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Down-regulation of peroxisome proliferator activated receptor γ coactivator 1 α induces oxidative stress and toxicity of 1-(4-Chlorophenyl)-benzo-2,5-quinone in HaCaT human keratinocytes

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Abstract

Peroxisome proliferator activated receptor γ coactivator 1 α (PGC-1 α) is a transcriptional coactivator that is known to regulate oxidative stress response by enhancing the expression of antioxidant genes. We have shown previously that 1-(4-Chlorophenyl)-benzo-2,5-quinone (4-CIBQ), a quinone-metabolite of 4-monochlorobiphenyl (PCB3) induces oxidative stress and toxicity in human skin keratinocytes, and breast and prostate epithelial cells. In this study, we investigate whether PGC-1 α regulates oxidative stress and toxicity in 4-CIBQ treated HaCaT human keratinocytes. Results showed significant down-regulation in the expression of PGC-1 α and catalase in 4-CIBQ treated HaCaT cells. Down-regulation of PGC-1 α expression was associated with 4-CIBQ induced increase in the steady-state levels of cellular reactive oxygen species (ROS) and toxicity. Overexpression of *pgc-1 α* enhanced the expression of catalase and suppressed 4-CIBQ induced increase in cellular ROS levels and toxicity. These results suggest that *pgc-1 α* mediates 4-CIBQ induced oxidative stress and toxicity in HaCaT cells presumably by regulating catalase expression.

Keywords

PCB3-quinone; PCB3; polychlorinated biphenyls; PGC-1 α ; oxidative stress; catalase

1. Introduction

Peroxisome proliferator activated receptor γ coactivator 1 (PGC-1) is a member of the transcriptional coactivator family that includes PGC-1 α , PGC-1 β , and PGC-1-related coactivator (Lin et al., 2005). PGC-1 α , encoded by *ppargc1a* gene is the best characterized

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Authors' disclosure statement

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member of the PGC-family. PGC-1 α was first identified as a coactivator of transcription factor peroxisome proliferator activated receptor γ (PPAR γ) regulating the PPAR γ mediated adaptive thermogenesis in adipocytes (Puigserver et al., 1998). To date, PGC-1 α has been shown to act as a cofactor for numerous transcription factors and nuclear receptors, *e.g.*, PPAR $\alpha/\beta/\delta$, nuclear respiratory factors (NRF-1 and NRF-2), Yin-yang 1, Forkhead box O1a (FoxO1a), and estrogen-related receptors (Lin et al., 2005). The transcription factors and nuclear receptors dock at the leucine rich motifs (LxxLL), which then activates the N-terminal transcriptional domain of PGC-1 α resulting in the recruitment of histone acetyltransferase (CBP/p300) and initiation of gene transcription (Lin et al., 2005; Puigserver et al., 1999; Puigserver and Spiegelman, 2003). PGC-1 α has been shown to be involved in the detoxification of reactive oxygen species (ROS) by enhancing the expression of antioxidant genes (St-Pierre et al., 2006; St-Pierre et al., 2003; Xiong et al., 2010). Overexpression of *pgc-1 α* has been reported to increase the mRNA levels of manganese superoxide dismutase (MnSOD) and glutathione peroxidase 1 (GPx1) in mouse C2C12 myotubes (St-Pierre et al., 2003). Xiong *et al* (2010) have demonstrated that the interaction of PGC-1 α with FoxO1a enhances the expression of catalase, while silencing of *pgc-1 α* decreases the expression of catalase in thoracic aortas vascular smooth muscle cells of Sprague-Dawley rats. A lower level of MnSOD, catalase, and GPx1 in *pgc-1 α ^{-/-}* mouse embryonic fibroblasts was reported to be more prone to hydrogen peroxide induced toxicity compared to *pgc-1 α ^{+/+}* mouse embryonic fibroblasts (St-Pierre et al., 2006). These previous reports suggest that the oxidative stress response properties of *pgc-1 α* could be due to its ability to enhance the expression of antioxidant genes.

Polychlorinated biphenyls (PCBs) are a class of 209 persistent environmental pollutants. 4-Monochlorobiphenyl (PCB3) is a semi-volatile PCB congener that has been detected in human blood, commercial paints, and the environment (DeCaprio et al., 2005; Hu and Hornbuckle, 2009; Hu et al., 2010; Martinez et al., 2012). 1-(4-Chlorophenyl)-benzo-2,5-quinone (4-CIBQ) is a quinone metabolite of PCB3 (McLean et al., 1996). Using electron paramagnetic resonance spectrometry, a previous study has demonstrated the formation of a semiquinone radical in 4-CIBQ treated MCF-10A human mammary epithelial cells, which correlated with an increase in hydrogen peroxide levels and toxicity (Venkatesha et al., 2008). 4-CIBQ treatment has also been shown to increase cellular ROS levels causing toxicity in human keratinocytes, and breast and prostate epithelial cells (Xiao et al., 2014; Xiao et al., 2013; Zhu et al., 2009). This study investigates whether PGC-1 α regulates 4-CIBQ induced oxidative stress and toxicity by modulating antioxidant gene expression. Results show that treatment with 4-CIBQ inhibits PGC-1 α and catalase expression, which correlates with an increase in cellular ROS levels and toxicity of HaCaT human keratinocytes. Overexpression of *pgc-1 α* inhibits 4-CIBQ-induced decrease in catalase expression, and increase in ROS levels and toxicity. These results suggest that the 4-CIBQ-induced oxidative stress and toxicity of HaCaT cells are mediated by a significant down-regulation in the expression of transcriptional coactivator *pgc-1 α* and its target gene, catalase.

2. Materials and methods

2.1 Chemicals, reagents, and antibodies

1-(4-Chlorophenyl)-benzo-2,5-quinone (4-CIBQ) and its parental compound 4-monochlorobiphenyl (PCB3) were provided by the Synthesis Core of the Iowa Superfund Research Project. These compounds were synthesized and purified as described previously (Amaro et al., 1996; Lehmler and Robertson, 2001). Gas chromatography analysis showed more than 98% purity of 4-CIBQ and PCB3. MitoSOX Red reagent was purchased from the Molecular Probes (Eugene, OR). Antisera against human catalase was obtained from the Athens Research and Technology (Athens, GA); β -actin antibody (sc47778) was purchased from the Santa Cruz Biotechnology (Santa Cruz, CA); PGC-1 α antibody (sc-5816; Santa Cruz Biotechnology) and Lamin A/C antibody (cst4777S; Cell Signaling Technology) were kindly provided by Drs. Rhonda A. Souvenir and Jennifer L. Casey, Department of Internal Medicine, University of Iowa.

2.2 Cell culture and treatment

Spontaneously immortalized human skin keratinocytes (HaCaT) provided by Dr. Norbert Fusenig (German Cancer Research Center, Heidelberg, Germany) (Boukamp et al., 1988) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum at 37 °C in a humidified incubator with 5% CO₂. HaCaT cells were used in this study because skin is one of the target tissues for exposure to airborne PCBs. Dimethyl sulfoxide (DMSO) was used to prepare stock solutions of 4-CIBQ (Venkatesha et al., 2008). Asynchronous cultures were treated with 0.1 - 3.0 μ M of 4-CIBQ for 24 h in serum-free DMEM followed by the addition of serum-containing regular medium. The concentration of PCBs used in this study is based on a previous report of 0.003 - 6.5 μ M PCBs that were detected in the blood of individuals living in Anniston, Alabama (Hansen et al., 2003). Control cells were treated with the equivalent amount of DMSO (0.1%, v/v).

2.3 Clonogenic survival assay

A clonogenic cell survival assay was used to measure toxicity (Xiao et al., 2013; Zhu et al., 2009). Untreated control and 24 h of 4-CIBQ treated cells were replated using appropriate dilutions designed to plate single cell. Replated cells were continued in culture in PCB-free medium for 14 days and then fixed in 70% ethanol. Ethanol-fixed cells were visualized by staining with 0.5% Coomassie Brilliant Blue G-250. A colony that contains 50 or more cells was counted. Plating efficiency (PE) and survival fraction (SF) were calculated using the following equations: $PE = (\text{number of colonies counted} / \text{number of cells seeded}) \times 100$; $SF = (\text{number of colonies counted} / \text{number of cells seeded}) \times PE$. PE represents survival of cells that were not treated with PCB. SF represents survival of cells that were treated with PCB; SF was calculated after correction for PE and then relative to untreated control cells.

2.4 Flow cytometry assays

Cellular ROS levels were determined by measuring the oxidation of MitoSOX Red following a published protocol (Xiao et al., 2013; Zhu et al., 2009). Control and 4-CIBQ treated cells were incubated with 2 μ M MitoSOX Red at 37 °C for 20 min. The fluorescence

of MitoSOX Red was measured using Becton–Dickinson FACScan flow cytometer (excitation 488 nm, emission 585 nm). The mean fluorescence intensity (MFI) of 10,000 events was analyzed for each sample and corrected for auto-fluorescence. Fold change in MFI of 4-CIBQ treated cells was calculated relative to the MFI of cells that were treated with DMSO alone.

Cell viability was determined by propidium iodide (PI) exclusion assay following a published protocol (Venkatesha et al., 2008). Control and 4-CIBQ treated cells were collected and washed with cold PBS. Cell suspensions were incubated with PI (1 µg/mL) in cold PBS. The PI fluorescence was measured using FACScan flow cytometer (excitation 488 nm, emission 585 nm). Data from 10,000 events were analyzed to calculate the percentage of PI-positive (non-viable) and PI-negative (viable) cells using WinMDI software.

2.5 cDNA synthesis and quantitative RT-PCR assay

Total RNA of control and 4-CIBQ treated cells was extracted using TRIzol (Invitrogen, Carlsbad, CA). ND1000 Nanodrop spectrophotometer (Nanodrop, Wilmington, DE) was used to measure the concentration and purity of isolated RNA. One microgram of RNA was reverse transcribed using cDNA Archive Kit (Applied Biosystems, Carlsbad, CA). Two microliters of the cDNA reaction were used to perform real-time PCR amplification using Power SYBR Green PCR Master Mix and StepOnePlus™ System (Applied Biosystems, Carlsbad, CA). The primer-pair sequence of individual genes were: *pgc-1α* (NM_013261.3) forward primer: 5'-GCATGAGTGTGTGCTCTGT-3' and reverse primer: 5'-CAGCACACTCGATGTCACTC-3', amplicon size: 133 bp; *catalase* (NM_001752.3) forward primer: 5'-CATCCAGAAGAAAGCGGTCAA-3' and reverse primer: 5'-CCAAGTGAGATCCGGACTGC-3', amplicon size: 140 bp; and *β-actin* (NM_001101.3) forward primer: 5'-TCACCATTGGCAATGAGCGGTT-3' and reverse primer: 5'-AGTTTCGTGGATGCCACAGGACT-3', amplicon size: 89 bp. Changes in mRNA levels were calculated as follows: $\text{Ct} = \text{Ct (4-CIBQ treated cells)} - \text{Ct (control cells)}$; relative expression = $2^{-\text{Ct}}$.

2.6 Immunoblotting assay

Nuclear extracts of control and 4-CIBQ treated cells were prepared following the protocol described by He *et al* (2013). Nuclear and total cellular protein extracts were used to measure PGC-1α and catalase protein levels. Equal amounts of nuclear (40 micrograms) and cellular (60 micrograms) protein extracts were separated on 12% SDS-PAGE and electro-transferred to nitrocellulose membrane. The blots were incubated with antibodies to human PGC-1α (1:200) and catalase (1:1000). Horseradish peroxidase conjugated secondary antibodies, Pierce enhanced chemiluminescence Plus reagent (Thermo Scientific, Rockford, IL), and Typhoon FLA 7000 (GE Healthcare, Waukesha, WI) were used for visualization of immune-reactive polypeptides. Lamin A/C and β-actin protein levels were used for comparison of PGC-1α and catalase protein levels, respectively. Quantitation of results was performed using ImageJ software. Fold-change was calculated after correction for loading controls in individual samples and then relative to cells that were not treated with 4-CIBQ.

2.7 Catalase activity assay

Spectrophotometric assay: catalase activity was measured spectrophotometrically by the method of Beers and Sizer (1952) and results are presented as mkunit per mg protein. “*k*” is a pseudo-first order rate constant derived from the kinetic data as described by Aebi (1984). This method measures exponential disappearance of hydrogen peroxide (10 mM) at 240 nm in the presence of cell homogenates. The equation used to calculate rate constant (*k*) is as follows: $A_{60s} = A_{\text{initial}} e^{-kt}$ (Spitz et al., 1990).

Native gel electrophoresis assay: one hundred microgram of total protein lysates were separated by 12% native polyacrylamide gel electrophoresis (Woodbury et al., 1971). The gel was incubated with 0.003% hydrogen peroxide for 10 min and then stained in a solution containing 1% ferric chloride and 1% potassium ferricyanide for 20 min at room temperature. The bands were visualized under bright fluorescent light and analyzed using ImageJ software. Fold change was calculated relative to cells that were not treated with 4-CIBQ.

2.8 Overexpression of *pgc-1α*

Human *pgc-1α* was transiently overexpressed in HaCaT cells using human PGC-1α plasmid DNA (Addgene, plasmid No. 10974; Cambridge, MA). This plasmid was generated by the laboratory of Dr. Toren Finkel at the National Institute of Health (Ichida et al., 2002). HaCaT cells were cultured to 70-80% confluence and then transfected with vector-alone and human PGC-1α cDNA containing plasmid DNAs (pcDNA4-myc-PGC-1α) for 48 h using Lipofectamine 3000 (Life Technologies, Grand Island, NY). Transfected cells were sub-cultured and treated with 4-CIBQ. The transgene expression was verified by measuring *pgc-1α* mRNA and protein expression using quantitative RT-PCR and immunoblotting assays.

2.9 Statistical analysis

One-way analysis of variance followed by Tukey post-test (SPSS 21.0 software) was performed to evaluate statistical significance of results. Results are presented as mean ± standard deviation. Results from at least $n = 3$ with $p < 0.05$ were considered significant.

3. Results

3.1 4-CIBQ treatment down-regulates *pgc-1α* expression in HaCaT cells

Transcriptional coactivator *pgc-1α* has been shown earlier to enhance antioxidant gene expression in response to oxidative stress (Austin and St-Pierre, 2012). We have shown previously that 4-CIBQ treatment induces oxidative stress and toxicity in HaCaT human keratinocytes (Xiao et al., 2014; Xiao et al., 2013). To determine whether *pgc-1α* regulates 4-CIBQ induced oxidative stress and toxicity, q-RT-PCR and immunoblotting assays were performed to measure *pgc-1α* mRNA and protein levels in 4-CIBQ treated HaCaT cells (Fig. 1). Results showed a dose-dependent decrease in *pgc-1α* mRNA levels: approximately, 50% decrease in 0.1 μM and 70% decrease in 3.0 μM 4-CIBQ treated cells (Fig. 1A). Because PGC-1α is primarily localized in the nucleus (Puigserver et al., 1998), an immunoblotting assay was performed to measure PGC-1α protein levels in the nuclear

extracts isolated from control and 4-CIBQ treated cells. Consistent with the down-regulation of *pgc-1a* mRNA expression, 4-CIBQ treatment decreased PGC-1 α protein levels (Fig. 1B and 1C). It is interesting to note that treatment with PCB3, the parental compound of 4-CIBQ did not alter *pgc-1a* mRNA expression in the effective range of 4-CIBQ (Fig. 1D). These results suggest that 4-CIBQ treatment down-regulates *pgc-1a* expression in HaCaT cells.

3.2 4-CIBQ treatment decreases catalase expression and activity in HaCaT cells

A previous study reports that *pgc-1a* regulates the expression of catalase in thoracic aortas vascular smooth muscle cells of Sprague-Dawley rats (Xiong et al., 2010). To determine whether the 4-CIBQ-induced inhibition in PGC-1 α expression (Fig. 1) is associated with changes in its target gene expression, catalase expression was measured using q-RT-PCR, immunoblotting and activity assays (Fig. 2). 4-CIBQ treatment showed a dose-dependent decrease in catalase mRNA levels: approximately 50% decrease in 0.1 μ M 4-CIBQ treated cells, which further decreased to approximately 70-80% in 1.0 and 3.0 μ M 4-CIBQ treated cells (Fig. 2A). Catalase protein levels decreased approximately 50% in 1.0 μ M and 40% in 3.0 μ M 4-CIBQ treated cells (Fig. 2B and 2C). Results from a spectrophotometric assay showed that while catalase activity was comparable in control and 0.1 μ M 4-CIBQ treated cells, its activity decreased approximately 40% in 1.0 and 3.0 μ M 4-CIBQ treated cells (Fig. 2D). These results demonstrate a significant down-regulation of catalase expression correlating with a decrease in PGC-1 α expression in 4-CIBQ treated HaCaT cells.

3.3 Overexpression of *pgc-1a* inhibits 4-CIBQ induced down-regulation of catalase expression, oxidative stress and toxicity in HaCaT cells

To determine whether PGC-1 α regulates 4-CIBQ induced oxidative stress and toxicity, *pgc-1a* was overexpressed in HaCaT cells prior to treatment with 4-CIBQ. Cells were transiently transfected with vector-alone or human *pgc-1a* cDNA containing plasmid DNAs. Results from measurements of *pgc-1a* expression showed approximately 2.5-fold increase in *pgc-1a* mRNA levels and 3.3-fold increase in its protein levels (Fig. 3A and 3B). Control and *pgc-1a* overexpressing cells were then treated with 3.0 μ M 4-CIBQ for 24 h and harvested for analysis of catalase expression. As shown before (Fig. 2), 4-CIBQ treatment decreased catalase mRNA levels by approximately 60% (Fig. 3C). Interestingly, the same treatment only decreased catalase mRNA levels approximately 30% in *pgc-1a* overexpressing cells (Fig. 3C). Comparable results were also obtained with catalase protein levels (Fig. 3D and 3E). Results from a native gel activity assay showed that 4-CIBQ treatment decreased catalase activity by 60% in cells transfected with vector-alone, while the same treatment did not affect catalase activity in cells overexpressing *pgc-1a* (Fig. 3F). These results suggest that the overexpression of *pgc-1a* suppressed 4-CIBQ induced down-regulation of catalase expression.

The decrease in catalase activity is anticipated to increase cellular ROS levels resulting in oxidative stress and toxicity in 4-CIBQ treated HaCaT cells. A flow cytometry assay was used to measure cellular ROS levels in control and *pgc-1a* overexpressing cells treated with and without 3.0 μ M 4-CIBQ. Consistent with our previously published results (Xiao et al., 2014; Xiao et al., 2013), oxidation of MitoSOX Red increased approximately 7-fold in 4-

CIBQ treated vector-alone transfected cells (Fig. 4A and 4B). It is interesting to note that the same treatment minimally increased (less than 2-fold) MitoSOX Red oxidation in *pgc-1a* overexpressing cells (Fig. 4A and 4B). These results showed that *pgc-1a* overexpression suppresses 4-CIBQ induced down-regulation of catalase, which minimizes oxidative stress in HaCaT cells.

The decrease in oxidative stress was associated with less toxicity in 4-CIBQ treated *pgc-1a* overexpressing cells. Results from a flow cytometry assay showed approximately 30% PI-positive cells (indicative of dead cells) in 4-CIBQ treated vector-alone transfected cells (Fig. 4C). However, 4-CIBQ treatment resulted only 8% PI-positive cells in *pgc-1a* overexpressing cells (Fig. 4C). *pgc-1a* overexpression-related resistance of cells to 4-CIBQ treatment was also evident from results obtained from a clonogenic assay. 4-CIBQ treated vector-alone transfected cells showed approximately 90% cell death compared to approximately 60% cell death in 4-CIBQ treated *pgc-1a* overexpressing cells (Fig. 4D). These results suggest that down-regulation of *pgc-1a* expression mediates 4-CIBQ induced oxidative stress and toxicity of HaCaT cells presumably by suppressing catalase expression.

4. Discussion

Transcriptional coactivator PGC-1 α is believed to be a key regulator of cellular response to oxidative stress presumably due to its ability to enhance the expression of antioxidant genes (Austin and St-Pierre, 2012). 4-CIBQ, a quinone metabolite of environmental pollutant PCB3 has been shown to increase cellular ROS levels resulting in oxidative stress in human keratinocytes and epithelial cells (Venkatesha et al., 2008; Xiao et al., 2014; Xiao et al., 2013; Zhu et al., 2009). Results from this study show that the expression of PGC-1 α is down-regulated in 4-CIBQ treated HaCaT cells, which correlates with a decrease in catalase expression, and an increase in cellular ROS levels resulting in toxicity. Overexpression of *pgc-1a* suppressed 4-CIBQ induced down-regulation of catalase expression, oxidative stress and toxicity in HaCaT cells. These results support the hypothesis that PGC-1 α regulates catalase mediated protection of HaCaT cells from 4-CIBQ induced oxidative stress and toxicity.

Recent evidence suggests that PGC-1 α regulates oxidative stress response by enhancing antioxidant gene expression (Austin and St-Pierre, 2012). This property of PGC-1 α is in addition to its widely-studied role in regulating mitochondrial biogenesis and oxidative respiration (Lin et al., 2005; Puigserver and Spiegelman, 2003; Scarpulla, 2012). To determine whether PGC-1 α regulates 4-CIBQ induced oxidative stress, initially we measured PGC-1 α expression in 4-CIBQ treated HaCaT cells. 4-CIBQ treatment results in a dose-dependent decrease in *pgc-1a* mRNA expression (Fig. 1A). Although the same treatment decreased PGC-1 α protein levels, a direct correlation was not observed between PGC-1 α mRNA and protein levels (Fig. 1). The lack of a direct correlation between PGC-1 α mRNA and protein levels could be due to a post-translational mechanism regulating PGC-1 α protein levels that is independent of *pgc-1a* transcriptional control.

4-CIBQ induced decrease in *pgc-1a* expression was also associated with a decrease in its target gene expression, catalase mRNA, protein, and activity (Fig. 2). Because catalase

neutralizes hydrogen peroxide, a decrease in catalase expression is anticipated to result in oxidative stress. This hypothesis is consistent with our earlier observation of oxidative stress in 4-CIBQ treated HaCaT cells (Xiao et al., 2014; Xiao et al., 2013) and results shown in Figure 4A and 4B. We have shown previously approximately 30% increase in the rate of production of hydrogen peroxide in 4-CIBQ treated MCF10A human breast epithelial cells (Venkatesha et al., 2008). The increase in hydrogen peroxide levels was associated with a significant increase in the percentage of micronuclei and phosphorylated-H2AX that are indicative of DNA damage (Venkatesha et al., 2008). It is interesting to note that pre-treatment of MCF10A cells with PEG-catalase suppressed 4-CIBQ induced oxidative stress, DNA damage and toxicity (Venkatesha et al., 2008). Our results of *pgc-1 α* expression correlating with catalase expression are also consistent with literature reports of a decrease in catalase expression in siRNA-mediated depletion of *pgc-1 α* in C3H10T1/2 mouse embryo fibroblasts and thoracic aortas vascular smooth muscle cells of Sprague-Dawley rats (St-Pierre et al., 2006; Xiong et al., 2010). Taken together, these results show that 4-CIBQ induced decrease in *pgc-1 α* expression inhibits catalase expression resulting in oxidative stress and toxicity of HaCaT cells.

The observation of PGC-1 α correlating with 4-CIBQ induced oxidative stress and toxicity is further evident from the results in Figures 3 and 4. HaCaT cells transiently overexpressing *pgc-1 α* showed approximately 3-fold increase in *pgc-1 α* mRNA and protein levels (Fig. 3A and B). It is interesting to note that while the 4-CIBQ treatment decreased *pgc-1 α* and catalase expression in vector-alone transfected cells, such an inhibition was significantly less in 4-CIBQ treated *pgc-1 α* overexpressing cells (Fig. 3C-F). PGC-1 α overexpression significantly mitigated 4-CIBQ induced oxidative stress and toxicity of HaCaT cells. These results are also consistent with previous reports of catalase inhibiting oxidative damage of nucleic acid in skeletal muscle of *pgc-1 α* overexpressing mice (Wenz et al., 2009) and high glucose induced oxidative stress and apoptotic cell death in human umbilical vein endothelial cells (Valle et al., 2005). Taken together, these results suggest that *pgc-1 α* mediates 4-CIBQ induced oxidative stress and toxicity presumably by regulating catalase expression.

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Abbreviations

4-CIBQ	1-(4-Chlorophenyl)-benzo-2,5-quinone
GPx1	glutathione peroxidase 1
MnSOD	manganese superoxide dismutase

PCBs	polychlorinated biphenyls
PCB3	4-monochlorobiphenyl
PGC-1α	peroxisome proliferator activated receptor γ coactivator 1 α
PI	propidium iodide
PPAR	peroxisome proliferator activated receptor
ROS	reactive oxygen species

References

- Aebi H. Catalase in vitro. *Methods Enzymol.* 1984; 105:121–126. [PubMed: 6727660]
- Amaro AR, Oakley GG, Bauer U, Spielmann HP, Robertson LW. Metabolic activation of PCBs to quinones: reactivity toward nitrogen and sulfur nucleophiles and influence of superoxide dismutase. *Chem Res Toxicol.* 1996; 9:623–629. [PubMed: 8728508]
- Austin S, St-Pierre J. PGC1 α and mitochondrial metabolism--emerging concepts and relevance in ageing and neurodegenerative disorders. *J Cell Sci.* 2012; 125:4963–4971. [PubMed: 23277535]
- Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem.* 1952; 195:133–40. [PubMed: 14938361]
- Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol.* 1988; 106:761–771. [PubMed: 2450098]
- DeCaprio AP, Johnson GW, Tarbell AM, Carpenter DO, Chiarenzelli JR, Morse GS, Santiago-Rivera AL, Schymura MJ. Polychlorinated biphenyl (PCB) exposure assessment by multivariate statistical analysis of serum congener profiles in an adult Native American population. *Environ. Res.* 2005; 98:284–302. [PubMed: 15910784]
- Hansen LG, DeCaprio AP, Nisbet ICT. PCB congener comparisons reveal exposure histories for residents of Anniston, Alabama, USA. *Fresenius Environ Bull.* 2003; 12:181–190.
- He C, Ryan AJ, Murthy S, Carter AB. Accelerated development of pulmonary fibrosis via Cu,Zn-superoxide dismutase-induced alternative activation of macrophages. *J Biol Chem.* 2013; 288:20745–20757. [PubMed: 23720777]
- Hu D, Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. *Environ Sci Technol.* 2009; 44:2822–2827. [PubMed: 19957996]
- Hu D, Lehmler HJ, Martinez A, Wang K, Hornbuckle KC. Atmospheric PCB congeners across Chicago. *Atmos Environ.* 2010; 44:1550–1557.
- Ichida M, Nemoto S, Finkel T. Identification of a specific molecular repressor of the peroxisome proliferator-activated receptor gamma Coactivator-1 alpha (PGC-1 α). *J Biol Chem.* 2002; 277:50991–50995. [PubMed: 12397057]
- Lehmler HJ, Robertson LW. Synthesis of polychlorinated biphenyls (PCBs) using the Suzuki-coupling. *Chemosphere.* 2001; 45:137–143. [PubMed: 11572605]
- Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab.* 2005; 1:361–370. [PubMed: 16054085]
- Martinez A, Erdman NR, Rodenburg ZL, Eastling PM, Hornbuckle KC. Spatial distribution of chlordanes and PCB congeners in soil in Cedar Rapids, Iowa, USA. *Environ Pollut.* 2012; 161:222–228. [PubMed: 22230089]
- Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman BM. Activation of PPAR γ coactivator-1 through transcription factor docking. *Science.* 1999; 286:1368–1371. [PubMed: 10558993]
- Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α): transcriptional coactivator and metabolic regulator. *Endocr Rev.* 2003; 24:78–90. [PubMed: 12588810]

- Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. 1998; 92:829–839. [PubMed: 9529258]
- Scarpulla RC. Nucleus-encoded regulators of mitochondrial function: integration of respiratory chain expression, nutrient sensing and metabolic stress. *Biochim Biophys Acta*. 2012; 1819:1088–1097. [PubMed: 22080153]
- Spitz DR, Elwell JH, Sun Y, Oberley LW, Oberley TD, Sullivan SJ, Roberts RJ. Oxygen toxicity in control and H₂O₂-resistant Chinese hamster fibroblast cell lines. *Arch Biochem Biophys*. 1990; 279:249–260. [PubMed: 2350176]
- St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*. 2006; 127:397–408. [PubMed: 17055439]
- St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. *J Biol Chem*. 2003; 278:26597–26603. [PubMed: 12734177]
- Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M. PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res*. 2005; 66:562–573. [PubMed: 15914121]
- Venkatesha VA, Venkataraman S, Sarsour EH, Kalen AL, Buettner GR, Robertson LW, Lehmler HJ, Goswami PC. Catalase ameliorates polychlorinated biphenyl-induced cytotoxicity in nonmalignant human breast epithelial cells. *Free Radic Biol Med*. 2008; 45:1094–1102. [PubMed: 18691649]
- Wenz T, Diaz F, Hernandez D, Moraes CT. Endurance exercise is protective for mice with mitochondrial myopathy. *J Appl Physiol*. 2009; 106:1712–1719. [PubMed: 19286571]
- Woodbury W, Spencer AK, Stahman MA. An improved procedure using ferricyanide for detecting catalase isozymes. *Anal Biochem*. 1971; 44:301–305. [PubMed: 4109029]
- Xiao W, Sarsour EH, Wagner BA, Doskey CM, Buettner GR, Domann FE, Goswami PC. Succinate dehydrogenase activity regulates PCB3-quinone-induced metabolic oxidative stress and toxicity in HaCaT human keratinocytes. *Arch Toxicol*. 2014 [Epub ahead of print].
- Xiao W, Zhu Y, Sarsour EH, Kalen AL, Aykin-Burns N, Spitz DR, Goswami PC. Selenoprotein P regulates 1-(4-Chlorophenyl)-benzo-2,5-quinone-induced oxidative stress and toxicity in human keratinocytes. *Free Radic Biol Med*. 2013; 65:70–77. [PubMed: 23770201]
- Xiong S, Salazar G, San Martin A, Ahmad M, Patrushev N, Hilenski L, Nazarewicz RR, Ma M, Ushio-Fukai M, et al. PGC-1 alpha serine 570 phosphorylation and GCN5-mediated acetylation by angiotensin II drive catalase down-regulation and vascular hypertrophy. *J Biol Chem*. 2010; 285:2474–2487. [PubMed: 19940161]
- Zhu Y, Kalen AL, Li L, Lehmler HJ, Robertson LW, Goswami PC, Spitz DR, Aykin-Burns N. Polychlorinated-biphenyl-induced oxidative stress and cytotoxicity can be mitigated by antioxidants after exposure. *Free Radic Biol Med*. 2009; 47:1762–1771. [PubMed: 19796678]

4-CIBQ inhibits PGC-1 α and catalase expression in HaCaT cells.

pgc-1 α overexpression abrogates 4-CIBQ induced reduction in catalase expression.

pgc-1 α overexpression suppresses 4-CIBQ induced oxidative stress and toxicity.

Fig. 1.

pgc-1α expression is inhibited in 4-CIBQ treated HaCaT cells. Quantitative RT-PCR and immunoblotting assays were used to measure *pgc-1α* (A) mRNA and (B) protein levels in control and 4-CIBQ treated cells at 24 h after the addition of 4-CIBQ. Quantitation of results from the immunoblots are shown in (C). (D) *pgc-1α* mRNA expression in control and 24 h of 3.0 μM PCB3 treated cells. Fold change was calculated relative to untreated control cells. Asterisks represent statistical significance compared to cells that were not treated with 4-CIBQ; $p < 0.05$, $n = 3$; error bars represent standard deviation of three independent experiments.

Fig. 2.

4-CIBQ treatment decreases catalase expression and activity in HaCaT cells. Quantitative RT-PCR, immunoblotting and biochemical assays were used to measure catalase (A) mRNA expression, protein levels (B and C) and activity (D) in 4-CIBQ treated cells at 24 h after the addition of 4-CIBQ. Fold change was calculated relative to untreated control cells. Asterisks represent statistical significance compared to cells that were not treated with 4-CIBQ; $p < 0.05$, $n = 3$; error bars represent standard deviation of three independent experiments.

Fig. 3. Overexpression of *pgc-1α* suppresses 4-CIBQ induced down-regulation of catalase expression in HaCaT cells. Quantitative RT-PCR and immunoblotting assays were used to measure PGC-1α (A) mRNA and (B) protein levels in vector-alone and PGC-1α plasmid DNA transfected cells. Catalase expression in control and 4-CIBQ treated vector-alone and PGC-1α plasmid DNA transfected cells was measured using quantitative RT-PCR (C), immunoblotting (D and E), and native gel-electrophoresis based activity assay (F). Asterisks represent statistical significance compared to vector-alone transfected cells in absence of 4-

ClBQ treatment; # indicates statistical significance compared to vector-alone transfected cells in presence of 4-ClBQ treatment; $p < 0.05$, $n = 3$; error bars represent standard deviation of three independent experiments.

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Fig. 4. Overexpression of *pgc-1α* suppresses 4-CIBQ induced oxidative stress and toxicity in HaCaT cells. Flow cytometry measurements of MitoSOX Red oxidation were used to determine cellular ROS levels in control and 4-CIBQ treated vector-alone and *pgc-1α* overexpressing cells at the end of 24 h of treatment: (A) Representative histograms and (B) fold-change in MitoSOX Red oxidation. (C) Flow cytometry measurements of PI-positive cells and (D) a clonogenic assay was used to measure toxicity in control and 4-CIBQ treated vector-alone and *pgc-1α* overexpressing cells. Fold change was calculated relative to vector-alone transfected cells in absence of 4-CIBQ treatment. Asterisks represent statistical significance compared to vector-alone transfected cells in absence of 4-CIBQ treatment; # indicates statistical significance compared to vector-alone transfected cells in presence of 4-CIBQ treatment; $p < 0.05$, $n = 3$; error bars represent standard deviation of three independent experiments.