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Changes to Oxidative Stress Levels Following Exposure to Formaldehyde in Lymphocytes

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Abstract**—**Formaldehyde is the illegal chemical substance used for food preservation in fish and vegetable. It can promote carcinogenesis. Superoxide dismutases are the important antioxidative enzymes that catalyze the dismutation of superoxide anion into oxygen and hydrogen peroxide. The resultant level of oxidative stress in formaldehyde-treated lymphocytes was investigated. The formaldehyde concentrations of 0, 20, 40, 60, 80 and 120µmol/L were treated in human lymphocytes for 12 hours. After 12 treated hours, the superoxide dismutase activity change was measured in formaldehyde-treated lymphocytes. The results showed that the formaldehyde concentrations of 60, 80 and 120µmol/L significantly decreased superoxide dismutase activities in lymphocytes ($P < 0.05$). The change of superoxide dismutase activity in formaldehyde-treated lymphocytes may be the biomarker for detect cellular injury, such as damage to DNA, due to formaldehyde exposure.

Keywords**—**Formaldehyde, lymphocytes, superoxide dismutase activity.

I. Introduction

FORMALDEHYDE is a flammable chemical substance. It is a colorless gas with a strong and pungent odor at room temperature. Formaldehyde is a widely used chemical in many industries and household products. This compound is used in a variety of products such as paint, adhesives, cosmetics and pharmaceutical products. It is used in pressed-wood products such as particleboard, plywood and fiberboard. In addition, formaldehyde is commonly used as an industrial fungicide, germicide and disinfectant. It is a preservative in mortuaries and medical laboratories. Formaldehyde also occurs naturally in the environment. It is produced in small amounts by most living organisms as part of normal metabolic processes. Formaldehyde may be present in food artificially through contamination or in nature. Ingestion of formaldehyde may cause corrosion in the gastrointestinal tract. It may also cause ulcers and inflammation in the mouth and esophagus. Severe exposure to formaldehyde through ingestion may cause abdominal pain, diarrhea and possible hemorrhage in the stomach or intestines. Acute exposure to formaldehyde via inhalation causes irritation in the eyes, throat and nasal cavities. Prolonged or chronic exposure to formaldehyde via inhalation may also lead to labored breathing and lesions in the lungs, potentially causing irreparable damage to the lungs. Long-term exposure to formaldehyde can result in skin sensitization and is associated with an increased risk of cancer. Individuals with an allergy to formaldehyde are prone to have severe skin irritation; skin rash characterized by redness, cracked and dry skin. Formaldehyde causes nasopharyngeal cancer. Inhalation of formaldehyde during the early postnatal period is linked to some neurological diseases that occurs in adults [1]-[3].

Reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are constantly produced during metabolic processes in all living species. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. However, excessive ROS accumulation leads to cellular injury, such as damage to DNA, protein, and lipid membrane. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by a variety of antioxidant defense mechanisms such as superoxide dismutase (SOD). Superoxide dismutases (SOD, EC 1.15.1.1) are the important antioxidative enzymes that catalyze the dismutation of superoxide anion into oxygen and hydrogen peroxide. They are an important antioxidant defense in nearly all cells exposed to oxygen [4]. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and the Ni type, which binds nickel. Copper and zinc are most commonly used by eukaryotes. The cytosols of virtually all eukaryotic cells contain an SOD enzyme with copper and zinc (Cu-Zn-SOD). The Cu-Zn enzyme is a homodimer molecule. Cu-Zn-SOD available commercially is normally purified from the bovine erythrocytes. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive center. Superoxide dismutase (SOD) catalyzes the destruction of O_2^- free radical. It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals. SOD protects hyaluronate against depolymerization by free-radicals and indicated that exogenous SOD might have an anti-inflammatory effect. The O_2^- ion, which has been considered important in aging, lipid peroxidation and the peroxidative hemolysis of red blood cells, is formed by the univalent reduction of O_2 during various enzymatic reactions or by

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ionizing radiation. There is also superoxide radical formation during leukocyte phagocytosis. SOD deficiency might lead to Heinz body hemolytic anemia [5].

The affect of formaldehyde exposure on lymphocyte superoxide dismutase activity was investigated in this study (in vitro). The use of formaldehyde as a food preservative is prohibited in many countries such as Thailand but some Thai merchants add formaldehyde in vegetable and fish for preservation. The change of superoxide dismutase activity in formaldehyde-treated human lymphocytes may be the biomarker which is useful for detect cellular injury, such as damage to DNA, due to formaldehyde-preservative food consumption.

II. Materials and Methods

A. Cell Line

Human lymphocyte cell line was cultured in DMEM/F12 supplemented with 10% fetal calf serum, 2mM L-glutamine and antibiotics (100µg/ml streptomycin and 50U/ml penicillin) at 37°C in a humidified incubator containing 5% CO_2. Cells were trypsinized and sub-cultured at 1:3 ratio for routine maintenance and experiments.

B. Study the Level of Superoxide Dismutase Activity Change in Formaldehyde –Treated Lymphocytes

Cells (1x 10^6 cell/ml) of human lymphocytes were plated in the 12-well plates and incubated for 24 hours at 37°C in a humidified incubator containing 5% CO_2. After incubation, the formaldehyde concentrations of 0, 20, 40, 60, 80 and 120 µmol/L were added in the cell suspensions and incubated for 12 hours. After 12 hours, the lymphocyte proliferation of all formaldehyde concentration groups were observed under microscope and the activity change of superoxide dismutase enzyme were studied.

The activity change of superoxide dismutase in formaldehyde-treated lymphocytes was done by centrifugation the suspension cells 6 x 10^6 cells at 700 xg for 2 minutes and discarded supernatant. The cell pellet was washed with ice-cold PBS, centrifuged and discarded the supernatant. The cell pellet was resuspended in 0.5mL of cold 1x Lysis buffer (10 mM Tris, pH 7.5, 150mM NaCl, 0.1mM EDTA). The cells were lysed with homogenation and centrifuged at 12000 x g for 10 minutes and collected the cell lysate supernatant. Superoxide dismutase assay in formaldehyde-treated lymphocytes was done by OxiSelect™ Superoxide Dismutase Activity Assay kit (Cell Biolabs, Inc., San Diego, CA, USA.) provided by the manufacturer.

C. Statistical Analysis

The correlation between the formaldehyde concentration and superoxide dismutase activity was studied by ANOVA. $P<0.05$ was considered to be statistically significant.

III. Results

After 12 hours treated incubation, the lymphocyte proliferation of all formaldehyde concentration groups was observed under microscope. The lymphocyte proliferation was decreased when the formaldehyde concentration increased (Figs. 1 and 2). Formaldehyde is the toxic compound and can inhibit human lymphocyte proliferation.

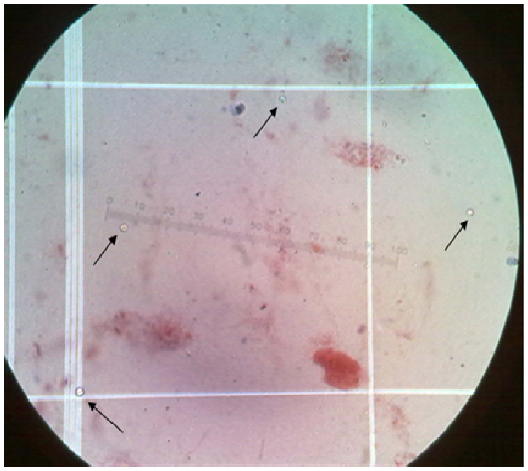

Fig. 1 The lymphocyte proliferation in the formaldehyde concentration of 60µmol/L experiment. The arrows indicated the living lymphocytes

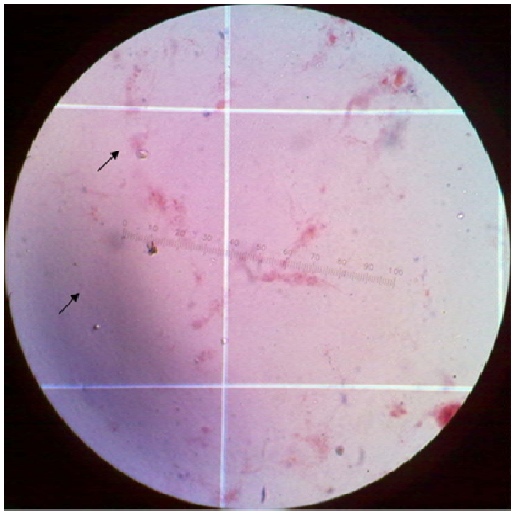

Fig. 2 The lymphocyte proliferation in the formaldehyde concentration of 120µmol/L experiment. The arrows indicated the living lymphocytes

In this study, formaldehyde significantly decreased lymphocyte superoxide dismutase activity. SOD activity levels significantly decreased when lymphocytes exposed formaldehyde concentrations at 60, 80 and 120µmol/L ($P<0.05$) comparing with the control group. The correlation between the formaldehyde concentrations in formaldehyde-

treated lymphocytes and the superoxide dismutase activities were shown in Table I.

TABLE I
THE CORRELATION BETWEEN THE FORMALDEHYDE CONCENTRATIONS IN FORMALDEHYDE-TREATED LYMPHOCYTES AND THE SUPEROXIDE DISMUTASE ACTIVITIES

The formaldehyde concentrations (µmol/L)	The superoxide dismutase activities (U/mg)
0 (control)	0.9±0.02
20	0.8±0.04
40	0.7±0.05
60	0.4±0.03*
80	0.3±0.01*
120	0.1±0.02*

* Formaldehyde significantly caused SOD activity change ($P<0.05$) comparing between the formaldehyde experiment groups and the control group

IV. DISCUSSION AND CONCLUSION

Formaldehyde is genotoxic and mutagenic to mammalian cell. Animal studies demonstrated that high concentrations of formaldehyde can cause irreversible damage to the nasal epithelium of rats and that in some cases rats exposed to these concentrations developed neoplasia [1]. The genotoxic of formaldehyde is the formation of DNA-protein crosslinks (DPC) in target tissues. *In vivo* experiments with rats and monkeys indicated that the rate of DPC formation is propotional to the tissue concentration of formaldehyde [6], [7]. The rate of formation of DPC can be regarded as a surrogate for the delivered concentration of formaldehyde and that the determination of DPC levels might improve human cancer risk estimates [2], [6], [8].

Prooxidant and antioxidant balance is vital for normal biological function of the cells and tissues [9]. Reactive oxygen species (ROS) including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical can be produced by endogenous source. ROS are important mediator of cellular injury and play a putative causing in oxidative stress. ROS-initiated stress can be regulated by cell defense mechanism including superoxide dismutases [10], [11]. SOD is the important enzymes in the antioxidant defense system, which is responsible for protecting tissues against the deleterious effects of ROS [12]. Oxidative stress occurs when the oxidative homeostasis is damaged [13]. Excessive ROS are generated and cause lipid peroxidation. Malondialdehyde (MDA), a marker of tissue injury, is one of most important products of lipid peroxidation, which interfere in protein biosynthesis by forming adducts with DNA, RNA and protein [14].

Formaldehyde caused oxidative stress and cellular injury in this study. The present findings suggest that formaldehyde exposure in lymphocytes affects the antioxidant system especially superoxide dismutase activity level. The use of formaldehyde as a food preservative is prohibited in Thailand but some Thai merchants add formaldehyde in vegetable and fish for preservation. Superoxide dismutase activity change in formaldehyde-treated human lymphocyte may be the biomarker which is useful for detect cellular injury, such as damage to DNA, due to formaldehyde-preservative food consumption.

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