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十溴联苯醚(BDE-209)对离体条件下小鼠肾脏和脑组织 SOD 活力和 MDA 含量的影响

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摘要： 十溴联苯醚(decabromodiphenyl ether,BDE-209)是目前应用最广泛的的溴系阻燃剂,其环境风险引起很大关注。本实验以小鼠肾脏和脑组织为实验材料,研究了离体条件下 BDE-209 的急性氧化损伤效应。BDE-209 染毒终浓度设置为 0，1，2，4 和 8 μg·mL^{-1},采用 NBT 和 TBA 法分别测定 SOD (superoxide dismutase) 活性和 MDA (malondialdehyde) 含量。结果显示,随着 BDE-209 染毒浓度的升高,小鼠肾脏和脑组织的 SOD 活性先升高后降低,较高染毒浓度组的 SOD 活性与对照组相比显著性降低; MDA 含量逐渐上升,并且与对照组相比较高染毒浓度组的 MDA 含量显著上升。以上结果说明,离体条件下 BDE-209 对小鼠肾脏和脑组织能够产生急性氧化应激,并导致脂质过氧化损伤。

关键词： 十溴联苯醚; 超氧化物歧化酶; 丙二醛; 氧化损伤; 小鼠; 肾脏; 脑
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Effects of Decabromodiphenyl Ether (BDE-209) on SOD Activities and MDA Contents in Mouse Kidney and Brain Tissues: An *in vitro* Study

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Abstract: As a most widely used brominated flame retardant, potential environmental risk of decabromodiphenyl ether (BDE-209) has been attracted more and more attention. In this experiment, the acute oxidative damage effect of BDE-209 on mouse kidney and brain tissues was performed. Mouse kidney and brain cells were exposed to different doses (0, 1, 2, 4, 8 μg·mL^{-1}) of BDE-209 *in vitro*, and enzymatic activity of superoxide dismutase (SOD) content of malondialdehyde (MDA) were determined. Our results showed that SOD activity in mouse kidney and brain tissue were activated at low doses and inhibited at high doses. In highest dose group, SOD activity was even lower than that of the control group. MDA content in kidney and brain cells gradually increased as BDE-209 concentration increased, which was significantly higher in the experimental groups than that in the control group. These results indicated that BDE-209 could induce acute oxidative stress on mouse kidney and brain tissue *in vitro*, resulting in lipid peroxidation damage.

Keywords: BDE-209; superoxide dismutase (SOD); malondialdehyde (MDA); oxidative damage; mouse; kidney; brain

Polybrominated diphenyl ethers (PBDEs) are widely used as brominated flame retardants carpet padding, which attracted great attention for their environmental risks. PBDEs were used in drape linings, some

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plastics, and polyurethane foam in chairs, sofas and mattresses [1-2]. PBDEs and their metabolites have been widely detected in various environmental media [3]. Several PBDEs show high environmental persistent and lipophilicity so that they have potential to be accumulated in the food chain [4]. In May 2009, some brominated PBDEs were formally listed in the "Stockholm Convention" [5] by United Nations Environment Programme (UNEP).

Recently, decabromodiphenyl ether (BDE-209) (Fig. 1) has been widely applied as a brominated flame retartant (BFR) in materials used for consumer products [6-7]. When BDE-209 was released into the environment, it transformed into polybrominated dibenzodioxins, polybrominated dibenzofurans and debrominated biphenyl ethers through photolysis, pyrolysis, biological, microbiological degradation and so on. These products more easily entried into the organism with greater toxicity than BDE-209. In recent years, the demand for BDE-209 is increasing, and relevant research on its toxicity is of great significance [8-9].

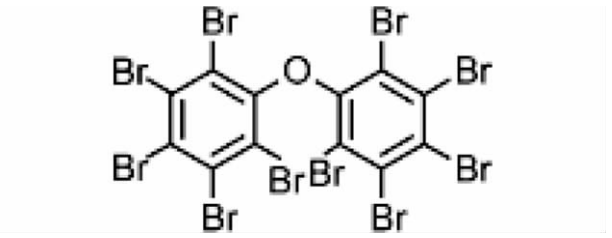

Fig. 1 Chemical structure of BDE-209

Under normal conditions, antioxidants eliminated excess oxygen free radicals to achieve a dynamic balance. However, under external stress, the level of oxygen free radicals in the cell increased, which could promote the enzyme activity in the antioxidation system to effectively remove the excess harmful substances. Once the oxygen free radical content exceeded a certain level, the free radicals cannot be efficiently removed by the antioxidation system. Excess free radicals accumulated and decreased the enzyme activity in antioxidation [10]. Superoxide dismutase (SOD) was the major antioxidant enzyme to remove superoxide anion radical generated in oxidation stress. The decline of SOD activity reduced the body's normal function on scavenging oxygen free radicals. MDA was the metabolite of oxidative damage, and its content reflected the changes of lipid peroxidation (LPO) level in body [11]. Therefore SOD and MDA have been used as biomarkers of oxidative damage.

In vitro assays are simpler, faster, and commonly used to screen for potential targets of toxicity with certain modes of action. In this study, in order to assess the toxicity of BDE-209, oxidative damage of mouse kidney and brain cells *in vitro* exposed to BDE-209 suspensions was examined.

1 Materials and methods

1.1 Experimental animals

Male SPF BALB/c mice used in the experiments were purchased from the experimental animal center in Hubei province. The animals were 9 weeks old with a body weight of about 25 g.

1.2 Reagents and apparatus

BDE-209 with the purity of ≥99%, were purchased from Labor Dr. Ehrenstorfer, Germany. Thiobarbituric acid (TBA) was purchased from Sinopharm Chemical Reagent Co. Ltd of China. Other reagents such as nitro blue tetrazolium (NBT), L-Met, riboflavin, EDTA were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd of China. Low-temperature refrigerated centrifuge (Eppendof-5415R, Germany), fluorescent microplate spectrophotometer (Bioteck, America) were used in this study.

1.3 Sample preparation and exposed

After sacrificed, the kidney and brain of BALB/c mice were separated quickly and washed with ice-cold saline. Then these tissues were minced with ophthalmic scissors (1 mm^3 of tissue). After filtered through 4 layers of lens cleaning paper, the cell suspension were obtained. The solution was diluted to a cell density of 10^7 cells·mL^{-1} and a portion of 0.5 mL was distributed into 1.5 mL centrifuge tubes. The ultrasonic dispersion of BDE-209 with concentration gradients of 0, 1, 2, 4, and 8 μg·mL^{-1}, repectively were added to the centrifugal tubes. For each centration level, 5 parallels were conducted accordingly. The centrifuge tubes were then put into a water bath and incubated at 37℃ for 1 h. After incubation, the cells in the centrifuge tubes were disrupted by repeated freezing and thawing for

three times. Finally, the cell suspension was centrifuged at 10 000 r·min^{-1} at 4℃ for 5 min and the supernatant was used to measure SOD and MDA.

1.4　SOD assay

NBT method [12] was used to measure SOD activity. Reagents were added in the following order: 1.5 mL saline, 0.3 mL met solution (130 mmol·L^{-1}), 0.3 mL NBT solution (750 μmol·L^{-1}), 0.3 mL EDTA-Na_2 solution (100 μmol·L^{-1}), 0.3 mL supernatant, and 0.3 mL riboflavin (20 μmol·L^{-1}). For the control, saline instead of supernatant was added into the tubes. The solution was mixed. Put a control tube in the dark and other tubes in the sunlight of 4 000 lx for 20 min. After reaction, the absorbances were measured at 560 nm using fluorescent microplate spectrophotometer. SOD activity was calculated according to:

$$U = (A_{ck} - A_E) \times V / (A_{ck} \times 0.5 \times w_{prot} \times V_t)$$

Where U is activity of SOD in U·mg$^{-1}_{prot}$, A_{ck} is absorbance of control tube in the sunlight, A_E is absorbance of sample tube, V is the total volume of the sample solution (3 mL), w_{prot} is the weight of the protein in each tube, and V_t is the volume of supernatant (0.3 mL).

1.5　MDA assay

MDA-TBA [13] adduct formed by the reaction of MDA with TBA at high temperatures (90 ~ 100℃) and acidic condition can be measured using fluorescent microplate spectrophotometer at 530 ~ 540 nm. To measure MDA formed in the cell suspension, 0.4 mL supernatant was transferred into a test tube, with added 2 mL 0.6% TBA solution. After boiled in the water bath for 15 min, the samples were cooled in cold water and centrifuged at 10 000 r min^{-1} for 10 min. The absorbance was measured at 450, 532 and 600 nm, respectively, and MDA content was calculated according to:

$$C = 6.45 (A_{532} - A_{600}) - 0.56 A_{450}$$

Where C is content of MDA in μmol·L^{-1}, A_{532}, A_{600} and A_{450} are absorbance at 450 nm, 532 nm and 600 nm, respectively.

1.6　Statistical analysis

The data were analyzed using Origin 7.5. T-test was carried out to analyze statistically significant differences between exposure groups and control groups. When p values were less than 0.05, differences were considered to be statistically significant; p value of less than 0.01 indicated the difference was very significant.

2　Results

2.1　Activity of SOD in kidney cells

Fig. 2 shows the effects of different concentrations of BDE-209 on SOD activity in mouse kidney cells. When concentrations of BDE-209 were more than 2 μg·mL^{-1}, SOD activities decreased. While in 8 μg·mL^{-1} group, SOD activity was significantly lower than that of the control group ($p < 0.05$).

2.2　Content of MDA in kidney cells

Fig. 3 shows the effects of different concentrations of BDE-209 on MDA content in mouse kidney cells. With increasing BDE-209 concentration, MDA content increased. In the 1 ~ 4 μg·mL^{-1} groups, the increases of MDA content were not significant. However, in the 8 μg·mL^{-1} group, the increase was significant ($p < 0.05$).

2.3　Activity of SOD in brain cells

Fig. 4 shows the effects of different concentrations of BDE-209 on SOD activity in mouse brain cells. When BDE-209 concentrations were more than 2 μg·mL^{-1}, SOD activity decreased with BDE-209 concentration rising. In the 8 μg·mL^{-1} group, SOD activity was significantly lower than that of the control group ($p < 0.01$).

2.4　Content of MDA in brain cells

Fig. 5 shows the effects of different concentrations of BDE-209 on MDA content in mouse brain cells. Except

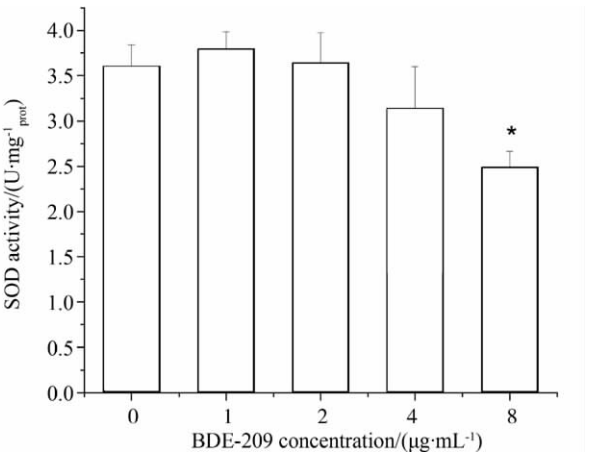

Fig. 2　Effects of BDE-209 on activity of SOD in mouse kidney cells

(*: $p < 0.05$, compared with control group)

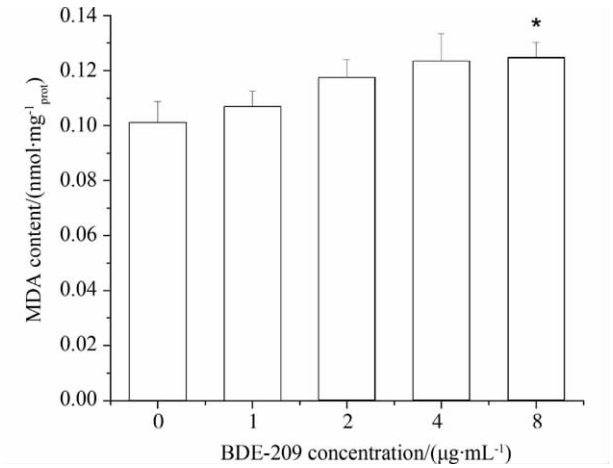

Fig. 3 Effects of BDE-209 on content of MDA in mouse kidney cells

(*: $p < 0.05$, compared with control group)

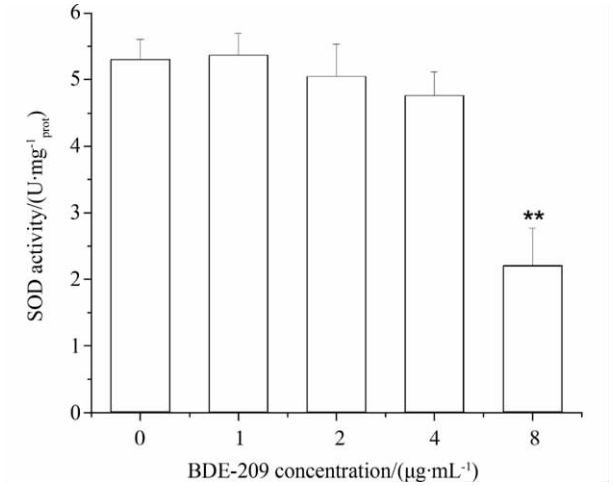

Fig. 4 Effects of BDE-209 on the activity of SOD in mouse brain cells

(**: $p < 0.01$, compared with control group)

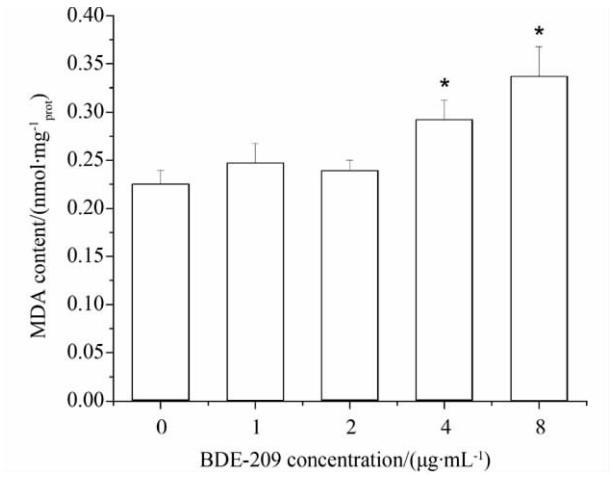

Fig. 5 Effects of BDE-209 on the content of MDA in mouse brain cells

(*: $p < 0.05$, compared with control group)

2 μg·mL^{-1} group, MDA content increased with BDE-209 concentration increased. In the 4 and 8 μg·mL^{-1} groups, the increases in MDA content were significant ($p < 0.05$) compared with the control group.

3 Discussion

Current studies focused on the toxicity of BDE-209 to liver [14], reproductive [15] and thyroid toxicity [16-17]. Comparedly, reports on oxidative damage were scarce. In liver mitochondria of *Carassius auratus* [18], with the concentration of BDE-209 increased, MDA, XOD tended to increase while T-AOC, SOD decreased, proving that T-AOC, XOD, MDA, SOD were closely related to BDE-209 concentration. However, to *Monopylephorus Limosus*, as BDE-209 exposure concentrations increased, the trend of SOD activity was from activation to inhibition [19]. This result is similar to the results of our experiment.

In our study, SOD activity in kidney and brain cells slightly raised with the increase of BDE-209 concentration. It is probably that the production of SOD generated in kidney and brain could remove excess ROS to protect the organism. When concentration of BDE-209 reached 2 μg·mL^{-1}, SOD activity gradually decreased. It suggested that the self-protection systems of the organism cannot work at high concentrations of BDE-209 so that the mouse kidney and brain structures were destroyed.

MDA content in kidney and brain cells gradually increased as BDE-209 concentration increased. Moreover, in high dose group, MDA content was significantly different to that in the control group. These results indicate that BDE-209 stress increases MDA content in mouse kidney and brain tissues to damage the membrane of cells. Basis on the above analysis, BDE-209 exposure can cause acute oxidative damage in mouse kidney and brain tissues. It was probably that free radicals generated from excess BDE-209 attacked amino and imino of amino acids. Unsaturated fatty acids of lipid bilayer produced the lipid peroxides such as MDA [20].

Obviously, BDE-209 induced more oxidative damage on the brain tissue than that on the kidney tissue, seen from Fig. 2 ~ 5. Because brain may be more easily oxidized than kidney by oxygen free radicals induced by BDE-209 [21], the brain tissue is more sensi-

tive than the kidney tissue to oxidative damage effects. Seen from Fig. 4 ~ 5, in the 4 $\mu g \cdot mL^{-1}$ dose group, compared with the control group, MDA content significantly increased, while SOD activity did not change significantly. The molecular mechanism may be that oxygen free radicals induced by BDE-209 firstly attacked the unsaturated fatty acid chain of lipid bilayer, then entered into the cells to attack antioxidant enzymes, resulting in oxidative damage. Antioxidant system in cytoplasm could not inhibit oxygen free radicals on lipid peroxidation, since it works only in cytoplasm [21].

In conclusion, BDE-209 exposure can cause significant oxidative damage in mouse kidney and brain tissue. There is a dose-effect relationship between the extent of damage and BDE exposure concentration. It is speculated that BDE-209 may produce excessive free radicals, leading to organ damage in mouse. Further related research on BDE-209 is need to be carried out, such as analytical methods of BDE-209, toxicological effects and biological action mode of adsorption on solid medium or suspended in water, toxicological effects of BDE-209 on human. In addition, the determination of BDE-209 levels in organisms and the environment should be carried out in the future work.

Biography of corresponding author: Yang Xu (1954—), male, professor. His research field is environmental toxicology.

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