Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Toxicology and Pharmacology 21 (2006) 153–167
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP
www.elsevier.com/locate/etap

The value of alternative testing for neurotoxicity in the context of regulatory needs

Sandra Coecke*, Chantra Eskes, Joanne Gartlon, Agnieszka Kinsner, Anna Price, Erwin van Vliet, Pilar Prieto, Monica Boveri, Susanne Bremer, Sarah Adler, Cristian Pellizzer, Albrecht Wendel, Thomas Hartung

European Centre for the Validation of Alternative Methods (ECVAM), Institute for Health & Consumer Protection, European Commission Joint Research Centre, Ispra (VA), Italy

Available online 24 August 2005

Abstract

Detection and characterisation of chemical-induced toxic effects in the central and peripheral nervous system represent a major challenge for employing newly developed technologies in the field of neurotoxicology. Precise cellular predictive test batteries for chemical-induced neurotoxicity are increasingly important for regulatory decision making, but also the most efficient way to keep costs and time of testing within a reasonable margin. Current in vivo test methods are based on behavioural and sensory perturbations coupled with routine histopathological investigations. In spite of the empirical usefulness of these tests, they are not always sensitive enough and often, they do not provide information that facilitates a detailed understanding of potential mechanisms of toxicity, thus enabling predictions. In general, such in vivo tests are unsuitable for screening large number of agents. One way to meet the need for more powerful and comprehensive tests via an extended scientific basis is to study neurotoxicity in specific cell types of the brain and to derive generalised mechanisms of action of the toxicants from such series of experiments. Additionally, toxicokinetic models are to be developed in order to give a rough account for the whole absorption, distribution, metabolism, excretion (ADME) process including the blood–brain barrier (BBB). Therefore, an intensive search for the development of alternative methods using animal and human-based in vitro and in silico models for neurotoxic hazard assessment is appropriate. In particular, neurotoxicology represents one of the major challenges to the development of in vitro systems, as it has to account also for heterogeneous cell interactions of the brain which require new biochemical, biotechnological and electrophysiological profiling methods for reliable alternative ways with a high throughput.

Keywords: Neurotoxicity; In vitro; Toxicokinetics; Regulatory toxicology

1. Introduction

Any modification to the structure and/or function of the nervous system (brain, spinal cord, body function controlling nerves) following exposure to a chemical, biological or physical agent can be considered as a neurotoxic insult. Functional changes due to neurotoxicity may result from exposures to industrial chemicals, cosmetic ingredients, pharmaceuticals, foods, food additives and naturally occurring substances.

Prediction of neurotoxic effects is a key feature in the toxicological profile of compounds and therefore required by many regulatory testing schemes (Crofton et al., 2004). Neurotoxicity can occur following both acute and chronic exposures to either neuronal or glial cell types. Acute toxic effects, can be observed even after a single exposure to a compound, and can be observed immediately or delayed. Chronic neurotoxic effects can be observed due to repeated, low-dose exposures.

The onset of neurotoxic effects can already occur in the developmental phase as during neurodevelopment, the blood–brain barrier is incomplete, and detoxification mechanisms are not as efficient as in the adult brain. This allows toxic effects to occur in foetuses and children that are not observed to the same extent in adults (Mayer, 2000; Costa et al., 2004; Tsuji et al., 2004; Wormley et al., 2004). Similarly, age-related variations in neurotoxic effects are also reported for elderly people who are prone to reduced defence mechanisms that decrease overall efficiency of organ function and leads to increased susceptibility to toxic substances (Hedlund et al., 2001; Thiruchelvam et al., 2003).

* Corresponding author. Tel.: +39 0332 78 98 06; fax: +39 0332 78 62 97.
E-mail address: sandra.coecke@jrc.it (S. Coecke).

1382-6689/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.etap.2005.07.006

Table 1
Current OECD/EC guidelines related to the assessment of neurotoxic effects

Initial testing for determining neurotoxic potential
Acute dermal toxicity [OECD 402, updated guideline, adopted 24 February 1987; EC B.3]
Acute inhalation toxicity [OECD 403, original guideline, adopted 12 May 1981; EC B.2]
Repeated-dose 28-day oral toxicity study in rodents [OECD 407, updated guideline, adopted 27 July 1995; EC B.7]
Repeated-dose 90-day oral toxicity study in rodents [OECD 408, updated guideline, adopted 21 September 1998; EC B.26]
Repeated-dose 90-day oral toxicity study in non-rodents [OECD 409, updated guideline, adopted 21 September 1998; EC B.27]
Repeated-dose dermal toxicity: 21/28-day study [OECD 410, original guideline, adopted 12 May 1981; EC B.9]
Subchronic dermal toxicity: 90-day study [OECD 411, original guideline, adopted 12 May 1981; EC B.28]
Repeated-dose inhalation toxicity: 28- or 14-day study (OECD 412, original guideline, adopted 12 May 1981; EC B.8)
Subchronic inhalation toxicity: 90-day study [OECD 413, original guideline, adopted 12 May 1981, EC B.29]
Prenatal developmental toxicity study [OECD 414, updated guideline, adopted 22 January 2001; EC B.31]
Acute oral toxicity—fixed dose procedure [OECD 420, updated guideline, adopted 20 December 2001; EC B.1bis]
Reproduction/developmental toxicity screening test [OECD 421, original guideline, adopted 27 July 1995]
Combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422, original guideline, adopted 22 March 1996]
Acute oral toxicity—acute toxic class method [OECD 423, updated guideline, adopted 20 December 2001; EC B.1] tris]
Acute oral toxicity: up-and-down procedure [OECD 425, updated guideline, adopted 20 December 2001]
Acute inhalation toxicity-fixed dose procedure [OECD 433, draft revised guideline, June 2004]
Acute dermal toxicity-fixed dose procedure [OECD 434, draft new guideline, May 2004]
Acute inhalation toxicity—acute toxic class (ATC) method [OECD 436, draft proposal new guideline, December 2004]
Carcinogenicity studies [OECD 451, original guideline, adopted 12 May 1981; EC B.32]
Chronic toxicity studies [OECD 452, original guideline, adopted 12 May 1981; EC B.30]
Combined chronic toxicity/carcinogenicity studies [OECD 453, original guideline, adopted 12 May 1981; EC B.33]
Specific guidelines for neurotoxicity testing
Neurotoxicity study in rodents [OECD 424, original guideline, adopted 21 July 1997; EC B.43]
Delayed neurotoxicity of organophosphorus substances following acute exposure [OECD 418, updated guideline, adopted 27 July 1995; EC B.37]
Delayed neurotoxicity of organophosphorus substances: 28-day repeated-dose study [OECD 419, updated guideline, adopted 27 July 1995; EC B.38]
Developmental neurotoxicity study [OECD 426, draft new guideline, September 2003]

Conventional toxicological animal-based tests adopt a holistic approach for hazard and risk assessment of chemical substances. Hazard identification uses compound knowledge to predict potential neurotoxic effects (Blaauboer et al., 1998). Neurotoxicity can occur directly due to toxicity to neurons and also indirectly via toxicity to surrounding cells, e.g. glia. Such indirect secondary events include impact on cell proliferation, triggering of the inflammatory cascade, activation or sensitisation to apoptotic signals, and enhanced susceptibility to endogenous (e.g. cytokines), exogenous (e.g. endotoxins) and immunological stimuli (Philbert et al., 2000; Jean Harry et al., 2003; Campbell et al., 2004; Tabakman et al., 2004). Risk assessment takes into account the probability of a substance to cause damage under relevant use conditions including, dose–response studies and also the assessment of exposures to compound mixtures. The toxic effects of the infinite number of different chemical mixtures and possible exposures are difficult to predict using experimental models, especially with variations occurring due to genetic polymorphism.

In vivo neurotoxicity methods listed in the OECD and EC test guidelines (see Table 1) are both expensive and time consuming. Implementation of alternative tests in screening strategies to assess neurotoxic effects of compounds will accelerate the rate at which compound knowledge and mechanistic data are produced.

Current in vitro models range in complexity from simple cell lines to complex brain reaggregate cultures which retain the three-dimensional structure of the respective tissue found in vivo. Some of these models represent future candidate test systems for inclusion in new testing strategies to fulfil regulatory needs. However, before embarking on their routine use for the current testing needs, all these new methods need to be validated according to general accepted validation principles (Hartung et al., 2005; OECD, 2004a).

2. Neurotoxicity testing as part of regulatory requirements

Prediction of neurotoxicity in humans is most commonly measured by relatively non-invasive neurophysiological and neurobehavioural methods that assess cognitive, sensory and motor functions (Anger, 2003). Neurotoxicological adverse drug reactions are described as being the second most frequent cause for withdrawal of pharmaceuticals. The implementation of a first tier for screening of adverse effects via in vitro methods is seen as being of prime importance in the assessment of adverse drug effects, on the one hand in order accelerate the development, and on the other hand, to improve safety. Both issues are central to keep the balance of costs for innovative approaches within a reasonable margin.

On 29 October 2003, the European Commission published its proposal for the regulation concerning registration, evaluation and authorisation of chemicals (REACH) (European Commission, 2003). This proposal addresses the lack of publicly available data on chemicals, one of the key issues for chemicals in Europe. It outlines a plan to test approximately 30,000 'existing substances' at an estimated cost of €1561 million (range €1180–2423 million, Pedersen et al., 2003). These chemicals are currently produced in volumes greater than 1 tonne/year and for which some essential human health and ecotoxicological data