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The Oxidative Damage Marker for Sodium Formate Exposure on Lymphocytes

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***Abstract*—**Sodium formate is the chemical substance used for food additive. Catalase is the important antioxidative enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). The resultant level of oxidative stress in sodium formate-treated lymphocytes was investigated. The sodium formate concentrations of 0.05, 0.1, 0.2, 0.4 and 0.6 mg/mL were treated in human lymphocytes for 12 hours. After 12 treated hours, catalase activity change was measured in sodium formate-treated lymphocytes. The results showed that the sodium formate concentrations of 0.4 and 0.6 mg/mL significantly decreased catalase activities in lymphocytes ($P < 0.05$). The change of catalase activity in sodium formate-treated lymphocytes may be the oxidative damage marker for detect sodium formate exposure in human.

***Keywords*—**Sodium formate, catalase activity, oxidative damage marker, toxicity.

I. Introduction

FOOD additives are substances added to food to preserve flavor or enhance its taste and appearance. These substances are also added to products such as pharmaceuticals, paints, biological samples, wood etc. to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product. Physical preservation entails refrigeration and drying. They are used in foods, cosmetics, and many other products. Artificial preservatives reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for preservation include dehydration, UV radiation, freeze-drying, and refrigeration [1].

Sodium formate (HCOONa) is the sodium salt of formic acid (HCOOH). It usually appears as a white deliquescent powder. It is a food additive (E237). It acts as a preservative, an anti-bacterial agent and a de-iceing agent. It is used in several fabric dyeing and printing processes. It is also used as a buffering agent for strong mineral acids to increase their pH. In structural biology, sodium formate can be used as a cryoprotectant for X-ray diffraction experiments on protein crystals, which are typically conducted at a temperature of 100K to reduce the effects of radiation damage.

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as vegetables, fruit or animals). Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7. The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide to water and oxygen each second. It does so according to the following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Any heavy metal ion can act as a noncompetitive inhibitor of catalase [2]. Furthermore, the poison cyanide is a competitive inhibitor of catalase at high concentrations of hydrogen peroxide. Arsenate acts as an activator [3].

In this study, catalase activity change was observed in lymphocytes exposed by sodium formate. The change of catalase activity in sodium formate-treated human lymphocytes may be the biomarker which is useful for detect cellular injury from oxidative damage, such as damage to DNA, due to sodium formate-food additive consumption.

II. Materials and Methods

A. Cell Line

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

B. Study the Level of Catalase Activity Change in Sodium Formate – 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 sodium formate concentrations of 0.05, 0.1, 0.2, 0.4 and 0.6 mg/mL were added in the cell suspensions and incubated for 12 hours. After 12 hours, the activity change of catalase enzyme was studied by centrifugation the suspension cells 6 x 10^6 cells at 700 x g 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

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0.5 mL of cold 1x Lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA). The cells were lysed with homogenation and centrifuged at 12000 x g for 10 minutes and collected the cell lysate supernatant. Catalase assay in sodium formate-treated lymphocytes was done by OxiSelect™ Catalase Activity Assay Kit, (Cell Biolabs, Inc., San Diego, CA, USA.) provided by the manufacturer.

C. Study the Toxic Effect of Sodium Formate on Lymphocyte Viability

The toxic effect of sodium formate on lymphocyte viability was also studied by trypan blue exclusion assay.

D. Statistical Analysis

The correlation between sodium formate concentration and catalase activity was studied by ANOVA. $P<0.05$ was considered to be statistically significant.

III. Results

A. The Level of Catalase Activity Change in Sodium Formate–Treated Lymphocytes

In this study, sodium formate significantly decreased catalase activity in lymphocytes. Catalase activity levels significantly decreased when lymphocytes exposed sodium formate concentrations at 0.4 and 0.6 mg/mL comparing with the control group ($P<0.05$). The correlation between sodium formate concentrations in sodium formate-treated lymphocytes and catalase activities were shown in Table I. The formate concentrations of 0.4 and 0.6 mg/mL were toxic for lymphocytes.

TABLE I
THE CORRELATION BETWEEN SODIUM FORMATE CONCENTRATIONS IN SODIUM FORMATE -TREATED LYMPHOCYTES AND CATALASE ACTIVITIES

Sodium formate concentrations (mg/mL)	Catalase activities (U/ml)
0 (Control)	85 ±0.03
0.05	83± 0.01
0.1	79±0.01
0.2	78±0.04
0.4	41±0.01*
0.6	34±0.04*

* Sodium formate significantly caused catalase activity change ($P<0.05$) comparing between the sodium formate experiment groups and the control group.

B. The Toxic Effect of Sodium Formate on Lymphocyte Viability

The toxic effect of sodium formate on lymphocyte viability was done by trypan blue exclusion assay. The sodium formate concentrations of 0.1 and 0.2 mg/mL were less toxic than the sodium formate concentrations of 0.4 and 0.6 mg/mL. The numbers of viable lymphocytes were found in 0.1 and 0.2 mg/mL sodium formate concentration groups more than 0.4 and 0.6 mg/mL sodium formate concentration groups. The viable cells of 0.1 and 0.2 mg/mL sodium formate concentration groups were shown in Figs. 1 and 2. The dead cells of 0.4 and 0.6 mg/mL sodium formate concentration groups were shown in Figs. 3 and 4.

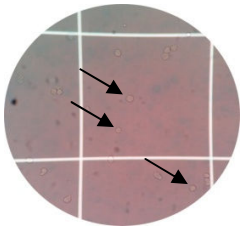

Fig. 1 The viable cells in 0.1mg/mL sodium formate concentration group

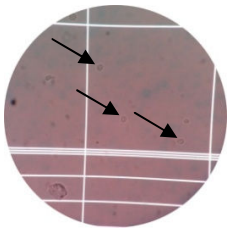

Fig. 2 The viable cells in 0.2 mg/mL sodium formate concentration group

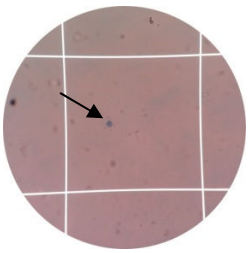

Fig. 3 The dead cell in 0.4 mg/mL sodium formate concentration group

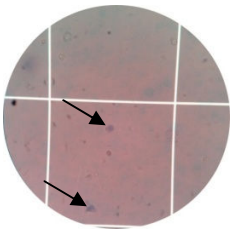

Fig. 4 The dead cells in 0.6 mg/mL sodium formate concentration group

IV. Discussion and Conclusion

Catalase occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Catalase can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. Catalase is usually located in a cellular, bipolar environment organelle called the peroxisome. Peroxisomes in plant cells are involved in photorespiration (the use of oxygen and production of carbon dioxide) and symbiotic nitrogen fixation (the breaking apart of diatomic nitrogen to reactive nitrogen atoms). Hydrogen peroxide is used as a potent antimicrobial agent when cells are infected with a pathogen. Catalase-positive pathogens, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Campylobacter*

jejuni, make catalase to deactivate the peroxide radicals, thus allowing them to survive unharmed within the host. Catalase contributes to ethanol metabolism in the body after ingestion of alcohol. It breaks down the fraction of alcohol in the body. Catalase involved in oxidative stress caused by the artificial sweetener aspartame in rat brain was studied. The use of aspartame could release methanol as one of its metabolite during metabolism. Aspartame could release methanol and induced oxidative stress in the rat brain. The blood methanol level was estimated and the free radical changes were observed in brain discrete regions by assessing the scavenging enzymes, reduced glutathione, lipid peroxidation (LPO) and protein thiol levels. There was a significant increase in LPO levels, superoxide dismutase (SOD) activity, GPx levels and catalase (CAT) activity with a significant decrease in GSH and protein thiol. Methanol and its metabolites may be responsible for the generation of oxidative stress in brain region. Catalase function was correlated with food additives such as aspartame and methanol metabolism in human [2].

Sodium formate is the sodium salt of formic acid. For commercial use, sodium formate is produced by absorbing carbon monoxide under pressure in solid sodium hydroxide at 160°C. Formic acid causes acidosis in human. The acidosis causes e.g. dilatation of cerebral vessels, facilitation of the entry of calcium ions into cells, loss of lysosomal latency and deranged production of ATP. The latter effect is to impede parathormone-dependent calcium reabsorption in the kidney tubules. Besides, urinary acidification is affected by formic acid. Its excretion causes continuous recycling of the acid by the tubular cell Cl^-/formate exchanger. Formic acid toxicity may cause histotoxic hypoxia. It affects organs with high oxygen consumption, e.g. kidney, brain and heart [4].

Sodium formate is toxic in human such as diuresis, methemoglobinemia, albuminuria, hematuria and cardiac depression. Dermal, ocular or inhalation of calcium formate or sodium formate can result in irritation. Ingestion of sodium formate may cause gastric irritation or ulcer. Formic acid disrupts mitochondrial electron transport and energy production by inhibiting cytochrome oxidase activity, the terminal electron acceptor of the electron transport chain. Cell death from cytochrome oxidase inhibition by formate is believed to result partly from depletion of ATP, reducing energy levels so that essential cell functions cannot be maintained. ATP depletion by formate comes partly from many studies showing decreased ATP synthesis in isolated mitochondria and decreased cellular ATP content in cultured neuronal cells exposed to formate. Since ATP is required for fundamental cellular functions such as operating energy-requiring ion pumps, ATP depletion may reduce cell viability by loss of ionic and volume regulatory controls. Inhibition of cytochrome oxidase by formate may also cause cell death by increased production of cytotoxic reactive oxygen species (ROS) secondary to the blockade of the electron transport chain.

There was the interesting research about antioxidants, ocular cells, formic acid and catalase. Treichel et al. [5] studied about antioxidants and ocular cell type differences in cytoprotection from formic acid toxicity in vitro. They found that retinal photoreceptors and retinal pigment epithelial (RPE) cells were sensitive to poisoning with methanol and its toxic metabolite formic acid. When exposed to formic acid in vitro, cultured cell lines from photoreceptors (661W) and the RPE(ARPE-19) were shown to accumulate similar levels of formate, but cytotoxic effects were greater in 661W cells. Catalase and glutathione were analyzed in the two retinal cell lines to determine whether differences in these antioxidant systems contributed to cell-type-specific differences in cytotoxicity. Cells were exposed to formic acid in the presence or absence of a catalase activity inhibitor, 3-amino1,2,4-triazole (AT), or a glutathione synthesis inhibitor, buthionine L-sulfoximine (BSO). Catalase protein, catalase enzyme activity, glutathione, glutathione peroxidase activity, cellular ATP, and cytotoxicity were analyzed. Compared to ARPE-19, the 661W cells showed lower antioxidant levels: 50% less glutathione, glutathione peroxidase and catalase protein, and 90% less catalase enzyme activity. In both cell types, formic acid treatment produced decreases in glutathione and glutathione peroxidase, and glutathione synthesis inhibition with BSO produced greater ATP depletion and cytotoxicity than formic acid treatment alone. Formate exposure produced decreases in catalase protein and activity in 661W cells, but increases in activity in ARPE-19. Treatment with the catalase inhibitor AT increased the formate sensitivity only of the ARPE-19 cells. ARPE-19 cells may be less susceptible to formate toxicity due to higher levels of antioxidants, especially catalase, which increased on formate treatment and which had a significant cytoprotective effect for the RPE cell line.

Formate may inhibit mitochondrial function resulting in decreased intracellular ATP and formate-induced neurotoxicity. In primary mouse neural cell cultures, formate (conc. 20 to 60 mM) was specifically neurotoxic; primarily affecting large polygonal neurons and higher concentrations of formate (conc. 120 mM) induced nonspecific cytotoxicity [6]. For study about formate-induced retinal toxicity in methanol-intoxicated rats, the researchers found that formate could induce inhibition of mitochondrial energy production, resulting in photoreceptor dysfunction and pathology [7].

The sodium salt “formic acid”; occurs widely in nature as its conjugate base formate. This anion is produced by reduction of carbon dioxide, catalyzed by the enzyme formate dehydrogenase. It is an inhibitor of the cytochrome oxidase complex, which is a vital component of the mitochondrial electron transport chain involved in ATP synthesis. Formic acid is a weaker inhibitor than cyanide and hydrosulphide anions. Formic acid is more toxic than other fatty acids, possibly owing to its enzyme-inhibiting activity. Formic acid is the toxic metabolite responsible for the metabolic acidosis and ocular toxicity observed in human methanol poisoning. Nonprimate species are ordinarily resistant to the accumulation of formate and the associated metabolic and visual toxicity. It produces retinal and optic nerve toxicity by disrupting mitochondrial energy production.

Nonprimates including mice, rats, rabbits, and dogs are resistant to methanol intoxication. In contrast, humans and nonhuman primates are uniquely sensitive to methanol poisoning. In humans and nonhuman primates, formate accumulates too much greater than endogenous concentrations, but it does not accumulate in resistant species (mice, rats, and rabbits). The species difference in formate accumulation is attributed to differences in hepatic tetrahydrofolate status. Hepatic tetrahydrofolate concentrations are significantly greater in rodents than in monkeys and humans. In rats, folate deficiency or selective reduction of tetrahydrofolate results in formic acidemia and metabolic acidosis. Recent studies had also demonstrated ocular toxicity in folate-deficient monkeys and tetrahydrofolate-deficient rats. In monkeys, folate deficiency increased formate accumulation and potentiates methanol toxicity, whereas treatment with folate derivatives decreased formate accumulation and could prevent or reverse the development of the methanol-poisoning syndrome. Rodents can remove formic acid at much higher rates than do humans can, and it is believed that the acidosis caused by formic acid is an important contributor to several manifestations of methanol toxicity [4].

In human, formic acid involves in methanol metabolism. Methanol metabolism in adults occurs primarily in the liver, but the early embryonic/fetal liver does not have similar detoxification functions and rely on other tissues for subsequent CHOH metabolism. Conversion of CH_3OH to formaldehyde (HCHO) in humans is catalyzed primarily by alcohol dehydrogenase 1 (ADHl). Alternative pathways of metabolism include catalase-mediated reactions, yielding HCHO and hydrogen peroxide. HCHO is converted to formic acid (HCOOH) by the nicotinamide adenine dinucleotide (NAD) dependent alcohol dehydrogenase 3 (ADH3). This reaction uses glutathione (GSH) as a cofactor and yields S-formylglutathione prior to thiol cleavage and HCOOH formation. Formic acid is converted to water and carbon dioxide via catalase.

High formate build up in the human body after excessive methanol intake could cause severe toxicity and may lead death. Undissociated formic acid readily crosses the blood brain barrier leading to central nervous system toxicity, aggressive alkaline therapy is required to maintain formic acid in the dissociated form.

Formic acid is essentially completely ionized at physiological pH. Formate ion distributes readily throughout total body water and under normal conditions appears in plasma or serum at concentrations of 0.02 to 0.25 mM. The concentration of formate in the erythrocytes of several species including humans is about twice that of plasma. The very rapid absorption of formate from both the peritoneal cavity and the gastrointestinal tract is consistent with its ease of distribution throughout total body water [2]. Formic acid is produced by the catabolism of several amino acids including serine, glycine, histidine, and tryptophan and by the recycling of methylthioadenosine from the polyamine biosynthesis pathway. Carbon atoms from formate are interconvertible via oxidation or reduction, respectively, of the cofactors CH_2-$H_4PteGlu_n$ and HCO-$H_4PteGlu_n$, which donate single carbon atoms used in pyrimidine and purine biosynthesis. In addition to its utilization for biosynthesis, formate is also oxidized to CO_2 and water, primarily by the action of formyltetrahydrofolate dehydrogenase on HCO-$H_4PteGlu_n$ [4].

Formic acid rapidly transfered across the placenta and thus had the potential to be toxic to the developing fetus. Formic acid decreases hCG secretion in the placenta, which may alter steroidogenesis and differentiation of the cytotrophoblasts, and this adverse effect could be mitigated by folate [8].

Sodium formate could reduce the hair follicle numbers in rats [9]. For study about glutathione depletion modulates sodium formate toxicity in cultured rat concept uses, it was shown that sodium formate exposure resulted in a high mortality rate of rat concept uses [10].

Formic acid at the lowest concentrations induced yeast cells death. Apoptosis analysis revealed that cells death was accompanied by activation of caspase. Minimal inhibitory concentrations of potassium carbonate and sodium bicarbonate induced *Candida* cells necrosis. Toxicity test with mammalian cell cultures showed that formic acid had the lowest effect on the growth of Jurkat and NIH 3T3 cells. A low concentration of formic acid induced apoptosis-like programmed cell death in the *Candida* yeast and had a minimal effect on the survivability of mammalian cells [11].

Sodium formate is toxic in human and animals. The present finding suggests that sodium formate exposure in lymphocytes affects the antioxidant system especially catalase activity level. The use of sodium formate as a food additive should be careful. Catalase activity change in sodium formate-treated lymphocytes may be the oxidative damage marker which is useful for detect oxidative stress level in human, due to sodium formate food additive consumption.

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