Arch Toxicol (2015) 89:265–267
DOI 10.1007/s00204-014-1442-0

LETTER TO THE EDITOR, NEWS AND VIEWS

Progress toward the development of in vitro model system for chemical-induced developmental neurotoxicity: potential applicability of stem cells

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Received: 15 December 2014 / Accepted: 17 December 2014 / Published online: 24 December 2014

Developmental neurotoxicity (DNT) has been the prime focus of our research group since it is one of the most crucial and significant parts of the neuroscience research arena. The restricted availability of live human fetal brain tissue and major ethical issues makes the field further challenging. So far, it has been best represented via the in vivo model systems; however, the inaccurate extrapolation of data to humans restricts its scope and applications. Therefore, there is a dire need for samples (cells and tissues) of human origin. Based on the OECD guidelines 426, rodent data are present for about 200 compounds, but there is a scarcity of data available for humans.

The developing brain is highly vulnerable to even minute doses of substances which are otherwise harmless for a fully developed mature brain. This is primarily because of the poorly developed blood–brain barrier, low oxygen levels and high mitotic rates. The entire process of brain development is quite complex, and in order to generate DNT models in vitro, cells and tissues of human origin are needed. Stem cells with their inherent potential for pluripotency and unique ability for unlimited proliferation serve as one of the best tools.

Stem cells can be made to differentiate into the neuronal lineages and can thereby sufficiently mimic cells of the developing brain. Human umbilical cord blood was employed for the isolation of stem cells as it is not only a rich source of stem cells, but also a waste material, therefore does not pose any major ethical dubious. $CD34^+$ hematopoietic stem cells (HSCs)/non-hematopoietic stem cells (non-HSCs) derived from cord blood are greatly committed toward the neuronal lineage and are very similar to the fetal neural stem cells.

In our so far published papers, we have successfully attempted to establish stem cell-based in vitro model systems for the study of the various aspects of DNT and have proven that human umbilical cord blood-derived stem cells (hUCBSCs) when differentiated into neurons serve as one of the classical tools for the same. These were employed to assess the developmental neurotoxicity potential of Monocrotophos (MCP), a known developmental neurotoxin. Differentiating cells were given MCP exposure at different stages of maturation, i.e., day 2, 4 and 8, and significant changes in the markers of differentiation, proliferation, injury and receptors were studied. Our group reported that there was a significant upregulation of the different MAPKs, apoptosis and neurogenesis markers and a down regulation in the various cell proliferation markers during the neuronal differentiation process. Expression analysis was done both at the transcriptional (^{q}RT-PCR) as well as translational levels (immunocytochemistry and Western blotting). As differentiation proceeded, there was a significant quantitative reduction in the expression of stems markers, i.e., CD133, MYC, NANOG, SHH, KLF4, SOX2, POU5F1 and FRAP1, whereas the differentiation marker expression was significantly increased except in case of NGF, CHRM2 and NR42A. The highest expression for differentiation markers, i.e., NFM, MBP, NFH, NCM, BDNF and STAT4, was seen on day 8. These results support that hUCBSCs successfully differentiated into the neuronal lineage. Post MCP exposure there was again a significant alteration in the various markers of apoptosis, oxidative stress, metabolism, etc. It was concurred from the results that MCP exposure leads to ROS generation, oxidative stress, depleted glutathione levels and even promoted apoptosis. Receptor studies further demonstrated that the

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acetylcholinesterase activity was reduced and acetylcholine levels were upregulated. MCP exposure was also capable of reducing the receptor binding specificity in case of cholinergic and dopaminergic receptors (Kashyap et al. 2014).

Xenobiotic metabolism and the constitutive expression and inducibility of cytochrome P450 s (CYPs) and their regulators in the developing brain were our next focus of research. There is negligible information available for the same so far. Our study employed the use of rifampin (universal inducer of CYPs), cimetidine (universal inhibitor of CYPs) and monocrotophos (known developmental neurotoxin). Differentiating neuronal stem cells were exposed to various combinations of the above mentioned substances, and expression analysis was done for the alteration in the expression of CYPs, receptors and phase II metabolizing enzyme Glutathione S-transferase P-1 (GSTP-1). Our major findings suggest that rifampin successfully induced the expression of major CYPs such as CYP1A1, CYP2B6, CYP2E1 and CYP3A4; however, it could not induce sufficient alterations in the expression of aryl hydrocarbon receptors (AHR), constitutive androstane receptor (CAR) and Glutathione S-transferase P-1 (GSTP-1). It was further observed that co-exposure with rifampin and monocrotophos had an additive effect on the induction of CYPs. Initially, cimetidine pretreatment did not majorly influence the CYP responsiveness against monocrotophos exposure, but by day 4, it was vice versa. Similarly, by day 4, exposure to cimetidine leads to a range of down-regulations in the expression of CYPs. All in all, our results indicated that undifferentiated cells (day 0) and early differentiated cells (day 2) are more susceptible and highly vulnerable to xenobiotics as compared to the fully differentiated mature cells. Our study comes up with strong evidence in favor of the xenobiotic metabolizing capability of developing neurons (Singh et al. 2012).

Having established differentiating neuronal stem cells as a potent in vitro tool for screening developmental neurotoxicity, we further probed the signaling cascades involved post monocrotophos exposure. In one of our recent publications, we reported the role of Akt in the regulation of xenobiotic-induced apoptosis and injury in hUCBSCs for the first time. To generate evidence in our favor, in silico molecular docking studies were performed to ensure whether MCP modulates the anticancer target protein kinase B (Pkb/Akt) and to probe the action mechanisms thus possible. The in silico studies were validated in vitro using expression analysis studies for markers of cell injury, apoptosis, selected CYPs and Akt signaling pathways. Our in vitro data strongly support the in silico finding which indicated that MCP substantially reduces Akt phosphorylation at both the domains, i.e., at position Thr308 and Ser473. GSK3β was inactivated by phosphorylated/activated Akt due to phosphorylation at Ser9. Results indicated that levels of phosphorylated GSK3β (Ser9) were lowered and levels of non phosphorylated GSK3β were unaffected. These results indicate a possible degradation of beta-catenin through an ubiquitin-dependent proteasome pathway which leads to cell death via apoptosis. The GSK3β activation also inhibits the Wnt/frizzled/disheveled (DSH) pathway and promotes growth and cell survival. The reduced levels of pAkt, Wnt and beta-catenin reported in our published study indicate the impaired Wnt/Akt/β-catenin signaling and induction of apoptosis in hUCBSCs subsequently. Levels of PTEN and PI3K were unaltered and confirm the involvement of other routes of pAkt inhibition in MCP-induced cell death signals in hUCBSCs (Kashyap et al. 2013).

We further explored the effect of 3-methylcholanthrene (MC) a well-characterized polycyclic aromatic hydrocarbon on developing neurons and stage-specific responses were studied. MC is an environmental contaminant and a known inducer of CYP1A1. In silico and in vitro approaches were run in parallel to strongly investigate the role of MC on aryl hydrocarbon receptors and xenobiotic metabolism and to confirm the neurotoxic potential of the same. The major findings of our publication were that MC induces the expression and activity of AHR, CYP1A1 and GSTP-1 significantly and reduces the expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) receptors and cAMP response element binding protein (CREB). All our experiments strongly suggested that MC in all probability not only interferes with neuronal transmission, but also inhibits the expression/activity of various developmental stage-specific neuronal markers. Our data once again throw light on the fact that early differentiating neurons are metabolically more active and more vulnerable to xenobiotic compounds and polycyclic aromatic hydrocarbons as compared to the fully differentiated mature neuronal cells (Singh et al. 2013).

In one of our unpublished studies, we have further demonstrated the molecular switching mechanism of TrkA/$p75^{NNT}$ signaling in MCP-induced neurotoxicity under both in vitro (cultured rat brain neural stem cell-derived neuronal cells) and in vivo (rat brain) conditions. We have thus tried to establish the neurotoxicity mechanisms of MCP and extract useful information that neurotoxicity induced by these pesticides is an important risk factor for neurodegenerative diseases. The exposure to these pesticides may further increase the intensity of neurodegeneration in the patients suffering from AD, PD and other neurodegenerative disorders.

Therefore, our research group has tried to cover the various in silico and in vitro as well as in vivo approaches so as to generate substantial reliable and reproducible data and to establish sophisticated in vitro models for screening,

assessing, analyzing and characterizing the various aspects of developmental neurotoxicity.

However, life is 3D not 2D; therefore, we have taken a step ahead and in our current laboratory scenario we are working toward the development of complete 3D tissue niche of neuronal tissue from human cord blood-derived stem cells using scaffolds so as to establish more efficient screening and predictive tools for developmental neurotoxicity. Hopefully in the near future, we shall come up with some innovative novel 3D human stem cell-based models and contribute generously toward the emerging field of developmental neurotoxicity.

References

Kashyap MP, Kumar V, Singh AK, Tripathi VK, Jahan S, Pandey A, Srivastava RK, Khanna VK, Pant AB (2014) Differentiating neurons derived from human umbilical cord blood stem cells work as a test system for developmental neurotoxicity. Mol Neurobiol [Epub ahead of print]

Kashyap MP, Singh AK, Kumar V, Yadav DK, Khan F, Jahan S, Khanna VK, Yadav S, Pant AB (2013) Pkb/Akt1 mediates Wnt/GSK3β/β-catenin signaling-induced apoptosis in human cord blood stem cells exposed to organophosphate pesticide monocrotophos. Stem Cells Dev 15:224–238

Singh AK, Kashyap MP, Jahan S, Kumar V, Tripathi VK, Siddiqui MA, Yadav S, Khanna VK, Das V, Jain SK, Pant AB (2012) Expression and inducibility of cytochrome P450s (CYP1A1, 2B6, 2E1, 3A4) in human cord blood $CD34^{+}$ stem cell-derived differentiating neuronal cells. Toxicol Sci 129:392–410

Singh AK, Kashyap MP, Kumar V, Tripathi VK, Yadav DK, Khan F, Jahan S, Khanna VK, Yadav S, Pant AB (2013) 3-methylcholanthrene induces neurotoxicity in developing neurons derived from human $CD^{34+}Thy^{1+}$ stem cells by activation of aryl hydrocarbon receptor. Neuromolecular Med 15:570–592