TCDD and TBBPAdisrupted on adipocyte and/or osteoblast differentiation in human mesenchymal stem cells

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Introduction

Obesity is caused by complex interactions among genetic, behavioral and environmental factors, and imbalance between caloric intake and expenditure is considered a key cause of the obesity epidemic. In addition, there is emerging evidence that exposure to environmental endocrine disrupting chemicals (EDCs) may also be an important contributor. These EDCs have been identified as environmental obesogens. Resently, Barker *et al*. proposed the developmental origins of health and disease (DOHaD) hypothesis, which was that various kinds of environmental factor such as nutrition and exposure of chemical substance during pregnancy and postnatal development period have an influence on adolescent and/or adult health and various kinds of disease outcome risks across the lifespan [1]. Moreover, there was high sensibility point against the EDCs, critical point, on embryonic and neonatal period [2]. It is suggested that chemical exposure on embryonic and neonatal period cause obesity and obesity related diseases.

Human mesenchymal stem cells (hMSCs) are multipotent cells isolated from bone and adipose tissue. Recently, it was reported that a risk of the osteoporosis rose to decrease the bone density with adipose deposits on bone marrow which was the place where the differentiation of the MSC having the differentiation ability to adipocyte and osteoblast was decided. Thus, there is close to osteoporosis relation to obesity, and it is suggested that to disrupt the differentiation of MSCs involves the induction and exacerbation of the both diseases. In the present study, we investigated the disruption effects of TBBPA and TCDD on the adipocyte or osteoblast differentiation of hMSCs.

Material and methods

1) *Cell cultures*

Human MSC (MSC-R14) was provided by the RIKEN BRC. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin and 3 ng/mL StemBeads FGF2 at 37 ˚C. The cytotoxicity of the chemicals and DMSO was determined using a tetrazolium-based colorimetric assay, the WST-8 kit, according to the manufacturer's protocol (data not shown).

2) *Adipogenetic induction and oil red O staining*

hMSCs were seeded on a 24-well plate. Two days after confluence (designated as Day 0), cells were treated with adipocyte differentiation medium containing 1 μM dexamethasone, 0.5 mM 3-isobuthyl-1-methylxanthine (IBMX), 200 μM indomethacin and 10 μg/mL insulin. After 3 days (Day 3), the media was replaced and maintained with 10 μg/mL insulin alone to Day 21. The differentiated cells were stained with oil red O to detect lipid droplets in adipocytes. After washing twice with phosphate buffered saline (PBS), cells were fixed with 4% paraformaldehyde at room temperature, and then stained with 3.3 mg/mL oil red O in 60% isopropanol for an hour. Cells were washed with PBS, and observed under an IX71microscope. Stained oil red O was eluted with isopropanol and optical absorbance was measured at a wavelength of 550 nm using a SPECTRA FLUOR for quantitative analyses.

3) *Osteogenetic induction and alizarin red S staining*

At Day 0, cells were treated with osteoblast differentiation medium containing 0.1 μM dexamethasone, 10 mM β-glycerophosphate and 50 μg/mL L-ascorbic acid, and maintained for 21 days. The differentiated cells were assessed by alizarin red S staining for the presence of calcium deposits. Briefly, the cells were fixed with ice cold 70% ethanol, rinsed with distilled water, and then stained with 40 mM alizarin red S dissolved in distilled water (pH 4.2; adjusted with 10% ammonium hydroxide) for 5 min. Cells were washed with distilled water and observed under an IX71microscope. After imaging, the dye was eluted with 10% acetic acid, and the absorbance was measured at 450 nm using a TriStar LB 941 microplate reader.

Result and discussion

To evaluate the effects of TBBPA and TCDD in alone or combination on hMSCs differentiation into adipocyte, we performed using oil red O staining, and quantitated the staining lipid droplet. As shown in Fig. 1, the number of lipid droplet increased in a dose-dependent manner in the presence of TBBPA. On the other hand, the number of lipid droplets decreased in a TCDD higher concentration cells. In the combination of TBBPA and TBBPA, the nunmber of lipid droplet dose-dependently inhibited by TCDD upregulated by TBBPA in a dose-dependent manner.

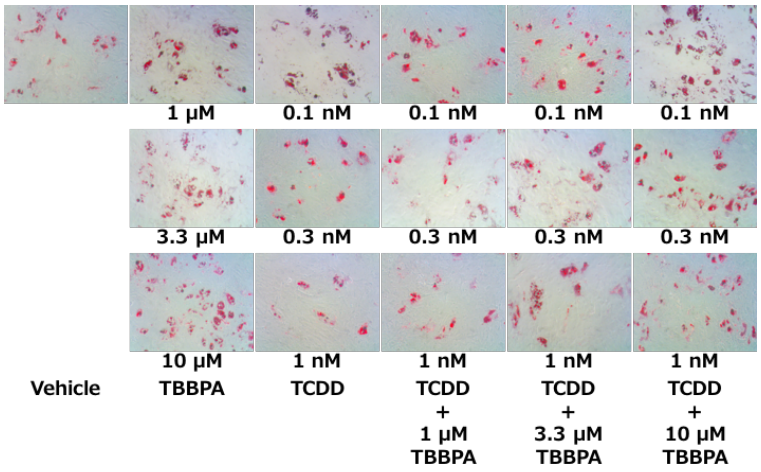

Fig. 1 Effect of TCDD and TBBPA on adipocyte differentiation in hMSCs

hMSCs were differentiated into adipocyte with TBBPA at 1, 3.3 and 10 μM and/or TCDD at 0.1, 0.3, and 1 nM. After 21 days, Lipid droplets were stained with oil red O.

To determine the effects of TBBPA and TCDD on hMSCs differentiation into osteoblast, we first examined alkaline phoapatase (ALP), an early osteoblastic marker, by staining and activity measurement. TBBPA did not influence on osteoblast differentiation On the other hand, the ALP staining and activity of TCDD-induced osteoblast decreased in a dose-dependent manner. Moreover, dose-dependent decrement in alizarin red staining was observed in the presense of TCDD (Fig. 2).

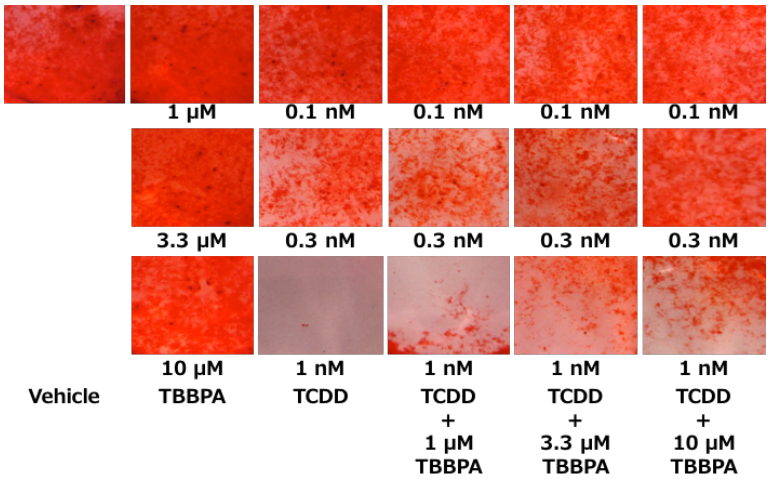

Fig. 2 Effect of TCDD and TBBPA on osteoblast differentiation in hMSCs

hMSCs were differentiated into osteoblast with TBBPA at 1, 3.3 and 10 μM and/or TCDD at 0.1, 0.3, and 1 nM. After 21 days, calcium deposits were stained with alizarin red S.

In conclusion, we showed that TBBPA and TCDD were disrupted the differentiation to adipocyte or osteoblast. Further studies are required to clarify the details of the molecular mechanisms by which pluripotency is disrupted by BFRa and dioxins.

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Reference

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2) Yoshida M. *et al*; *Toxicologic Pathology.*, 30, 357-64, 2002.