BIOMARKERS OF CERTAIN ENVIRONMENTAL NEUROTOXICANTS: AN OVERVIEW

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ABSTRACT: Environmental pollution led to the presence of various toxicants in the air, water, soil, food, etc. The resulting exposure of the population seldom causes overt symptoms so that the need for prevention of long-term damages is not always obvious. There are several groups of pollutants, which, like certain pesticides and heavy metals, attack the nervous system, thereby endangering the most valuable human resource. The effects of external agents on an organism, organ, or biological function can, theoretically, be detected by means of biomarkers, measurable parameters indicating internal exposure by a substance, its effect on organs or functions, or the susceptibility of the organism in question against the substance. In this paper, existing and proposed chemical-biochemical markers of certain neurotoxic pollutants are reviewed and the possibilities of developing neuro-functional markers discussed.

KEY WORDS: Biomarker, neurotoxicity, xenobiotic, insecticide, heavy metal

MANKIND AND THE ENVIRONMENT

Endeavors to alter the given natural environment have always been characteristic of humans. One can even say that civilization commenced, in a material sense, when people first managed to transform stones and pieces of wood or bone to primitive tools and were able to build some kind of shelter. The goal has been, all the time, to achieve better conditions of life (at least secure survival). The result was, and is – however, ambivalent – simply because humans, in their striving to become less dependent on the harsh laws of nature, did do a lot of environmental damage. Burning a forest to chase the game into a trap or to gain arable land easily got out of control; heating and lighting huts and caves with open fire resulted in massive "indoor" air pollution; and the mere number of human individuals, living in close vicinity in primitive settlements, increased the risk of transmissible infections. So, the interaction of human populations and the environment has more facets than in case of any other creature.

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Considering only those cases where human activity results in appearance of a chemical substance in the environment, we have to distinguish between unwanted emission and purposeful application. The former is equal to pollution of the air, water, soil, etc., including the deposition of waste, which is – or seems to be – inevitable at a given level of technology. Chemicals applied on some element of the environment with a known purpose include, first of all, the agrochemicals: pesticides, fertilizers, etc. Depending on the substance in question, and the self-purification capacity of the environmental medium into which it was emitted, chemicals have a higher or lower chance to be neutralized or decomposed, resulting in low or high persistence (Mellanby, 1992).

BIOMARKERS: CONCEPT AND HISTORY

According to a current definition, biomarkers are "measurements that indicate exposure to a chemical, the effect of such exposure, or susceptibility to effect (usually toxic) of such an exposure" (Hayes, 2001). Three types of biomarkers can be distinguished: i/ biomarkers of exposure – presence of an exogenous compound (or its metabolite or adduct) in the organism; ii/ biomarkers of effect – alterations in metabolite levels, presence of abnormal metabolites, altered functional (biochemical, neural etc,) capacities; and iii/ biomarkers of susceptibility. The latter indicate the actual or expectable reaction of the organism to an external chemical insult, determined by inherited and acquired factors (Grandjean et al., 1994).

The concept of biomarkers originated from clinical chemistry, with the primary goal of better describing the relation between exposure and disease (Silbergeld and Davis, 1994). Exposure biomarkers may give a more accurate estimation of the internal dose than environmental samples, questionnaires on consumption habits, etc. Any measurement of exposure is based on the internal dose determined in a biological sample. In case of the central nervous system (CNS) however, it is impossible to obtain human samples. In case of certain important neurotoxicants (e.g., organophosphates, see below) the relationship of chemical forms and effect is too complicated (Silbergeld, 1993). Hence, biomarkers of effect may be more practical in detecting the damage induced by environmental chemicals in the central and peripheral nervous system.

Nearly all existing effect biomarkers are of biochemical nature, for historical reasons and because the methods applied are well known and mostly at hand. But, even if a neurotoxicant has a known peripheral effect, like that of cholinesterase inhibitors on the cholinesterase activity of blood, this effect does not necessarily reflect what is going on in the CNS (Manzo et al., 1996). This gradually raised the interest in biomarkers based on functions of the nervous system, a promising field with limited practical outcome up to now.

PESTICIDES

Cultivating plants and breeding animals mean a deliberate deviation from the natural state of the ecosystem in concern (Yassi et al., 2001). Farmlands are ecosystems under external control where the high abundance of the cultivated species and the low occurrence of any unwanted organisms is kept up by human activity. Eliminating unwanted organisms (animals or plants) by causing fatal biochemical disturbance in them with external substances – simply, by poisoning them – has proven an efficient means of control. Most of the known pesticides have, however, insufficient target specificity: they are toxic not only to insects, microfungi, or weeds, but also to other organisms including humans. Although some fungi- and herbicide agents also turned out to be poisonous for humans, typically the pesticide group with the highest human health risk are the insecticides.

Modern insecticides, in contrast to, e.g., arsenic used in the past, are no systemic poisons. Their target within the arthropod organism is the nervous system. And, as the difference in the biochemistry of the nervous system of complex invertebrates (insects, mites, etc.) and higher order mammals (including humans) is strikingly little, any substance with high insect-killing potency bears some human health risk.

Organophosphates (OPs; organic derivatives of phosphoric or thiophosphoric acid; WHO, 1986) have been used as pesticides since the 50s and are important means of insect control even today. Thanks to their low persistence, OPs are not very ecotoxic. Their human toxicity is, however, rather high, resulting in occupational health risk during production, formulation, application, etc., and population-level indirect risk due to residues potentially present in food and drinking water.

Exposure biomarkers of OPs can be the agent molecules or their metabolites in a suitable biological sample. Such analyses are, however, of limited use, because *i/* the blood, and even more the urine, level of an OP may be very different from its level in the CNS (Manzo et al., 1996); *ii/* the unmetabolized, metabolically activated, and broken-down fractions of an OP are in an equilibrium with complex and inadequately known kinetics (Silbergeld, 1993); and *iii/* several OPs and metabolites can have the same physiological effect yet they are determined as separate chemical entities.

Biomarkers of effect can be more adequate in case of neurotoxic substances (Manzo et al., 1995; Silbergeld and Davis, 1994). For cholinesterase inhibitors including OPs, the standard test is measurement of acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE) activity in blood samples. Here it is assumed that biochemical events in the periphery give a sufficiently good mirror image of what is happening in the CNS. Neuropathy target esterase (NTE) for example, was detected in lymphocytes (Lotti, 1987). In all such "surrogate" biomarkers (Manzo et al., 1996), a crucial question is the existence of a direct relationship to CNS effects of the substances to be detected. In several cases, this criterion was not met. Dési et al. (1991) observed that acute effect of dimethoate, a widely used OP (WHO, 1989b), on cortical evoked potentials was not influenced by atropine. In an other experiment (Papp et al., 1996), the effects of dichlorvos (WHO, 1989a) on the cortical and the hippocampal activity (where the latter was known to be under cholinergic

modulation; Rovira et al., 1983) were of opposite direction. And it was seen both in animals (Dési, 1983; Gralewicz et al., 1991) and humans (Savage et al, 1988; Rosenstock et al., 1991) that neurological effects of OP exposure were of significantly longer duration than inhibition of blood or brain cholinesterase.

All that would suggest that measurement of the central or peripheral nervous activity itself would give better biomarkers of OP effect. The results up to now have been, however, ambiguous. Past and recent OP exposure of sprayers was in correlation with the reported neurological symptoms (dizziness, headache, etc.) but not with the measured vibration sense (London et al., 1998). In another study, OP exposure by the residues on cultivated plants did not alter electrophysiological indices of peripheral nerve activity (Engel et al., 1998). Muttray et al. (1996), on the contrary, found quantifiable EEG alterations after spraying an OP – at a dose, which left cholinesterase activity unchanged. Results of animal experiments (Dési and Nagymajtényi, 1999; Papp et al., 2001) also suggest that electrophysiological parameters have the potency of being effect biomarkers of OPs.

The PON1 gene seems to be a good predictor of individual OP sensitivity (Costa et al., 2003). The gene product, an enzyme, was named "paraoxonase" but its natural substrates are oxidized blood lipids.

HEAVY METALS

The presence of toxic heavy metals in the environment is largely due to human activity. Lead, the toxicity of which was first observed over two thousand years ago (Eaton and Robertson, 1994), can cause occupational and/or environmental exposure via air, drinking water, and food. Pb levels in blood and urine are good biomarkers of current or recent exposure while Pb deposited in bones indicates lifetime exposure (ATSDR, 1999a).

Pb intoxication has several known neurological sequelae (WHO, 1977). In lead smelter workers, parameters of the brainstem auditory evoked potential (BAEP) were altered in correlation with current and life-time Pb dose (Bleecker et al., 2003). In another study (Schwartz et al., 2001) blood Pb, but not tibial bone Pb, proved to be a good predictor of decreased performance in the WHO Neurobehavioral Core Test Battery. In a group of Chinese workers exposed to Pb, increased postural sway was in correlation with blood Pb while the parameters of BAEP were not (Yokoyama et al., 2002). These results indicate that reasonably chosen neurological and behavioral tests can be utilized as effect biomarkers of Pb (Lucchini et al., 2000), although in a meta-analysis Goodman et al. (2002) stressed the need for further prospective studies, performed to uniform standards, in order to obtain reliable markers. The relationship of childhood lead burden (e.g., Pb in dentine) and poor school performance was described (Needleman et al., 1990; Fergusson et al., 1997) but this was more the final outcome itself than its indicator.

Mercury in the environment has also a few natural sources but the levels inducing health risk are always man-made (ATSDR, 1999b). Hg also belongs to those heavy metals the neurotoxicity of which has been known for a long time. Hg can

have various chemical forms whereby inorganic and organic mercurials show different environmental behavior and toxic effects (WHO, 1990, 1991). Recent exposure to metallic and inorganic Hg can be reliably tested in urine samples while blood Hg level indicates exposure to organic Hg. For past exposures to methyl-Hg, hair samples can be used (ATSDR, 1999b).

BAEPs, as well as somatosensory and visual evoked potentials, of chloralkali workers exposed to Hg vapors were shown to be altered already where the Hg load of the body was still subclinical (Chang et al., 1995). This utilizable sensitivity of the somatosensory evoked potentials to Hg was previously also reported by Lamm and Pratt (1985), and that of BAEPs, by Discalzi et al. (1993).

Most of the cases of large-scale population exposure have been caused by food-borne methyl-Hg. The emitted form of Hg is usually inorganic, converted to methyl-Hg and other alkylated forms by environmental microorganisms (WHO, 1989c). In the Amazon basin, low-tech gold mining resulted in massive elementary Hg emission, leading to increased methyl-Hg levels of locally obtained food, mainly fish. Lebel et al. (1996) described decreased color discrimination, contrast sensitivity and peripheral visual field in exposed individuals at hair Hg level below the total hair Hg level given as limit of toxicity (WHO, 1990). In the same population, psychomotor motor deficits and reduced grip strength were observed (Dolbec et al., 2000).

CONCLUSION: BIOMARKERS IN MONITORING

To obtain an ongoing picture of health effects of environmental origin, some kind of monitoring is needed. With environmental monitoring, the external exposure is followed up, and compared to a threshold limit value (TLV). TLVs are determined on the basis of the final biological outcome (ACGIH, 1996) but cannot account for inter-individual differences (indicated by, e.g., biomarkers of susceptibility relating to metabolic capacities). Internal exposure (e.g., heavy metal levels in blood, bone, hairs etc.) is a better measure of the actual health risk, and is also a field of application of exposure biomarkers. The first effect within the organism, like reaction with a biomolecule (enzyme, channel protein, etc.) is determined by the exposure and the susceptibility (both within the scope of biomarkers) and can be a measure of the effective dose (blockage of cholinesterases by OPs or delta-aminolevulinic acid dehydrase by Pb). The final effect (CNS disorder due to cholinergic overweight, Pb-induced peripheral neuropathy) could also be followed up by appropriately chosen biomarkers (*Fig. 1*).

Use of existing biomarkers and development of new ones can largely contribute to improvements in environmental health. In case of the nervous system, functional (physiological) markers constitute a promising field of applied research.

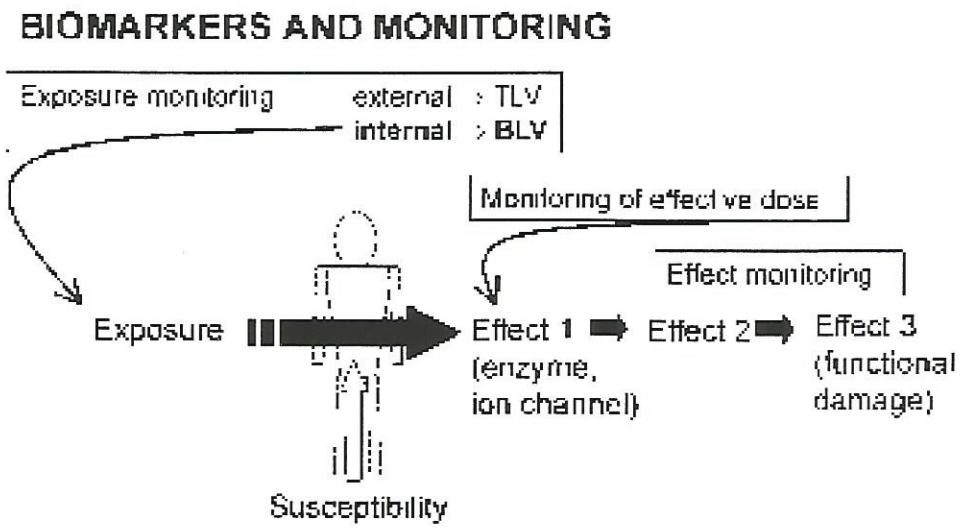

Fig. 1. Possible roles of biomarkers in the monitoring of environmental exposures. TLV: threshold limit value; BLV: biological limit value

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