Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 8 (4): 405-409
15th June, 2008

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Hussain Md. Shahjalal
Department of Biochemistry and Molecular Biology,
Jahangirnagar University,
Savar, Dhaka-1342, Bangladesh

Formaldehyde Content in the Rui Fish (*Labeo rohita*) in Bangladesh and Effect of Formaldehyde on Lipid Peroxidation in Rat Liver and Intestinal Tissues

[1]Md. Shahdat Hossain, [1]Mohammad Azizur Rahman,
[2]Tofael Kabir Sharkar and [1]Hussain Md. Shahjalal

This study was conducted to estimate formaldehyde levels in the rui fish (*Labeo rohita*) in Bangladesh. Results showed that formaldehyde levels were significantly higher in the imported rui fish than that of the fresh rui of local ponds [mean levels: 13.40±0.52 vs. 3.95±0.40 (nmol mg^{-1} of fish protein)]. The effect of formaldehyde on lipid peroxidation was also examined and found a significantly higher lipid peroxide levels in the liver and the intestinal tissues of the orally formaldehyde-administered rats (4.8 and 3.7 folds, respectively) than those of the control rats. A dose dependent increase of lipid peroxide value also was observed in rat liver and intestinal epithelial cell homogenates, when these were *in vitro* treated with different concentration of formaldehyde (0~500 μM). These results suggest that formaldehyde has considerable tissue damaging effects; thus formaldehyde consumption through fomalinated-fish should be avoided.

Key words: Formaldehyde, *Labeo rohita*, lipid peroxide

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[1]Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[2]Immunology Lab, Laboratory Sciences Division, ICDDR, B: Knowledge for Global Lifesaving Solutions, Mohakhali, Dhaka-1212, Bangladesh

INTRODUCTION

Thirty seven percent formaldehyde solution, known as formalin, is characterized as an inexpensive and effective preservative that rapidly penetrates the tissue. It is frequently used as one of the most common preservatives for fish. Besides, it is widely used as a disinfectant in many human medicines and cosmetics and as an antiseptic in veterinary drugs and biologicals and in fungicides, textiles and embalming fluids (Feick *et al*., 2006; Ross *et al*., 2002; IARC, 1982). In the urban area of Bangladesh, the most commonly consumed fish is rui (*Labeo rohita*), majority of which come from neighboring countries like India and Myanmar. These rui fish are usually treated with excess formalin prior to import. But the levels of formalin used in these fish and their possible harmful effects on body tissues are not known clearly. Some reports suggest that ingested formaldehyde causes inflammation of the linings of the mouth, throat and gastrointestinal tract and eventual ulceration and necrosis of the mucous lining of the gastrointestinal tract (Yanagawa *et al*., 2007; Sidhu and Sidhu, 1999; Owen *et al*., 1990) and in case of chronic exposure, formaldehyde has the potential to cause cancer and a variety of unknown pathology (Hildesheim *et al*., 2001; Vaughan *et al*., 2000; Wippermann *et al*., 1999). Thus, if the fish with high formaldehyde levels are consumed by human for a long period, they may encounter a host of biochemical, as well as, pathophysiological abnormalities, although, to what extent it may cause such abnormalities and subsequent health hazards have remained unclear.

The objectives of this study were to estimate the levels of formaldehyde in the rui fish (*Labeo rohita*) in Bangladesh and to examine the possible detrimental effects of formaldehyde in rat liver and intestinal tissues. Toxic compounds may cause cellular damage, which is often exhibited by the electron rich reactive species, called lipid peroxides (LPO) (Halliwell *et al*., 1992). Therefore, by measuring the levels of LPO, the extent of tissue damage caused by formaldehyde could simply be evaluated.

MATERIALS AND METHODS

Fish: The study was conducted from August, 2005 to February, 2006. Thirty-five imported rui fish (*Labeo rohita*) weighing ~2.5 kg were purchased from 7 different markets of Dhaka city. A similar number of fresh rui fish of similar size and weight were collected from 7 different ponds away from Dhaka city. Both the imported and the fresh rui fish were brought to the laboratory and stored on ice at temperature around 0°C.

Preparation of fish tissue homogenates and estimation of formaldehyde: Fish were cut into small pieces, bones and fins were separated. The tissue (2.0 g) was homogenized with 10 mL phosphate buffer (25 mM KH_2PO_4-NaOH, pH 7.4) using a polytron homogenizer (PT 1200C, Kinematica AG, Switzerland). The homogenates were centrifuged at 16000 x g for 20 min. (Eppendorf Centrifuge, Model: 5415D, Germany) and supernatants were collected. Formaldehyde was estimated in the supernatants using formaldehyde standards (25-200 μM) in phosphate buffer pH 7.4.

The samples/standards were mixed with 34.2 mM Purpald in 480 mM HCl and the reaction mixtures were incubated with continuous shaking for 60 min. at room temperature. The reaction was terminated by addition of 50 μL 7.8 M KOH solution. Thereafter, the reaction mixtures were incubated further for 10 min at room temperature and the reaction product was oxidized by 65.2 mM KOH (Johansson and Borg, 1988). The absorbance was measured at 550 nm (U best-30, JASCO, Japan). Phosphate buffer pH 7.4 was used as blanks.

Rats: A total of 28 *Long Evans* male rats weighing ~250 g were recruited. They were housed in an animal room at ~25°C, under 12 h dark-light cycles (light 8:00-20:00 h; dark 20:00-08:00 h) before use.

Oral administration of formaldehyde to rats and preparation of liver and intestinal tissue homogenates: Of the 28 rats, 14 were orally administered to a single dose of formaldehyde (100 mg). The remaining 14 rats were administered to a single dose of 0.50 mL phosphate buffer pH 7.4. These rats were considered as formaldehyde un-administered control rats. After 15 min. rats were anesthetized using pentobarbital; whole liver and small intestine were removed. These tissues (2.0 g) were, separately homogenized with 10 mL phosphate buffer pH 7.4 using a polytron homogenizer. The resulting homogenates were centrifuged at 16000 x g for 20 min to remove the unbroken tissues or cells and the supernatants were subjected to lipid peroxide (LPO) estimation.

Preparation of liver and intestinal epithelial cell homogenates of control rat and *in vitro* treatment with formaldehyde: The liver and the small intestine of the formaldehyde un-administered control rats were isolated. The small intestine was perfused with ice-cold phosphate buffer pH 7.4 in waxed-Petri dish, segmented into small pieces of 7~8 cm, cleaned and epithelial cells were collected by scraping with a stainless metallic loop. These

epithelial cells and the liver tissue (2.0 g of each) were separately homogenized with 10 mL phosphate buffer pH 7.4 using a polytron homogenizer. Thereafter, the homogenates (50.0 μL of each) were treated with 0, 62.5, 125, 250 and 500 μM formaldehyde for 1 h and were subjected to LPO estimation.

Estimation of LPO: LPO levels were estimated by the thiobarbituric acid (TBA) test (Ohkawa *et al*., 1979) with slight modification. In brief, the homogenates were mixed with 0.02% butyl hydroxytoluene to inhibit spontaneous oxidation. To each 50 μL of homogenate sample, 300 μL of 8.1% sodium dodecylsulphate (SDS), 2.0 mL of 0.04% TBA in 20% acetic acid (pH 3.5) and 500 μL of distilled water were added. The mixtures were incubated at 95°C for 1 h. After cooling with tap water, 1.0 mL distilled water and 2.5 mL of n-butanol-pyridine (15:1, v/v) were added and the mixtures were shaken vigorously for about 20 min. After centrifugation at 1000 x g for 10 min, the absorbance of the upper organic layer was determined at 530 nm. Malonyldialdehyde levels were calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane.

Protein assay: Homogenized tissues were heated at 80°C for one hour in 0.2 N NaOH to solubilize the protein content. Each aliquot was centrifuged at 2000 x g for 30 min. The supernatants were used for protein assay according to the method of Lowry *et al*. (1951).

Statistical analysis: Results are expressed as mean±SEM (Standard Error of Mean). For two-group differences, data were analyzed by Unpaired t-test. For more than two-group differences, data were analyzed by one-way ANOVA. ANOVA was followed by Fisher's protected least square differences (PLSD) for post hoc comparisons. The statistical program used was StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, USA). $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Rui fish (*Labeo rohita*) imported in Bangladesh from neighboring countries are usually preserved with formalin. Therefore, it is assumed that the imported rui fish may retain formaldehyde in their tissues. In Bangladesh, reports are not available about the levels of formaldehyde in these preserved fish. Most of the people of Bangladesh are not aware about the health hazards of formaldehyde consumption with fish. This study was conducted to examine the levels of formaldehyde in the rui fish, as well as, to evaluate the possible detrimental effects of formaldehyde in rat liver and small intestinal tissue.

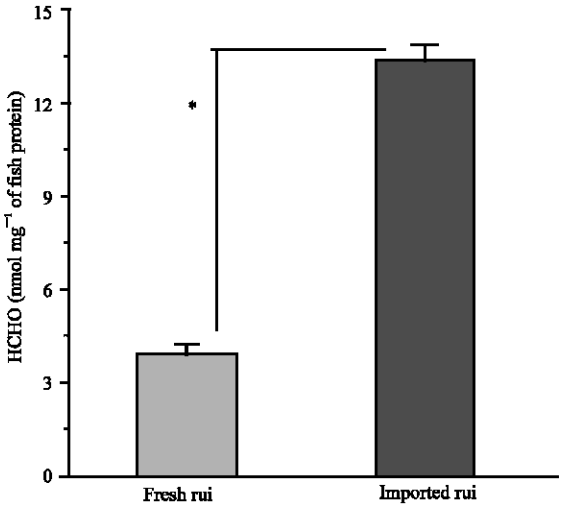

Fig. 1: Formaldehyde levels in fish tissue. Results are expressed as mean±SEM (n = 35). Unpaired t-test was performed for data analysis. *$p<0.05$ was considered statistically significant

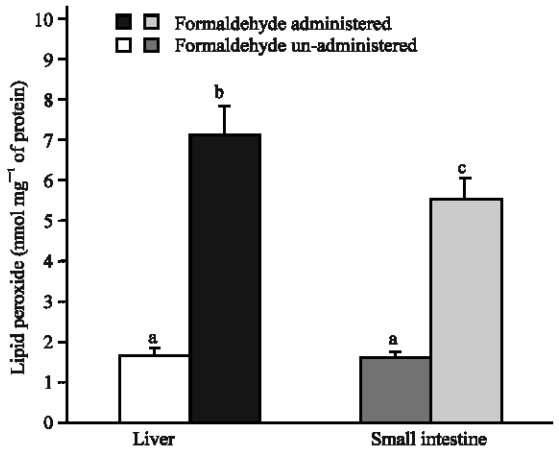

Fig. 2: Effect of orally administered formaldehyde on lipid peroxidation in rat liver and small intestinal tissues. Results are expressed as mean± SEM (n = 14). Bars with different notations are significantly different at $p<0.05$. One-way ANOVA was performed for data analysis. ANOVA was followed by Fisher's protected least square differences (PLSD) for post hoc comparisons

The imported rui fish and the fresh rui fish of local ponds examined are very similar in size, shape, color and appearance. The imported rui fish had significantly higher formaldehyde levels (~3.4 folds) than that of the fresh rui of local ponds (Fig. 1), indicating that excessive formalin was used for preservation of the rui fish prior to

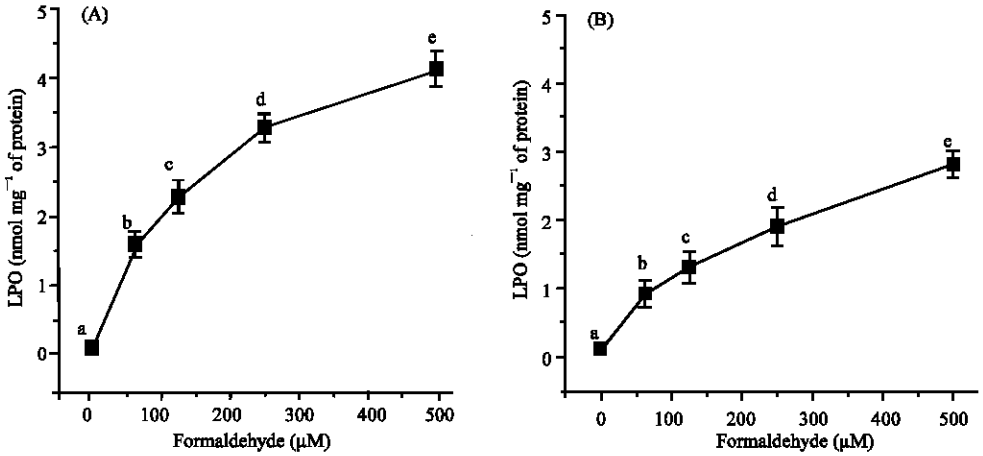

Fig. 3: Dose dependent effect of *in vitro* formaldehyde treatment on lipid peroxidation in rat liver tissue (A) and intestinal epithelial cells (B). Each symbol indicates mean±SEM (n = 14). Symbols with different notations are significantly different at $p<0.05$. LPO: Lipid peroxide. One-way ANOVA was performed for data analysis. ANOVA was followed by Fisher's protected least square differences (PLSD) for post hoc comparisons

import. Formaldehyde was also present to some extent in the fresh rui fish. The storage of fish on ice might be responsible for it, as formaldehyde is obtained in frozen fish by means of enzymatic reactions (Bianchi *et al*., 2007).

In this study, the extent of tissue damage caused by formaldehyde was also evaluated by measuring the levels of LPO. The liver and small intestinal tissues of the orally formaldehyde-administered rats had significantly higher LPO levels than that of the liver and intestinal tissues of the control rats (Fig. 2). The increase in liver and in small intestine was 4.8 vs. 3.7 folds as compared to control, indicating that formaldehyde has considerable tissue-damaging effects in liver, as well as, in small intestine.

The effects of *in vitro* formaldehyde treatment on LPO in rat liver tissue and intestinal epithelial cells were also examined. Different concentrations of formaldehyde (0~500 μM) were used in this purpose. Results show that the LPO levels were dose dependently increased ($p<0.05$) in both the liver tissues (Fig. 3A) and the intestinal epithelial cells (Fig. 3B), although the effect is more prominent in the liver tissues than in the small intestinal cells. Thus, the *in vitro* effect of formaldehyde on lipid peroxidation supports the *ex vivo* effect. Now, the question is, why LPO levels were increased in liver and small intestine after formaldehyde treatment.

The production of LPO and other oxidative species like H_2O_2, superoxide radicals ($O^{\bullet}_2$), hydroxyl radicals ($\bullet OH$) is an intrinsic phenomenon of normal cellular metabolism. The oxidative species are neutralized by endogenous antioxidative enzymes like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) (Benov *et al*., 1990), as well as, by antioxidant substrates like reduced glutathione (GSH) (Kidd, 1997), vitamin E (Dundar and Aslan, 2000) and other free radical scavengers. When the production of oxidative species exceeds the endogenous protections of CAT, GPx, SOD, GSH, the resulting damage to cellular constituents is known as oxidative stress. After formaldehyde administration, it might have acted upon antioxidants and reduced the activities of antioxidative enzymes and/or antioxidant substrates; otherwise the LPO levels could have not been raised.

Formaldehyde metabolism in the body requires a number of enzymes. One of which is formaldehyde dehydrogenase (FDH). It is present in all animal tissues tested (Achkor *et al*., 2003). FDH system is active in both the cytosol and the mitochondria. The cytosolic form is dependent on reduced glutathione (GSH). Thus, the increased LPO levels in the liver and small intestine after formaldehyde treatment is consistent to the fact that GSH is exploited biochemically in order to metabolize formaldehyde.

Although, LPO levels were increased after formaldehyde treatment, it is much higher in liver than in small intestinal tissues in both the *ex vivo* and *in vitro* experiment. The exact reasons for this variation are not clearly understood. The intestinal tissues serve principally as the site for absorption of nutrients, water and both beneficial and potentially harmful xenobiotics. In the rat, it is about 90 cm long and its luminal surface is composed of monolayer of enterocytes with numerous finger-like projections of villus. It is, therefore, speculated that formaldehyde that was orally administered to the rats

had limited time-exposure in the intestine as compared to that in the liver, the ultimate first-pass metabolic reservoir. Thus, after oral administration, the oxidative stress of formaldehyde was lower in the intestine when compared to that in the liver, despite its higher antioxidative defense than the former. We conclude that after oral administration formaldehyde affects mainly the liver where its catabolism occurs, with a concomitant increase in the extent of cell damage.

The enzymatic catabolism of formaldehyde occurs prevalently in the liver with the expense of antioxidants. This might be related with the more significant effect of formaldehyde on the oxidative stress in the liver tissues than in the small intestinal cells in *in vitro* experiment, as antioxidants are exploited biochemically in order to metabolize formaldehyde. However, further study will be required to know the actual reason.

As formaldehyde affects a diverse biochemical process of the body tissues, people should avoid consumption of formalin-preserved fish, especially rui fish (*Labeo rohita*) that come in Bangladesh from neighboring countries. The government should take necessary measures to prevent the use of formalin as fish preservative and also generate awareness in the consumers about the harmful effects of formaldehyde.

ACKNOWLEDGMENT

This study was supported, in part, by a Grant-in-Aid from Jahangirnagar University, Savar, Dhaka, Bangladesh [Grant No. 1234 (140), 2005].

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