I) of mice. Data was shown as mean $\pm$ S.E. (n = 7-9). *: $p < 0.05$ compared with control.

Oxidative stress by formaldehyde exposure

Fig. 4. Changes in NO_3^- levels in urine (A), plasma (B), lung (C), liver (D) and brain (E) following low dose FA exposure. Data was shown as mean $\pm$ S.E. (n = 8). *: $p < 0.05$ compared with control.

the effect of FA exposure on the inflammatory response (Fig. 5). In plasma of mice exposed to low dose of FA, IL-6 levels did not show any significant changes during these experimental periods. We then tried to clarify whether FA pre-exposure would affect NO_3^- levels following LPS injection. After 24 hr high dose FA exposure (3 ppm), mice were injected with LPS (5 mg/kg) and blood, urine and tissues were examined after another 4 hr. NO_3^- levels in the plasma and liver were significantly lower than in that of the room air-exposed control mice. On the other hand, there was no difference in NO_3^- levels in lung and brain (Fig. 6).

We hypothesized that FA exposure might have induce

an anti-stress as a mechanism for preconditioning. Then we measured an anti-oxidative stress marker SOD activity in plasma, urine and liver homogenate 24 hr after FA (3 ppm) exposure. SOD activity levels in the plasma and urine increased significantly 24 hr after FA exposure (plasma, from 10.6 ± 0.9 to 14.5 ± 0.7 U/ml, $p < 0.05$; urine, from 19.3 ± 1.2 to 31.7 ± 2.7 U/ml, $p < 0.05$), but not in liver homogenate (Fig. 7).

DISCUSSION

In this study, we have assessed the effect of FA inhalation on oxidative stress in mice using an HPLC system

Fig. 5. Concentration of IL-6 in plasma following low dose FA exposure. IL-6 concentration (pg/ml) was detected using a B9 bioassay. Data was shown as mean $\pm$ S.E. (n = 8).

Fig. 6. Levels of NO₃⁻ 4 hr after LPS injection with or without FA pre-exposure for 24 hr. NO₃⁻ levels in the plasma and liver significantly decreased in mice pretreated with FA. Data was shown as mean $\pm$ S.E. (n = 7). *: $p < 0.05$ compared with control (room air exposure mice).

Fig. 7. SOD activity in plasma and urine and liver following 24 hr FA exposure. Data was shown as mean $\pm$ S.E. (n = 10-12).

that is approximately 100 times more sensitive than ELISA or colorimetric methods (Kasai, 2003; Shimoi *et al.*, 2002). We measured the levels of oxidative stress markers 8OHdG and NO_3^- in blood, urine and other tissues for FA exposures up to 24 hr. Finally, we examined synergic effects and changes in inflammatory response caused by LPS injections in mice exposed to FA. We also measured SOD activity to detect anti-stress system with or without FA inhalation.

For the initial experiments, we used a gaseous concentration of 0.1 ppm FA, a very low concentration that is near the criterion value of the Ministry of Health, Labour and Welfare in Japan (<http://www.mhlw.go.jp/bunya/roudoukijun/anzeneisei17/index.html>). Our results showed that over the time period of 24 hr, the NO_3^- levels and 8OHdG/dG ratios in tissues and urine significantly decreased compared to control. Conversely, the levels of 8OHdG/dG ratios and NO_3^- in plasma increased in the same time period within 24 hr. We first expected that FA exposure increases oxidative stress like as previous reports (Kum *et al.*, 2007a; Zhou *et al.*, 2006) and from our primitive *in vitro* experiments using FA in dG solution which designed to predict levels of 8OHdG/dG and had suggested a significant increase in 8OHdG/dG (data not shown). However, the response differed between sampling tissues.

We then measured IL-6, a marker for inflammatory response in plasma, to test the hypothesis that inflammation in mice would increase following exposure to FA. However, IL-6 levels in plasma did not change significantly over the course of the experiment. Morphological changes also did not show any critical differences between the experimental groups (data not shown). These results suggest that while mice were affected by FA exposure, the inflammatory response or tissue damages was not induced. The phenomenon where some people who are sensitive to FA subsequently develop other allergies or immune diseases is known as sick-building syndrome. We then tested whether FA inhalation changes the sensitivity of immune and inflammatory responses in mice NO_3^- levels in plasma and were lower in mice exposed to FA than in mice not exposed to FA. This result suggests that FA inhalation either decreased NO production or increased NO scavenging, which may have induced an anti-stress system as a mechanism for preconditioning. Preconditioning is a phenomenon in which non-lethal stresses such as short periods of ischemia or use of some anesthetics or chemicals induce a stress-responsive cascade in the brain, heart and other organs. Other studies have shown that increased stress responses result in the protection of host organs and tissues from lethal stress-

es (Collins *et al.*, 2009; Pong, 2004; Zhao, 2007). It has been recently reported that NO and ROS trigger a phase of preconditioning that lasts 3 to 4 days and protects against both infarction and stunning in heart (Bolli, 2000). NO and ROS are thought to induce a complex cascade of signaling events including production of aldose reductase, Mn-SOD, and other cardioprotective proteins (Bolli, 2000). Gülec *et al.* (2006) found that prolonged exposure to FA at 10 ppm caused increased SOD levels but did not alter NO or lipid peroxidation in the hearts of rats. Therefore, we have tried to measure SOD activity in plasma, urine and liver 24 hr after FA exposure. As expected, plasma and urine SOD activities significantly increased the mice exposed to FA to compare with control. These results suggest that short periods and low level of FA expose gives a stress but it does not induce inflammatory and resulted in preconditioning-like phenomenon. Interestingly, although alterations of the oxidative markers differed between the tissues, responses of the SOD activity tend to consistent. The exactly reasons why the oxidative responses were specific in the tissues were not sure. However, it reported recently that manganese SOD (Mn-SOD) levels differed from tissues using the Mn-SOD gene deficient mice (Shimizu and Shirasawa, 2010). Indeed, it could contribute distribution of FA and/or metabolite the oxidant between the tissues. Moreover, we could not determine whether longer periods of FA exposure or an increased dose of FA tolerant to similar with the present study. We should consider the tolerance between rodents and humans against FA stress as well. Further studies could be required to determine whether the FA expose is beneficial or not.

In conclusion, our results show that exposure to a low dose of gaseous FA influences oxidative stress, and that the effects vary in different tissues. It may be that inhalation of FA has resulted in triggering of a low level protective cascade.

REFERENCES

- Archer, S. (1993): Measurement of nitric oxide in biological models. *FASEB J.*, **7**, 349-360.
- Bolli, R. (2000): The late phase of preconditioning. *Circ. Res.*, **87**, 972-983.
- Brüne, B., von Knethen, A. and Sandau, K.B. (1998): Nitric oxide and its role in apoptosis. *Eur. J. Pharmacol.*, **351**, 261-272.
- Cheng, G., Shi, Y., Sturla, S.J., J alas, J.R., McIntee, E.J., Villalta, P.W., Wang, M. and 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.
- Cogliano, V.J., Grosse, Y., Baan, R.A., Straif, K., Secretan, M.B. and El Ghissassi, F. (2005): Meeting report: summary of IARC mon-

- ographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. *Environ. Health Perspect.*, **113**, 1205-1208.
- Collins, M.A., Neafsey, E.J., Mukamal, K.J., Gray, M.O., Parks, D.A., Das, D.K. and Korthuis, R.J. (2009): Alcohol in moderation, cardioprotection, and neuroprotection: epidemiological considerations and mechanistic studies. *Alcoh. Clin. Exp. Res.*, **33**, 206-219.
- Conaway, C.C., Whysner, J., Verna, L.K. and Williams, G.M. (1996): Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation. *Pharmacol. Ther.*, **71**, 29-55.
- Ezratty, V., Bonay, M., Neukirch, C., Orset-Guillossou, G., Dehoux, M., Koscielny, S., Cabanes, P.A., Lambrozo, J. and Aubier, M. (2007): Effect of formaldehyde on asthmatic response to inhaled allergen challenge. *Environ. Health Perspect.*, **115**, 210-214.
- Fujimaki, H., Kurokawa, Y., Kunugita, N., Kikuchi, M., Sato, F. and Arashidani, K. (2004): Differential immunogenic and neurogenic inflammatory responses in an allergic mouse model exposed to low levels of formaldehyde. *Toxicology*, **197**, 1-13.
- Gorge, M.P. (1998): How cytotoxic is nitric oxide? *Exp. Nephrol.*, **6**, 12-16.
- Güleç, M., Songur, A., Sahin, S., Ozen, O.A., Sarsilmaz, M. and Akyol, O. (2006): Antioxidant enzyme activities and lipid peroxidation products in heart tissue of subacute and subchronic formaldehyde-exposed rats: a preliminary study. *Toxicol. Ind. Health*, **22**, 117-124.
- Guevera, I., Iwanejko, J., Dembińska-Kieć, A., Pankiewicz, J., Wanat, A., Anna, P., Gołabek, I., Bartuś, S., Malczewska-Malec, M. and Szczudlik, A. (1998): Determination of nitrite/nitrate in human biological material by the simple griess reaction. *Clin. Chim. Acta*, **274**, 177-188.
- Halliwell, B. (1997): Antioxidants and human disease: a general introduction. *Nutr. Rev.*, **55**, S44-S52.
- IARC (1995): Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 62, Lyon, Wood Dust and Formaldehyde.
- Kadiiska, M.B. and Mason, R.P. (2000): Acute methanol intoxication generates free radicals in rats: an ESR spin trapping investigation. *Free Radic. Biol. Med.*, **28**, 1106-1114.
- Kasai, H. (2003): A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system. *J. Radiat. Res.*, **44**, 185-189.
- Kum, C., Kiral, F., Sekkin, S., Seyrek, K. and Boyacioglu, M. (2007a): Effects of xylene and formaldehyde inhalations on oxidative stress in adult and developing rats livers. *Exp. Anim.*, **56**, 35-42.
- Kum, C., Sekkin, S., Kiral, F. and Akar, F. (2007b): Effects of xylene and formaldehyde inhalations on renal oxidative stress and some serum biochemical parameters in rats. *Toxicol. Ind. Health*, **23**, 115-120.
- Lino dos Santos Franco, A., Domingos, H.V., Damazo, A.S., Breithaupt-Faloppa, A.C., de Oliveira, A.P., Costa, S.K., Oliani, S.M., Oliveira-Filho, R.M., Vargaftig, B.B. and Tavares-de-Lima, W. (2009): Reduced allergic lung inflammation in rats following formaldehyde exposure: long-term effects on multiple effector systems. *Toxicology*, **256**, 157-163.
- Loft, S., Fischer-Nielsen, A., Jeding, I.B., Vistisen, K. and Poulsen, H.E. (1993): 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *J. Toxicol. Environ. Health*, **40**, 391-404.
- Lu, Z., Li, C.M., Qiao, Y., Yan, Y. and Yang, X. (2008): Effect of inhaled formaldehyde on learning and memory of mice. *Indoor Air*, **18**, 77-83.
- 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. and Jiskoot, W. (2004): Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. *J. Biol. Chem.*, **279**, 6235-6243.
- Naya, M. and Nakanishi, J. (2005): Risk assessment of formaldehyde for the general population in Japan. *Regul. Toxicol. Pharmacol.*, **43**, 232-248.
- Oyanagui, Y. (1984): Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Biochem.*, **142**, 290-296.
- Pilger, A. and Rüdiger, H.W. (2006): 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int. Arch. Occup. Environ. Health*, **80**, 1-15.
- Pong, K. (2004): Ischaemic preconditioning: therapeutic implications for stroke? *Expert Opin. Ther. Targets*, **8**, 125-139.
- Shimizu, T. and Shirasawa, T. (2010): Anti-aging research using Mn-SOD conditional knockout mice. *Yakugaku Zasshi*, **130**, 19-24.
- Shimoi, K., Kasai, H., Yokota, N., Toyokuni, S. and Kinae, N. (2002): Comparison between high-performance liquid chromatography and enzyme-linked immunosorbent assay for the determination of 8-hydroxy-2'-deoxyguanosine in human urine. *Cancer Epidemiol Biomarkers Prev.*, **11**, 767-770.
- Songur, A., Sarsilmaz, M., Ozen, O., Sahin, S., Koken, R., Zararsiz, I. and Ilhan, N. (2008): The effects of inhaled formaldehyde on oxidant and antioxidant systems of rat cerebellum during the postnatal development process. *Toxicol. Mech. Methods*, **18**, 569-574.
- Speit, G., Zeller, J., Schmid, O., Elhajouji, A., Ma-Hock, L. and Neuss, S. (2009): Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. *Mutat. Res.*, **677**, 76-85.
- Tréz, L., Hullán, L., Szarvas, T., Csiba, A. and 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.
- Tsukahara, S., Yamamoto, S., Tin-Tin-Win-Shwe, Ahmed, S., Kunugita, N., Arashidani, K. and Fujimaki, H. (2006): Inhalation of low-level formaldehyde increases the Bcl-2/Bax expression ratio in the hippocampus of immunologically sensitized mice. *Neuroimmunomodulation*, **13**, 63-68.
- Yagi, S., Takaki, A., Hori, T. and Sugimachi, K. (2002): Enteric lipopolysaccharide raises plasma IL-6 levels in the Hepatoportal vein during non-inflammatory stress in the rat. *Fukuoka Acta Med.*, **93**, 38-52.
- Yamada, K. and Nabeshima, T. (1997): Simultaneous measurement of nitrite and nitrate levels as indices of nitric oxide release in the cerebellum of conscious rats. *J. Neurochem.*, **68**, 1234-1243.
- Zhao, H. (2007): The protective effect of ischemic preconditioning against ischemic injury: from the heart to the brain. *J. Neuroimmune Pharmacol.*, **2**, 313-318.
- Zhou, D.X., Qiu, S.D., Zhang, J., Tian, H. and Wang, H.X. (2006): The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. *Asian J. Androl.*, **8**, 584-588.