TOXICOLOGICAL HIGHLIGHT

Use of Gene Expression of Neural Markers in Cultured Neural Cells to Identify Developmental Neurotoxicants

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The neurotoxic effects on humans of 201 chemicals, mostly solvents, pesticides, industrial compounds, and metals, have been documented (not including drugs, food additives, animal toxins, and biogenic substances). The number of compounds that are neurotoxic in experimental animal models is much higher than this (Grandjean and Landrigan, 2006). Neurotoxicity is usually manifested as altered behavior or alterations to motor and learning cognitive processes. These are often a consequence of alterations in gamma-aminobutyric acid (GABA) and glutamate neurotransmission and of neurodegenerative processes.

People are typically exposed to neurotoxic compounds over long periods of time and even throughout their entire lives. Prolonged exposure to low concentrations of persistent neurotoxic environmental pollutants can lead to neuronal dysfunction, especially during neural development. Therefore, there is concern about the possible effects of chronic exposure to low concentrations of chemicals, particularly during periods of neurodevelopment. It is accepted that the developing human brain is much more susceptible to toxic agents than is the adult brain. Furthermore, because the human brain has a prolonged period of postnatal maturation, continuing through adolescence, neurotoxins have considerable opportunity to disrupt the functioning of the nervous system (Bondy and Campbell, 2005). Sensory and cognitive deficits, potentially induced by some metals such as lead, manganese, and mercury compounds (Walker et al., 2007), may result in subclinical toxicity. Neurodevelopmental effects are usually recognized in humans after large-scale prospective epidemiological studies. These have then led to the recognition of several industrial chemicals as developmental neurotoxicants: lead, mercury, arsenic, polychlorinated biphenyls, etc. Among pharmaceuticals, the antiepileptic drug valproic acid is recognized as a teratogen; however, concern is growing as to its neurodevelopmental toxicity (Ornoy, 2009).

Humans are also exposed to environmental contaminants that accumulate in food chains. Pesticide residues have been reported in ~50% of fruit, vegetables, and cereals grown in Europe (amounting to about 300 biocides in food products) (Commission of the European Communities, 2007); urine in the majority of the population in the United States (Mage et al., 2004); and human adipose tissue, serum, and placenta in agricultural areas (López-Espinosa et al., 2007). Experimental laboratory studies have led to increasing concern as to whether pesticides currently used can cause neurodevelopmental toxicity (Björting-Poulsen et al., 2008).

Chemicals are tested for developmental neurotoxicity to provide data on neuronal functional deficits and morphological effects on the developing nervous system that result from prenatal or early postnatal exposure in rats (Organisation for Economic Cooperation and Development, 2007). The U.S. National Academies have recommended the use of predictive strategies based on in vitro toxicity assays that predict cellular level effects and can be extrapolated to effects on individuals.

In the present issue of Toxicological Sciences, Hogberg et al. (2009a) present seminal findings that gene expression in an in vitro model of differentiating neural cells could be used as a sensitive tool for the initial identification of developmental neurotoxicants. The selected in vitro model, primary cultures of mixed neuronal-glial cells, was prepared from cerebella from 7-day-old Wistar rat pups. This model has been characterized for crucial stages of neurodevelopment (glial proliferation, neuronal differentiation, and morphological and functional maturation) by measuring the levels of neurotransmitters and of messenger RNA (mRNA) and protein of selected markers after different numbers of days in vitro (Hogberg et al., 2009a,b; Mundy et al., 2008; Sonnewald et al., 2006). In this work, Hogberg et al. (2009a) evaluate gene expression at the mRNA level for the neuronal markers NF-68 and NF-200 (covering the initial neurite outgrowth and the later stages of morphological maturation, respectively), ionotropic glutamate N-methyl D-aspartate 1, and gamma-aminobutyric acid A subunit delta...
receptors (NMDA-R and GABA_A-R: main neuronal excitatory and inhibitory receptors, respectively), and for the glial markers glial fibrillary acidic protein (GFAP) and S100 beta protein. Additionally, the gene expression of markers of neural progenitor cells (Nestin and Sox10) is evaluated. Four neurotoxic chemicals were selected: methylmercury and lead (recognized as developmental neurotoxicants in humans), valproic acid (recognized as teratogenic in humans), and trimethyl tin. Aspirin was used as a negative control that lacks neurotoxic effects. The neuronal cultures were exposed to the chemicals after 1 day in vitro for up to 12 days in vitro and mRNA evaluation took place at 1, 4, 8, and 12 days in vitro. Each chemical was tested at three nontoxic concentrations or at concentrations below concentration producing 20% loss of cell viability (LC_{20}) values based on the Alamar Blue mitochondrial reduction assay. The mRNA levels for the neuronal markers were downregulated after exposure to methylmercury chloride, valproic acid, and trimethyl tin, while expression of the astrocytic markers was unchanged. In contrast, lead chloride decreased the expression of the astrocytic marker GFAP, but only the neuronal marker NMDA-R was altered. In agreement with these results, neurons are more sensitive to methylmercury-induced toxicity and accumulate more methylmercury than glial cells (Kaur et al., 2006), while lead selectively accumulates in glia. Gene expression measurements also detected more specific effects as valproic acid decreased the mRNA level for GABA_A-R much earlier than it decreased that for NMDA-R. This may reflect the mechanism of action of this antiepileptic drug, which modifies neuronal GABA concentrations (Löschler, 1999). GABA_A-R was also more sensitive to the neurotoxic effects of methylmercury and trimethyl tin than NMDA-R; GABA_A-R was affected sooner. The high sensitivity of GABA_A-R with respect to NMDA-R has also been observed in neuronal cultures after short periods of exposure to chemicals (Galofré et al., 2009). Indeed, changes in NMDA-R that are secondary to long-term impairment of GABA_A-R have also been reported in cultured neurons exposed to the pesticide dieldrin (Babot et al., 2007).

As for the nestin gene, it was upregulated by methylmercury chloride, valproic acid, and trimethyl tin but not by lead chloride. Nestin is expressed in cells during development and is replaced by specific intermediate filaments (neurofilaments in neurons and GFAP in astrocytes) as neurodevelopment progresses. Its expression is also reinduced in activated astrocytes after neuronal damage. Counting neurons, astrocytes, and microglia in these cultures suggests that the observed increase in nestin mRNA may reflect increased proliferation of astrocytes and/or reexpression in activated astrocytes. A recent study of the development of an in vitro strategy to predict human acute systemic toxicity found that the genomic biomarkers NF-H, GFAP, MBP, HSP32, and caspase-3 to be very sensitive end points for neurotoxic agents. Additionally, functional assays for GABA_A-R and cell membrane potential improved the in vitro predictability of human acute toxicity (Forsby et al., 2009).

I hope that the work by Hogberg et al. presented in this issue will foster toxicogenomic studies to define a profile of genes that could be used to identify developmental neurotoxicants, through changes in their expression. The use of genomics in toxicology may also take advantage of medium—high-throughput toxicogenomic technologies to simultaneously evaluate changes in biological processes using cells. This would increase our understanding of toxicity pathways. By analyzing changes in the gene expression profile of cells exposed to toxicants, it will be possible to find genes/gene clusters that are commonly or specifically challenged in response to damage associated with developmental neurotoxicants. Finally, cultured cells of human origin should be incorporated into the genomic strategy for developmental neurotoxicity testing. However, such human cells will still require further development before it can be demonstrated that they express the whole array of genes defined.

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REFERENCES


