NeuroToxicology 29 (2008) 546–555

Contents lists available at ScienceDirect

NeuroToxicology

Use of non-mammalian alternative models for neurotoxicological study

Randall T. Peterson [a], Richard Nass [b], Windy A. Boyd [c], Jonathan H. Freedman [c], Ke Dong [d], Toshio Narahashi [e,*]

[a] Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[b] Department of Pharmacology and Toxicology, Center for Environmental Health, Indiana University School of Medicine, Indianapolis, IN 46202-5120, USA
[c] Laboratory of Molecular Toxicology and National Toxicology Program, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, USA
[d] Pesticide Research Center, Michigan State University, East Lansing, MI 48824, USA
[e] Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Received 17 January 2008
Received in revised form 28 March 2008
Accepted 1 April 2008
Available online 25 April 2008

Keywords:
C. elegans
Parkinson's
Zebrafish
Neurotoxicological studies
Insects
Insecticides

ABSTRACT

The field of neurotoxicology needs to satisfy two opposing demands: the testing of a growing list of chemicals, and resource limitations and ethical concerns associated with testing using traditional mammalian species. National and international government agencies have defined a need to reduce, refine or replace mammalian species in toxicological testing with alternative testing methods and non-mammalian models. Toxicological assays using alternative animal models may relieve some of this pressure by allowing testing of more compounds while reducing expense and using fewer mammals. Recent advances in genetic technologies and the strong conservation between human and non-mammalian genomes allow for the dissection of the molecular pathways involved in neurotoxicological responses and neurological diseases using genetically tractable organisms. In this review, applications of four non-mammalian species, zebrafish, cockroach, *Drosophila*, and *Caenorhabditis elegans*, in the investigation of neurotoxicology and neurological diseases are presented.

1. Introduction

There was a time when non-mammals were thought to be far from ideal materials for the study of biomedical sciences because they are phylogenically too distant from humans. However, it has now become abundantly clear that some non-mammals are not only convenient materials but are also endowed with physiological and pharmacological properties common to humans. Thus, several such species have become very popular alternative organisms and are being used extensively as models. Here we would like to present a few such examples: *Drosophila*, *Caenorhabditis elegans*, cockroach, and zebrafish. Each of them is now being used not only for genetics, biochemistry, physiology and pharmacology of the nervous system, but also for neurotoxicology. This article summarizes the symposium on "Use of Non-Mammals for Neurotoxicological Study" which was held as part of the 11th Meeting of the International Neurotoxicological Association in 2007.

Zebrafish are amenable to high-throughput screening in small molecule discovery and cardiac toxicology. Zebrafish small molecule screening takes advantage of the small size, chemical permeability, and optical transparency of the zebrafish embryo. Transgenic lines expressing fluorescent proteins in specific neuronal subpopulations have also been developed, which could facilitate screening. Cardiotoxicity is perhaps the most thoroughly tested zebrafish toxicity to date. Zebrafish screens have also been used to discover novel compounds that suppress the effects of genetic vascular defects.

The nematode *C. elegans*, another useful neurotoxicological model, has been used to study Parkinson's disease and manganism. The nematode's nervous system is highly conserved with mammals, and contains almost all of the known signaling and neurotransmitter systems found in vertebrates. In addition, the means to screen potential neurological and developmental toxicants using *C. elegans* have been developed in a medium-throughput setting. Assays are designed to assess chemical sensitivity to specific endpoints including growth, reproduction, movement, and feeding. Several additional toxicological assays are currently under development including green fluorescent protein-based, stress-responsive transgenic *C. elegans*.

Mammalian Na^+ channels consist of a large pore-forming α-subunit and several small auxiliary β-subunits in various tissues and cell types. In *Drosophila melanogaster*, however, the *para* appears to be the only gene that encodes a functional sodium

* Corresponding author. Tel.: +1 313 503 8284; fax: +1 312 503 5349.
E-mail address: narahashi@northwestern.edu (T. Narahashi).

0161-813X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2008.04.006

channel. Insects employ extensive alternative splicing and RNA editing to generate many functionally diverse sodium channel variants from a single sodium channel gene. Most of these alternative splice sites are conserved in *D. virilis*, the house fly *Vssc1*, and the cockroach *BgNa$_v$*. The cockroach sodium channel gene *BgNa$_v$* undergoes extensive alternative splicing and RNA editing to produce functionally distinct sodium channel variants. Interestingly, variants BgNa$_v$1-1 and BgNa$_v$2-1 showed different sensitivities to pyrethroids. BgNa$_v$2-1 channel variant is 100-fold less sensitive to deltamethrin than the BgNa$_v$1-1 variant. This is the first example of involvement of alternative splicing of a sodium channel gene in differential sensitivity to neurotoxins.

In many cases, insects and mammals have the same type of target site for an insecticide, but with differential sensitivity. It has become increasingly clear that invertebrates including insects and *C. elegans* have inhibitory glutamate-gated chloride channels (GluCls) which are not present in mammals and which are highly sensitive to insecticides. Because of the presence only in invertebrates, GluCls are a potentially important target of insecticides. At least three types of currents were recorded in response to 100 μM glutamate: a fast-desensitizing current, a slow-desensitizing current, and a mixed type of current. Methods have recently been developed for recording them differentially. Slow-desensitizing currents could be inhibited selectively by trypsin, whereas fast-desensitizing currents were blocked selectively by soybean trypsin inhibitor or polyvinylpyrrolidone. The slow-desensitizing GluCls were much more sensitive to the blocking action of fipronil than the fast-desensitizing GluCls with IC_{50}s of 10 nM and 800 nM, respectively. Fipronil is known to be degraded to fipronil sulfone via biotic/abiotic oxidation and to a desulfinyl photoproduct via photolysis. Fipronil sulfone blocked both slow- and fast-desensitizing GluCls, the former being slightly more sensitive than the latter.

2. Potential applications for zebrafish in neurotoxicology (R.T.P.)

The zebrafish, a favorite model organism for developmental geneticists, has been shown to be amenable to high-throughput screening in applications including small molecule discovery and cardiac toxicology (Zon and Peterson, 2005) (Fig. 1). Recent developments in zebrafish small molecule screening were reviewed to explore the possibility that the approach could be applied to neurotoxicity testing.

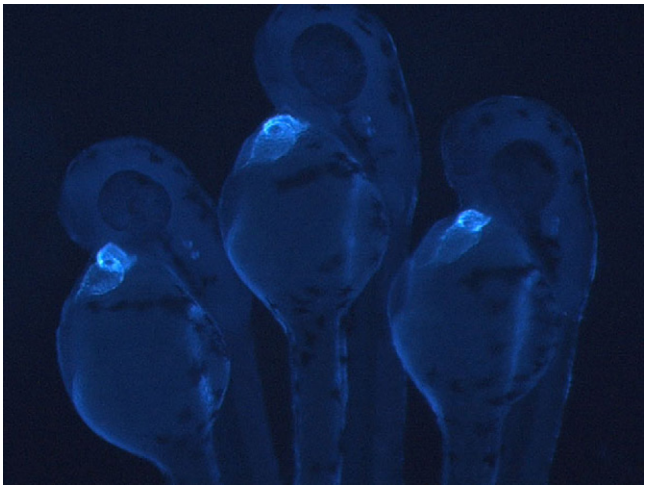

Fig. 1. Transgenic zebrafish expressing a blue fluorescent protein from the cardiac myosin light chain 2 promotor. Image courtesy of Peter Schlueter.

Zebrafish small molecule screening takes advantage of the small size, chemical permeability, and optical transparency of the zebrafish. Embryonic and larval stages of the zebrafish can be grown in 96- or 384-well assay plates, exposed to small molecules by adding the compounds to the water in the wells, and the effects can be observed in the transparent embryos using microscopy. The first small molecule screens performed employed wild-type zebrafish and visual screening to identify obvious morphological defects (Peterson et al., 2000; Sternson et al., 2001; Spring et al., 2002; Shafizadeh et al., 2004; Moon et al., 2002; Khersonsky et al., 2003). These screens identified defects in numerous organ systems including the central nervous system (CNS). Phenotypes identified in this way were generally severe, and for the CNS ranged from loss or expansion of brain ventricles to truncation of the telencephalon to severe neuronal necrosis. While these studies demonstrate the ability of this approach to identify small molecules that cause severe developmental neurotoxicities, it is doubtful that such screens could reliably identify subtle neurotoxicities that are not manifest in obvious morphological changes. More sophisticated assays will likely be necessary. Vital dyes like acridine orange have been reported to stain apoptotic cells in zebrafish and may help detect subtle neurotoxicities (Parng et al., 2004). Transgenic lines expressing fluorescent proteins in specific neuronal subpopulations have also been developed, which could facilitate screening. Numerous functional and behavioral assays, including assays of vision, hearing, touch responsiveness, memory, anxiety, and startle habituation have been developed and could also be useful for identifying neurotoxicants that do not cause obvious developmental phenotypes (Brockerhoff et al., 1995; Bang et al., 2002; Fetcho et al., 1998; Peitsaro et al., 2003). It is possible that a panel of several high-throughput morphological and functional assays could be used to screen broadly for neurotoxicants.

Increasing the number and sophistication of high-throughput neuronal assays for zebrafish will be of little value if zebrafish and human neurotoxicities do not correlate. Much work remains to be done to determine the extent to which zebrafish toxicities are predictive, but initial data from other organ systems are encouraging. Cardiotoxicity is perhaps the most thoroughly tested zebrafish toxicity to date. In an assay for drug-induced bradycardia, 22 of 23 compounds known to cause human QT prolongation were detected among 100 tested compounds, suggesting a high degree of correlation between zebrafish and human cardiotoxicity (Milan et al., 2003). Similar types of studies focused on neurotoxicity would be very useful but have not been reported. However, some individual compounds have been reported to have predictable neurotoxicities in zebrafish, including ethanol, 6-hydroxydopamine, acrylamide, MPTP, and pentylenetetrazole (Parng et al., 2007; McKinley et al., 2005; Baraban et al., 2005).

Beyond screening for neurotoxicants, might the zebrafish high-throughput platform be useful for identifying neuroprotectants? Zebrafish screens have been used to discover novel compounds that suppress the effects of a genetic vascular defect (Peterson et al., 2004; Hong et al., 2006). Similar screens have discovered a small molecule that suppresses the effects of a mutation that causes a cell cycle defect in zebrafish (Stern et al., 2005). This approach could be applied to neuroprotection by exposing thousands of zebrafish en masse to a neurotoxicant, then screening in high-throughput for novel small molecules that block the neurotoxic effects of the toxicant. As preliminary evidence that such an approach may be feasible, several known neuroprotectants have been shown to protect zebrafish from L-hydroxyglutaric acid neurotoxicity (Parng et al., 2006), and in a separate study, l-deprenyl and nomifensine were shown to protect zebrafish from MPTP-induced neurotoxicity (McKinley et al., 2005).