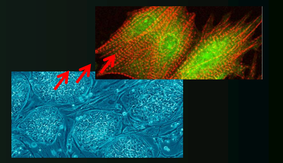
EFFECT OF ENVIRONMENTAL CHEMICAL EXPOSURES ON ADULT HUMAN CARDIAC PROGENITOR CELL VIABILITY AND DIFFERENTIATION

Kaberi Das, Christopher Lau, and Kevin Dreher

Toxicology Assessment Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC



Introduction

Background: Over the past several decades cell and developmental biology, as well as regenerative medicine, research have undergone a revolution with the discovery of stems cells and their impact on organ homeostasis, repair, susceptibility to disease, and overall longevity. Currently, the heart is currently no longer considered to be a static organ but a dynamic one containing cardiac stem (CSCs) and progenitor cells (CPCs) involved in its injury/repair and organ maintenance over time.

Abstract:. However, the impact which exposures to environmental pollutants and/or chemicals have on CSCs and/or CPCs development and function over the various life stages is unknown. To address this uncertainty a scalable assay using adult human inducible pluripotent stem cell-derived CPCs is being developed to determine the effects of various environmental chemicals on their viability and differentiation. CPC cytotoxicity and differentiation were determined by quantitative fluorescent analysis (LI-COR Odyssey CLx Imaging System) using Red Dot1 nuclear and anti-cardiac troponin T antibody in-cell western staining, respectively. Optimal induction of CPCs into cardiomyocytes (CMs) was obtained by treatment with both Wnt (10 µM XAV939) and activin/TGFβ (2.5 µM SB431542) differentiation inducers for 48h (5 - 10% cardiac troponin T^+ with no treatment vs. 50 – 60% cardiac troponin T^+ with inducers). CPCs were exposed to vehicle controls or triclosan, bisphenol A, $CeCl_3$ each at 0.125 to 12.5 µM or a saline extract of residual oil fly ash (ROFA-L) at 0.02 to 20 µg/ml concentrations in the presence of CPCs differentiation inducers for 48h. CPC cultures were stained with Red Dot1 and anti-cardiac troponin T at 6 days post-exposure. Exposure to vehicle (saline or dimethyl sulfoxide) did not affect viability or differentiation of CPCs to CMs. Exposure of CPCs to $CeCl_3$ produced a dose dependent decrease in their ability to differentiate into CMs with no effect on cytotoxicity. In contrast to a previous study with murine stem cells, exposure of adult human CPCs to triclosan, or ROFA-L, led to a dose dependent increase in CPCs cytotoxicity. Bisphenol A had no effect on human CPCs cytotoxicity or differentiation to CMs. These results indicate that a variety of environmental chemicals, at low *in vitro* concentrations, can impact adult human CPCs in several ways including decreased viability and\or differentiation to CMs. In addition, there may exist species and\or developmental differences between current models that assess the impact of environmental chemicals on CSC or CPC viability or differentiation potential. (This abstract does not represent EPA policy)

Materials and Methods

CPCs: Adult human inducible pluripotent stem cell-derived CPCs were obtained from CDI (Cellular Dynamics International, Inc., Madison, WI, USA) and plated as well as maintained according to the manufacturer's protocol.

***In Vitro* Exposures:** CPCs were exposed to either: bisphenol A (BPA); cerium chloride ($CeCl_3$); triclosan (TCS); or perfluoroalkyl compounds such as PFNA, PFOA, PFOS, PFHxS, PFBS. Chemicals were obtained from Sigma-Aldrich, Milwaukee, WI. All chemicals except for $CsCl_3$ were prepared in dimethyl sulfoxide (DMSO) while $CsCl_3$ was prepared in saline. CPCs were also exposed to extracts prepared from either diesel exhaust particles (DEP) or residual oil fly ash (ROFA). DEP extract (DEP-extr) was prepared in DMSO while ROFA leachate (ROFA-L) was prepared in saline (SAL). CPCs were exposed to various concentrations of chemicals, emission particle extracts, or vehicle (saline or DMSO) in the presence of inducers for 48 hr. Cells were then maintained in inducer-free and chemical-free medium up to 6 days at which time CPC cultures were evaluated for cytotoxicity and differentiation.

CPC Cytotoxicity and Differentiation: CPC cytotoxicity was determined using Red Dot1 nuclear staining (Biotium. Inc., Fremont, CA). In-cell western staining, as described by K. J. Chandler et al., PLoS ONE 6(6):e18540, 2011; PMID: 21666745, was used to assess differentiation to cardiomyocytes (CM) using anti-cardiac troponin T (TnT) (Thermo Fisher Scientific, Pittsburgh, PA) antibody while undifferentiated CPCs levels were assessed usinganti-NKX2.5 (My Biosource Inc., San Diego, CA).

Bioimage Analysis: Cytotoxicity and in-cell western analyses were quantified using the LI-COR Biosciences, Odyssey CLx Imaging System and measuring relative fluorescent units (RFU) for Red Dot1 nuclear stain at 700 nm and anti-NKX 2.5 or anti-TnT staining at 800 nm. Fluorescent emission intensities or units were corrected for background fluorescence prior to being normalized to total cells per well.

Results

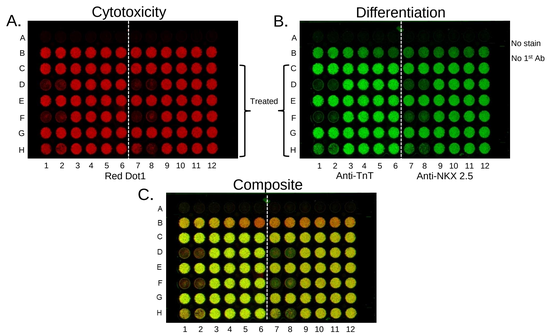


Figure 1. Detecting and quantifying chemical and environmental pollutant effects on adult human CPC viability and CM differentiation.

Result: Adult human CPCs were prepared as described in Materials and Methods. A) Representative image of CPCs assessed for cytotoxicity by staining with Red Dot1 and measuring relative fluorescent units (RFU) at 700 nm; B) Representative image of CPCs assessed for undifferentiated CPC levels using anti-NKX2.5 or differentiated CM using anti-TnT in cell western and measuring RFU at 800 nm; C) Composite image of Figures A and B.

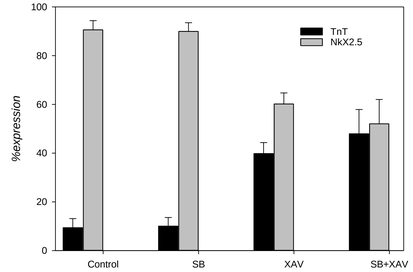


Figure 2. Determining optimal assay conditions for CPC to CM differentiation. Studies were undertaken to ensure optimal conditions for differentiation of CPCs to CMs prior to evaluating the effects of chemicals and environmental pollutants. CPCs were treated with either: inducer vehicle control (DMSO), SB (2.5 µM SB431542), XAV (10 µM XAV939), or SAB+XAV (2.5 µM SB431542 + 10 µM XAV939) inducers for 48 hours. Cells were maintained in inducer-free medium for 6 days at which time CPC cultures were evaluated for NKX 2.5 or TnT expression as described in Materials and Methods. Values are means +/- SE, N=6 from triplicate cultures.

Result: Spontaneous CM differentiation (control, no inducers) was found to average 10% cardiac troponin T^+ cells. Optimal CM differentiation induction was found to occur using both inducers and ranged between 50 – 60% cardiac troponin T^+ cells.

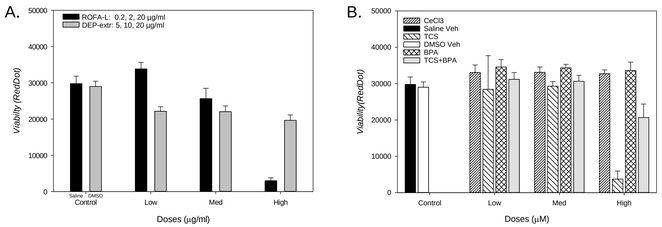


Figure 3. Effect of primary combustion particle extracts and chemical exposures on adult human CPC viability. (A) CPCs were exposed to: (A) ROFA-L in saline or DEP extracted with DMSO; or (B) CeC_{l3} (0.125,1.25, 12.5 µM), TCS (2.5, 5, 10 µM), BPA (0.125, 1.25, 12.5 µM), or BPA+TCS (0.062, 0.625, 6.25 µM) for 48 hours in the presence of differentiation inducers. Cells were then maintained in inducer-free and chemical-free medium up to 6 days at which time CPC cultures were evaluated for cytotoxicity by nuclear staining with Red Dot1 and scanned using the Li-COR Biociences, Odyssey CLx measuring RFU 700 nm. Values represent means ± SE, N=6 from triplicate cultures.

Result: (A) extracts of combustion particles ROFA-L and DEP-extr decreased CPC viability with ROF-L having the greatest effect; (B) only the chemicals TCS and TCS+BPA were found to decrease CPC viability at the 10 and 6.25 µM doses, respectively.

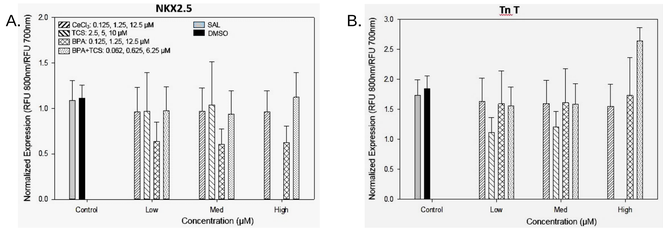


Figure 4. Effect of chemical exposures on adult human CPC levels and CM differentiation. CPCs were exposed as described in Figure 3 and subsequently analyzed by in Cell Western staining using (A) using anti-NKX2.5 antibodies, or (B) differentiated CMs, using anti-TnT antibodies. In Cell Westerns were scanned using the Li-COR Biociences, Odyssey CLx measuring RFU 800 nm. Values represent means ± SE, N=6 from triplicate cultures.

Result: TCS at 10 µM decreased both NKX2.5 and TnT expression; BPA decreased both NKX2.5 and TnT expression at all doses; TCS+BPA mixture increased TnT expression at 6.25 µM.

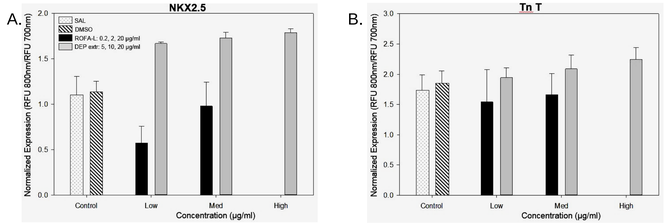


Figure 5. Effect of primary combustion particle extracts on adult human CPC levels and CM differentiation. CPCs were exposed as described in Figure 3 and analyzed as described in Figure 4. Values represent means, ± SE, N=6 from triplicate cultures.

Result: ROFA-L decreased NKX2.5 expression at all concentrations tested and TnT expression at 20 µg/ml while DEP-extract increased NKX2.5 and TnT expression at all concentrations tested.

Summary

Various environmental chemicals, including those associated with air pollution, were found to have significant effects on adult human CPC viability and differentiation:

Chemical	CPC Viability	CM Differentiation/TnT	CPC Level/NKX2.5
$CeCl_3$	-	-	-
TCS	+ (↓)[1]	+ (↓)[3]	+ (↓)[2]
BPA	-	-	+ (↓)[3]
BPA+TCS	+ (↓)[2]	+ (↑)[2]	-
ROFA-L	+ (↓)	+ (↓)[2]	+ (↓)[3]
DEP extr.	+ (↓)	-	+ (↑)

+, exposure induce an effect which increased (↑) or decreased (↓) the indicated endpoint.
-, no effect observed at any concentration tested.
1. in contrast results obtained using a murine ESC > CM model, 10 µM TCS was cytotoxic to adult human CPCs.
2. effect observed only at highest concentration tested.
3. effect observed at non-cytotoxic concentrations.

PFNA, PFOA, PFOS, PFHxS, and PFBS did not have any effect on adult human CPS viability or CM differentiation when tested at 25, 50, and 100 µM concentrations.

Conclusion

These results indicate that a variety of environmental chemicals, including those associated with air pollution related combustion emission particles, can impact human cardiac cell biology and potentially effect the heart's regenerative capacity leading to increased disease susceptibility. Specifically, environmental chemicals alter adult human CPCs in several ways including decreased viability and\or differentiation to CMs. In addition, there may exist species and\or developmental differences between current models that assess the impact of environmental chemicals on CSC or CPC viability or CM differentiation potential. Additional key conclusions of this research are its:

Relevancy\Impact: established model to evaluate the effects of chemicals and environmental pollutants on *human adult* cardiac cell developmental toxicology and associated adverse health outcome pathways;

Sensitivity\Physiology: the adult human CPC model detects responses at relatively low *in vitro* concentrations and is currently being developed to assess differentiated CM functionality;

Throughput\Cost: the adult human CPC model and related endpoints are scalable and may be multiplexed to increase throughput as well as provide increased bio-information generation at lower costs.

Corresponding Lead Investigator: Kevin L. Dreher, M.S., Ph.D., US EPA, ORD, National Health and Environmental Research Laboratory, Research Triangle Park, NC 27711, Dreher.kevin@epa.gov or kvdreh@gmail.com