EFFECTS OF LEAD EXPOSURE ON PROLIFERATION AND DIFFERENTIATION OF STEM CELLS ISOLATED FROM BONE MARROW AND DENTAL ORIGIN

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INTRODUCTION

Lead (Pb^{2+}) is regarded as one of the most toxic substances among heavy metals. Pb^{2+} causes serious illness which resulted in physiological, biochemical and behavioural dysfunctions in humans, especially in children (Needleman, 2004; Clarkson et al., 1990). It can be exposed to the human body through food intake as well as through many environmental elements such as dust, air, and water. The common route of exposure of Pb^{2+} is in the blood and eventually it is deposited in the hard tissues such as bone and teeth. The deposition in the latter tissue is permanent (Delves and Campbell, 1993). Several factors are identified which influences the Pb^{2+} deposition in teeth. These include types of teeth and the presence of caries. Pb^{2+} is distributed with the highest concentration in the circumpulpal dentin which is located in the innermost layer of dentin, adjacent to the dental pulp. The mechanism by which Pb^{2+} is deposited within the matrix of the primary and circumpulpal dentin is not clear (Lansdown et al., 1986). Nevertheless, it has been suggested that the deposition of Pb^{2+} in teeth is probably due to the similar oxidation number of both Ca^{2+} and Pb^{2+} ions. Stem cells play an important role in maintaining the homeostasis and function of tissues and play a pivotal role in dealing with invaders such as Pb^{2+}. These effects have been documented in previous work only through the use of dental cells (Thaweboon et al., 2002). Little is known, however, on the behaviour of dental stem cells towards Pb^{2+} .

AIM

To investigate the effects of lead on the proliferation and differentiation of stem cells originating from bone marrow (BM-MSCs), pulp of deciduous (SCDs), permanent (DPSCs) teeth and periodontal ligaments (PDLs).

METHODOLOGY

TEETH EXTRACTION
- Ethic Clearance Number: DF C01107/0066(L)

ISOLATION
BM-MSCs (Age : 24-35)
SCDs (Age : 3-12)
DPSCs (Age : 24-35)
PDLSCs (Age : 24-35)

Pb^{2+} PREPARATION
5 different Pb^{2+} (Cat No 203580, Sigma Aldrich) concentrations (160, 80, 40, 20, and 10 µM/mL) were used in this study, with a 50% difference between each concentration

NON-TREATED → TREATED

PROLIFERATION
- Morphology
- Growth Curve
- Senescence

NON-TREATED → TREATED

DIFFERENTIATION
- Osteogenesis
- Chondrogenesis
- Adipogenesis

RESULTS

PROLIFERATION

Morphology of BM-MSCs, SCDs, PDLs and DPSCs exposed to Pb^{2+}

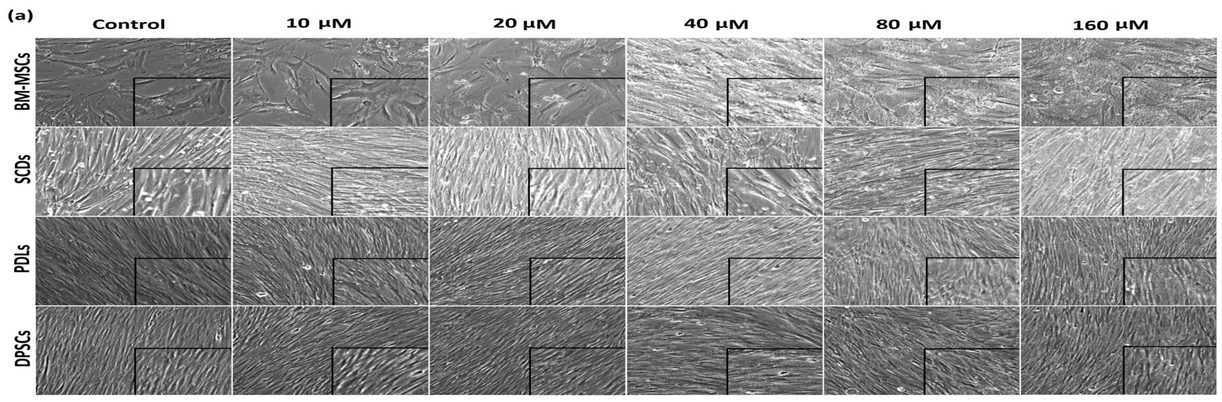

Figure1: Cytotoxic effect of Pb^{2} to BM-MSCs, SCDs, PDLs and DPSCs (Phase contrast microscope; 10x of magnification) in presence of various concentrations of Pb^{2+} (n=3).

- BM-MSCs, SCDs, DPSCs and PDLs cultures maintained a fibroblastic cell-like morphology in controlled conditions and a similar observation was seen in cells exposed to 10 µM of Pb^{2+}.
- The cells line began to loose their cell texture as the concentration of Pb^{2+} increased to 160 µM. BM-MSCs shows the worst followed by SCD, DPSCs and PDLs.

Qualitative SA-β-Gal activity of BM-MSCs, SCDs, PDLs and DPSCs

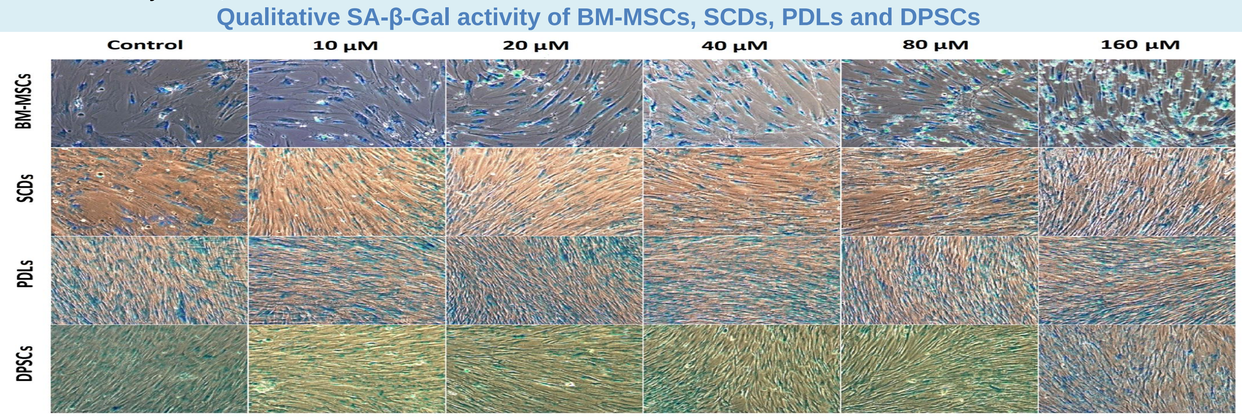

Figure2: Conventional qualitative SA-β-gal assay by X-gal staining of BM-MSCs, SCDs, DPSCs and PDLs upon exposure to various concentrations of Pb^{2+} (n=3).

- BM-MSCs show the highest percentage of SA-β-Gal activity followed by SCDs, DPSCs and PDLs in a concentration dependent manner.
- At the lowest concentration of Pb^{2+} (10 µM), there was no significant ($p>0.05$) inhibition of the proliferation rate of any of the cell lines.
- However between 20 -160 µM of Pb^{2+} a drastic inhibition of cell growth was observed in BM-MSCs.
- As for stem cells derived from dental sources, only a small variation in SA-β-Gal activity was observed between control and 160 µM treated groups ($p>0.05$).

DIFFERENTIATION

Trilineage Capacity of BM-MSCs,SCDs, PDLs and DPSCs

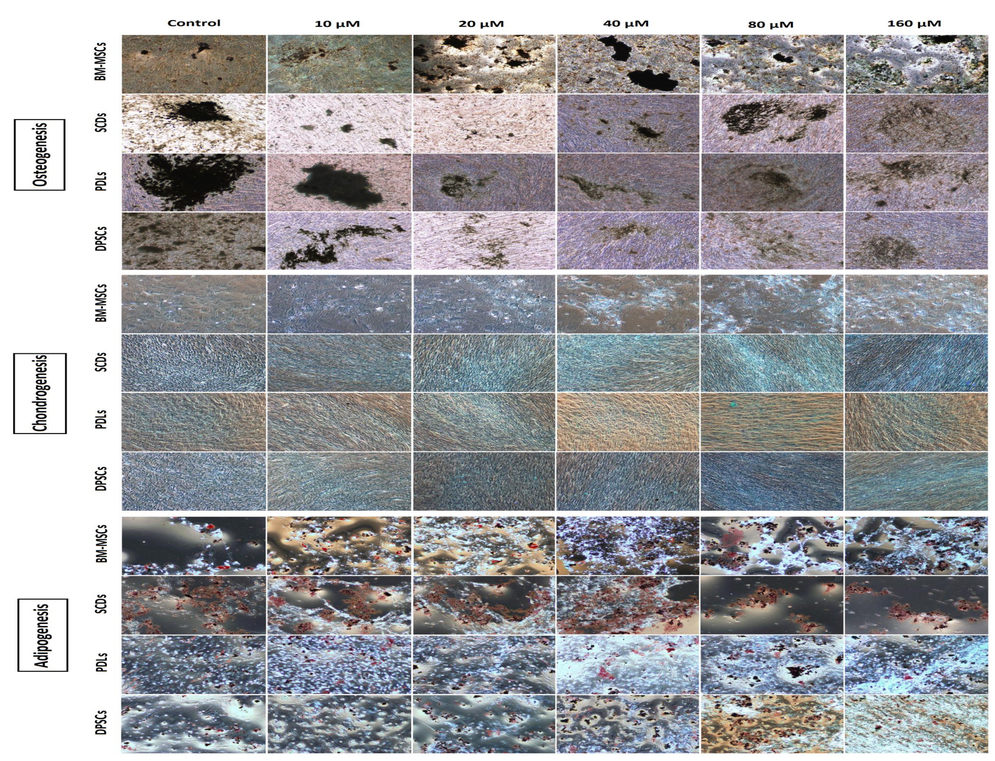

Figure 3: Differentiation of BM-MSCs, PDLs, DPSCs at various concentration of Pb^{2+} (n=3).

- Osteogenic differentiation (von Kossa staining - 21 days) : BM-MSCs showed a weak deposition of calcium in the mineralized matrix starting at a concentration of 40 µM. Whereas, cell lines from dental sources were only affected at 160 µM.
- Chondrogenic differentiation (alcian Blue - 21 days) : No differences were noted between the control and treatment groups in all cell lines .
- Adipogenic differentiation (oil Red O - 21 days) : BM-MSCs showed less accumulation of lipid vacuoles of cells exposed from 80 up to 160 µM. Whereas for dental derived stem cell, the lipid vacuoles were still obvious even at 160 µM.

DISCUSSIONS

- Pb^{2+} exposure inhibited adhesion with the highest being in BM-MSCs followed by SCDs, DPSCs and PDLs as well as it increased the biological aging of the cells in a dose-dependent manner.
- In this study we identified that the concentration of Pb^{2+} (40 to 160 µM) suppressed the proliferation of stem cells rather than inducing cell death. One possible explanation is that platelets are present in significant amounts in stem cells or bone marrow mononuclear cell cultures (Assmus et al., 2010; Prokopi et al., 2009) and Pb^{2+} is thought to influence platelets or lysate-based platelets by regulating the levels of growth chemotactin factors (Muller et al., 2011). Thus, we speculate that Pb^{2+} inhibits growth factors related to the proliferation of stem cells resulting in slow growth of cells.
- All the cell lines from various sources were capable of differentiating into osteoblast, chondrocytes and adipocytes as observed in the control samples.
- Osteogenic differentiation occurred when all the cell lines were exposed to Pb^{2+} , even at the highest concentration of Pb^{2+} (160µM). However, BM-MSCs showed reduce differentiation capacity compared to dental derived stem cell. Pb^{2+} follows the movement of Ca2+ in the body as it utilises the same ion transporter as calcium, acting like a competitive inhibitor (Alissa and Ferns, 2011). But we postulate that the demineralisation process is slow in teeth allowing the cells of dental origin to adapt for more resistance to Pb^{2+} toxicity. The genes such as alkaline phosphatase and osteocalcin has been supressed by Pb^{2+} (Long et al., 1990). We speculate/ suggest that Pb^{2+} can inhibit osteoblast differentiation due to disturbance of osteogenic pathways.
- Our results showed that when exposed to Pb^{2+},chondrogenic differentiation was not observed in all cell lines. This concurred with findings reported by Zuscik et al. (2007) and Cormouchee (2005).

CONCLUSIONS

- Our study revealed that lead exposure affects the proliferation rate and induces alteration in differentiation of stem cells of dental origin.
- Dental derived stem cells have a potential as screening model for heavy metal since it has demonstrated their ability to withstand the toxicity of Pb^{2+} better than BM-MSCs

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