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Research Article

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The protective effect of folic acid on oxidative stress induced by lead acetate in PC12 cells

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Abstract: Folic acid (FA) plays an important role in cell metabolism. In this study, we primarily looked at the protective effect of FA on PC12 cytotoxicity induced by lead acetate. PC12 cells were exposed to lead-free medium and 10μmol/L lead-acetate culture solution for 24h. Medium with FA proportions of 0mg/L, 0.5mg/L, 5mg/L, and 50mg/L continued to function. After 24h, 48h, and 72h, we collected PC12 cells, and detected cell proliferation or inhibition with the MTT method. We extracted cell protein and detected the activities of SOD, MDA, and GSH-PX in each group. The immunofluorescence method was used to study the expression of caspase-3 protein. Changes of $A\beta_{40}$ and $A\beta_{42}$ in the cell-culture fluid were detected with ELISA. The results showed that lead exposure inhibited cell growth ($P < 0.05$), increased intracellular MDA content, SOD activity, and caspase-3 protein expression ($P < 0.05$), and decreased GSH-PX activity and $A\beta_{40}$ protein expression ($P < 0.05$). FA intervention can increase cell proliferation, enhance the activity of SOD and GSH-PX ($P < 0.05$), and reduce cell MDA content and caspase-3 protein expression ($P < 0.05$). There was no statistically significant difference between $A\beta_{40}$ and $A\beta_{42}$ content between the groups ($P > 0.05$). The study found that FA could be a highly effective approach against lead-associated PC12 cell toxicity.

Key words: Lead; Folic acid; PC12 cells; Oxidative stress; Apoptosis

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1 Introduction

Lead (Pb) is one of the most common of the heavy metals that are toxic to the human body. Recent epidemiological and toxicological studies report that lead exposure can cause a variety of diseases, including hypertension, kidney disease, neurodegenerative disease, and cognitive impairment, and has been proposed as a potential cause of Alzheimer's disease (AD) (Boskabady, et al., 2018, Sun, et al., 2019). A large number of studies have confirmed that free radicals constantly carry out numerous chemical reactions in the human body, and are responsible for energy transport and other functions. For example, SOD is a metalloprotease which can catalyze disproportionation of reactive oxygen species to remove oxygen in the body. Free radicals inhibit and reduce harmful lipid peroxidation, detecting SOD, this demonstrates the body's ability to scavenge free radicals and antioxidants (Bresciani, et al., 2013, de Mendonça, et al., 2016). MDA is a product formed by the reaction of excess free radicals in the body and unsaturated fatty acids on the cell membrane, and causes serious damage to the body. Measuring levels of MDA content allows determination of the degree of lipid peroxidation and oxidative damage in the body. Meanwhile, GSH-PX is a selenium-containing anti-

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oxidant enzyme which can catalyze hydrogen peroxide, prevent harmful free radical reactions, and protect the structure and function of cell membranes, thereby protecting the normal physiological functions of the body. Oxidative stress can be seen as an imbalance between reactive oxygen species (ROS) production and the response of antioxidant proteins. Recent studies have demonstrated that reactive oxygen species and resulting oxidative stress play a pivotal role in apoptosis(Kim, et al., 2015). Caspase-3 is a key proenzyme in cell apoptosis, and some progress has been made in using it in treatment of cancer, heart failure, and neurodegenerative diseases (Asadi, et al., 2021). It was found that exposure of mothers to Pb in drinking water may alter the expression of Calsyntenin-2 (Clstn2) and Calsyntenin-3(Clstn3) in off spring. Lead-induced learning deficit may be partly related to the altered expression of Calsyntenin protein(Li, et al., 2021). The central nervous system is the main target of lead poisoning, as proved by research showing that perinatal lead exposure can change calsyntenin-2 and calsyntenin-3 expression in the hippocampus and also cause learning deficits in weaned mouse pups(Li, et al., 2021). Colacurcio, D J et al. study found that the formation of Aβ oligomers and fibrils played a central role in the pathogenesis of Alzheimer's disease (AD). $A\beta_{42}$ and $A\beta_{40}$ are the main forms of Aβ in the brain, and $A\beta_{42}$ is the main component of amyloid plaques, but the overall abundance of $A\beta_{40}$ is several times that of $A\beta_{42}$(Gu and Guo, 2021). The group also discovered that age-related plaques and neurofibrillary tangles in the brains of AD mice were contaminated with lead. Gu He et al. found that continuous lead exposure could increase the formation of Aβ fibers in mouse brains, indicating that there is a definite link between lead exposure and AD (Wu, et al., 2020).

Folic acid (FA), also known as vitamin B_9, is a water-soluble vitamin (Sijilmassi, 2019). It is also an indispensable vitamin for the circulation of the central nervous system (Zhou, et al., 2020). Since the human body cannot synthesize this vitamin, it must rely on dietary intake. Deficiencies may be related to diseases such as neural tube defects, AD, pregnancy complications, and various cancers (van Gool, et al., 2018, Hinterberger and Fischer, 2013). Studies have found that FA supplementation can prevent apoptosis induced by oxidative stress and inhibit ROS levels by down-regulating vascular peroxidase 1 (VPO1) as a result of changes in DNA methylation, thus protecting endothelial cells from damage (Cui, et al., 2018).In rats, lead exposure can increase expression levels of HO-1 and CHOP and cause damage to the spleen. FA down-regulates expression levels of HO-1 and CHOP proteins through the NrF2/HO-1 and GRP78/CHOP pathways, alleviating lead-induced oxidative stress and endoplasmic reticulum-induced spleen damage (Li, et al., 2021). FA can also reduce the oxidative stress response and inhibit the apoptosis of astrocytes, thereby preventing telomere DNA oxidative damage and telomere wear(Li, et al., 2019). However, it has not been shown whether FA has neuroprotective effects against lead induced toxicity in neuronal PC12 cells.

PC12 cells, derived from a pheochromocytoma tumor of the rat adrenal medulla, have been extensively used as a cell model to study lead neurotoxicity (Westerink and Ewing, 2008). In this study, we explored the internal mechanism of lead exposure in PC12 cells and evaluated the neuroprotective effect of FA on lead-induced neurotoxicity in these cells.

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2 Materials and methods

Sijiqing Premium Fetal Bovine Serum (FBS), Trypsin and BCA protein concentration determination kits were purchased from Beijing Solarbio Technology Co., Ltd. DMEM/F-12 medium was purchased from Hyclone, USA. FA, MTT, trypsin, and dimethyl sulfoxide were obtained from Sigma-Aldrich (Missouri, USA). GSH, SOD, and GSH-PX kits were purchased from the Nanjing Institute of Bioengineering (Jiangsu, China). Amyloid Peptide (1-40) and Amyloid Peptide (1-42) ELISA kits were purchased from Shanghai Fusheng Industrial Co., Ltd. The other chemicals and reagents used were of analytical grade.

2.1 Cell culture and treatment

PC12 cells were donated by Zhengzhou University School of Basic Medicine (Zhengzhou, China). The cells were cultured in prepared DMEM/F12 cell-culture medium (containing 10% fetal bovine serum). The temperature, relative humidity, and relative concentration of CO_2 in the CO_2 incubator were 37℃, 90%, and 5%, respectively. The culture medium was replaced every two days, and the cells were selected as logarithmic growth cells of the same generation.

When PC12 cells were cultured for 80%, it indicated that the cell growth was normal and in a better state. We try psinized the cells and used a cell-counting plate to count the them and prepare a single cell suspension with a cell concentration of 5×10^5/mL, we then added 100μL of the cell suspension to each well of a 96-well plate. We also added 200μL of PBS to the sides of the plate to prevent excessive evaporation of the medium.

Our previous experimental study revealed that when PC12 cells were treated with 0.1, 1, 10μmol/L, and 100μmol/L of PbAc, 10μmol/L of PbAc was the minimum dose required to cause change in PC12 cells, so we selected this concentration to build the lead exposure model. At the same time, according to the literature, 10μmol/L of lead acetate (the optimal concentration for inducing apoptosis, as confirmed by expression levels of Bax and Bcl-2) can induce rat pheochromocytoma (PC12) cells to produce a bystander effect on neighboring cells (Guo, et al., 2014).

The PC12 cells were seeded in 96-well plates at a density of 5×10^5 cells per well. The lead-exposed group was added with 10μmoL/L lead acetate medium, and the lead-free group was added with the same volume of medium. After culturing for 24 h, we added different concentrations of FA to the lead-exposed group (0, 0.5, 5, 50 mg/L), and labeled the groups as P, G0.5, G5, and G50. We did the same for the lead-free group and labeled the subgroups as C, F0.5, F5, and F50. After 24, 48, and 72 h in a cell incubator, we added MTT solution (20μl) and maintained the cells at 37° C, incubating them for 4h. We then removed the medium and added 150μl of DMSO to dissolve. Absorbance was measured at 490 nm with an enzyme-linked immunoassay. The CPIR (cell proliferation inhibition rate) was measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

$$CPIR = [(A\ OD / B\ OD) - 1] \times 100\%$$

Note: A represents the experimental group; B represents the control group.

2.2 Determination of SOD, MDA, and GSH-PX activity

We took 4 disposable culture flasks and labeled them C, F, P, and G, indicating the control group, 50mg/L FA group, 10μmoL/L (PbAc) group, and 10μmoL/L (PbAc) +50mg/L FA group, respectively. We then added 4ml of medium and waited until the cells were stable in the culture flask after growth; after which we discarded the original medium, added 4ml of medium containing 10μmoL/L PbAc in groups P and G, and added the same volume of medium in groups C and F. We discarded it after culturing in a cell incubator for 24 h in the edium. Groups C and P received an additional 4ml of medium, and groups F and G received 4ml of FA with a concentration of 50 mg/L. After 72 h, the culture flask was taken out, the medium was discarded, and the protein was washed twice with 2ml PBS; then the total protein of the PC12 cells was extracted. The medium was collected and SOD, MDA, and GSH-PX activity were determined using a commercially available assay kit (Nanjing Jiancheng, China) based on the manufacturer's protocol.

2.3 Immunofluorescence

Immunofluorescence staining was used to detect the expression of the caspase-3 protein in the PC12 cell-culture medium. We fixed cells in 4% paraformaldehyde for 15 min at room temperature, washed them with PBS each time for 5 min, repeat the dip 3 times, and used 0.3% triton X-100 for 20 min at room temperature to break cell membranes. Next, we blocked the cells with 10% goat serum for 30 min, washed and dried them, add diluted caspase-3 protein, and incubated the cells overnight at 4°C in an incubator. On the second day, we added caspase-3 protein anti-rabbit IgG (1:100, cy3 anti-rabbit), placed the cells in a 37℃ thermostat for 2h, and then washed them with PBS for 3 minutes, a total of 3 times. DAPI staining was carried out at room temperature, and we used a microscope to collect images to observe the location of the caspase-3 protein in the cells. Finally, we used Image-Pro Plus7.0 software to analyze protein expression in the cells.

2.4 ELISA

After the cells were cultured for 72h, they were taken out, according to the instructions for use of the rat amyloid beta 1-40/1-42 ($A\beta_{1\text{-}40/1\text{-}42}$) enzyme-linked immunosorbent assay kit, the content of $A\beta_{1\text{-}40/1\text{-}42}$ in the cell culture medium was detected. The content of Aβ1-40 and Aβ1-42.

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2.5 Statistical Analysis

Caspase-3 protein expression in the cells was analyzed with Image-Pro Plus7.0, and a one-way analysis of variance was performed on each set of data with SPSS 16.0. The data is expressed in the form of mean ± standard deviation ($\bar{x} \pm s$), and the test significance level is α=0.05.

3 Results

3.1 Effects of lead exposure on growth and development of PC12 cells

It can be seen from Figure 1 that the 72h OD value of group C (the control group) was greater than the 48h OD value or the 24h OD value, indicating that within 72h, as time increased, PC12 cells continued to proliferate. The F50 group had its highest OD value at 72h, indicating that the number of cells was largest at that point. At all the time points (24h, 48h, or 72h), OD value was positively correlated with the dose of FA, showing that with an increase of FA, the number of PC12 cells multiplied. Among groups, the OD value of group P was the lowest, indicating that the number of cells in this group was the least. Although the lead only acted on the cells for 24h, the culture medium was changed after 24h; However the OD values of the cells were almost the same after 24h, 48h, and 72h, indicating that the lead caused damage to the cells, damage caused by lead to cells is irreversible by continuing to culture PC12 cells with complete medium. Reversed. The OD value of the G group was greater than that of the P group, and with increasing FA concentration, OD value showed an upward trend. At 72h, the OD value of the G50 group was greater than that of the G0.5, G5, or P groups, indicating the presence of lead. After damage is caused to PC12 cells, adding FA to the culture medium will increase the number of cells, and with greater doses of FA, the number of PC12 cells will gradually increase. We speculate that FA has a promoting effect on PC12 cells damaged after lead exposure and can increase the number of cells.

Figure 2 shows that at 24h, compared with the C group, the cell growth rates of the G and F50 groups were significantly increased ($P < 0.05$), and the F50 group had the highest cell growth rate, which was significantly greater than the rest of the groups. Compared with the P group, the cell growth rate in the G group was significantly increased ($P < 0.05$), and the cell growth rate in the G50 group was greater than that in the G0.5 and G5 groups, but there was no significant difference between the three groups. compared with P, G0.5, G5, G50 ($P < 0.05$),

At 48h, the cell-growth rate of the F50 group was significantly increased ($P < 0.05$) compared with the control group, and had reached its highest level. The F0.5 and F5 groups also exhibited promotion of cell growth, but there was no significant difference between them ($P > 0.05$). In the P group, cell growth was inhibited. Compared with the P group, the cell-inhibition rate of the G50 group was significantly lower ($P < 0.05$).

At 72h, compared with the control group, group F was promoting cell growth, groups P and G inhibited cell growth, the cell growth rate of group P was significantly lower ($P < 0.05$), the cell-growth rates of the G5 and G50 groups were significantly lower ($P < 0.05$), and the cell-growth rate of the F50 group was significantly higher ($P < 0.05$), reaching its highest level. There was no significant difference between group G0.5 and group P ($P > 0.05$), but the inhibition rate of group P was significantly higher than that of the G5 and G50 groups ($P < 0.05$).

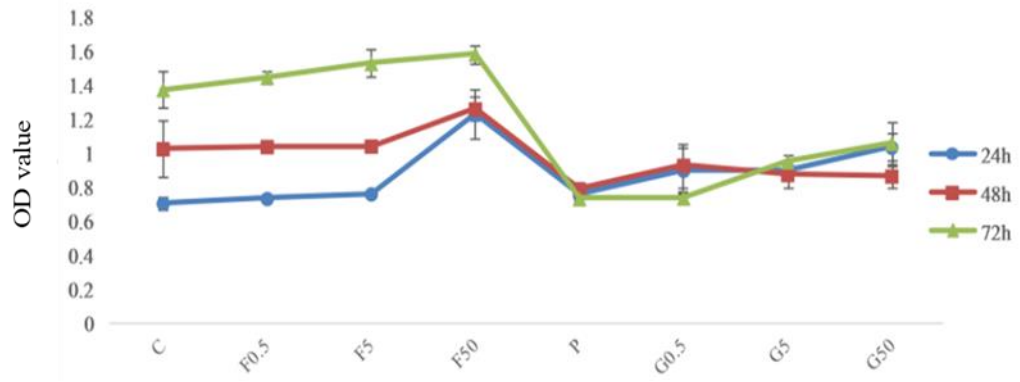

Figure 1. MTT detection of the effect of FA on the OD value of PC12 cells at different times under lead exposure（n=6）

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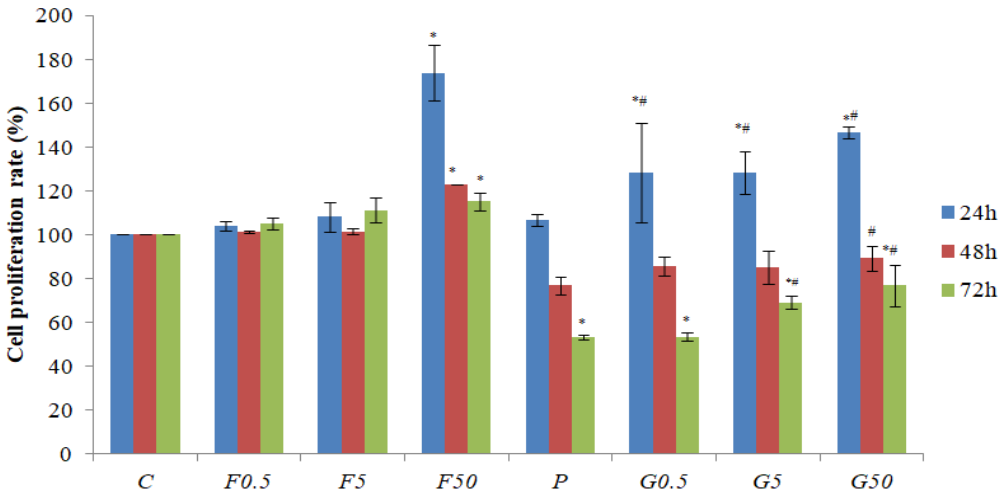

Figure 2 The effect of FA on PC12 cell proliferation under lead exposure（n=6）; (*P < 0.05, a significant difference compared with group C; #P < 0.05, a significant difference compared with group P). Note: Group C is the control group; F0.5, F5, and F50 groups are 0.5, 5, and 50 mg/L FA respectively, and P group is the 10μmoL/L (PbAc) group. The G0.5, G5 and G50 groups were 0.5, 5, and 50 mg/L FA+10μmoL/L (PbAc) respectively.

3.2 Effect of FA on the antioxidant capacity of PC12 cells exposed to lead

One can see from Figure 3 that compared with the C group, the SOD activity of the F group was lower, but the difference between the two was not significant (P > 0.05). The SOD activity of groups P and G increased significantly (P < 0.05), and group G had the highest activity. Compared with group P, the activity of SOD in group G was significantly higher (P < 0.05).

The MDA activity of groups F, P, and G was significantly higher (P < 0.05) than in group C, and the MDA content of group P was the highest. After adding FA, the MDA activity of group G was significantly lower than that of group P (P < 0.05).

Again comparing against group C, GSH-PX activity was significantly improved in groups F and G (P < 0.05), and significantly lower in group P (P < 0.05). Compared with the P group, the GSH-PX activity was significantly increased in the G group (P < 0.05).

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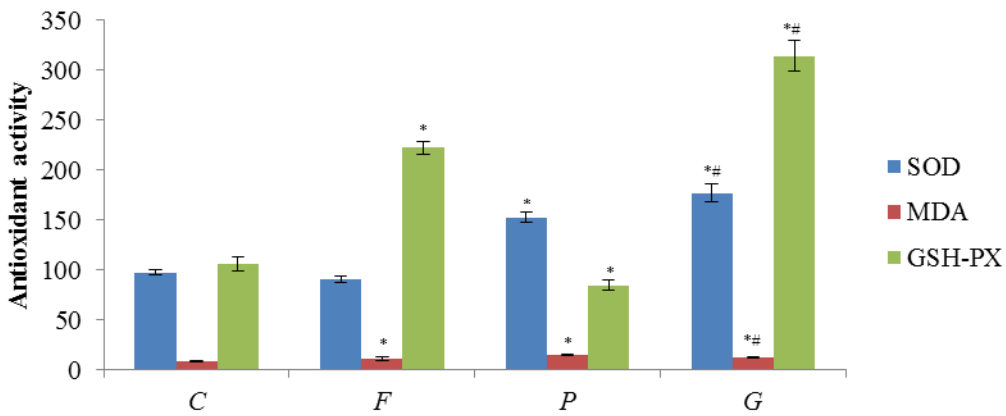

Figure 3 The effect of FA on the antioxidant capacity of PC12 cells exposed to lead（n=6）. (* P < 0.05, indicates a significant difference compared with group C; # P < 0.05, indicates a significant difference compared with group P). Note: C, F, P, and G represent the control group, 50mg/L FA group, 10μmoL/L (PbAc) group, and 10μmoL/L (PbAc) + 50mg/L FA group, respectively.

3.3 Effect of FA on lead-induced caspase-3 protein expression in PC12 cells

The expression, distribution, and metastasis of Caspase-3 were assessed with immunofluorescence analysis. The positive immunofluorescence reaction of Caspase-3 protein was expressed as red in the cytoplasm, and the nucleus was stained blue by DAPI. Figure 5 shows that there were fewer Caspase-3 protein positive immunofluorescence reactions in the C and F groups, while the P and G groups had relatively more. The nuclei of cells in the P group were broken, while the cytoplasm was concentrated and the boundaries were not clear. The Caspase-3 protein originally in the cytoplasm overlapped with the nuclei, indicating that the cells had begun to undergo apoptosis. The nuclei in group G were still intact, but it can be seen that the Caspase-3 protein in the cytoplasm had overlapped the nuclei. Calculated from Figure 4-1 (Table 1), the integral optical density value and mean fluorescence intensity of Caspase-3 protein positive reactants in PC12 cells in group P were significantly greater than those in other groups; compared with group C, the positive reactants of Caspase-3 protein in group G were significantly increased. However, compared with the P group, it was significantly decreased. Compared with group C, the positive reaction of Caspase-3 protein was also significantly increased in group F, however, compared with the C group, the positive reaction of Caspase-3 protein in the F group was also significantly increased, but mainly in the cytoplasm, indicating that the high concentration of folic acid acts on the PC12 cells for a long time, and it is also damaged. Caspase-3 protein in group G. was significant, compared with group P, they were also significantly

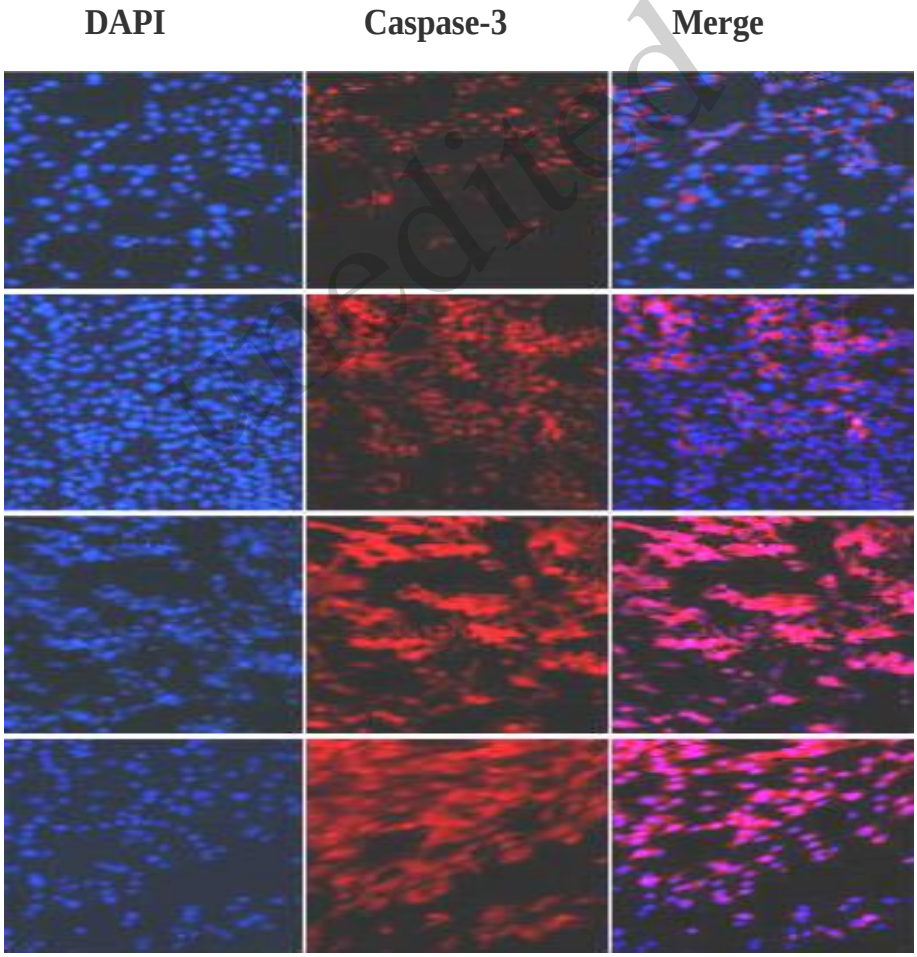

Figure 4 The effect of FA on the immunofluorescence expression of Caspase-3 protein in PC12 cells exposed to lead（n=3）.

Table 1 Data analysis of Caspase-3 protein immunofluorescence in PC12 cells exposed to lead（n=3）

Group	Integrated optical density	Average fluorescence intensity value
C	507.44±7.10	0.92±0.07
F	4101.72±122.23*	1.00±0.05*
P	15063.32±1759.60	1.24±0.10
G	9509.75±498.23*#	1.08±0.03*#

Table 1. (*$P < 0.05$, indicates a significant difference compared with group C; # $P < 0.05$, indicates a significant difference compared with group P). See (Figure 4) legend.

3.4 Effect of FA on Aβ40 and Aβ42 in PC12 cell-culture medium with lead

First, we analyzed the Aβ40 content in the cell-culture medium of each group, as shown in Figure 5. The Aβ40 content in the cell-culture medium of group F was higher than in group C, but the difference was not significant ($P > 0.05$); the Aβ40 content in the cell-culture medium of group P decreased significantly ($P < 0.05$) Although Aβ40 content decreased, the difference was not statistically significant ($P > 0.05$). Next, we analyzed the Aβ42 content in the cell-culture medium of each group, and found no significant differences between the C, F, P, and G groups ($P > 0.05$).

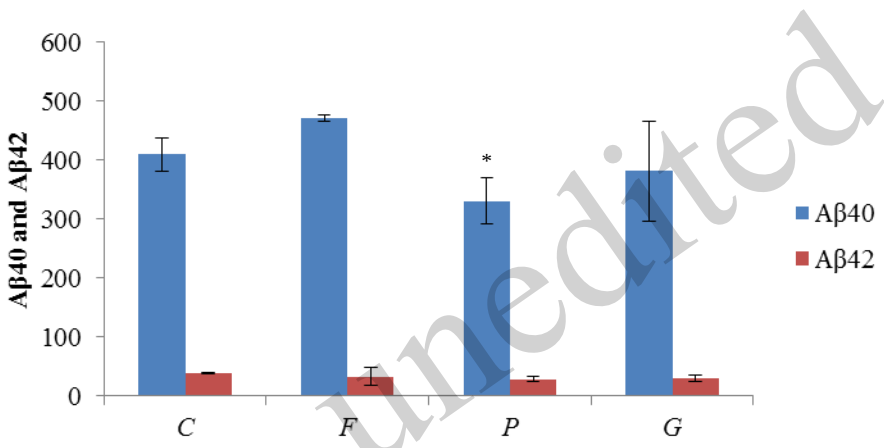

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Figure 5. The effect of FA on Aβ40 and Aβ42 protein expression in PC12 cell-culture medium under lead exposure（n=3). (*$P < 0.05$, indicates a significant difference compared with group C). See Figure 4 legend.

4 Discussion

Lead is a heavy metal that is harmful to the human body. It can cause damage to all organs and systems of the body, and the damage to the nervous system is even more serious. Hu Guiping used different concentrations of lead to poison PC12 cells and found that lead could inhibit the viability of PC12 cells and that the inhibition rate was positively correlated with the concentration (Gui-ping, et al., 2015). Quan et al. administered FA as an intervention to lead-poisoned young rats and found that it had a certain protective effect on the damaged neurons of lead-exposed rats (Quan, et al., 2015). However, there is no research on the effect of FA on PC12 cells exposed to lead in vitro. Therefore, we constructed a PC12 lead exposure model and explored the effects of FA on the proliferation, apoptosis, and antioxidant status of PC12 cells under lead exposure. After damage is caused to PC12 cells, adding FA to the culture medium will increase the number of cells, and with greater doses of FA, the number of PC12 cells will gradually increase. We speculate that FA has a promoting effect on PC12 cells damaged after lead exposure and can increase the number of cells. Our experimental results confirm that lead exposure can significantly inhibit the growth of PC12 cells, which is consistent with the results of Aminzadeh et al (Aminzadeh and Salarinejad, 2019). FA can promote proliferation of PC12 cells damaged by lead exposure, but the cell growth rate decreases with time.

A large number of studies have shown that free radicals play an important role in maintaining the normal

physiological functions of cells, by participating in cell differentiation and immune response, learning and memory, and by killing parasites and bacteria, Free radicals are also responsible for the transmission of energy(Vina, et al., 2013). Under normal conditions, the body has its own free radical scavenging system, allowing free radicals to carry out their tasks effectively. The natural control of free radicals, in a balanced state, prevents them from interfering with human health(Sies, 2015) . However, if a person becomes ill, the free radicals in the body will not be controlled (Schieber and Chandel, 2014);the number will rise sharply, leading to the occurrence of oxidative stress and lipid peroxidation of cell membranes, which will cause serious harm to the body (Yu and Schellhorn, 2013). For example, it can lead to some degenerative diseases in the elderly (Losada-Barreiro and Bravo-Diaz, 2017), cancer (Steenland, et al., 2019), and cardiovascular disease (Senoner and Dichtl, 2019). The body's free radical scavenging system includes enzymes and non-enzymes. Representative enzymes include SOD, GSH-PX, and CAT. SOD can "clean up" oxygen free radicals in the body and convert them into hydrogen peroxide, which is harmful to the body (Sakamoto and Imai, 2017), while GSH-PX and CAT continue the process by decomposing hydrogen peroxide (Liang, et al., 2017), so that the final product of the reaction is water, thereby protecting the body's normal physiological activities. Based on this knowledge, we speculate that FA operates by improving and increasing the antioxidant enzyme activity of PC12 nerve cells after lead exposure, removing harmful free radicals generated after exposure to lead, and reducing lipid peroxidation, thereby protecting cells.

Caspase-3 protein is a key protein in regulating cell apoptosis. When cells undergo apoptosis, Caspase-3 in the cytoplasm can be hydrolyzed and activated by certain enzymes, and enter the nucleus to perform its function. There is also evidence that most Caspase-3 substrates are present in the nucleus (Moncsek, et al., 2015). Zhao experimentally found that FA can increase the expression of pERK1/2 in neuronal cells in rats with focal cerebral ischemia and reduce the apoptosis rate of neuronal cells in rats, and that increasing the dose of FA can further protect neuronal cells(Zhao, et al., 2016). Wu found that long-term FA supplementation can significantly prevent cardiomyocyte apoptosis in type 2 diabetic rats (Wu, et al., 2008). Yang and other researchers found that combined intervention with FA and VB12 promotes down-regulation of Caspase-3 and Caspase-8 protein expression in the myocardial tissue cells of diabetic rats, relieves cell apoptosis, and protects myocardial tissue from damage(Yang, et al., 2016).Our study revealed that the cell nuclei in the lead-exposed group were in a broken state, a large amount of cytoplasm was concentrated, and cell boundaries were unclear. Meanwhile, the positive reaction of Caspase-3 protein increased significantly, indicating that the cells had begun to undergo severe apoptosis. However, the nucleus and cytoplasm of the lead exposure group with 50 mg/L of FA remained normal, while the caspase-3 protein in the cytoplasm had overlapped the nuclei. Compared with the control group and the 50mg/L FA group, the group showed a significant increase in caspase-3 protein positive reactants. We speculate that the cells received the apoptosis signal but had not yet undergone apoptosis, indicating that FA can prevent and delay apoptosis of PC12 cells. However, compared with the control group, caspase-3 protein positive reactants were also significantly increased in the FA 50mg/L group, but were mainly present in the cytoplasm, which means that high concentrations of FA acted on PC12 cells for a long time, there was still lead-induced damage.

One of the main pathological features of AD patients is senile plaque (Peric and Annaert, 2015). Amyloid β is the main component of senile plaques, which are produced by the breakdown of amyloid precursor protein (Colacurcio, et al., 2018). Aβ is a soluble dimer with strong cytotoxicity and can induce apoptosis of nerve cells. It has two main residue subtypes, $A\beta_{40}$ and $A\beta_{42}$.$A\beta_{40}$ is composed of 40 amino acid residues, and $A\beta_{42}$ is composed of about 42 amino acid residues (Nixon, 2017). Because Aβ mainly exists as monomers, oligomers, and fibers, and $A\beta_{42}$ has two more amino acid residues than $A\beta_{40,}$ the latter aggregates more easily to form senile plaques. We found that the content of $A\beta_{40}$ in the PC12cell-culture medium of the lead exposure group decreased significantly, while in the FA group it increased, but not significantly. However, there was also no significant difference in the $A\beta_{42}$ content in various groups. Therefore, it is our opinion that lead can most likely inhibit the production of extracellular $A\beta_{40}$. This may be because lead can induce production of more insoluble Aβ oligomers in cells, thereby reducing extracellular Aβ content. FA may promote excretion of harmful soluble $A\beta_{40}$ from the body, increase $A\beta_{40}$ content in cell-culture fluid, and protect the body. However, whether FA affects $A\beta_{40}$ or $A\beta_{42}$ in PC12 cells under lead exposure is a question that will require further research and experimentation.

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5 Conclusions

Lead can induce lipid peroxidation and oxidative stress in cells, while inhibiting cell growth and promoting cell apoptosis. Folic acid can protect PC12 cells damaged by lead exposure, and may increase the intracellular activity of SOD and GSH-PX, bringing MDA content down and then inhibiting or delaying expression of Caspase-3 protein to protect damaged cells.

Abbreviations

FA, folic acid; Pb, lead; AD, Alzheimer's disease; Aβ, amyloid β-protein; $A\beta_{1-40}$, Amyloid Peptide (1-40) ; $A\beta_{1-42}$, Amyloid Peptide (1-42) ; GSH, glutathione; GSH-PX, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; CAT, catalase; ROS, reactive oxygen species; Caspase-3, Cysteine aspartate protease 3; Caspase-8, Cysteine aspartate protease 8; VPO1, Vascular peroxidase 1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase 1; GRP78, glucose-regulated protein 78/binding immunoglobulin protein; CHOP, C/EBP-homologous protein; DAPI, 4',6-diamidine-2-phenylindole; pERK1/2, ERK1/2, Extracellular signals regulate kinase phosphorylation;VB12,Vitamin B12; PC12, Rat adrenal pheochromocytoma; MTT, 3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl Tetrazolium Bromide

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Author contributions

All authors in the paper have made great contributions to the manuscript, and Ning Li and Mingming Li participated in the design of the experiment, statistical analysis of data, manuscript writing and literature retrieval. Xianqing Huang, Lianjun Song and Mingwu Qiao carried out thesis design, literature retrieval and manuscript preparation. Tianlin Wang and Tiange Li participated in literature search, data collection and data analysis. Xiuhong Zhu and Can Cui provided assistance with data collection and manuscript editing. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Ning Li, Mingming Li, Xianqing Huang, Lianjun Song, Mingwu Qiao, Tianlin Wang,Tiange Li, Xiuhong Zhu and Can Cui declare that they have no conflicts of interest.

This article does not contain any human or animal experimental studies conducted by any of the authors.

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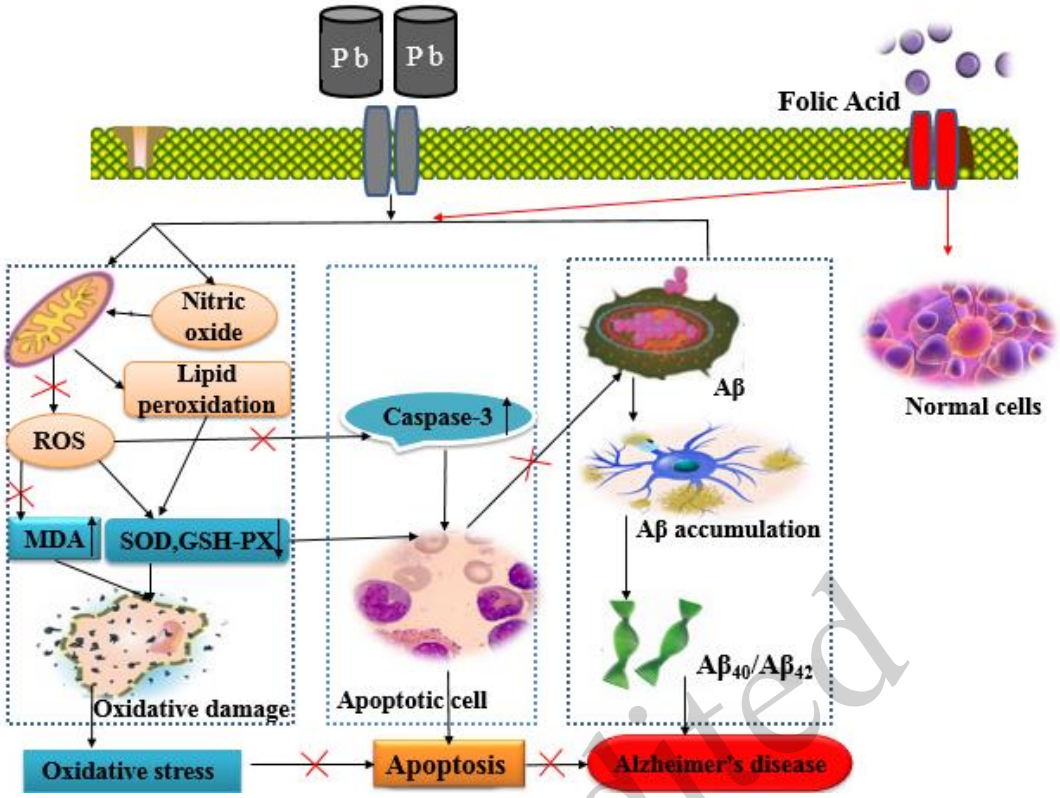
Graphical summary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Oxidative damage
Caspase-3
Apoptotic cell
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Aβ accumulation
Aβ40/Aβ42
Normal cells
Oxidative stress
Apoptosis
Alzheimer's disease

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