EXPERIMENTAL STUDY

Vitamin E Reversed Apoptosis of Cardiomyocytes Induced by Exposure to High Dose Formaldehyde During Mice Pregnancy

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Summary

In this study, we investigated the protection effect of Vitamin E (Vit E) on formaldehyde (FA) exposure during pregnancy induced apoptosis of cardiomyocytes, and used an HL-1 cell line to confirmed the findings in vivo.

Pregnant mice received different doses of FA (0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 0.1 μg Vit E, or 1.5 mg/kg + 0.1 μg Vit E). TUNEL staining was used to reveal the apoptosis in cardiomyocytes, and SOD, MDA, GSH, Livin, and Caspase-3 in cardiomyocytes were detected by ELISA, RT-PCR, and Western blot. For in vitro study, HL-1 cells were treated with vehicle, 5 μmol/L FA, 25 μmol/L FA, 50 μmol/L FA, 10 mg/L Vit E, and 50 μmol/L FA+ 10 mg/L Vit E, respectively. CCK-8 assay and flow cytometry were used to evaluate cell vitality and apoptosis. A high dose of FA exposure led to cytotoxicity in both pregnant mice and offspring, as TUNEL staining revealed a significant apoptosis of cardiomyocytes, and the alternation in SOD, GSH, MDA, Livin, and Caspase-3 was found in cardiomyocytes. 0.1 μg Vit E could reverse high doses of FA exposure induced apoptosis of cardiomyocytes in both pregnant mice and offspring. The in vitro study revealed that FA exposure induced a decrease of cell viability and increased cell apoptosis, as well as oxidative stress in HL-1 cells with alternation in SOD, GSH, MDA, Livin, and Caspase-3.

This study revealed a high dose of FA induced oxidative stress and apoptosis of cardiomyocytes in both pregnant mice and offspring, and Vit E supplement during pregnancy reversed the systemic and myocardial toxicity of FA.

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Key words: Formaldehyde, cardiomyocytes, pregnancy, Oxidative stress, Vitamin E

Formaldehyde (FA) is an important environmental pollutant in domestic and outdoor environments as it is used in construction, textiles, furniture, hospitals, laboratories, and industries, which poses a significant threat to public health. In China, the levels of FA in the occupational environment were often higher than the national standard (Chinese occupational exposure limit for FA is 0.5 mg/m³), and the occupational and domiciliary exposure of FA induced multiple adverse health effects. As a low molecular organism with high water solubility, FA reacts with proteins and nucleic acid, or with low molecular weight substances. FA could cause DNA damage and DNA repair delay after UV irradiation, and directly trigger inflammation. The International Agency for Research on Cancer has classified FA as a carcinogenic agent for nasopharyngeal cancer and leukemia. As studies showed that FA also revealed reproductive and developmental toxicity and that exposure during pregnancy increased the rate of teratogenicity, deformity, and spontaneous abortion, as well as low birthweight in offspring, and FA exposure during pregnancy was also found to induce defects in the immune and respiratory system in both maternal mice and offspring. Genotoxicity, as DNA and chromosomal damage, and cytotoxicity, as cell death or apoptosis, induced by FA was considered as the underlying mechanism of the toxicity of FA.

Reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anions, and hydroxyl radicals, play an important role in oxidative stress caused cell and DNA damage, and may contribute to a variety of disorders. Superoxide dismutase (SOD), glutathione (GSH), and other enzymatic antioxidants, such as glutathione peroxidase, were the most important constituents of the antioxidant system to scavenge harmful ROS and repair oxidized and damaged molecules. In the cardiovascular system, ROS are implicated in the pathogenesis of atherosclerosis, reperfusion injury, hypertension, and heart failure, as increased oxidative stress induced apoptosis and necrosis of myocytes. Overproduction of ROS, or low levels of available antioxidants, can cause...
DNA, proteins, and lipids damage, which implies that ROS overload during pregnancy may induce developmental toxicity and result in defects in many tissues, such as myocytes in the offspring. FA exposure can increase ROS in many tissues. Previous studies reported that FA could decrease the activity of SOD resulting in an increase of malondialdehyde (MDA), which is representative of lipid peroxidation. These results indicate that FA exposure induced an increase of ROS and may be an important underlying mechanism of FA induced cell damage.

Apoptosis is a cellular response to a wide range of toxic substances, and necrosis in cardiac tissue is often found to follow apoptosis. Apoptosis in myocytes is the most important event in the molecular mechanisms of cardiac injury. Livin and Caspase-3 are important molecules participating in apoptosis. In vitro studies have proven that exposure of FA could induce oxidative stress and trigger apoptosis progression via activating apoptosis-initiator Caspase-9 and apoptosis-effector Caspase-3/7.

Vitamin E (α-tocopherol) is an essential lipid soluble antioxidant, acting as a potent peroxyl radical scavenger, cleaning hydroperoxyl radicals in the lipid milieu. Anti-oxidant treatment has been found to be cytoprotective both in vitro and in vivo.

In this study, we hypothesised that FA exposure during pregnancy may increase oxidative stress and induce apoptosis of cardiomyocytes in maternal mice and their offspring, and Vit E may alleviate oxidative stress induced by FA.

Methods

Animals: Pregnant KM/NIH mice were obtained from the Institute of animal experiment center, University of Qingdao, and housed in a room (12/12-hour light-dark cycle, 21 ± 2°C), with free access to food and water. All of the animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experiments were approved by the Institutional Animal Care Committee, the affiliated Hospital of Qingdao University.

Experimental design and exposure to formaldehyde: 42 pregnant mice were randomly divided into 6 groups (n = 7 per group): Saline group, 0.5 mg/kg FA group, 1.0 mg/kg FA group, 1.5 mg/kg FA group, 0.1 μg Vit E group, 1.5 mg/kg FA + Vit E group. 0.5 mg/kg FA group, 1.0 mg/kg FA group, 1.5 mg/kg FA group, 1.5 mg/kg FA + Vit E group received intraperitoneal injection of 40% (w/w) FA aqueous solution (Shanghai Shenggong, F1912) with a dose of 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, and 1.5 mg/kg respectively. 0.1 μg Vit E group only received an intraperitoneal injection of 0.1 μg Vit E. For 1.5 mg/kg in the FA + Vit E group, the mice were given 0.1 μg of Vit E (0.1 μg Vit E in 0.1 mL saline, i.p., Accu Standard, ACC-VIT-012N) 0.5 hour before FA exposure. For Saline group, the animals were given 0.1 mL saline, i.p. All of these were performed during pregnancy days 13 to 20. Body weights were recorded every day. On the pregnancy day 21, cesarean sections were performed on all of the animals. The total number born, the number of stillborn fetuses, live fetuses, and deformed fetuses of each mother mouse were recorded, and the birth weight of each of the offspring was also recorded. All of the mothers and offspring were executed on the same day.

TUNEL assay and hematoxylin eosin (HE) staining: TdT-mediated dUTP nick-end labeling (TUNEL) staining labels fractured double-stranded DNA in apoptotic cells. A paraffin section of 20 μm of cardiac muscle tissue was used for the evaluation of apoptosis by TUNEL staining. The in situ cell death detection kit-POD (Roche, 11684817910) was used to determine apoptotic cells according to the manufacturer’s protocols. Counting for TUNEL-positive cells was conducted on 3-6 × 100 magnifications sections each group. The Hematoxylin and Eosin Staining Kit (Yeasea, 60524ES60) was used for HE staining in cardiac muscle according to the manufacturer’s protocols.

Quantitative real-time reverse transcription PCR (qRT-PCR): Total RNA was extracted from myocardial tissue or HL-1 cells in different experiment groups by using the Trizol (TAKARA biotechnology, China). cDNA was synthesized using 1 μg total RNA via reverse transcription kit (DBI Bioscience, USA). Primers used in qRT-PCR included: β-actin, 5'-CATTGGCTGACAGGATGCAGA-3' (sense), 5'-CTGCTGGAAGGTGGACGTA-3' (antisense); Livin 2, 5'-AGACCCAGCCTGAAGATGC-3' (sense), 5'-ACAGCTACAGTAGGCAACC-3' (antisense); Caspase-3, 5'-GCTGGAAGGTGGACGTA-3', 5':GACTGGATGAACCACCAG-3' (antisense); BNP, 5'-TTGAGCGCTCGAAATGG-3' (sense), 5'-CAGACCTCTTGACCATTTTG-3' (antisense). SyberGreen qRT-PCR master Mix (DBI Bioscience, USA) was used for qRT-PCR amplification. Cycling conditions included denaturing at 95°C for 2 min followed by annealing at 40 repeats of 94°C for 20 s and extension at 58°C for 20 s. The relative quantity of mRNA was normalized by β-actin and calculated in the delta-delta method from threshold cycle numbers. On the basis of exponential amplification of target genes, as well as a calibrator, the amount of amplified molecules at the threshold cycle is given by 2−ΔΔCt.

ELISA assay: Levels of SOD, GSH, and MDA in myocardial cells or HL-1 cells in different experiment groups were measured using an antigen-based sandwich ELISA. Mouse SOD ELISA Kit (Fanke, FK-F12115), Mouse GSH ELISA Kit (Fanke, FK-F11514), Mouse MDA ELISA Kit (Fanke, FK-F11633) were used, and the absorbance was then measured at 450 nm using a microplate spectrophotometer (Thermo, Multiskan GO).

Western blotting assay: Forty mg myocardial tissues or HL-1 cells in different experiment groups were placed in buffer with phosphatase and protease inhibitors (Bioss). Twenty μl of total protein was separated on 12% Tris-glycine polyacrylamid gels (Pagen). Samples were transferred to PVDF membranes (Pall Corporation), and incubated with rabbit anti-Livin (Sigma-Aldrich, L9544-25 UL), rabbit anti-Caspase-3 (CALBIOCHEM, 235412-100 ULorbi101550), rabbit anti-GAPDH (ABCLONAL, AC 001). HRP Goat anti-Rabbit IgG (BOSTER, BA1054) was used as secondary antibodies. The specific protein band intensities were quantified by Image-Pro Plus 6.0.
Cell preparation and culture: HL-1 mice cardiac ventricular myocytes were obtained from Sixin Biotechnology Company, Shanghai, China. HL-1 cells were cultured in a Claycomb medium with 10% FBS, 1% penicillin/streptomycin, 1% norepinephrine, and 1% l-glutamine, in an atmosphere with 5% CO_2 and at 37°C. When the cells reached ~80% confluence, the HL-1 were used for subsequent experiment. The HL-1 myocytes were divided into six groups: the control group, the 5 μmol/L FA group, the 25 μmol/L FA, the 50 μmol/L FA group, 10 mg/L Vit E group, and the 50 μmol/L FA+ 10 mg/L Vit E group. In the 5 μmol/L FA group, the 25 μmol/L FA, the 50 μmol/L FA group, HL-1 cells were incubated with FA aqueous solution (Shanghai Shenggong, F1912) with a concentration of 5 μmol/L, 25 μmol/L, 50 μmol/L, respectively. Ten mg/ L Vit E group cells incubated with 10 mg/L Vit E. For 50 μmol/L FA, 10 mg/L Vit E group, cells were cultured with FA aqueous solution with concentration of 50 μmol/ L and Vit E with a concentration of 10 mg/L. For the control group, cells were cultured with 0.1 mL saline.

Cell viability assay: Cell viability was determined by using the Dojindo Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan). Cells in different experiment groups were seeded into a 96-well plate at a density of 1 × 10^4 cells per well. After 24 or 48 hours, CCK-8 solution (10 μl in each well containing 100 μl of medium) was added. Plates were incubated at 37°C for 4 hours, and the absorbance at 490 nm was then measured. All experiments were done in triplicate and performed three times.

Cell apoptosis analysis by flow cytometry: Cell apoptosis assays were detected via apoptosis detecting kit (Invitrogen, Burlington, Canada). HL-1 cells of different experiment groups (5 × 10^4 cells per group) were disposed with trypsase, and then cells were washed and centrifuged at 1000 rpm for 10 minutes. Cells were resuspended in PBS and incubated in binding buffer containing 5 μL Annexin V and 5 μL PI and put in the dark for 15 minutes according to the kit instructions. After incubation, samples were immediately analyzed by flow cytometry (Beckman Coulter, Epics XL).

Immunofluorescent: The cells were made permeable with 0.5% Triton X-100 for 30 minutes. After being washed with PBS for three cycles, 5 minutes for each cycle, the cells were blocked in 10% goat serum for 15 minutes. Primary rabbit polyclonal antibodies to Livin (Abcam, diluted 1:50) were then added and the cells were incubated overnight at 4°C in 1% goat serum. Staining was performed by incubating the cells with fluorescein isothiocyanate secondary antibody (Alexa Fluor594-conjugated) for 1 hour. After incubation with the secondary antibody, cells were washed and then stained with 4, 6-diamino-2-phenyl indole (DAPI) for 5 minutes at room temperature. After three cycles of 5-minute washes with a PBS buffer, the slides were fixed and imaged with the fluorescent microscopy at 400 × magnification.

Statistical analysis: Data are expressed as the means ± SEM, and one-way ANOVA followed by the Tukey’s post-hoc test for multiple comparisons were used by the Graph Pad software V.5. P-values less than 0.05 were considered statistically significant.

Results

Vitamin E reversed high dose FA exposure induced apoptosis of cardiomyocytes in maternal mice and offspring: A TUNEL assay was used to determine the level of cardiomyocytes apoptosis. HE staining demonstrated that FA exposure resulted in cytopathic effects in cardiomyocytes in both maternal mice and their offspring (Figures 1A, 2A). For maternal mice, in FA exposure groups, the TUNEL-positive cell was observed in the cardiomyocytes tissue (Figure 2B), and quantitative data showed that the percentage of apoptotic cells was significantly increased along with increase of FA dose, while Vit E reversed FA exposure induced apoptosis of cardiomyocytes (Figure 2C, P < 0.05). While in the offspring, only in 1.0 mg/kg group and 1.5 mg/kg group was found significantly increased in the TUNEL-positive cell (Figure 1B), and Vit E could reverse this trend (Figure 1C, P < 0.05).

FA exposure during pregnancy increased oxidative stress in cardiomyocytes in maternal mice and their offspring: To investigate whether the increased cardiomyocytes apoptosis after FA exposure during pregnancy was related with increased oxidative stress, we tested the level of SOD, GSH, and MDA in cardiomyocytes in maternal mice and their offspring by ELISA. The results revealed that, in both maternal mice and their offspring, SOD and GSH in cardiomyocytes cells were significantly decreased (Figure 3A, B, D, E, P < 0.05), and MDA significantly increased after FA exposure (Figure 3C, F, P < 0.05), while in the 1.5 mg/kg FA+ Vit E group, supplements of Vit E reversed the high dose FA exposure induced alteration of SOD, GSH, and MDA (Figure 3A-F). There is no significant difference between Vit E group and Saline group.

FA exposure during pregnancy increased expression of apoptosis related gene Livin and Caspase-3 in cardiomyocytes in maternal mouse and offspring: To evaluate myocardial injury, mRNA expression of gene natriuretic peptide B (BNP) was observed. In both maternal mice and the offspring of the 1.5 mg/kg FA group, BNP was higher expressed compared with Saline group (Figure 4A, P < 0.05). We also detected the gene and protein expression of Livin and Caspase-3 in cardiomyocytes in pregnant mouse and offspring to reveal the underlying mechanism of FA induced cardiomyocytes apoptosis. In both maternal mice and their offspring, in the 0.5 mg/kg FA group, the mRNA level of Livin significantly decreased, while Vit E reversed FA exposure induced low expression of Livin (Figure 4B, P < 0.05). The mRNA expression pattern of Caspase-3 in the mothers and offspring was different. In the offspring, only those in the 1.5 mg/kg FA group showed significantly increased Caspase-3 mRNA, and Vit E reversed the effect of 1.5 mg/kg FA exposure (Figure 4C, P < 0.05). There is no significant difference about mRNA expression of Livin and Caspase-3 between the Vit E group and the Saline group in the offspring. In the maternal mice, mRNA of Caspase-3 significantly increased in the 0.5 mg/kg FA group, the 1.0 mg/kg FA group, and the 1.5 mg/kg FA group compared with the Saline group, and Vit E floated the effect of the 1.5 mg/kg
Figure 1. Vit E reversed high dose FA exposure induced apoptosis of cardiomyocytes in offspring. A: Representative photomicrographs of HE staining showing histological changes in response to FA exposure in offspring. B: Representative photomicrographs of TUNEL staining positive cells in response to FA exposure in offspring. C: Percentage of TUNEL staining positive cells in offspring. Scale bar = 50 μm (low-power images). n = 3 per group. One-way ANOVA, Tukey’s post-hoc test, *P < 0.05, **P < 0.01.

Figure 2. Vit E reversed high dose FA exposure induced apoptosis of cardiomyocytes in maternal mouse. A: Representative photomicrographs of HE staining showing histological changes in response to FA exposure in maternal mouse. B: Representative photomicrographs of TUNEL staining positive cells in response to FA exposure in maternal mouse. C: Percentage of TUNEL staining positive cells in maternal mouse. Scale bar = 50 μm (low-power images). n = 3 per group. One-way ANOVA, Tukey’s post-hoc test, *P < 0.05, **P < 0.01.

FA exposure increased oxidative stress and expression of apoptosis related gene Livin and Caspase-3 in HL-1 cells: A TUNEL assay had revealed that a high dose of FA exposure induced an increase of apoptosis in cardiac muscle tissue in both pregnant mice and their offspring, however the type of apoptotic cell was unclear. To investigate whether FA exposure could induce apoptosis and oxidative stress in cardiomyocytes but not other types of cells, we used an HL-1 myocardial cell line to identify the effects of FA exposure. The results revealed that the viability of the HL-1 cell in the 25 μmol/L FA group and the 50 μmol/L FA group were significantly decreased compared with the control group and the 5 μmol/L FA group, while Vit E could reverse the effects of 50 μmol/L FA exposure (Figure 5A, P < 0.05). In the 5 μmol/L FA group, the 25 μmol/L FA group, and the 50 μmol/L FA group, the level of SOD and GSH in cardiomyocytes cells was significantly decreased, while MDA was significantly increased, and a supplement of Vit E reversed the high...
FA exposure during pregnancy increased oxidative stress in cardiomyocytes in maternal mouse and offspring. A-C: Level of SOD, GSH, and MDA in offspring. D-F: Level of SOD, GSH and MDA in maternal mouse. n = 3–5 per group. One-way ANOVA, Tukey’s post-hoc test, *P < 0.05, **P < 0.01.

dose FA exposure induced alternation of SOD, GSH, and MDA (Figure 5B, P < 0.05). Flow cytometry revealed that the percentages of early cell apoptosis (right lower quadrant) was significantly increased in the 5 μmol/L FA group (5.9%), the 25 μmol/L FA group (4.3%), and the 50 μmol/L FA group (10.9%) compared with the control group (0.7%), and late cell apoptosis (right upper quadrant) was markedly increased in the 25 μmol/L FA group (12.0%) and the 50 μmol/L FA group (18.4%) compared with the control group (4.2%). Vit E reversed the increase of both early cell apoptosis (6.6%) and late cell apoptosis (9.0%) induced by 50 μmol/L FA exposure (Figure 5C). Besides, mRNA level of BNP in 50 μmol/L FA group was significantly increased when compared with the 50 μmol/L FA + Vit E group (Figure 5D, P < 0.05). In the 25 μmol/L FA group, 50 μmol/L FA group, the mRNA level of Livin significantly decreased and Caspase-3 increased, and Vit E reversed the FA exposure induced alteration (Figure 5E, P < 0.05). There is no significant difference among the NC group, Vit E group, and the 50 μmol/L FA + Vit E group. Western blotting and immunofluorescent confirmed these results (Figure 5F and Figure 6).

Discussion

In this study, we investigated the effects of Vitamin E on apoptosis of cardiomyocytes induced by exposure to high doses of FA during pregnancy. We found that a high dose FA exposure resulted in cytotoxicity in both maternal mice and their offspring, as TUNEL staining revealed a significant apoptosis of cardiomyocytes, and alternation in SOD, GSH, MDA, Livin, and Caspase-3 was found in cardiomyocytes. A dose of 0.1 μg Vit E could reverse a high dose of FA exposure induced apoptosis of cardiomyocytes in both maternal mice and their offspring. Cytology experiments with an HL-1 cell line confirmed the effect that the high dose of FA exposure increased oxidative stress and cell apoptosis, and decreased the viability of HL-1 cells.

To evaluate the systemic effects of FA exposure on both maternal mice and their offspring, the body weight of the maternal mice was weighed during FA exposure, and the number of total births, live births, and malformations of every maternal mouse, as well as the body weight of the newborns, were recorded. The results revealed FA exposure induced a significant decrease of body weight in maternal mice and their offspring in the 1.5 mg/kg group, the 1.5 mg/kg group, and the 1.5 mg/kg FA + Vit E group. In the 1.5 mg/kg group, the number of total births, live births, and body weights of newborns were significantly lower compared with the Saline group, and Vit E reversed the alternation induced via a 1.5 mg/kg FA injection (Supplemental Table).

FA is a low molecular weight chemical with high
water solubility, which can react with proteins and nucleic acids, or with low molecular weight substances and could cause DNA damage. These features implied the developmental toxicity of FA. Previous studies reported that FA exposure during pregnancy could induce dysfunction in the development of the immune system in offspring of FA exposed mother mice, and a supplement of antioxidants, such as Vit C, could reverse FA induced immunosuppression in the offspring. Our study confirmed the developmental toxicity of FA.

Apoptosis is a phenomenon of cellular death that is regulated by caspases, which are an aspartate-specific protease. Livin is an endogenous caspase inhibitory factor belonging to an inhibitory apoptosis protein family, and Livin can directly inhibit the caspases-3 family. In this study, we found that in FA exposure groups, Livin mRNA and protein decreased while the level of caspases-3 increased in the mothers and offspring. These results indicated that FA exposure could trigger apoptosis.

Oxidative stress was closely related with cell apoptosis, and previous studies reported that exposure to FA increased lipid peroxidation, and suppressed the antioxidant system. Previous studies have reported that the FA suppressed SOD activities. In this study, we observed that SOD and GSH levels decreased while MDA levels significantly increased, which revealed a higher lipid per-
FA exposure increased oxidative stress and expression of apoptosis related gene Livin and Caspase-3 in HL-1 cells. A: Cell viability assay of in HL-1 cell. B: Level of SOD, GSH, and MDA in HL-1 cell. C: Cell apoptosis in HL-1 cell. D: Level of Livin and Caspase-3 mRNA in HL-1 cell. E: Representative band of Livin and Caspase-3 in HL-1 cell. One-way ANOVA, Tukey’s post-hoc test, *P < 0.05, **P < 0.01, ***P < 0.001.

oxidation after FA exposure, and Vit E reversed these alternation; an in vitro experiment confirmed Vit E could reverse FA induced cell apoptosis. SOD and non-enzymatic antioxidants such as Vit C, GSH, and Vit E play dominant roles in antioxidant defense. Previous studies reported that, FA exposure could significantly decrease the activity of SOD, and in our study, we found that FA exposure could also decrease the level of SOD. Vit E reacts with membrane phospholipid bilayers to stop the chain reactions initiated by hydroxyl radicals too. It has been reported in various studies that the increase in MDA owing to lipid peroxidation was reduced by administering Vit E, and our study was in accordance with these studies.

In our study, we found that Vit E may act as an antioxidant to protect the myocardial cell from FA induced apoptosis. A study by Ogutcu et al. has reported that as supplements of Vit E significant decreased, it increased MDA levels in diazinon-treated rats. Several randomized controlled trials revealed that daily supplements of Vit E would significantly reduce the risk of cardiovascular death and non-fatal myocardial infarction, which may attribute to the role of Vit E in prevention of atherosclerosis by the inhibition of oxidation of low-density lipoprotein. Our study was in accordance with these findings, as both in vivo and in vitro, the Vit E could significantly decrease the oxidative stress induced by FA, and reduced the FA exposure induced myocardial apoptosis. These finding indicated a role of Vit E in the protection of the cardiovascular system as an antioxidant.

It should be noted that, in our study, TUNEL assays revealed that in the offspring of FA exposed mice, a significant increase of apoptosis in myocardial tissue was found in the 1.0 mg/kg group and the 1.5 mg/kg group, compared to the 0.5 mg/kg FA group and the Saline group. While in maternal mice, 0.5 mg/kg FA group showed a significant increase of TUNEL-positive cells in myocardial tissue. A possible explanation of this phenomenon is that when exposed to a low dose of FA, the maternal mouse buffered the oxidative stress induced by the FA to protect the fetus. While, when exposed to a high dose of FA, supplements of exogenous antioxidants, such as Vit E, would reverse the oxidative stress via replenishing the insufficiency of endogenic antioxidant.

In conclusion, in our study we found that FA exposure during pregnancy showed significant developmental toxicity in offspring. FA induced oxidative stress and apoptosis of cardiomyocytes in both maternal mice and their offspring, and Vitamin E supplements during pregnancy reversed the systemic and myocardial toxicity of FA.

Disclosures

Ethical approval: All animal experiments were carried out strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals.

Conflict of interest: The authors declare no conflict of
Figure 6. Expression and distribution of Livin protein in HL-1 cell detected by immunofluorescence assay (magnification at 400×).

interest.

References


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Supplemental Files
Supplemental Table
Please see supplemental files: https://www.jstage.jst.co.jp/article/ihj/58/5/58_16-279/_article/supplement