TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2013) 43: 52-56

doi:10.3906/sag-1206-58

The effects of formaldehyde intoxication on the inducible nitric oxide synthase expression and nitric oxide level in the liver tissue of rats

Ergün UÇMAKLI[1,*], Ferah ARMUTCU[2], Alaattin ÖZTÜRK[3]

[1]Department of Pathology, Sema Training and Research Center, Fatih University, İstanbul, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Fatih University, Ankara, Turkey
[3]Department of Surgery Clinics, Sema Training and Research Center, Fatih University, İstanbul, Turkey

Received: 18.06.2012 • **Accepted:** 01.08.2012 • **Published Online:** 18.01.2013 • **Printed:** 18.02.2013

Aim: To investigate inductive activity of formaldehyde (FA) on the expression of inducible nitric oxide synthase (iNOS) and level of nitric oxide (NO) in the liver of rats.

Materials and methods: Sixteen male Wistar albino rats of the same age (3 months old) were divided into 2 experimental groups, with 8 rats as the control and 8 rats formaldehyde-treated (FAt). The FAt group received intraperitoneal injection of 10 mg/kg FA for 10 days. Activity of iNOS was shown with immunohistochemical stain kits, and the concentration of NO level was measured by spectrophotometric method in the liver tissue.

Results: The FAt group had increased iNOS expression and NO levels in liver tissue compared to controls.

Conclusion: FA increased iNOS expression and NO production in liver tissue of rats.

Key words: Formaldehyde, nitric oxide, iNOS, rat liver

1. Introduction

Formaldehyde (FA), which is a one-carbon very reactive compound, can react with different cellular components, such as lipids, proteins, and nucleic acids (1). Humans are exposed to FA every day because it is found in different kinds of medicine and industrial products such as building materials, cosmetics, cigarette smoke, photochemical smog, and even various fruits (2). It can also be physiological formed by the metabolism of some amino acids or methylamine, and it can contribute to biological methylation by folic acid (3,4). After intraperitoneal, oral, or inhaler administration, FA rapidly diffuses into many tissues, including the brain, testis, and liver (5). Formaldehyde can cause gene mutations, carcinogenesis, neurodegenerative disorders, digestive symptoms, and spermatogenic inhibition (6–8). Many studies have already been done to learn the pathogenesis of FA toxicity, but answers are not clear yet. Nitric oxide (NO) is one of the crucial regulators of numerous biological processes, including vascular tone, transfer of nerve impulses, inflammation, thrombosis, motor activity, secretion, and macrophage-mediated immunity. NO is produced by nitric oxide synthase (NOS) activity from arginine and it has double-sided effects. It is synthesized from the terminal guanidine nitrogen of the semiessential amino acid L-arginine, which is converted to L-citrulline in a complex reaction involving molecular oxygen and NADPH as cosubstrates; and enzyme-bound heme and flavin nucleotides including FAD and FMN, and tetrahydrobiopterin, as cofactors; and it is catalyzed by nitric oxide synthases (9). Three major isoforms of NOS, which are each encoded by different genes, have so far been identified: endothelial NOS (eNOS) and neuronal NOS (nNOS), which are expressed constitutively and are collectively termed constitutive nitric oxide synthase (cNOS); and induced NOS (iNOS) by endotoxin and inflammatory mediators, such as cytokines (10–12). The aim of the present study was to investigate the inductive activity of FA on the expression of iNOS and levels of NO in the liver of rats.

2. Materials and methods

2.1. Animals and design

Three-month-old Wistar albino rats, weighing between 250 and 300 g, were obtained from the Fatih University Medical Faculty Experimental Research Center and housed

* Correspondence: eucmakli@gmail.com

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. Twelve male rats were randomly allotted into 1 of 2 experimental groups: control and FA-treated (FAt) groups. Each group consisted of 6 animals. The FAt group received intraperitoneal injection of 10 mg/kg FA (37% formaldehyde, Merck, Darmstadt, Germany) for 10 days at the same time in the morning (13). After the treatment, the animals were sacrificed and liver tissues were removed for biochemical and immunohistochemical investigations.

2.2. Biochemical analysis

2.2.1. NO determination

As NO measurement is very difficult in biological specimens, tissue nitrite and nitrate were estimated as an index of NO production. The method for the nitrite and nitrate levels in the tissues was based on the Griess reaction (13). Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite+nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules with a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions of sodium nitrite. Linear regression was done using the peak area from the nitrite standard. The resultant equation was then used to calculate the unknown sample concentrations. Results were expressed as micromoles per gram protein in the tissues. Protein assays in the samples were done by the method of Lowry et al. (14).

2.2.2. Immunohistochemical detection of iNOS in tissue samples

Sections of 5 μm in thickness were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 3% H_2O_2 in deionized water for 5–10 min. Nonspecific binding sites were blocked by incubating the sections in 10% normal rabbit serum for 10–15 min. The sections were then incubated with polyclonal rabbit anti-iNOS (dilution 1:25) or anti-NF-kB p65 (dilution 1:100) overnight at 4 °C, followed by incubation with biotinylated goat-antirabbit IgG at room temperature for 10–15 min. After phosphate buffered saline (PBS) rinses of 3 × 3 min, the horseradish-peroxidase-conjugated streptavidin solution was added and incubated at room temperature for 10–15 min. The antibody binding sites were visualized by incubation with a diaminobenzidine–H_2O_2 solution. The sections incubated with PBS instead of the primary antibody were used as negative controls. Brown-yellow granules in cytoplasm or nuclei were recognized as positive staining for iNOS. Activity of iNOS tissues was shown by commercial iNOS immunohistochemical stain kits. Streptavidin–biotin complex was used. Cytoplasmic stainings with iNOS were revealed to be positive.

2.3. Statistical analysis

All statistical analyses were carried out using SPSS for Windows, version 11.0. All data were presented as mean ± standard deviation (SD). Dual comparisons between groups that presented significant values were evaluated with the Mann–Whitney U test. The differences were considered to be significant at $P < 0.05$.

3. Results

In the examination of hematoxylin and eosin (H&E)-stained sections, hepatocytes in control group rats were observed to be normal; no sign of Kupffer cell hyperplasia and inflammation was encountered and portal areas appeared normal (Figure 1a). In the examination of H&E-stained preparations of FAt rat liver tissue, mild edema, mild degeneration in hepatocytes, and Kupffer cell hyperplasia were observed (Figure 1b). In the immunohistochemical examination (Figures 2a, 2b, and 3), intracytoplasmic and perinuclear localization of brown-colored iNOS expressions were observed in

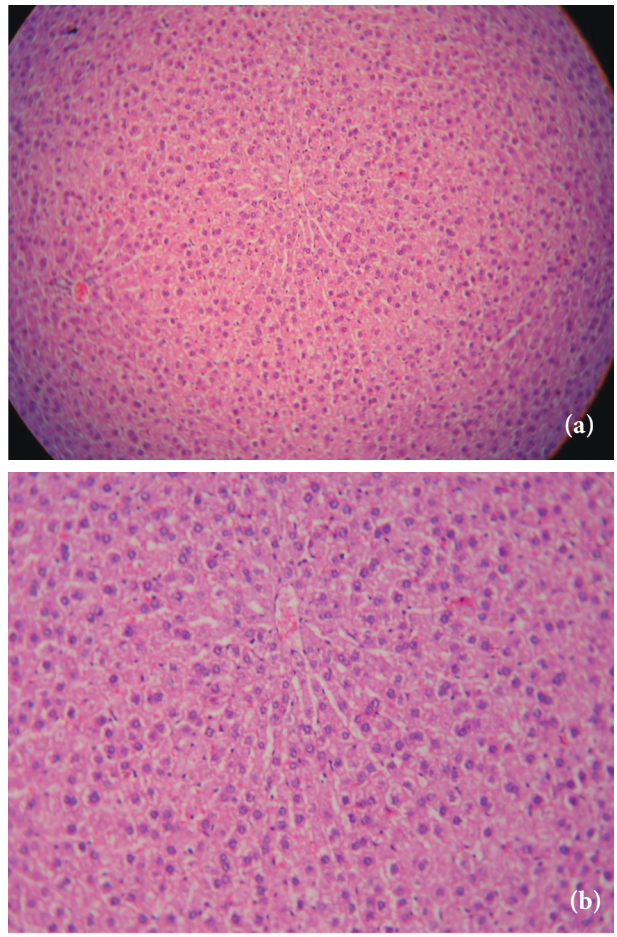

Figure 1. Observations of liver tissue: a) in control group, normal liver tissue of rat was observed (H&E 40×); b) in FAt group, nuclear degeneration of hepatocytes and hyperplasia of Kupffer cells was observed (H&E 100×).

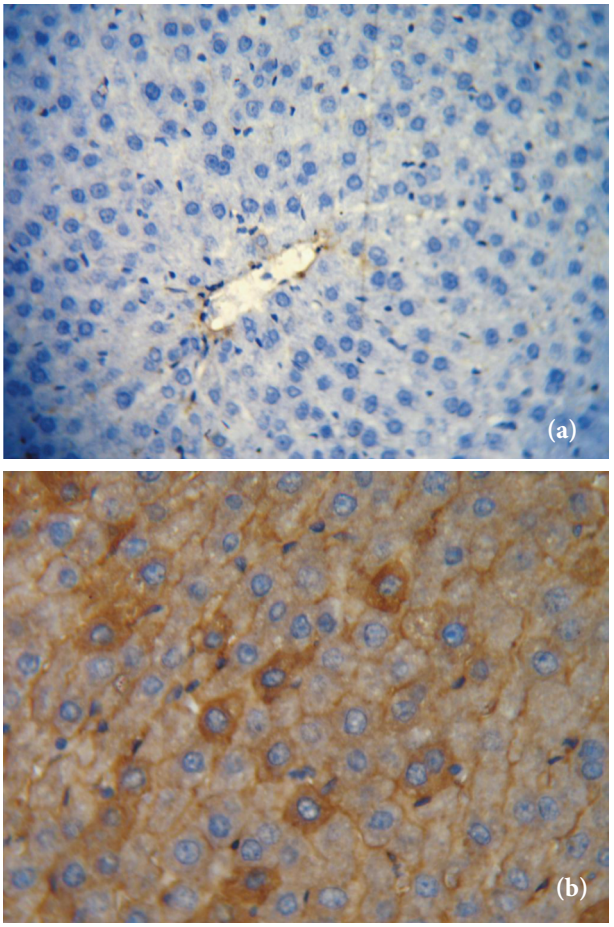

Figure 2. Immunohistochemical examination: a) in control group, cords, hepatocytes, and portal area were noted to be normal, and immunohistochemical staining was negative (200×); b) in FAt group, intracytoplasmic and perinuclear localization of brown-colored iNOS expressions were observed (400×).

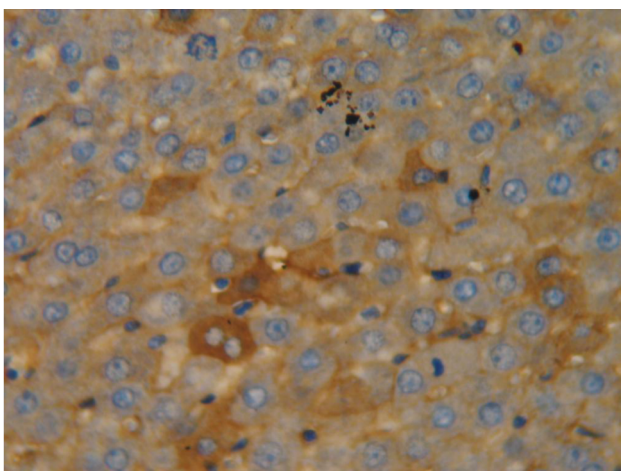

Figure 3. Intracytoplasmic and perinuclear localization of brown-colored iNOS expressions were observed in FAt group rat liver tissues (400×).

FAt rat liver tissues (Figures 2b and 3). However, in liver tissues of control group rats, no sign of any expressions were observed (Figure 2a). The semiquantitative results of tissue iNOS immunohistochemical staining are given in the Table. Tissue iNOS expression in the FAt group was found to be higher compared to the control group. The Table indicates the mean values and standard deviations of the concentrations of tissue NO in the FAt and control groups. The level of NO in the tissue was significantly increased in the FAt group as compared to the control group.

4. Discussion

Inducible nitric oxide synthase, which is an isoform of the NOS enzyme, is found in a wide variety of tissues, including the liver (15). In this study, compared to the control group in iNOS expression, an increase in the amount of liver cells in the FAt group was observed with immunohistochemical study. While there was no expression monitored in hepatocytes of the control group, iNOS expression was monitored in intracytoplasmic and perinuclear areas of hepatocytes in the FAt group. This also shows that FA has an effect on iNOS. Furthermore, the amount of NO in the liver tissues of the FAt group was detected to be significantly higher than in the control group. Increased activity of iNOS is assumed to be the reason for this. Therefore, an increment in the amount of NO is consistent with immunohistochemical findings.

FA can cause severe tissue injury by producing reactive oxygen species (16). In a literature review, no solid understanding of the impact of FA on the tissue expression of iNOS was encountered. In several different experimental studies on animal models, it was reported that FA caused an increase in the amount of NO in tissue and serum. Gulec et al. claimed that FA caused the amount of NO in and liver tissues to increase (17). In addition, the results of the present study are supported by the articles recently written by Franklin et al. They demonstrated an association between inhaled FA concentrations and exhaled NO levels in their study performed in children with no previous airway damage and atopy (18). The mechanism of the effect of FA on the expression of iNOS is not exactly clear. We assume that this effect occurs in 1

Table. The liver NO levels (mean ± standard deviation) and semiquantitative iNOS expression in rat tissues.

	Control (n = 6)	FAt (n = 6)
NO (μmol/g protein)	0.126 ± 0.016	0.198 ± 0.038*
iNOS expression	+	+++

*P < 0.005 compared to control group.

of 2 ways. The first is that FA or its metabolites act like a stimulator/activator that induces iNOS protein synthesis directly. The fact that FA is a water-soluble molecule, which easily diffuses into membranes and directly cross-reacts with DNA-protein chains, supports this approach (19). Nevertheless, this approach should be supported with cell cultures and/or advanced analysis methods. The second way may be through cytokines (20).

Several studies showed that cytokines such as TNF-α and IL-1β are effective on iNOS mRNA synthesis and iNOS activity or expression (20,21). The researchers reported that FA also has a stimulating impact on cytokines, which affects iNOS metabolism (22,23). Therefore, the increase in liver cell expression iNOS is thought to be mediated by cytokines. The liver is an organ that metabolizes many toxic/nontoxic compounds, including FA. In this study, edema and degeneration findings were observed in hepatocytes by examining the liver tissues of the FAt group with H&E staining.

Indeed, in other studies of light and electron microscopy, the following observations were reported in the liver cells of experimental animals exposed to FA: flat endoplasmic reticulum hypertrophy and hyperchromatic nucleus (24), rough endoplasmic reticulum and mitochondrial damage (25), and impairment of membrane integrity (26). It was also reported that FA caused damage to intrahepatic and extrahepatic bile ducts in addition to hepatocytes (27). Moreover, it was asserted that FA led to similar cellular changes in other organs (28,29). Along with all these histopathologic findings, it was also reported that FA caused increase in the activities of enzymes that were used as indicators of liver tissue damage, such as serum aspartate transaminase, alanine transaminase, and alkaline phosphatase, and decrease in the amount of albumin and total protein (30).

In conclusion, this study showed that FA caused an increase in iNOS expression in hepatocytes and an increase in the amount of NO in liver tissues.

References

1. Cheng G, Shi Y, Sturla SJ, Jalas JR, McIntee EJ, Villalta PW et al. Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem Res Toxicol 2003; 16: 145–52.
2. Metz B, Kersten GF, Hoogerhout P, Brugghe HF, Timmermans HA, De Jong A et al. Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem 2004; 279: 6235–43.
3. Conaway CC, Whysner J, Verna LK, Williams GM. Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation. Pharmacol Ther 1996; 71: 29–55.
4. Trezl L, Hullan L, Szarvas T, Csiba A, Szende B. Determination of endogenous formaldehyde in plants (fruits) bound to L-arginine and its relation to the folate cycle, photosynthesis and apoptosis. Acta Biol Hung 1998; 49: 253–63.
5. Smith AE. Formaldehyde. Occup Med 1992; 42: 83–8.
6. Soffritti M, Maltoni C, Maffei F, Biagi R. Formaldehyde: an experimental multipotential carcinogen. Toxicol Ind Health 1989; 5: 699–30.
7. Kilburn KH. Neurobehavioural impairment and seizures from formaldehyde. Arch Env Health 1994; 49: 37–44.
8. Chowdhury AR, Gautam AK, Patel KG, Trivedi HS. Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. Indian J Physiol Pharmacol. 1992; 36: 162–8.
9. Kaplan İ, Aydın Y, Bilen Y, Genç F, Keleş MS, Eroğlu A. The evaluation of plasma arginine, arginase, and nitric oxide levels in patients with esophageal cancer. Turk J Med Sci 2012; 42: 403–9.
10. Akar İ, Rahman A, Çolak MC, Üstündağ B, Özercan İH, Uysal A. Immunohistochemical evaluation of the effects of nebivolol on intimal hyperplasia following endothelial injury. Turk J Med Sci 2011; 41: 53–63.
11. Maffei CM, Mirels LF, Sobel RA, Clemons KV, Stevens DA. Cytokine and inducible nitric oxide synthase mRNA expression during experimental murine cryptococcal meningoencephalitis. Infect Immun 2004; 72: 2338–49.
12. Wen J, Rirbeiro R, Zhang Y. Specific PKC isoforms regulate LPS stimulated iNOS induction in murine microglial cells. J Neuroinflammation 2011; 8: 38–40.
13. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium reduction method. Clin Chem 1990; 36: 1440–3.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 183: 265–75.
15. McNaughton L, Puttagunta L, Martinez-Cuesta MA, Kneteman N, Mayers I, Moqbel R et al. Distribution of nitric oxide synthase in normal and cirrhotic human liver. Proc Natl Acad Sci U S A 2002; 99: 1716–7.
16. Gurel A, Coskun O, Armutcu F, Kanter M, Ozen OA. Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. J Chem Neuroanat 2005; 29: 173–8.
17. Gulec M, Gurel A, Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol Cell Biochem 2006; 290: 61–7.
18. Franklin P, Dingle P, Stick S. Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med 2000; 161: 1757–9.

19. Speit G. The implausibility of systemic genotoxic effects measured by the comet assay in rats exposed to formaldehyde. J Proteome Res 2006; 5: 2523–4.

20. Förstermann U, Kleinert H. Nitric oxide synthase: expression and expressional control of the three isoforms. Naunyn Schmiedebergs Arch Pharmacol 1995; 352: 351–64.

21. Horie A, Nagai K, Ohkura S, Ohama T, Komatsu H, Sato K. Proinflammatory cytokines suppress the expression level of protease-activated receptor-2 through the induction of iNOS in rat colon. J Vet Med Sci 2009; 71: 1609–15.

22. Yabuuchi K, Maruta E, Minami M, Satoh M. Induction of interleukin-1 beta mRNA in the hypothalamus following subcutaneous injections of formalin into the rat hind paws. Neurosci Lett 1996; 207: 109–12.

23. Persoz C, Achard S, Leleu C, Momas I, Seta N. An in vitro model to evaluate the inflammatory response after gaseous formaldehyde exposure of lung epithelial cells. Toxicol Lett 2010; 195: 99–105.

24. Cikmaz S, Kutoglu T, Kanter M, Mesut R. Effect of formaldehyde inhalation on rat livers: a light and electron microscopic study. Toxicol Ind Health 2010; 26: 113–9.

25. Strubelt O, Younes M, Pentz R, Kühnel W. Mechanistic study on formaldehyde-induced hepatotoxicity. J Toxicol Environ Health 1989; 27: 351–66.

26. Ku RH, Billings RE. Relationships between formaldehyde metabolism and toxicity and glutathione concentrations in isolated rat hepatocytes. Chem Biol Interact 1984; 51: 25–36.

27. Dumont M, D'Hont C, Moreau A, Mbape H, Feldmann G, Erlinger S. Retrograde injections of formaldehyde into the biliary tree induce alterations of biliary epithelial function in rats. Hepatology 1996; 24: 1217–23.

28. Lino-Dos-Santos-Franco A, Domingos HV, de Oliveira AP, Breithaupt-Faloppa AC, Peron JP, Bolonheis S et al. Differential effects of formaldehyde exposure on the cell influx and vascular permeability in a rat model of allergic lung inflammation. Toxicol Lett 2010; 197: 211–8.

29. Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T et al. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 1997; 22: 239–54.

30. Beall JR, Ulsamer AG. Formaldehyde and hepatotoxicity: a review. J Toxicol Environ Health 1984; 14: 11–21.