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$ZnSO_4$ rescued vimentin from collapse in DBP-exposed Sertoli cells by attenuating ER stress and apoptosis

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ABSTRACT

Sertoli cells (SCs) provide physical and nutritional support for spermatogenesis. Dibutyl phthalate (DBP) is a plasticizer that has male reproductive toxicity. The collapse of vimentin in DBP-exposed SCs is thought to induce the sloughing of spermatocytes from seminiferous tubules. In this study, we explored methods to rescue vimentin from collapse in DBP-exposed SCs. DBP not only induced the hyperphosphorylation of vimentin but also triggered endoplasmic reticulum (ER) stress and apoptosis in SCs. Treatment with BAPTA-AM, an antagonist of Ca^{2+}, significantly decreased the level of phosphorylated vimentin, while LY294002, an inhibitor of Akt1, did not. ER stress and apoptosis remained at high levels, and the distribution of vimentin was not improved. $ZnSO_4$ treatment did not decrease the level of phosphorylated vimentin. However, after treatment, ER stress and apoptosis were obviously inhibited, and the distribution of vimentin was reconverted. These results indicated that $ZnSO_4$ could alleviate the collapse of vimentin by attenuating ER stress and apoptosis. This study suggested that an appropriate zinc supply might be a choice to alleviate DBP-induced adverse reproductive effects.

1. Introduction

DBP is one of the dominant organic contaminants in air (5.9–1630 ng/m^3) (Jo et al., 2016), dust (11.9–9930 μg/g) (Blanchard et al., 2014), rivers (0.009–47.7 μg/g) (Liu et al., 2014), food (0.7–85 ng/g) (Birnbaum and Schug, 2014; Cao et al., 2016) and personal care products (0.01–27,400 μg/g) (Guo and Kannan, 2013). The estimated daily DBP intake of humans ranges from 0.02 to 38.46 μg/kg bw/day (Cao et al., 2016). Previously, we investigated concentrations of DBP in the rivers of Chongqing City in China, and the DBP concentration ranged from 1.316 μg/dm^3 to 2.178 μg/dm^3 (Cui et al., 2009). According to Dobrzynska's (2016) statistics, the concentration of DBP in Europe and the United States ranged from 0.01 to 622.9 μg/dm^3 in surface water, from < 0.008 to 0.75 mg/m^3 in the work place, and from 36 to 50 mg/kg in home dust; furthermore, its concentration was approximately 143 μg/kg bw/day in occupational exposure and ranged from 2 to 10 μg/kg bw/day in general population exposure (Dobrzynska, 2016). Concentrations of DBP and its metabolite MBP in human urine are associated with poor sperm quality (Chen et al., 2017; Thurston et al., 2016; Wang et al., 2015). Recently, we detected the urinary concentrations of MBP (83.40 ng/mL) in Chongqing men (MARHCS cohort study), and the results indicated that MBP was the most dominant contaminant in PAEs. Although the detected concentration of MBP was less than the reference dosage indicated by the United States Environmental Protection Agency (US EPA), MBP exposure exhibited a significantly negative correlation with semen viability (Chen et al., 2017). Thus, it was necessary to investigate its mechanism in animal and cellular studies. The sloughing of spermatocytes from seminiferous tubules during spermatogenesis is one of the phenomena induced by DBP, according to rat gavage studies (Aly et al., 2016; Bao et al., 2011; Salazar et al., 2004).

Spermatogenesis takes place in the seminiferous tubules of the testis. SCs are the major somatic cells that constitute the seminiferous tubules. SCs provide physical and nutritional support for spermatocytes (Alves et al., 2013). During spermatogenesis, Sertoli and germ cells crosstalk through paracrine signaling, and different stages of spermatocytes move from the basal to adluminal compartment (Cheng and Mruk, 2010). This process is coordinated by the restructuring of the ectoplasmic specialization-blood testis barrier-hemidesmosome (ES-BTB-HD) axis. In the desmosome structure, integral membrane proteins,

Abbreviations: Akt1, v-akt murine thymoma viral oncogene homolog 1; BAPTA-AM, acetoxymethyl ester of 1, 2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; BTB, blood-testis barrier; DBP, dibutyl phthalate; ER, endoplasmic reticulum; ES, ectoplasmic specialization; Grp78, glucose-regulated protein 78; HD, hemidesmosome; HSPA5, heat shock 70-kDa protein 5; MARHCS, Male Reproductive Health in Chongqing College students; MBP, monobutyl phthalate; Msc2, meiotic sister chromatid recombination 2; PAEs, phthalic acid esters; SCs, Sertoli cells; TUNEL, terminal-deoxynucleotidyl transferase-mediated nick end labeling

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Table 1
List of primary and secondary antibodies used in this study.

Antibody	Source	kDa	Dilution	Vendor	Catalog #
Vimentin	Mouse	57	1:1000	Santa Cruz Biotechnology, CA, USA	sc-6260
p-Vimentin	Rabbit	57	1:500	Santa Cruz Biotechnology, CA, USA	sc-16674-R
p-Akt1	Mouse	60	1:1000	Santa Cruz Biotechnology, CA, USA	sc-293125
Bax	Rabbit	23	1:500	Boster Biological Technology Co., Ltd., Wuhan, China	BA0315
Bcl2	Rabbit	26	1:500	Boster Biological Technology Co., Ltd., Wuhan, China	BA0412
HSPA5	Rabbit	70	1:500	Boster Biological Technology Co., Ltd., Wuhan, China	BA4293
β-Actin	Mouse	42	1:2000	Beyotime Institute of Biotechnology, Haimen, China	AA128
Mouse	Goat	–	1:2000	Beyotime Institute of Biotechnology, Haimen, China	A0208
Rabbit	Goat	–	1:2000	Beyotime Institute of Biotechnology, Haimen, China	A0216

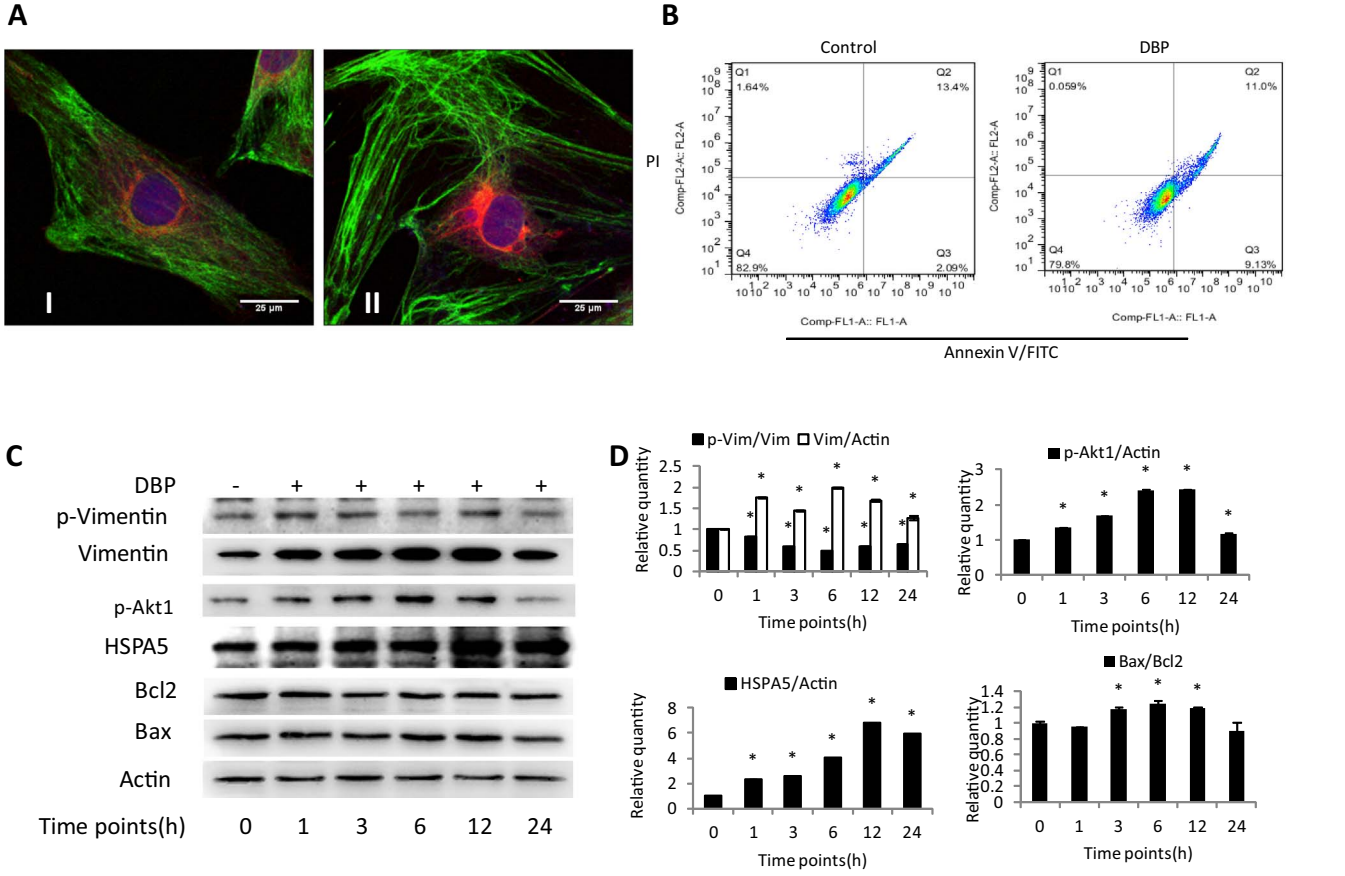

Fig. 1. Effects of DBP on the distribution and expression of vimentin and HSPA5 and apoptosis. Part A presents the distribution of vimentin (green) and HSPA5 (red) in control (I) and DBP-treated SCs (II) at 24 h (original magnification: 200×). Part B shows the apoptotic results (flow cytometry) detected by Annexin V/PI staining. Part C presents the western blotting results of p-vimentin, vimentin, p-Akt1, HSPA5, Bcl2 and Bax. Part D is the quantitative analysis of part C done using ImageJ software. $p < 0.05$ was considered statistically significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

such as desmogleins and desmocollins, are anchored to vimentin *via* the cytolinker desmoplakin (Cheng et al., 2011). Vimentin in SCs was regulated cyclically during stages of spermatogenesis, and it is essential for the adaptation of SCs to neighboring cells (Aumuller et al., 1988; Mali et al., 1987). Phosphorylation is the key factor that affects the assembly and distribution of vimentin (Cogli et al., 2013; Eriksson et al., 2004; Goto et al., 2002). Altered phosphorylation and distribution of vimentin are thought to lead to the escape of spermatocytes from seminiferous tubules, ultimately causing apoptosis (Alam et al., 2010; Kleymenova et al., 2005). Our previous studies indicated that inhibition of PPARα and Smad2/3 could attenuate DBP-induced hyperphosphorylation and the redistribution of vimentin in DBP-exposed SCs to some extent (Zhang et al., 2014; Zhang et al., 2015).

Besides the hyperphosphorylation and redistribution of vimentin in SCs, DBP can also induce ER stress and apoptosis in the testis (Zhang et al., 2016b). Ca^{2+}, Akt1 and Zinc are closely associated with ER stress, apoptosis and vimentin redistribution. Intracellular Ca^{2+} is mainly stored in the ER lumen. The ER controls various cellular signaling transductions in response to stress by releasing Ca^{2+}. Overloaded Ca^{2+} in the mitochondria induces mitochondrial swelling and apoptosis (Bahar et al., 2016). Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) not only mediates ER stress and apoptosis (Bracken et al., 2016) but also modulates the phosphorylation of vimentin in SCs (Ando et al., 1991; Spruill et al., 1983). Akt1 enhances HSPA5 accumulation by helping protein stability and inhibiting apoptosis (Dai et al., 2010). Additionally, Akt1 also modulates the phosphorylation of vimentin (Zhu et al., 2011). Zinc and Msc2 (zinc transporter protein) are required for ER function (Ellis et al., 2004). Zinc deficiency induces ER stress and promotes apoptosis (Kim et al., 2016; Sunderman, 1995). DBP exposure increased the urinary excretion of zinc and markedly decreased the zinc content in the testes of rat (Cater et al., 1977). In addition, zinc can directly bind to vimentin at residue cysteine 328 and