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Individual and combined hepatocytotoxicity of DDT and cadmium *in vitro*

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Abstract

The organochlorine insecticide dichlorodiphenyltrichloroethane (DDT) and heavy metal cadmium (Cd) are widespread environmental pollutants. They are persistent in the environment and can accumulate in organisms. Although the individual toxicity of DDT and Cd has been well documented, their combined toxicity is still not clear. Since liver is their common target, in this study, the individual and combined toxicity of DDT and Cd in human liver carcinoma HepG2 and human normal liver THLE-3 cell lines were investigated. The results showed that DDT and Cd inhibited the viability of HepG2 and THLE-3 cells dose-dependently and altered lysosomal morphology and function. Intracellular reactive oxygen species and lipid peroxidation levels were induced by DDT and Cd treatment. The combined cytotoxicity of DDT and Cd was greater than their individual cytotoxicity, and the interaction between Cd and DDT was additive on the inhibition of cell viability and lysosomal function of HepG2 cells. The interaction was antagonistic on the inhibition of cell viability of THLE-3 cells. These results may facilitate the evaluation of the cumulative risk of pesticides and heavy metal residues in the environment.

Keywords

Organochlorine pesticide, heavy metal, combined toxicity, oxidative stress, hepatic cell line, lysosome, hepatotoxicity

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Introduction

Chemical pesticides and heavy metals are two important industrial products, which are also the major sources of the environmental pollution. Dichlorodiphenyltrichloroethane (DDT) is an effective chemical pesticide, which was once largely produced and widely used around the world (Kaushik and Kaushik, 2007). However, DDT is a persistent organic pollutant and many countries have banned DDT's agricultural application (Boul et al., 1994). Nevertheless, DDT has been used in indoor spraying for malaria control for decades in Africa, which has protected millions of people from malaria infection (Bouwman et al., 2011; Mabaso et al., 2004). Although the use of DDT is restricted or even prohibited nowadays, DDT residues are found in soil, water, and agricultural products worldwide (Mendes et al., 2016; Tsakiris et al., 2015; Veljanoska-Sarafiloska et al., 2013). DDT has prominent multi-organ toxicity to humans and animals. DDT can induce hepatotoxicity (Ben et al., 2000), neurotoxicity (Eriksson et al., 1990, 2000), lung toxicity (Narayan et al., 1984), and reproductive toxicity (Magnarelli et al., 2009). DDT can also be carcinogenic (Alavanja et al., 2004). Thus, the presence of DDT

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poses a significant risk to the health of humans and wild animals.

Cadmium (Cd) is a widely used heavy metal, which is also one of the most common industrial pollutants in the environment. However, Cd can accumulate in the human body and persist for a long time (Järup et al., 1998), which may cause serious damage to multiple organs such as kidney (Satarug and Moore, 2004), liver (Bucio et al., 1995), and nervous system (Méndez-Armenta et al., 2003). In addition, Cd causes acute liver injury and induces end-stage damage in kidneys, at least in humans (Hyder et al., 2013; Matović et al., 2015; Nambunmee et al., 2018; Rinaldi et al., 2017). Cd also contributes to the development of cancer (Waalkes, 2000).

Oxidative stress plays an important role in Cd-induced hepatotoxicity. Cd treatment affects electron transfer (Müller and Ohnesorge, 1984); stimulates reactive oxygen species (ROS) production (Lopez et al., 2006) and lipid peroxidation; decreases ATP/ADP ratio (Müller, 1986); inhibits the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) in rats (Jamall and Smith, 1985; Ognjanovic et al., 2008; Ossola and Tomaro, 1995); and reduces the level of the antioxidant glutathione (GSH) (Urani et al., 2005). Oxidative stress is also involved in the hepatotoxicity of DDT (Tomiyama et al., 2003). DDT can inhibit electron transfer, uncouple oxidative phosphorylation, disturb mitochondrial membrane potential (Narayan et al., 1984), and induce ROS production (Khan and Cutkomp, 1982).

It was found that oxidative stress could be accumulated in cells to lead lysosomal dysfunction (Tai et al., 2017). It has been reported that Cd induced lysosomal damage and might mediate cell death (Fotakis et al., 2005). In addition, incubation of DDT could disrupt lysosomal membranes in liver of rats and fish (Köhler et al., 2002; Rogers et al., 1976). Thus, it is also important to study the combined effect of Cd and DDT on lysosomal morphology and function since both DDT and Cd are persistent chemical materials and could coexist in the environment with people probably simultaneously exposed to the two chemicals from food, water, soil, and other environmental media or agricultural products (Mansour et al., 2009; Abdallah and Morsy, 2013; Gaw et al., 2008; Huang et al., 2011; Zhu et al., 2012). However, different toxicants may interact with each other and cause different toxicity compared with their individual toxicity. A generally accepted concept is that when the combined toxicity is greater or less than the sum of their individual toxicity, the interaction between the two toxic chemicals is termed as synergistic or antagonistic, respectively (Crain et al., 2008; Eaton and Gilbert, 2008; Moser et al., 2003). When the combined toxicity equals the sum of their individual toxicity, the interaction between the two chemicals is additive (Eaton and Gilbert, 2008; Moser et al., 2003). Although the individual toxicity of Cd and DDT has been well documented, their combined toxicity has not been thoroughly investigated. Therefore, we chose DDT, which is the most widely used organochlorine pesticide with persistent toxicity, and Cd, which is a toxic heavy metal, as the test industrial toxicants to investigate their combined toxic effects on hepatocytes *in vitro*.

Materials and methods

Materials

HepG2 cells were donated by Dr Guangbiao Zhou in Institute of Zoology, Chinese Academy of Sciences. THLE-3 cells were kindly provided by Dr Yue-Ling Zhang from Shantou University (Guangdong, China). Cadmium chloride ($CdCl_2$, hereafter called "Cd" for short), propidium iodide (PI), dichlorofluorescein diacetate (DCFH-DA) fluorescent probe, Dulbecco's modified Eagle's medium (DMEM), and Hoechst 33258 was obtained from Sigma-Aldrich Co (St Louis, Missouri, USA). DDT was obtained from Riedel-de Haen AG (Seelze, Germany). Fetal calf serum was purchased from HaoYang Biological Manufacture Co. (Tianjin, China). Penicillin and streptomycin were purchased from North China Pharmaceutical Group Corporation (Beijing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sanland Corporation (Xiamen, China). Coomassie Brilliant Blue R250 and dimethyl sulfoxide (DMSO) were obtained from Amresco LLC (Solon, Ohio, USA). Total SOD assay kit and Lyso-Tracker Red were obtained from Beyotime Institute of Biotechnology (Jiangsu, China). Neutral red was obtained from Shanghai Third Chemical Factory (Shanghai, China).

Cell culture

HepG2 cells and THLE-3 cells were cultured in DMEM and RPMI-1640 medium, respectively, and both of them were supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

MTT assay and neutral red uptake assay

Cd and DDT were dissolved in double-distilled water and DMSO, respectively, and then diluted with DMEM solution. The MTT reagent and the neutral red staining reagent were dissolved in phosphate-buffered saline (PBS) and then diluted with DMEM solution; the working solutions were filtered through a 0.22-μm membrane filter before use.

HepG2 cells and THLE-3 cells were exposed to Cd (0–10 μM for HepG2 cells and 0–40 μM for THLE-3 cells, respectively), DDT (0–100 μM and 0–200 μm, respectively), or their mixtures for 48 h. In the MTT assay, 20 μL of MTT solution (5 mg/mL) was added to each well and the final MTT concentration was 0.5 mg/mL. Then the cells were incubated at 37°C for 4 h. The absorbance was measured at 570 nm with a Benchmark microplate reader (Bio-Rad, Hercules, CA, USA). Each treatment was carried out in triplicate and each experiment was repeated at least four times.

In the neutral red uptake assay, neutral red solution (50 μg/mL) was added to every well and incubated at 37°C for 2 h. The cells were washed with PBS twice, and the dye was extracted with extract solution (1% (v/v) glacial acetic acid and 50% (v/v) ethanol). The absorbance was measured at 570 nm with the microplate reader. Each treatment was carried out in triplicate, and each experiment was repeated at least four times.

Lyso-Tracker Red staining

HepG2 cells were treated with 0.25, 0.5, and 1 × IC_{50} value of Cd, DDT, and Cd plus DDT for 48 h, and then the intracellular lysosomes were visualized by Lyso-Tracker Red staining. The nuclei were counterstained with Hoechst 33258. The red fluorescence was observed by using a confocal microscope LSM710 (Zeiss, Germany) with an excitation wavelength 577 nm and emission wavelength 590 nm. The blue fluorescence was observed with excitation wavelength 350 nm and emission wavelength 460 nm. Each treatment was carried out in duplicate, and the experiment was repeated twice.

ROS assay and lipid peroxidation assay

HepG2 cells were exposed to different concentrations (0.125, 0.25, 0.5, 1, and 2 × IC_{50}) of Cd, DDT, and Cd plus DDT for 8 h, for ROS production was induced at the highest level 8 h after treatment. Then, the intracellular ROS level was detected by DCFH-DA fluorescent probe, and the fluorescence was measured with a Bio-Tek Synergy 4 fluorescent microplate reader (Winooski, Vermont, USA). Malondialdehyde (MDA) level was measured to reflect the lipid peroxidation level in the cells by the method of Wu and Cederbaum (2001).

Measurement of antioxidants and antioxidant enzymes

Briefly, HepG2 cells were exposed to different concentrations (0.25, 0.5, and 1 × IC_{50}) of Cd, DDT, and Cd plus DDT for 48 h. Then cells were scraped using a rubber policeman and collected by centrifugation (1000 *g*, 4°C, 10 min). Cell crude extract was obtained by sonication. SOD activity was measured using SOD assay kit according to the manufacturer's instruction.

HepG2 cells were exposed to different concentrations of Cd, DDT, and Cd plus DDT for 48 h. Then, CAT activity was measured as described by Sinha (1972). Intracellular GSH level was assayed as described by Look et al. (1997). GR activity was measured as described by Dringen and Gutterer (2002).

Statistics

SPSS 18.0 software (SPSS, Inc., Chicago, Illinois, USA) was employed for the statistical evaluations. Data are presented as mean ± standard error. Analysis of variance test was performed to determine significant differences among the groups, followed by Tukey's multiple comparison test. The differences were considered significant with the value of $p < 0.05$.

Results

Cell viability

HepG2 cells were treated with different concentrations of Cd and DDT for 48 h. Then the cell viability was measured by MTT assay. As shown in Figure 1(a) and (b), Cd and DDT alone treatments inhibited cell viability in a dose-dependent manner. The IC_{50} values of Cd and DDT were 4.21 μM and 57.26 μM, respectively (Table 1). The cell viability IC_{50} values were

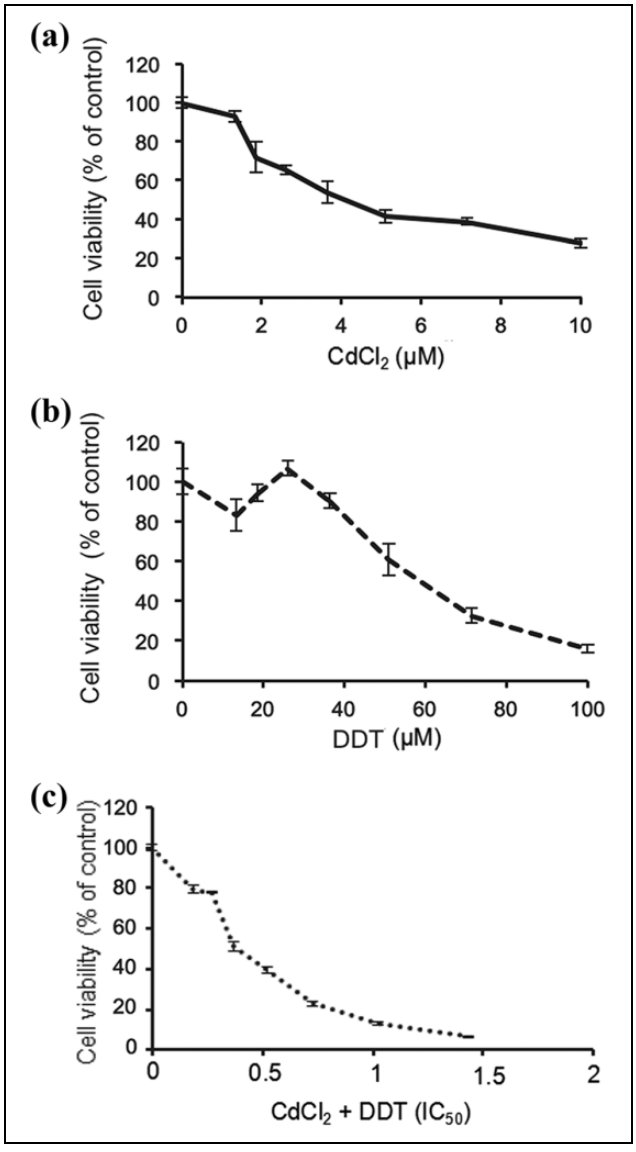

Figure 1. Effect of cadmium and DDT on cell viability of HepG2 cells. HepG2 cells were treated with different concentrations of $CdCl_2$ (a), DDT (b), or $CdCl_2$ plus DDT (c) for 48 h. Cell viability was measured by MTT assay. Data were calculated as percentage of corresponding control values and presented as mean $\pm$ SD ($n = 4$). DDT: dichlorodiphenyltrichloroethane; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

used as the assigned IC_{50} values for the following studies. Treatment with mixtures of Cd and DDT at concentrations with equal toxicity also decreased cell viability in a dose-dependent manner (Figure 1(c)). The IC_{50} values of combined Cd and DDT were 2.41 μM and 32.86 μM, respectively (Table 1).

The interaction between different chemicals with regard to their toxicity may be additive, antagonistic, or synergistic. Generally speaking, when the combined toxicity is greater or less than the sum of their individual toxicity, the interaction between the two toxic chemicals is termed as synergistic or antagonistic, respectively. When the combined toxicity equals the sum of their individual toxicity, the interaction between the two chemicals is additive. To quantitatively evaluate the relationship between Cd and DDT, the additive index (AI) was calculated by using the following equation: $S = A_m/A_i + B_m/B_i$ (Marking and Dawson, 1975), where A and B represented IC_{50} of two different chemicals; i and m indicated the IC_{50} for the individual and mixture, respectively; S was the sum of toxic effects. If $S = 1.0$, AI = 0 (or the 95% confidence interval of AI overlaps 0), the interaction between A and B was considered as additive; if $S < 1.0$, AI $= 1/S - 1$, the interaction was synergistic; $S > 1.0$, AI $= 1 - S$, the interaction was antagonistic. As shown in Table 1, the 95% confidence interval of the AI value was from −0.32 to 0.00, indicating that the interaction of Cd and DDT on HepG2 cell viability appeared to be additive. In other words, the combined toxicity of Cd and DDT equaled the sum of their individual toxicity on HepG2 cell viability.

The combined effects of Cd and DDT were also characterized in a human normal liver cell line THLE-3 (Figure 2). The IC_{50} values of Cd and DDT were 16.87 μM and 59.39 μM, respectively (Table 2). Thus, the normal liver cell line THLE-3 cells were less sensitive to $CdCl_2$ compared with liver carcinoma cell line HepG2 cells, and these two cell lines had similar sensitivity to DDT. The IC_{50} values of combined Cd and DDT were 11.10 μM and 39.08 μM, respectively

Table 1. Effect of cadmium and DDT on cell viability of HepG2 cells.

Toxicants	IC_{50} (μM) and 95% confidence intervals		AI[a] and its 95% confidence interval
	Individually	In combination	
$CdCl_2$	4.21 (3.89–4.55)	2.41 (2.25–2.58)	−0.15 (−0.32 to 0.00)
DDT	57.26 (53.98–60.74)	32.86 (30.68–35.20)	

DDT: dichlorodiphenyltrichloroethane; IC_{50}: half inhibitory concentration.
[a]AI represents marking additive index (Marking and Dawson, 1975).

(Table 2). The 95% confidence interval of the AI value was from −0.49 to −0.15, indicating that the interaction of Cd and DDT on THLE-3 cell viability appeared to be antagonistic.

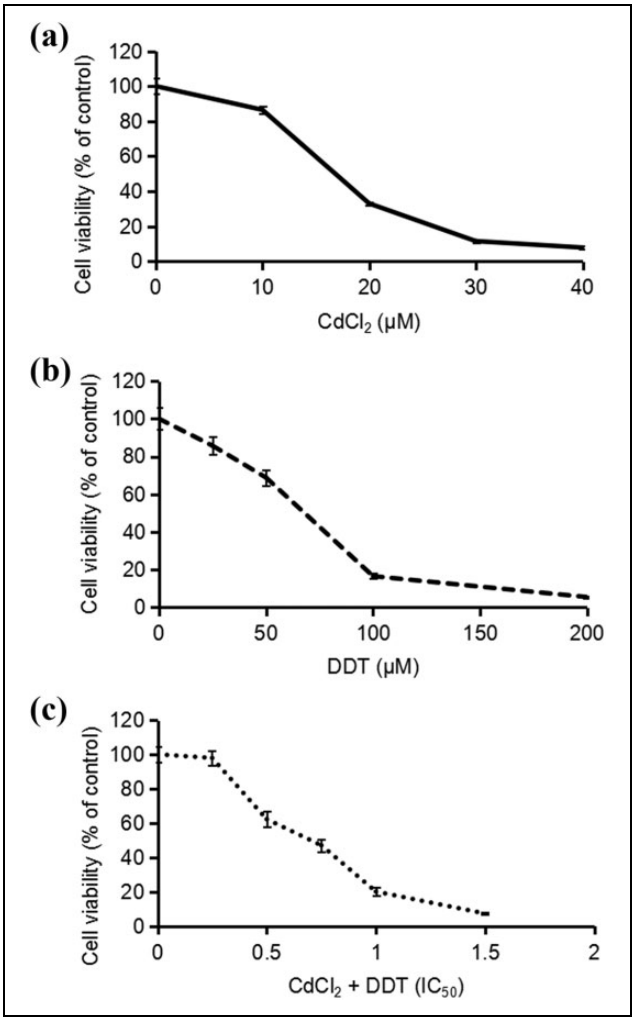

Figure 2. Effect of cadmium and DDT on cell viability of THLE-3 cells. THLE-3 cells were treated with different concentrations of $CdCl_2$ (a), DDT (b), or $CdCl_2$ plus DDT (c) for 48 h. Cell viability was measured by MTT assay. Data were calculated as percentage of corresponding control values and presented as mean ± SD ($n = 4$). DDT: dichlorodiphenyltrichloroethane; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Lysosome morphology and function

Lysosomes are important subcellular organelles that digest waste/damaged macromolecules and organelles. Staining HepG2 cells with Lyso-Tracker Red showed that lysosomes' number and volume in Cd- and DDT-treated cells increased compared with control (Figure 3(a)). However, the ability of lysosomes to absorb neutral red decreased dose-dependently after DDT and Cd treatment (Figure 3(b)). The inhibition by Cd on lysosomal function was much more pronounced than that of DDT. The combination of Cd and DDT had a stronger effect on lysosomal function than Cd and DDT alone. The interaction between Cd and DDT on the lysosomal function appeared to be additive because the effect of their combination equaled the sum of their individual effects.

ROS production and lipid peroxidation level

Oxidative stress plays an important role in the toxicity of Cd and DDT. Therefore, we determined whether the intracellular ROS level was induced by Cd, DDT, and their mixture. As shown in Figure 4(a), treatment with low concentrations of DDT ($0.25 \times IC_{50}$ and $0.5 \times IC_{50}$) and Cd ($0.5 \times IC_{50}$) increased ROS levels, whereas treatment with high concentrations of DDT decreased ROS (Figure 4). At lower concentrations ($0.125 \times IC_{50}$ and $0.25 \times IC_{50}$), the combination treatment induced slightly higher ROS levels compared to individual treatment. At higher concentrations ($1 \times IC_{50}$ and $2 \times IC_{50}$), the combination treatment produced less ROS compared to individual treatments.

Oxidative stress often causes lipid peroxidation. MDA, the product of lipid peroxidation, was measured to reflect the level of lipid peroxidation. Our results showed that high concentrations ($1 \times IC_{50}$) of

Table 2. Effect of cadmium and DDT on cell viability of THLE-3 cells.

Toxicants	IC_{50} (μM) and 95% confidence intervals		AI[a] and its 95% confidence interval
	Individually	In combination	
$CdCl_2$	16.87 (16.35–17.41)	11.10 (10.27–11.93)	−0.31 (−0.49 to −0.15)
DDT	59.39 (54.69–64.35)	39.08 (36.17–41.99)	

DDT: dichlorodiphenyltrichloroethane; IC_{50}: half inhibitory concentration.
[a]AI represents marking additive index (Marking and Dawson, 1975).

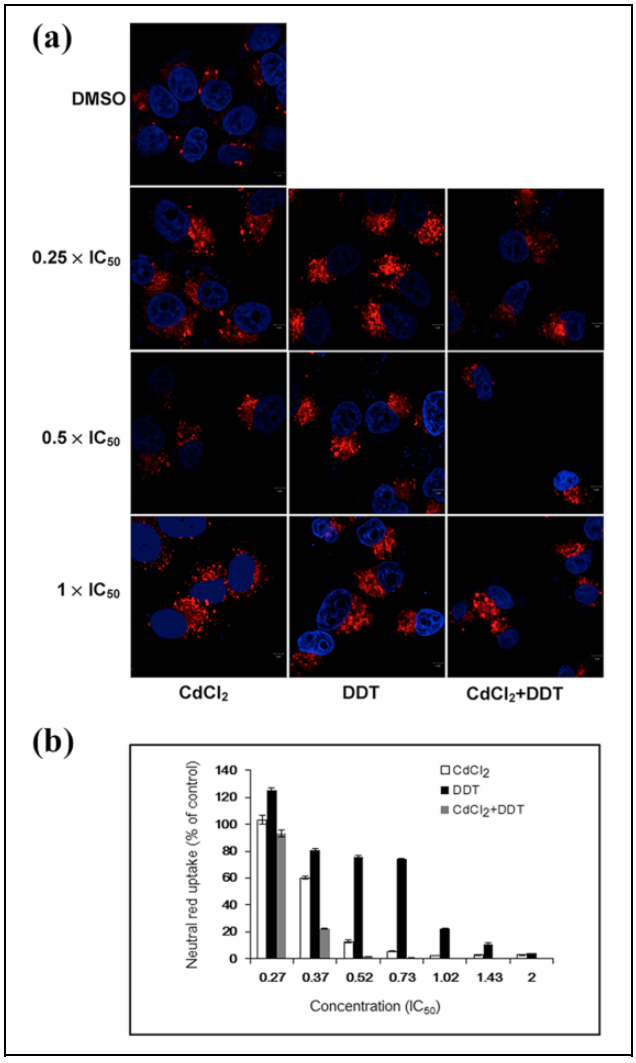

Figure 3. Effect of cadmium and DDT on lysosomes of HepG2 cells. HepG2 cells were treated with DMSO, different concentrations of $CdCl_2$, DDT, and $CdCl_2$ plus DDT for 48 h. (a) Lysosomes were stained with Lyso-Tracker Red (red fluorescence). Nuclei were stained with Hoechst 33258 (blue fluorescence). The cells were observed under confocal microscope SLM710 with scale bar as 5 μm; (b) lysosomal function was detected with neutral red uptake assay. Results were calculated as the percentage of corresponding control values and presented as mean ± SD ($n = 4$). DMSO: dimethyl sulfoxide; DDT: dichlorodiphenyltrichloroethane.

Cd, DDT, and their combination increased MDA levels significantly (Figure 4(b)). At high concentration ($1 \times IC_{50}$), the MDA level induced by the combination of Cd and DDT was higher than the MDA level induced by the two individual chemicals. At lower concentrations ($0.25 \times IC_{50}$ and $0.5 \times IC_{50}$), DDT had no effect on MDA level and the combined chemicals had a similar effect on MDA level as Cd alone.

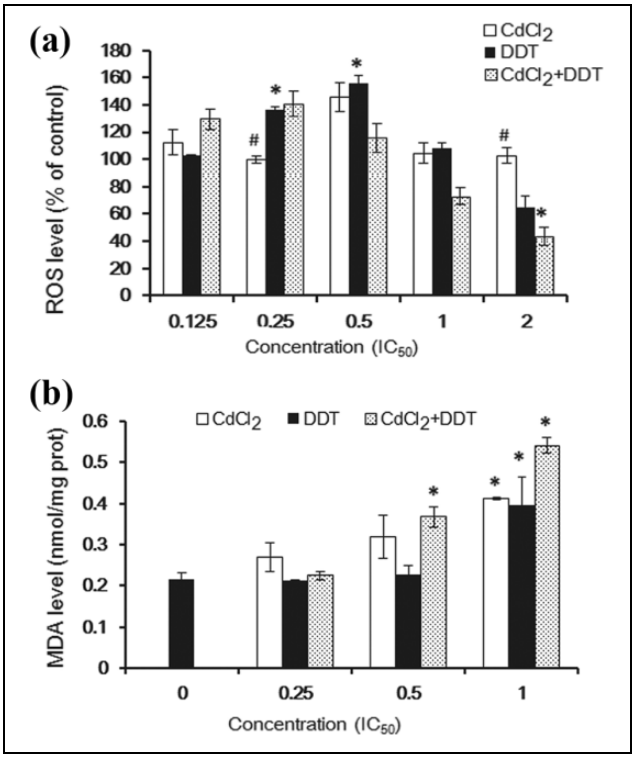

Figure 4. Individual and combined effect of cadmium and DDT on ROS production and lipid peroxidation. (a) HepG2 cells were treated with DMSO, $CdCl_2$, DDT, and $CdCl_2$ plus DDT for 8 h. Then intracellular ROS was detected by DCFH-DA fluorescent probe. Data were calculated as percentage of corresponding control values and presented as mean ± SD ($n = 3$). The control ROS level is 161 ± 13.6 AU. *$p < 0.05$, compared with control; $^{\#}p < 0.05$, compared with the combined treatment. (b) HepG2 cells were treated with DMSO, $CdCl_2$, DDT, and $CdCl_2$ plus DDT for 48 h. Then intracellular MDA level was detected. The data were presented as mean ± SD ($n = 4$). *$p < 0.05$, compared with control. DDT: dichlorodiphenyltrichloroethane; ROS: reactive oxygen species; DMSO: dimethyl sulfoxide; MDA: malondialdehyde.

Activity of antioxidant enzymes and the level of GSH

SOD, CAT, and GR are important intracellular antioxidant enzymes, which protect cells from oxidative damage. GSH is an important reducing agent, which could maintain the intracellular reducing environment. High concentrations ($1 \times IC_{50}$) of Cd plus DDT treatment increased GR activity and GSH levels (Figure 5(a) and (b)), which are consistent with the decrease of ROS in the high concentration combination treatment group (Figure 4). Cd, DDT, and Cd plus DDT treatment had no significant effect on SOD and CAT activity (data not shown).

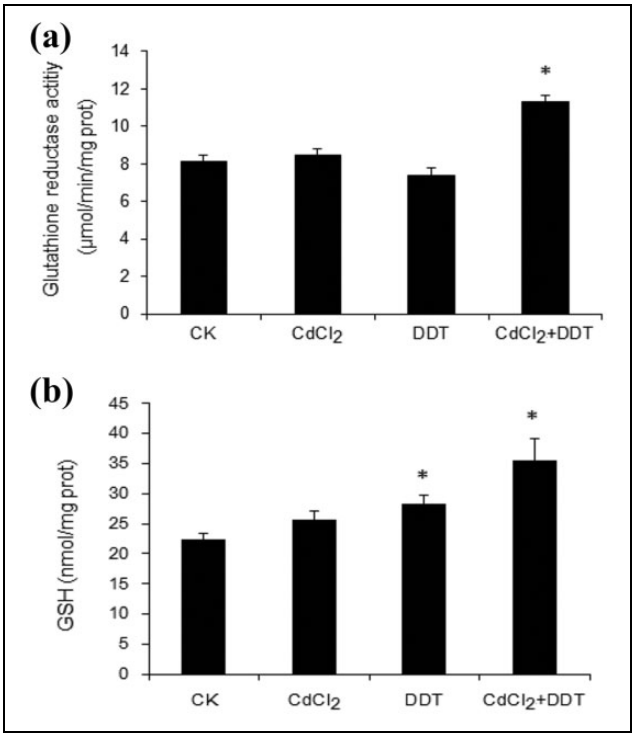

Figure 5. Effect of cadmium and DDT on antioxidant enzymes and antioxidants in HepG2 cells. HepG2 cells were treated with DMSO, $CdCl_2$, DDT, and $CdCl_2$ plus DDT at the concentrations (μM) of their respective $1 \times IC_{50}$ values for 48 h, then activity of GR (a) and level of GSH (b) were detected. The data were presented as mean ± SD ($n = 4$). *$p < 0.05$, compared with control. DDT: dichlorodiphenyltrichloroethane; DMSO: dimethyl sulfoxide; GR: glutathione reductase; GSH: glutathione.

Discussion

We know that organochlorine pesticides and heavy metals are widely distributed in the environment and commonly found together in water, soil, agricultural products, and even foods (Abdallah and Morsy, 2013; Gaw et al., 2008; Kumar et al., 2018; Muir et al., 1992). For example, soil, water, and other environmental media are contaminated with both the organochlorine pesticide DDT and heavy metal Cd in different countries around the world, as indicated by the elevated residue levels in aquatic organisms, medicinal plants, soils, and food products (Abdallah and Morsy, 2013; Hovinga et al., 1993; Huang et al., 2011; Kumar et al., 2018; Zhu et al., 2012). We need to pay more attention to the combined toxic effect of Cd and DDT, although the individual toxicity of Cd and DDT has been well documented. It has been reported that Cd and DDT increased intracellular ROS levels, inhibited enzymatic activity of SOD and CAT, depleted GSH contents, and enhanced lipid peroxidation (Bucio et al., 1995). Our current results showed that, compared with their individual effect, the combined effect of Cd and DDT was more profound on inhibiting cell viability, altering lysosomal function as well as inducing ROS and lipid peroxidation (Figures 1 and 3 to 5). HepG2 cells are the main choice for the study of chemical-induced hepatotoxicity. Although there are some defects in the performance of liver function, the HepG2 cell line is still the preferred choice in the study of cell viability or cell subcellular structure (Garside et al., 2014). The interaction between Cd and DDT was additive on inhibition of cell viability and lysosomal function in HepG2 cells because the AI value included zero and the effect of their combination equaled the sum of their individual effects. However, the interaction of the two chemicals found in this study was antagonistic on inhibition of cell viability in THLE-3 cells because the AI value was negative. THLE-3 cell line used in this study is a kind of hepatic epithelial cells. It is known that toxic chemicals could enter the hepatic sinusoids when they are distributed in the liver with the blood in the body, and the toxic chemicals could progressively damage the sinusoidal epithelial cell wall (Shehab et al., 2016). Thus, the THLE-3 cell line was used in this *in vitro* study to investigate the hepatotoxicity of the two toxic chemicals. The different interaction patterns of DDT and Cd in between HepG2 cells and THLE-3 cells may be due to the different metabolic capacities and expression patterns of target proteins, which may also explain the different sensitivity of THLE-3 cells and HepG2 cells to Cd (Shah et al., 2014). However, the detailed underlying mechanism merits further investigation.

Lysosomes function as detoxification organelles, which degrade waste/damaged proteins and organelles (Perera and Zoncu, 2016). It has been reported that Cd and DDT induced lysosomal damage and might mediate cell death (Fotakis et al., 2005; Rogers et al., 1976). In this study, we tested the cell apoptosis by PI staining and found that after treatment with high concentration of the chemicals ($2 \times IC_{50}$) Cd plus DDT for 48 h, only about 10% of total cells were PI positive (data not shown), indicating that the cell membrane integrity was not prominently compromised after Cd and DDT treatment. However, the lysosomes were found enlarged and their function decreased in the cells exposed to Cd and DDT (Figure 3). These results indicated that the decrease of cell viability

was associated with the lysosomal damage induced by the chemicals.

Previous studies have shown that Cd and DDT could affect energy metabolism and redox enzymes. For example, Miccadei and Floridi (1993) suggested that Cd inhibited the electron flow through NAD-linked substrates and complex 2 in the respiratory chain. DDT was reported to reduce NADH oxidase activity and increase the level of free radicals (Byczkowski, 1976). Cd and DDT were also reported to deplete GSH content (Dehn et al., 2005; Singhal et al., 1987). In the current study, we found that $CdCl_2$ and DDT did not induce SOD and CAT activity (data not shown) and did not deplete GSH content (Figure 5), which was inconsistent with the previous reports. The lower concentrations and shorter time used in our study may be one of the possible reasons. In addition, the combination of Cd and DDT treatment significantly induced GSH and GR activity (Figure 5). This result suggested that the mixture of Cd and DDT may alter GSH production probably by stimulation of GR activity. However, the underlying mechanism of how the mixture of Cd and DDT induced GR activity merits further investigation.

In addition, our results showed that low concentrations of DDT induced ROS but not MDA, indicating that DDT may affect oxidation of proteins or other macromolecules other than MDA (Figure 4). And the induction of oxidative stress by DDT was not due to the depletion of GSH because DDT did not reduce GSH levels (Figure 5). Intriguingly, neither Cd individually nor in combination with DDT increased ROS levels at $0.5 \times IC_{50}$. Only exposure to DDT alone at $0.5 \times IC_{50}$ caused an increase in ROS (Figure 4). The underlying mechanism of combined toxicity of DDT and Cd merits further investigation.

In conclusion, we found that the combination of Cd and DDT inhibited cell viability and lysosomal function, and induced intracellular ROS level and lipid peroxidation in HepG2 cells. This result may facilitate the evaluation of the cumulative risk of these pollutants. However, further study is merited to elucidate the detailed mechanism underlying the combined toxicity of these two industrial chemicals.

Authors' note

Ying-Jian Sun and Qing-Juan Cao contributed equally to this article.

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Declaration of conflicting interests

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