Resource report

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Development of functional assays in lymphoblastoid cell lines

Dr Shavanthi Rajatileka, Dr Alix Groom, Dr Susan Ring

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CLOSER
UCL Institute of Education
20 Bedford Way
London
WC1H 0AL
United Kingdom

Tel: +44 (0)20 7331 5102
Email: closer@ucl.ac.uk
Web: www.closer.ac.uk
Twitter: @CLOSER_UK
YouTube: CLOSER

Aims

Lymphoblastoid cell lines (LCLs) can be used to investigate disease mechanisms and assess the effect of environmental exposures on cell lines from individuals of different genetic backgrounds. For example cell lines from individuals with different genetic risk scores for conditions such as obesity or schizophrenia could be grown and gene expression, epigenetic and proteomic differences compared. The aim of this section of the work package was to set up proof of principle experiments which could then potentially be rolled out to cell lines from individuals with specific genetic backgrounds from the CLOSER cohorts.

HEAVY METAL TOXICITY; THE EFFECTS OF LEAD, ARSENIC, COPPER AND ZINC ON CELLULAR FUNCTION

Background

Individuals are exposed to a wide range of toxic compounds through diet, the environment they live in and occupational exposure. Within the environment a number of heavy metals have reached harmful levels particularly when combined with other factors that increase the metals' absorption, retention and toxicity.

Heavy metals often occur in mixtures with other heavy metals and limited research has been undertaken looking at the effects of metal mixes on health outcomes. Lead and arsenic pose a potential risk due to their persistence in the environment. Although copper and zinc are essential for cell function they are toxic in excess. Therefore, the effects of lead, arsenic, copper and zinc on LCL proliferation, methylation and transcriptomic profiles were investigated. A workflow for the pilot and future work is depicted in Figure 1.

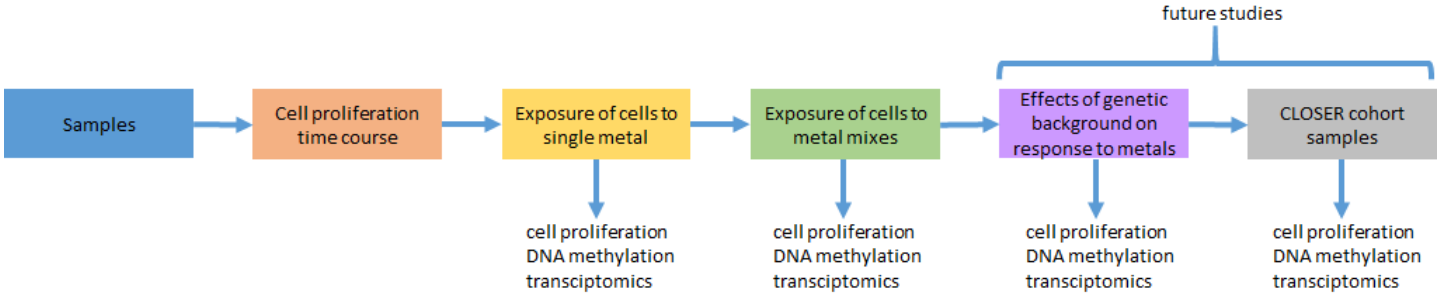

Figure 1: Workflow for analysing effects of metals on cellular function

Cell proliferation time course

Initially a cell proliferation time course was undertaken to determine how long to expose cells to the heavy metals as well as how many cells to seed. Overgrowth of cells in culture would have an effect on cell viability irrespective of any exposure. The cells would also need to remain healthy without feeding during this time period as adding fresh media could influence cell proliferation rates. Cultures from 2 different LCLs were used throughout this pilot 'C0046' (female) and 'C0057' (male) originally purchased from the European Collection of Authenticated Cell Cultures (ECACC). For these cells lines it was found that seeding 1.5 x 10^7 cells in a T75 flask was sufficient for sampling to take place at 24hr, 48hr, 78hr and 96hr after seeding whilst preventing overgrowth of the cultures. The cells remained healthy at 96hr as ascertained by viable cell count of the cultures.

Exposure of cells to metals

Cells were exposed to copper and zinc at levels associated with a dietary overdose and to lead and arsenic at levels relevant to industrial exposure (see Table 1). The effects of each metal individually and as mixtures were analysed in the 2 LCLs for 96 hours. Every 24 hours the control and exposed

cultures were sampled to assess cell proliferation and at the end of the exposure samples were also taken to extract DNA and RNA.

heavy metal treatment	metal compound	concentration µM
copper	copper sulphate ($CuSO_4$)	250
zinc	zinc sulphate ($ZnSO_4$)	200
arsenic	sodium arsenite ($NaAsO_2$)	3.8
lead	lead nitrate ($Pb(NO_3)_2$)	1.6
copper/zinc	copper sulphate and zinc sulphate	250/200
arsenic/lead	sodium arsenite and lead nitrate	3.8/1.6
copper/zinc/arsenic/lead	copper sulphate, zinc sulphate, sodium arsenite and lead nitrate	250/200/3.8/1.6

Table 1: Heavy metals and the concentration of compounds which cells were exposed to

Cell proliferation

The proliferation of cells during exposure to metals was assessed by viable cell count. Table 2 shows a comparison of heavy metal treatment against untreated cells showing whether proliferation increased, decreased or was unaffected by the treatments. Exposure to copper

	time point							
heavy metal treatment	24hrs		48hrs		72hrs		96hrs	
copper	↔	↔	↔	↓	↓	↓	↓	↓
zinc	↔	↔	↓	↓	↓	↓	↓	↓
lead	↔	↔	↑	↑	↑	↑	↑	↑
arsenic	↑	↔	↑	↑	↑	↑	↓	↓
copper/zinc	↔	↔	↔	↓	↓	↓	↓	↓
arsenic/lead	↔	↔	↑	↓	↑	↓	↑	↓
copper/zinc/arsenic/lead	↔	↔	↓	↓	↓	↓	↓	↓

Table 2: LCL cell count levels compared to untreated LCLs. Key: female cell line; male cell line; ↔ no difference in cell count; ↑ increase in cell count; ↓ decrease in cell count

and zinc in isolation had a negative effect on cell count, however exposure to arsenic and lead increased cell proliferation after 24hrs of exposure. Other studies have also shown that arsenic increases cell proliferation[1,2]. When cells were exposed to all 4 heavy metals simultaneously an increase in cell proliferation was not observed.

DNA methylation

To assess whether exposure of LCLs to heavy metals affects DNA methylation DNA was extracted from the cells 96hrs after exposure. The DNA was analysed using the Infinium HumanMethyaltion450K BeadChip (illumina) platform. This work is being drafted for publication. In summary, strong associations were observed for all heavy metals with DNA methylation. The number of CpG associations (methylation sites) with the individual metals is in Table 3.

heavy metal	CpG sites associated with exposure
copper	39
zinc	38
lead	1134
arsenic	8

Table 3: Number of CpG sites associated with heavy metal exposure.

Data generated from this pilot is being used to identify potential stable biomarkers of exposure that can then be investigated further using data for known chemical exposure in ALSPAC study participants.

Transcriptomic profile

To determine if heavy metal exposure affected gene expression it was the intention to undertake array-based transcriptome expression analysis using an Affymetrix platform. RNA was extracted from cells that had been exposed to either heavy metal(s) or the control (untreated) after 96 hours. The quality of the RNA was assessed and the majority of samples were found to have a very low RNA Integrity Number (RIN). This indicates that there is substantial RNA degradation which limits the downstream analyses that can be undertaken. It is difficult to determine the cause of this RNA degradation. Possibilities are that this is attributed to the potential presence of heavy metal(s) in the RNA sample and/or initiation of apoptosis by the cells due to exposure.

Outcomes

Although only 2 cell lines were used for this pilot due to the number of exposures analysed and the experiments run in triplicate this generated a substantial amount of work. It would not be feasible to scale up this protocol to analyse LCLs that have been stored by the CLOSER cohorts. However, this study has shown the potential of LCL work to identify markers that can then be interrogated on a larger scale.

References

[1]Rajdeep Chowdhury, Raghunath Chatterjee, Ashok K. Giri, Chitra Mandal, Keya Chaudhuri, Arsenic-induced cell proliferation is associated with enhanced ROS generation, *Erk* signalling, and *CyclinA* expression. Toxicology Letters 198(2): 263-271, 2010

[2]Todd A. Stueckle, Yongju Lu, Mary E. Davis, Liying Wang, Bing-Hua Jiang, Ida Holaskova, Rosana Schafer, John B. Barnett, Yon Rojanasakul, Chronic occupational exposure to arsenic induces carcinogenic gene signalling networks and neoplastic transformation in human lung epithelial cells. *Toxicol Appl Pharmacol* 261(2): 204-216, 2012