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Neurotoxicology: From the Whole Animal to Molecules

Introduction

Neurotoxicity induced by a chemical in vivo is usually produced when the target site within the nervous system is exposed to a sufficient amount of the chemical or its toxic metabolite(s) for a duration of time that is sufficient to result in biological changes. These may be seen as changes in behavior, neurochemistry, neurophysiology, or neuropathology.

There are several special considerations that should be given to the assessment of adverse effects on the nervous system, compared with other body organs or systems. First, the nervous system controls all physiological functions of the body, including those of the cardiovascular, digestive, and respiratory systems. Second, neurons in the brain are formed before birth and cannot be regenerated, as can other cellular components. Third, the interconnections among neurons within a nervous pathway or among different pathways and other organs are very complex and sophisticated. All these contribute to the difficulties in predicting the nature of neurotoxicity that may be induced by a neurotoxicant and in delineating the site and mechanisms of action of a neurotoxicant.

Neurotoxicants are agents that produce neurotoxicity by direct actions on the structure or function of the central nervous system or peripheral nervous system, or both. A chemical that exerts an indirect consequence similar to neurological changes secondary to the effect induced by acting on other organs should not be considered a neurotoxicant. For example, carcinogens, such as polycyclic aromatic hydrocarbons, aflatoxins, and dimethyl nitrosamine, induce cancers that could make patients exhibit some degree of behavorial abnormality because of severe sickness. However, these compounds themselves are not considered to be neurotoxicants.

Environmental chemicals, such as organophosphatases and carbamate pesticides, alcohols, lead, and mercury, are known to directly affect the structure or function of the nervous system. However, the effects of neurotoxic agents are not specific only to the nervous system and, depending on the extent of exposure, neurotoxicants can also affect other organs or systems. Even for neurotoxicants with relatively well-known mechanisms of action, there is still a lack of specificity. For example, cholinesterase-inhibiting pesticides are often known to produce other biological responses [7]. The complexity of the nervous system, the many potential endpoints that may be affected, the sensitivity of some neurological endpoints, the difficulties in detecting them methodologically, and the potential for masking such effects by other toxicological responses, all make the identification of neurotoxicity of chemicals a complicated task.

Sites of Action by Neurotoxicants

One major site of action of neurotoxicants is the synapse [4, 8]. The neurotoxic chemicals can act on presynaptic sites of neurotransmission, e.g. sites for synthesis of neurotransmitter, storage, release, reuptake, autoreceptors, and metabolism. They could also act on postsynaptic sites of the neurotransmission, e.g. receptors for the neurotransmitter, its degradation, signal transduction and pulse conduction. Any of these sites affected by a neurotoxicant could potentially result in neurotoxicity. With the complexity of the nervous systems, it is often difficult

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to pinpoint where is the exact site of action of a specific neurotoxicant. Neurotoxicity induced by neurotoxicants can be by direct or indirect actions on the nervous systems. For example, the CNS is protected by the blood-brain barrier (BBB), formed by endothelial cells surrounding capillaries that supply the brain and interact with astrocytes. The BBB allows only certain smaller molecular-sized substances, such as certain nutrients, amino acids, hormones, fatty acids, peptides, and carbohydrates that require active transport systems to reach the brain [22]. Lipophilic molecules may enter the brain by simple diffusion. However, not all the areas of the brain are equally protected by the BBB. Certain regions, such as the postrema area and circumventricular area, lack the protection of the BBB. Furthermore, in the young, the BBB is not well developed. Chemicals that affect the BBB can lead to neurotoxicity by itself or to other substances as a result of entry to the brain.

Factors that Influence Neurotoxicity

Numerous factors are known to influence the severity of neurotoxicity in different subjects. For a neurotoxicant, the physiochemical property, form of preparation, dose and concentration, exposure frequency, route of administration, and metabolic rate of the agent would significantly affect the degree of neurotoxicity after the subjects have been exposed to the agent. Variations in biological systems, such as age, sex, genetics, state of health, and nutritional or dietary factors, are also important parameters. Environmental factors, such as physical location and temperature also deserve consideration. Examples of neurotoxicity affected by various factors can be found in several excellent references [6, 7, 11, 12].

Acute and Chronic Exposures to Neurotoxicants

Acute exposures to neurotoxicants are usually related to accidental or intentional exposures. Symptoms of acute neurotoxicity are usually more obvious and easier to be detected. However, slowly developing neurotoxicity for chronic exposures to low doses or subtoxic doses of neurotoxicants is difficult to detect. Chronic exposure is a long-term process that can also be complicated by numerous factors. Symptoms induced by a neurotoxicant following acute or chronic exposure are often not identical. For example, the neurotoxicity of organophosphorous cholinesterase inhibitors is due to their irreversible inhibition of acetylcholinesterase. These chemicals produce acute symptoms such as anxiety, restlessness, insomnia, confusion, slurred speech, ataxia, tremor, and convulsion. However, these symptoms can disappear, even when the cholinesterase activities have not appreciably recovered. The lack of correlation between inhibition of acetylcholinesterase and signs of neurotoxicity of exposure to toxic organophosphorus compounds [10, 13] inevitably has led to the conclusion that other systems must also be involved in the toxic manifestations evoked by exposure to these compounds.

Assessment of Neurotoxicity

Neurotoxicity induced by neurotoxicants, regardless of the sites of action (i.e. central nervous system or peripheral nervous system; CNS or PNS), direct or indirect actions on the nervous system, and specificity of action on target sites, can be detected in terms of changes in four areas: behavior, neurochemistry, neuropathology, and neurophysiology. These four different disciplines are described in the following.

Behavioral Sciences

Behavioral changes following acute or chronic exposure to neurotoxicants are sensitive and rapid indices of neurotoxicity [1, 5, 9, 18, 20, 23, 26–28]. A series of tests has been widely used by neurotoxicologists for screening of neurotoxicity. The methods designed for neurobehavioral testing are based on changes in motor function, sensory function, reactivity, learning and memory, and naturally occurring behaviors [9, 27].

Neurochemistry

Most of the chemicals that produce neurotoxicity act on the biochemical processes of the CNS and PNS, either through a general action or by specific mechanism at the molecular or cellular level. Although the biochemical mechanisms of most known neurotoxicants are not well understood, certain agents have been relatively extensively studied. One of the best examples is a group of chemicals commonly referred to as organophosphorus cholinesterase inhibitors. The well-known organophosphorus insecticides, such as parathion, chlorpyrifos, diazinon, disulfoton, malathion, phorate, and terbufos, are some of the examples. The major action of these insecticides is their potent irreversible inhibitory action of acetylcholinesterase (AChE) and other esterases. Some of the organophosphorus cholinesterase inhibitors also produce delayed neurotoxicity called organophosphate-induced de-

layed neuropathy (OPIDN) [14–17, 19, 24]. The target site for these compounds to induce OPIDN is a membrane-bound nerve cell protein called neurotoxic esterase or neuropathy target enzyme (NTE). The characteristics of OPIDN are a dying back of long myelinated nerve axons, especially in the sciatic nerve and within the spinal cord. Some organophosphorus compounds (e.g. tri-*o*-cresyl phosphate; TOCP) that are not insecticides are also potent inhibitors of NTE.

Neurophysiology

Measuring changes in neurophysiology using the electrophysiological approach is one of several means to study neurotoxicity. Electrical signals that are generated by nerve and muscle cells are associated with ionic fluxes across the cell membranes. A variety of neurotoxins excite these cells. This excitation occurs by changing membrane potential caused by membrane permeability changes to different cations such as Na^+, K^+, and Ca^{2+}. Marine neurotoxins, such as tetrodotoxin and saxitoxin, have been well demonstrated to block Na^+ channels [2, 21, 25]. Brevetoxins, toxins isolated from *Ptychodiscus brevis*, which depolarize nerve and muscle membrane, also act on Na^+ channels as their target site [29]. Isolated rat phrenic nerve hemidiaphragm, the frog sciatic nerve and satorius muscle, the crayfish neuromuscular junctions, and electroplax of the electric eel are generally used for electrophysiological investigations of neurotoxicants.

Neuropathology

Neuropathological investigation is one of the essential aspects of neurotoxicology. The objectives of neuropathology are to furnish information on the topography or location of the lesions and to define the nature and characteristics of the damage of the nervous systems caused by neurotoxicants [3]. The observation in neuropathological findings may provide valuable correlation with the results obtained from behavioral, neurochemical, and electrophysiological studies. For example, OPIDN is often seen in neuropathological changes in the sciatic, peroneal, and tibial nerves. The pathological findings can be correlated with the inhibition of NTE and neurological symptoms such as ataxia and paralysis.

Goals for Investigation in Neurotoxicology

With the increasing knowledge of neurotoxicology and the need to evaluate the public health significance of the presence of chemicals in our environment, it is essential to investigate the potential neurotoxicity that may be induced by these chemicals. The goals for the study of chemically induced neurotoxicities include the following:

(a) Identification of toxicants that are potentially neurotoxicants.

(b) Detection of the nature of neurotoxicity induced by neurotoxicants.

(c) Determination of specific mechanisms of action involved.

(d) Correlation of neurotoxicity with possible mechanisms of action.

(e) Development of predictive testing techniques.

(f) Design of suitable regimens for the prevention and treatment of neurotoxicity potentially induced by neurotoxicants.

(g) Development of a reliable database for risk assessment and risk management.

Future Perspectives

Further advances in elucidating the mechanisms by which neurotoxic chemicals exert their effects on the nervous systems should be emphasized. Information obtained from investigations can lead to the development of sensitive, reliable and simple detecting methods, e.g. in vitro testing, for potential neurotoxicants. The problems associated with chemical-drug or chemical-chemical interactions which may exaggerate or potentiate neurotoxicity, and the potential hazards induced by low-dose chronic exposure, should also be emphasized. For better evaluation of neurotoxicity of drugs or chemicals, several points are listed below which deserve closer attention:

(a) Investigation on the mechanisms of action of neurotoxic agents.

(b) Investigation on multiple systems involved in the actions of neurotoxic chemicals.

(c) Alteration in susceptibility after repeated exposure to neurotoxic chemicals.

(d) Investigation of drug interactions, chemical-drug or chemical-chemical interactions.

(e) Development of in vitro systems for neurotoxicity evaluation.

(f) Employment of techniques in molecular biology for the study of molecular mechanisms of neurotoxicity induced by drugs and chemicals.

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