Chemosphere 167 (2017) 10–18

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Oxidative stress intensity-related effects of cadmium (Cd) and paraquat (PQ) on UV-damaged-DNA binding and excision repair activities in zebrafish (*Danio rerio*) embryos

Li-Bin Ling, Yung Chang, Chia-Wei Liu, Po-Ling Lai, Todd Hsu*

*Department of Bioscience and Biotechnology and Center of Excellence for the Oceans, National Taiwan Ocean University, No.2, Pei-Ning Rd., Keelung 20224, Taiwan, Republic of China*

## HIGHLIGHTS

- UV-DDB 2 gene expression was activated in Cd and PQ-exposed zebrafish embryos.
- Cd and PQ triggering weak oxidative stress stimulated UV-damaged-DNA excision repair.
- PQ triggering strong oxidative stress inhibited UV- and cisplatin-damaged-DNA repair.
- Up- or down-regulation of excision repair correlated with 6-4PP binding activity.

## ARTICLE INFO

*Article history:*
Received 1 July 2016
Received in revised form
13 September 2016
Accepted 15 September 2016

Handling Editor: David Volz

*Keywords:*
Cadmium
Nucleotide excision repair
Oxidative stress
Paraquat
UV
Zebrafish

## ABSTRACT

Our earlier studies showed the inhibitory effects of cadmium (Cd) and paraquat (PQ) on the gene expression of DNA mismatch recognition proteins in zebrafish (*Danio rerio*) embryos. This study explored the effects of Cd and PQ on nucleotide excision repair (NER) capacity in zebrafish embryos. Exposure of embryos at 1 h post fertilization (hpf) to 3–5 μM Cd or 30–100 μM PQ for 9 h induced a 2–3-fold increase of oxidative stress, while a 6.5-fold increase of oxidative stress was induced by 200 μM PQ. Real-time RT-PCR detected a down-regulated xeroderma pigmentosum C (XPC) and an up-regulated UV-DDB2 gene expression in mildly-stressed embryos, whereas 8-oxoguanine DNA glycosylase (OGG1) gene expression increased with PQ exposure levels. NER of UV-damaged DNA was enhanced in weakly oxidant-stressed embryos as shown by a transcription-based DNA repair assay, yet repair activities of both UV and cisplatin-damaged DNA were inhibited in embryos exposed to 200 μM PQ. Band shift assay showed a suppression of cyclobutane pyrimidine dimer (CPD) binding activity in all stressed embryos. In contrast, (6-4) photoproduct (6-4PP) recognition activity was weakly stimulated except in embryos exposed to 200 μM PQ, revealing a link of NER capacity to 6-4PP binding. Our results showed that Cd and PQ imposed similar inducing effects on UV-DDB2 gene expression, NER of UV-damaged DNA and 6-4PP binding activity in zebrafish embryo under low levels of oxidative stress and NER capacity could be inhibited if the intensity of oxidative stress increased to a critical level.



## 1. Introduction

Cadmium (Cd) is a toxic transition metal widely present in rivers, estuaries, near shore waters, and marine sediments because of the discharge of Cd compounds in industrial activities (Yuan et al., 2004; Mangal et al., 2016). The waterborne Cd level in seriously polluted water areas may reach 1 mg/L (9 μM) (Ma et al., 2008). Cd may cause human kidney damage through consumption of contaminated water and food (Wang et al., 2016) and Cd is already classified as a human carcinogen (IARC, 1993). Exposure of rats to Cd aerosol was found to suppress the expression of 8-oxoguanine DNA glycosylase (OGG1) in the lung, therefore promoting the accumulation of 8-oxoguanine due to reduced capacity of base excision repair (BER) (Potts et al., 2003). Cd has also been shown to inhibit the oxidative DNA cutting activity in the gill cells of the mussel *Mytilus edulis* L. in the presence of increased levels of lipid peroxidation products (Emmanouil et al., 2007). A significant level of DNA strand breaks remained detectable 7 days after transferring Cd (10–100 μg/L)-exposed neonates and embryos of the snail *Potamopyrgus antipodarum* to clean water, strongly

* Corresponding author.
*E-mail address:* toddhsu@mail.ntou.edu.tw (T. Hsu).

http://dx.doi.org/10.1016/j.chemosphere.2016.09.068
0045-6535/

suggesting the inhibition of DNA repair enzymes by Cd (Vincent-Hubert et al., 2012). Hence, oxidative stress and inhibition of DNA damage repair have been proposed as major factors underlying Cd genotoxicity in mammalian cells (Giaginis et al., 2006; Joseph, 2009) and aquatic organisms (Emmanouil et al., 2007).

Paraquat (PQ) is a fast-acting and broad-spectrum herbicide used extensively for weed control and this herbicide is very toxic to humans (Sun et al., 2016) as PQ undergoes a redox cycling reaction that produces tissue-damaging reactive oxygen species (ROS) within the cells (Senator et al., 2004; Lushchak, 2016). Contamination of PQ in aquatic environment through water runoff has been reported because of the high water solubility of PQ (Moustakas et al., 2016). PQ is known to induce DNA single strand breaks and lipid peroxidation in a rabbit kidney cell line (Senator et al., 2004). Exposure of the aquatic macrophyte *Cymodycea nodosa* to PQ at 2 to 1000 μM was shown to cause a significant reduction in photosynthetic capacity, but the maximal levels of photochemical change and hydrogen peroxide production were induced by PQ at 200 μM (Moustakas et al., 2016).

Zebrafish (*Danio rerio*) has been extensively employed as a model organism for studying development-regulated gene expression (Lindeman et al., 2010) as well as for assessing the hazardous effects of aquatic toxicants on waterborne animals (Fraysse et al., 2006; Jezierska et al., 2009). Zebrafish contains nearly all genes involved in different DNA repair pathways in eukaryotes such as BER, DNA mismatch repair (MMR), and nucleotide excision repair (NER), making this fish species also a good system to investigate the response of each repair system to environmental toxicants (Pei and Strauss, 2013). We have cloned DNA mismatch recognition proteins MutS homolog 2 (MSH2) and MSH6 from zebrafish and mRNAs encoding these two factors were shown to express at high levels in zebrafish at early embryonic stages (Yeh et al., 2003; 2004). Our further studies indicated the potential of Cd at sublethal levels to disturb MMR in zebrafish embryos by down-regulating *msh2/msh6* expression (Hsu et al., 2010; 2013). Cd-generated ROS were believed to function as signaling molecules in Cd-induced gene inhibition because Cd at 3–5 μM and the ROS-inducing herbicide PQ at 200 μM caused similar levels of MSH gene inhibition (Hsu et al., 2013).

Irradiation of UV on DNA induces the formation of cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs) between two adjacent pyrimidine bases (Chan et al., 1985; Mitchell and Nairn, 1989). Helix-distorting CPDs and 6-4PPs are removed from UV-damaged DNA primarily by NER in which DNA damage recognition, incision/excision of the lesion-containing fragment and repair synthesis are carried out by a coordinated action of many polypeptides (Nouspikei, 2009; Spivak, 2015). In addition to its ability to remove CPDs and 6-4PPs, NER is a versatile DNA repair system which also eliminates helical structures distorted by a variety of chemical agents such as those induced by the chemotherapeutic agent cisplatin (Bowden, 2014). NER is composed of two distinct subpathways known as transcription-coupled NER (TC-NER) that allows preferential repair of lesions present in the transcribed strand of active genes (Hanawalt and Spivak, 2008; Hu et al., 2015) and global genomic NER (GG-NER) which removes lesions throughout the whole genome (Gillet and Schärer, 2006; Hu et al., 2015; Spivak, 2015).

GG-NER is initiated by the binding of two independent damage recognition protein complexes to helix-distorting lesions, one involving xeroderma pigmentosum C (XPC)/RAD23B/Centrin2 protein (Pascucci et al., 2011) and the other employs UV-damaged DNA-binding protein (UV-DDB) complex composed of DDB1 (p127) and DDB2 (p48) as the damage sensing molecule (Sugasawa, 2006; Scrima et al., 2008). The damage recognition activity of UV-DDB complex depends on the contact between DDB2 and dipyrimidine photolesions as shown by structural analysis of UV-DDB bound to 6-4PP-containing DNA (Scrima et al., 2008). DDB2 is encoded by XPE gene and mutations in XPA to XPG gene in human is known to cause XP genetic disorders characterized by skin hypersensitivity to sunlight and a high incidence of skin cancer (Sugasawa, 2006). Experimental evidences indicate that UV-DDB is a GG-NER factor specialized for the detection of UV-induced DNA lesions (Scrima et al., 2008), while XPC is a key general sensor that recognizes different types of DNA adducts arising from UV irradiation, genotoxic chemicals and reactive metabolic byproducts (Clement et al., 2010).

A high expression of UV-damaged-DNA binding activity recognizing both CPDs and 6-4PPs in zebrafish embryos was stably detected in our previous studies when an oligonucleotide carrying a CPD or a 6-4PP was used as the binding target in band shift assay (Hsu et al., 2002; Lai et al., 2006; 2012). Due to the importance of NER in eliminating UV-induced dipyrimidine photoproducts, this study explored the effects of Cd and PQ on UV-damaged-DNA binding activity, NER capacity and the gene expression of NER-related damage recognition factors in zebrafish embryos to determine if oxidative stress regulates NER activities. As interaction between NER and BER was identified in a previous study based on the stimulation of OGG1 activity after the addition of XP-C complex to XP-C defective cell extracts (Pascucci et al., 2011), effects of Cd and PQ on the gene expression of OGG1 in zebrafish embryos were also examined.

## 2. Materials and methods

### 2.1. Collection of zebrafish embryos

Zebrafish (*Danio rerio*) were maintained at 28.5 °C in a glass tank equipped with a water circulating system under 14 h light/10 h dark cycle. Two female and 2 male adult zebrafish with age between 4 and 18 months after fertilization were transferred to a stainless metal cage placed in a plastic box containing water and the spawning of eggs was triggered by giving light stimulation in the morning. Fertilized eggs were then transferred to Petri dishes and incubated in the embryo medium (19.3 mM NaCl, 0.23 mM KCl, 0.13 mM $MgSO_4.7H_2O$, 0.2 mM $Ca(NO_3)_2$, 1.67 mM Hepes, pH7.2) (Kimmel et al., 1995) and embryo development was staged according to the time period (h) post-fertilization (hpf) based on microscopic examination of morphological criteria (Kimmel et al., 1995).

### 2.2. Exposure of zebrafish embryos to Cd and PQ

As the major goal of this study was to explore the effects of oxidative stress induced by cd and PQ on NER at both gene expression and protein activity levels, zebrafish embryos were exposed to $CdCl_2$ at 3–5 μM or PQ at 200 μM that have been shown to impose similar inhibitory effects on *msh2/msh6* expression after a 9-h exposure (Hsu et al., 2013). Some embryos were exposed to PQ at 30–100 μM to determine if NER responded differently to lower levels of oxidative stress. Exposure of embryos to oxidative stressors was performed by transferring 100 embryos to a Petri dish containing 30 mL embryo medium and embryos at 1 hpf were exposed to $CdCl_2$ or PQ at the indicated concentrations for 9 h. Cd and PQ were freshly diluted in distilled water before adding to the embryo medium. When reduced somite number and loss of transparency were taken as lethal toxicity indicators, no lethal effects of Cd and PQ at the concentrations used in this study on zebrafish embryos were observed as shown in our previous report (Hsu et al., 2013). The maintainence of zebrafish and exposure of zebrafish embryos to toxicants were carried out in accordance with