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Decabromodiphenyl ether (BDE-209) enhances foam cell formation in human macrophages via augmenting Toll-like receptor 4-dependent lipid uptake

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ABSTRACT

Growing epidemiological evidence is substantiating an association between exposure to persistent organic pollutants (POPs) and incidence of atherosclerosis. Decabromodiphenyl ether (BDE-209) is a new POP which presents extensively in human populations; whether this contaminant is potentially arteriosclerotic remains unclear. In this study, we investigated the effects of BDE-209 on macrophage-derived foam cell formation, a hallmark of early atherosclerosis, using THP-1-derived macrophages incubated with oxidized low-density lipoprotein (oxLDL) as a foam cell model. The results showed that 6.25, 12.5 and 25.0 μM of BDE-209 significantly enhanced lipid accumulation inside the foam cells, in a dose-dependent manner. Further mechanism assays suggested that BDE-209 significantly increased the expression of Toll-like receptor 4 (TLR4), a signal transducing integral membrane protein mediating lipid uptake in macrophages, at both the mRNA and protein levels. In contrast, there was no significant changes for several key regulators involving in lipid efflux, lipogenesis, and lipid oxidation in macrophages. Furthermore, the augmented lipid accumulation was almost completely abrogated by treatment with an anti-TLR4 antibody. Together, these data illustrate that BDE-209 enhances oxLDL-induced macrophage foam cell formation via augmenting TLR4-dependent lipid uptake in the cells.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are flame-retardant chemicals that are added into a variety of consumer products, including textiles, plastics, wire insulation and automobiles, to decrease flammability (de Wit, 2002). These chemicals can be released to the environment during the use and disposal of the PBDE-containing products, and can accumulate in biota due to their highly lipophilic properties (Alaee et al., 2003; de Wit, 2002). Three commercial PBDE products, i.e., c-PentaBDE, c-OctaBDE and c-DecaBDE, have been used, with c-DecaBDE being the largest volume of usage (Earnshaw et al., 2013). Decarbromodiphenyl ether (BDE-209) is the major component of c-decaBDE (Alaee et al., 2003), and has been identified as a ubiquitous pollutant in the environment (English et al., 2016; Verreault et al., 2018). Because of its environmental and human health concerns, BDE-209 has been recently listed in the Stockholm Convention as a persistent organic pollutant (POP) substance (Stockholm Convention, 2017). Despite of the restriction of use, release of BDE-209 from existing products and the environmental reservoirs (e.g., sediment and soil) will continue for many years. Recent studies also revealed that BDE-209 still presented in the blood serum of the general population, at ng/g lipid weight level (Huang et al., 2014; Fromme et al., 2016; Jeong et al., 2018; Ji et al., 2017). This observation raises public concerns about the potential adverse impact of this chemical on human health. However, the toxicity aspects of BDE-209 for humans are still limited; most of which focused on neurological development, the endocrine system and carcinogenicity (Darnerud et al., 2001; Darnerud, 2003; Hardy et al., 2009; Costa and Giordano, 2011; Czerska et al., 2013; Vuong et al., 2018; Chen et al., 2018; He et al., 2018).

Atherosclerosis is a non-communicable disease that encompasses a group of disorders of the heart and blood vessels (Labarthe, 2011). In recent years, atherosclerosis underlies most cardiovascular diseases

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(CVD), the leading cause of death and disability worldwide (Pagidipati and Gaziano, 2013). Recent epidemiological and laboratory evidence suggests that the exposure to environmental POPs is statistically associated with the incidence of multiple CVD, directly or indirectly via promotion of CVD risk factors and associated diseases (Barrett, 2012; Alharbi et al., 2018; Gupta et al., 2018; Henríquez-Hernández et al., 2017; Singh and Chan, 2018). Although there has been no reports on the impacts of BDE-209 on CVD so far, epidemiological studies suggested that some lower brominated PBDE congeners, e.g., BDEs-28, -47, −99 and −100, and a polychlorinated biphenyl (PCB) congener, CB-209, in human blood were associated with some markers or risk factors of CVD. Gump and co-workers demonstrated the associations between the levels of BDEs-28, -47, −99, and −100 in whole blood of children and CVD risk factors, including cardiovascular responses to acute stress and relevant psychological variables (hostility and depression) (Gump et al., 2014). They found that BDE-28 was significantly associated with greater heart rate and lower total peripheral resistance during acute psychological stress and greater anger; meanwhile, BDEs-47 and -100 was significantly associated with lower diastolic blood pressure and BDEs-28 and -100 was associated with shorter pre-ejection period. Moreover, Lind et al. (2012) reported that circulating levels of CB-209 in the elderly were significantly associated with the markers of CVD, including the prevalence of overt carotid plaques, the intima-media thickness (IMT), and gray scale median of the intima-media complex (IM-GSM). Circulating level of CB-209 in the elderly was also revealed to be significantly associated with the increases in total serum cholesterol and LDL-cholesterol, a risk factor for atherosclerosis and other CVD (Penell et al., 2014). BDE-209 is structurally similar to these lower brominated PBDE congeners and CB-209, and usually presents at higher levels than these chemicals in human blood (Huang et al., 2014; Fromme et al., 2016). This warrants an in-depth analysis of the impact of BDE-209 on the health of human cardiovascular system.

During the initiation of atherosclerosis, one of the key processes is the macrophage-derived foam cell formation, a result of the continuous uptake modified low-density lipoprotein (LDL) and the excessive lipoprotein-derived cholesterol accumulation inside the cells (Moore and Tabas, 2011; Moore et al., 2013). The lipid homeostasis in macrophages is mainly regulated by several key regulators mediating the uptake, efflux, synthesis, and oxidation of lipid in the cells (Moore et al., 2013). In various vascular diseases, however, nutrients such as fatty acids and glucose, endogenous toxins, e.g., oxidized LDL (oxLDL), and environmental pollutants may disregulate the signaling pathways of these regulators and lead to an imbalance of the uptake, metabolism, or removal of lipid in macrophages, thereby increasing the risk of foam cell formation and the development of atherosclerosis (Moore and Tabas, 2011; Moore et al., 2013; Crow et al., 2008; Ross et al., 2014). Although many studies have reported that nutrients and endogenous toxins can impair lipid homeostasis in macrophages, little research focused on the role of environmental toxicants in this context (Crow et al., 2008; Ross et al., 2014; Rao et al., 2014).

In the present study, we sought to investigate whether BDE-209 promotes the formation of macrophage-derived foam cells and to explore the molecular mechanisms involved, using the uptake of oxLDL in THP-1 derived macrophages as a foam cell model. Our study provides evidence that BDE-209 augments macrophage-derived foam cell formation, which is an early marker of atherosclerosis. We also demonstrate an enhancement of lipid accumulation induced by BDE-209 that is mediated by TLR4-dependent lipid uptake in the macrophages.

2. Materials and methods

2.1. Chemicals and reagents

BDE-209 (95.9% purity) and dimethyl sulfoxide (DSMO) were obtained from Dr. Ehrenstorfer GmbH (Germany) and Sigma-Aldrich (USA), respectively. BDE-209 was dissolved in DMSO to make a stock solution of 2 mM. The stock was further diluted for testing.

Table 1
Primer sequences used for quantitative real-time PCR.

gene	forward sequence	reverse sequence
SR-A	TCCTCGTGTTTGCAGTTCTC	GCAATTCTTCGTTTCCCACT
CD 36	TTGAACTTCTGGGCAAATG	TGGGGATGCCTTCAAACAC
VLDLR	GGTCAGACTGGGGCGAGCCA	GCTGGCAGGCAGAGATATTC
TLR4	GGTGATTGTTGTGGTGTCCCA	AGTGTTCCTGCTGAGAAGGCG
ABCA1	GTATTTTTGCAAGGCTA	CCAGTTACATTTGACAA
ABCG1	CCCTCAGAATGCCAGCAGTT	CCGAGACACACACCGACTTG
SR-BI	GGCGGTGATGATGGAGAAT	TGAAGAGCCCAGAGTCGGA
HMGCR	TGACCTTTCCAGAGCAAGC	CCAACTCCAATCACAAGACATTC
HMGCS	CATTAGACCGCTGCTATTCT	AGCCAAAATCATTCAAGGTA
FAS	GCCGCCATCTACAACATC	GTCTTCCACACTATGCTCAG
SCD1	CACCCAGCTGTCAAAGAGAAGG	AGGACGATATCCGAAGAGGTGG
CPT1	ATGAGTCGTGCCACCAAGAT	AAGAGGCCTCACCGACTGTA
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC

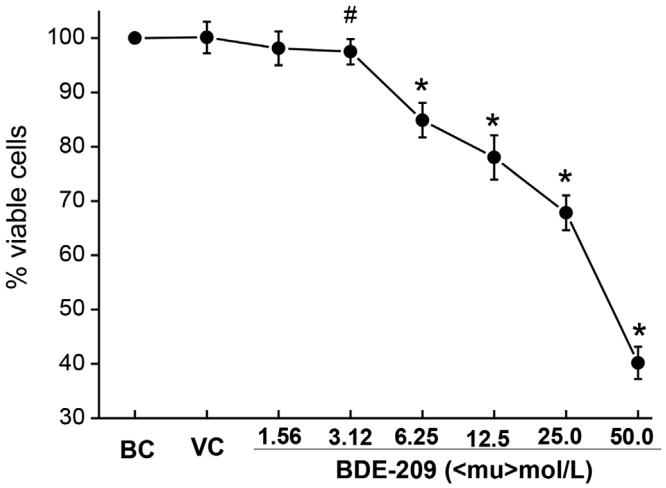

Fig. 1. Cytotoxicity of BDE-209 on THP-1-dervied macrophages after 24 h exposure. Three independent experiments were performed and the data are expressed as mean ± SE% of control cells. BC, blank control; VC, vehicle control. * indicates significant difference ($p < 0.05$) when compared with BC. # represents the no observed effect concentration (NOEC).

2.2. Cell culture

The THP-1 human monocyte-derived cell line was from the American Type Culture Collection (ATCC, USA). The THP-1 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 20 g/ml streptomycin and 20 IU/ml penicillin at 37 °C, under a saturated 5% CO_2 humidified environment. Differentiation into macrophages was achieved by treating the cells with phorbol 12-myristate 13-acetate (PMA, 200 ng/ml) for 48 h. Cell transfections were performed with the SuperFect fragment (Qiagen; USA) according to the manufacturer's instructions. The cells were incubated for 24 h after transfection and used for the future experiments.

2.3. Determination of cell viability

Cell viability was determined by the cell counting kit-8 (CCK-8) test, using a CCK-8 cell proliferation test kit (Betboy, China). THP-1 macrophages were seeded at density of 2×10^4 cells/well on 96-well microtiter plates. Cells were incubated with BDE-209 at increasing concentrations (1.56, 3.12, 6.25, 12.5, 25 and 50 μM). The maximum concentration of DMSO in the culture medium is 2.5%. Thus, the culture medium containing a final concentration of 2.5% of DMSO was also cultured in parallel, serving as a control. After 24-h of incubation at