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Neural stem cells in lead toxicity

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Abstract. – Lead (Pb) exposure in the early stages of neurodevelopment results in long-lasting alterations that ultimately cognitive function and behaviour. The prime targets of lead toxicity are the multipotent neural stem cells (NSCs). The present review will discuss the basic molecular physiology involved in the toxicity mechanisms induced by lead and its resultant counter effects on nervous system and physiology. The article shall help researchers working in the area to design new drugs and therapeutics for the efficient management of neuro-toxic states especially upon prenatal exposure to lead.

Key Words:
Pb neurotoxicity, Neural stems cells, Cognitive function.

Introduction

Lead (Pb) is persistent in the environment and remains an important public health concern in the whole world[1]. Moreover, the mean blood Pb levels have been recently reported to be 13 μg/dL in China[2], 15 μg/dL in Bangladeshi urban populations[3] and 11 μg/dL in urban Indian children[4]. However, as economically lesser-developed countries tackle Pb phase-outs from gasoline and products like paint, new challenges are arising. Moreover, Pb is also common in electronic waste, and this waste at present is the rapidly growing disposal challenge for the whole world. For instance in Guiyu, a poorly regulated electronic waste destination in China, 70.8% of children have blood Pb levels above the 10 μg/dL[5].

The biological factor responsible for Pb prenatal exposure is its ability to freely cross the placenta in order to directly affect fetus development[6]. A longitudinal study showed prenatal Pb exposure was associated with lower mental development index (MDI) at two years of age[7]. In another study, Pb exposure in children under 7 years old was associated with lower IQ scores at 11-13 years of age[8]. Dramatically, a recent study also revealed that adults in Boston aged 28-30 had IQ's statistically significantly inversely associated with blood Pb at 6 months, 4 year and 10 years of age; and at each time point blood Pb averaged below 10 μg/dL[9].

Neural Stem Cells (NSCs) and the Lead Exposure

In early embryogenesis, the ectoderm thickens and folds to create the neural tube, which is the precursor of the central nervous system. The cells of the neural tube are multipotent stem cells that can differentiate into any of the cell types of the central nervous system, and are referred as NSCs[10,11]. NSCs begin to proliferate rapidly in weeks 5-6 of gestation and continue to do so until the end of the first trimester[12]. In the second trimester, these cells start to migrate and differentiate primarily into neurons, astrocytes, and oligodendrocytes. The early stages of development, especially in the nervous system, are believed to be particularly vulnerable to toxicant insult[13].

In predictive models of 24-month MDI outcomes, Pb exposure assessed in maternal blood during the first trimester was the most statistically significant predictor of lead toxicity as compared to maternal blood during second trimesters or third trimesters[14]. The timeline presented by Hu et al[14] suggested a potential role for NSCs in the etiology of Pb-related cognitive deficits in early life. Laboratory experiments showed Pb slows proliferation of NSCs *in vivo*[15] and *in vitro*[16]. In addition, Pb exposure in rats altered dendrite morphology in NSCs differentiating into neurons, but did not influence their cell-type fate[17]. While these studies reveal marked effects on NSCs under Pb exposure, the mechanisms underlying the effects remain unknown.

While the existence of an *in utero* critical time point is supported by many studies cited here, it is worth noting that in a cohort of Yugoslavian children, blood Pb at 4, 5 and 7 years of age is more predictive of IQ than in utero exposure assessed in maternal blood[18]. Differences in assessing the Pb exposure, population-specific confounders and differences in outcome measurements might describe the discrepancy. The toxico-kinetics of the passage of Pb from mother to fetus are not well

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described, and the exact timing of fetal exposure and assessment might complicate association studies at this exposure time point. Moreover, lead associated oxidative stress is seen in the form of elevated malondialdehyde (an oxidative stress biomarker) levels in brains of rats exposed pre- and perinatally to Pb acetate in drinking water[19]. A similar trend was noticed in the blood of people with occupational Pb exposure[20].

Important Mechanisms of Lead Toxicity

Many promising putative toxicological mechanisms of Pb have been described, and most notable among them are related to Pb's non-competitive antagonism of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is a ligand-gated ion channel that plays an essential role in brain development, synaptic plasticity and long-term potentiation (LTP). LTP, is the enhanced postsynaptic response following high frequency stimulation and is believed to be the central mechanism of learning and memory. Furthermore, calcium serves as a second messenger in a variety of signaling cascades that promote LTP. One such signaling cascade activates the cAMP element binding transcription factor (CREB), which drives the expression of brain-derived neurotrophic factor (BDNF)[21]. This pathway has been linked to the Pb-induced reduction of BDNF expression which ultimately results in Pb-associated cognitive deficits[22]. However, the relevancy of the Pb-NMDA relationship in NSCs is still less clear.

Recent Genetic Alterations due to Lead Toxicity

A microarray study[23] in the recent past on a 21 day-old mouse brain revealed a total of 350 genes affected by Pb-exposure, which was enriched for pathways pertaining to focal adhesion, extracellular matrix receptor interaction, Fc epsilon R1 signaling, glycan structures-biosynthesis 1, purine metabolism, Nglycan biosynthesis, and VEGF signaling. Another microarray study[24] by the same research group reported significant differential expression patterns in rats with peri- and postnatal Pb exposure, which differed by the timing of exposure and gender. Furthermore, the pathway analysis in another study[25] identified deregulation of pathways about ion binding, regulation of RNA metabolic processes and positive regulation of macromolecule biosynthetic processes. Pb has been also observed to elicit both a unique set of expression changes as well as changes shared among zebrafish embryos exposed to other environmental toxicants[26]. Expression changes included induction of chaperone proteins, like heat shock protein 70 (Hsp70) and oxidative stress-related proteins including glutathione-s-transferase omega 1 (GSTO1). Expression changes among zebrafish embryos vary by embryonic stage and included many genes involved in neuronal development[22].

Role of SPP1 in Lead-Induced Neurotoxicity

SPP1 is, in fact, the only known secreted protein whose gene is targeted by NRF2 which is another target of Pb[27]. There are many reports[27,28] confirming NRF2 activation and upregulation of NRF2 targets by Pb. Notably, however, some transcription profiling of Pb-exposed animals[29] did not include several NRF2 targets among the top hits. This is likely due to the numerous secondary effects of Pb in animals undergoing long-term exposure. These secondary effects might crowd out the primary cellular transcriptional response. Furthermore, compensatory regulation of the NRF2 pathway might bring down the level of NRF2 activation upon long-term exposure.

SPP1 is a pleiotropic extracellular glycoprotein with emerging roles as a potent neuroprotectant. SPP1 in the brain is upregulated in several morphological stress conditions including hypoxic ischemia, cortical lesion and subarachnoid hemorrhage[29-31]. Stimuli known to regulate SPP1 expression included pro-inflammatory mediators, hormones, growth factors and mechanical stressors reviewed by Mazzali et al[32]. NRF2 activation is consistent with many of these conditions and exposures. However, it is important to note that transcription factor binding sites for AP1 and NF-κB have also been validated in the promoter of SPP1[32], which are also consistent with upregulation upon many stress conditions. SPP1 appears to be a hub for some conditions and understanding its effect on the developing brain is an important aspect to understand its putative roles in modulating Pb neurotoxicity and other stress responses. On the other hand, SPP1 has not been previously shown to be upregulated by Pb[33]. However, enrichment of the networks related to MAPKs, extra-cellular matrix receptor and focal adhesion are consistent with the involvement of Pb in the SPP1-FAK signaling axes. Furthermore, vasospasm, is another common complication of subarachnoid hemorrhage observed following SPP1 treatment[34]. Following unilateral entorhinal cortical lesion, SPP1 is a pro-synaptogenic factor

in the recovery phase and SPP1 knockout animals showed delayed cognitive recovery[30]. In rat neural progenitor cells, exogenous recombinant SPP1 has been reported to increase proliferation via Akt signaling[35,36]. So, SPP1 might have a role in the developing brain in addition to its roles in stress responses. The data supporting SPP1 as a neuroprotective factor in stress conditions is consistent with the paradigm of NRF2 activation as a cytoprotective response to oxidative stress. SPP1 is the only secreted protein identified as a target of NRF2, and is a possible mechanism of cell-to-cell communication of oxidative stress conditions.

However, the benefits of SPP1 signaling are not without exceptions. In conflict with other reports on the subject, one report showed no benefit in a mouse preterm brain injury model with recombinant SPP1 treatment[37]. SPP1 is also upregulated and associated with poor prognosis in many cancers including glioma patients[38]. The implications of NRF2 targeting of SPP1 are of broader interest than Pb exposure, since the NRF2 response is central to many environmental stressors, neurodegenerative diseases and cancers.

Conclusions

Numerous aspects of lead toxicity have been explored. Various molecular details provided new drug targets for the better management of drug toxicity. Further, studies are still required for pronounced efficiency in neuroprotection against lead toxicity.

Conflict of Interest

The authors declare no conflicts of interest.

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