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Chronic effects of low level exposure to anticholinesterases — a mechanistic review

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Abstract

High dose exposure to anticholinesterases which results in symptomatic poisoning can have lasting consequences due to the trauma of intoxication, excitotoxicity, secondary hypoxic damage, and (for some agents) a delayed onset polyneuropathy (OPIDN). The potential effects of low level exposure are less well defined. The most reliable data comes from controlled clinical trials with specific agents. A single dose of sarin or repeated doses of metrifonate or mevinphos, have produced only transient adverse effects at doses causing substantial acetylcholinesterase inhibition. Other data comes from epidemiological surveys. These have often used more sensitive indices than the clinical studies, but are less reliable due to the difficulty of defining exposure and matching control and exposed populations. Subtle, mainly cognitive, differences between exposed and non-exposed populations are sometimes seen. Low level exposure can cause a reversible down-regulation of cholinergic systems, and a range of non-cholinesterase effects that are structure-specific, and do not always parallel acute toxicity. Novel protein targets sensitive to low level exposure to some organophosphates are known to exist in the brain, but their functional significance is not yet understood.

Keywords: Organophosphorus pesticides; Acetylcholinesterase inhibitors; Cognitive function

1. Introduction

A number of reviewers (Eyer, 1995; Steenland, 1996; Jamal, 1997) have discussed the possibility that low-level (i.e. acutely asymptomatic) exposure to cholinesterase inhibitors may lead to adverse health effects, but have reached disparate conclusions. This review is an attempt to re-evaluate published studies in a mechanistic context. An extended version of this review is available (IEH, 1998).

The major cholinesterase inhibitors to which humans are exposed are organophosphorus and carbamate esters used as pesticides (Marrs, 1993; Richardson, 1995) or medicines, plus some either as industrial chemicals or contaminants. Other organophosphorus esters have been used as warfare agents (Marrs et al., 1996). Most of these esters are effective covalent inhibitors of acetyl-

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cholinesterase, and their acute toxicity is dominated by the pharmacological consequences of accumulation of excess acetylcholine in the brain, autonomic system, and neuromuscular junction. These acute effects are reasonably well understood, both mechanistically and in terms of risk management. They can be effectively monitored by clinical signs, and inhibition of red cell acetylcholinesterase provides a sensitive and specific index of exposure.

The more difficult question of possible adverse consequences of low level exposure will be addressed in three ways: by downward extrapolation from higher dose exposures; by direct studies of low dose exposures; and from mechanistic knowledge.

2. Effects of high dose exposures

Much of the public concern about long-term and low level toxicity from anticholinesterases is a result of the known potential of these agents to cause acute toxicity, and of some to cause delayed neuropathy (OPIDN). However, an important caution when extrapolating down to threshold level toxicity is that although high dose poisoning is unambiguous, lesser cholinergic signs, such as headache, memory problems, gastrointestinal disturbance and anxiety are also seen in other unrelated conditions heavily influenced by psychosocial pressures (Spurgeon et al., 1996), and cease to be specific in cases where exposure is poorly defined.

Acute intoxication with anticholinesterases produces a complex mixture of muscarinic and nicotinic signs which vary in relative severity with target organ tissue, dose, and agent (Marrs, 1993). An 'intermediate syndrome' has also been described as a late complication of some cases of severe acute poisoning (Senanayake and Karalliede, 1987). It is characterized by a specifically proximal muscle weakness lasting 5–18 days which can lead to respiratory failure. A further effect produced by some, but not all, organophosphorus esters is organophosphorus ester-induced delayed polyneuropathy (OPIDPN). This is characterized by a delayed onset 10–14 days after exposure (Richardson, 1995). All commercial agents to which man might be exposed produce OPIDPN only after a cholinergic crisis. The lesion is a highly selective distal axonopathy affecting the distal axons of sensory, motor, and autonomic neurons, and the longer central tracts. The lower motor neuron axonopathy is reversible, but not that of the upper motor neuron (Vasilesceu and Floresceu, 1980).

Within the central nervous system excitotoxicity has been described as a consequence of frank poisoning. Pathogenesis probably involves a combination of hypoxia and cholinergically mediated excitotoxicity with secondary recruitment of glutamatergic excitotoxicity (Solberg and Belkin, 1997). This is a high dose effect, which has only been produced by exposures sufficient to produce clear signs of poisoning. In a series of suicidal poisonings with organophosphorus pesticides (Wadia et al., 1974), of 21 surviving cases with neurological deficits, none had signs that persisted beyond 3 days. A larger study of 639 cases of acute sarin poisoning as a result of the terrorist attack in Tokyo, four of 111 cases of severe or moderate poisoning showed a (possibly non-specific) post-traumatic stress disorder 3 months after poisoning (Okumara et al., 1993).

Rosenstock et al. (1991) examined cases of accidental pesticide poisoning 10–34 months after intoxication. Unfortunately it was not possible to measure pre-morbid ability and so their lower dexterity, attention, and visual motor skills may have pre-dated the poisoning; although the similar vocabulary scores and incidence of minor pesticide-related illness in the poisoned and control groups would argue against this. More recently larger studies have shown similar effects in formerly poisoned workers (Savage et al., 1988; Steenland et al., 1994), some of which could be correlated with the severity of the original poisoning. In contrast, most clinical studies show a general lack of persisting effect, even at high doses. Sustained clinical use of metrifonate causing substantial cholinesterase inhibition has produced few if any adverse effects (Aden Abdi et al., 1990; Becker et al., 1996). The differing long-term consequences reported in the epidemiological studies are probably the result of clinical investigators avoiding traumatic dose levels, and also of some

of the epidemiological studies generating artifactual effects due to poor population matching.

3. Effects of low dose exposures

Studies divide into two groups: those where effects were measured in the absence or in the presence of current low dose exposure.

Of studies where assessment was not complicated by effects of current exposure, a major cross-sectional epidemiological study (Stephens et al., 1995; Beach et al., 1996) compared sheep dippers who regularly used organophosphates, but had never been poisoned, with quarry workers. Syntactic reasoning was slower in the exposed group, and this difference could be related to cumulative exposure. Subjective memory and simple reaction time showed no differences, but symbol-digit substitution performance and general psychological health were worse, although these could not be related to exposure. Other co-variables (such as responses to economic pressure or social isolation) may have had a causal role in these latter outcomes. Sprayers (Fiedler et al., 1997) who had used organophosphorus pesticides for over 27 years without poisoning showed no difference in a number of tests of concentration, visual-motor function, verbal and visual memory and verbal function. Simple reaction time was 11% slower in the exposed group, but the effect could not be linked to an exposure estimate. It was clear that no gross cognitive effect was seen in this group of moderately exposed workers, but the low participation rate in both this and the previous study means that these results should be interpreted with caution. A careful neuropsychological study of orchard sprayers with 7 years exposure to azinphos-methyl at a level sufficient to produce a small number of acute poisonings within the sample (Daniell et al., 1992), found no differences which could be attributed to occupation or correlated with pesticide exposure measures, although the relatively small sample size would have limited the power of the study to detect minor decrements. Finally, a study of accidental occupational exposure to the warfare agent sarin (Duffy et al., 1979) found eight of the 151 EEG indices to be affected. It is not clear if this represented a direct action on the brain, or a consequence of anxiety.

Studies where previous and current exposure could not be distinguished are less useful. One of farm and public health workers exposed to organophosphorus and carbamate pesticides over an average of 5 years (Ramos et al., 1986) found a reduced score on a test of manual dexterity which could be correlated with years of employment. The exposed workers had no psychological or neurological problems. In contrast, a neuropsychological and EEG study of a poorly defined population (Metcalfe and Holmes, 1969) found increased reported forgetfulness and difficulty in thinking, as well as other effects probably related to current exposure. A linked EEG investigation found more slow activity (an effect completely different to that seen with sarin (Duffy et al., 1979)). Unfortunately only general conclusions were reported in an anecdotal manner. In a later study, occupational pesticide exposure was associated with differences in EEG spectrum and neuropsychological performance (Korsak and Sato, 1977), but there was no correction of data for social status or educational attainment. A psychological study of farm workers and sprayers (Levin et al., 1976) showed exposure-related increases in work-related tension, sleep disturbance, restlessness, and nervousness — but not depression. Farmers and sprayers (Rodnitzky et al., 1975) who had recently used organophosphorus pesticides showed unchanged verbal recall, vigilance, complex reaction time, linguistic performance and proprioception. Agricultural workers with subsymptomatic organophosphorus pesticide poisoning over the previous 5 years showed a better serial digit test score, but no other differences (Ames et al., 1995). It is interesting to note that these same measures were, however, changed after more severe poisoning (Savage et al., 1988).

Other studies have been able to separate the effect of acute exposure from any chronic effect: thus groups of mixers, loaders and sprayers showed an exposure-dependent decrement in neuropsychological tests during, but not after exposure (Durham et al., 1965). A study which was

made of workers using diazinon showed a small but significant decrease in performance after work (Maizlish et al., 1987), but again gave no evidence for a persisting effect. Farmers and residents exposed to organophosphorus pesticides showed a post-season performance similar to that of the non-exposed group (Richter et al., 1992), and significantly better scores for anger and hostility. However, during exposure, scores in all but two tests were significantly reduced.

A geographically-based study (Stoller et al., 1965) found that schizophrenia was more common in areas where organophosphorus pesticide sales were higher, but no individual case could be linked to pesticide use. Depression was reported in a survey of greenhouse workers currently exposed to moderate (and occasionally high) levels of organophosphates, and other pesticides (Parrón et al., 1996), and a high suicide rate was also reported in this area. Local reports of mood disorders were of a higher incidence, but unfortunately the suicide rates were not age adjusted, nor were they broken down by occupational group, so it is not clear if pesticide exposure (high or low level), intensive work, or ready availability of pesticides for suicide was the cause of the increased suicide rate.

When the many low exposure studies are summarized, a number of cautions mentioned by individual authors need to be borne in mind. The main difficulty is that exposures are variable and poorly categorized. In many cases exposure was inextricably associated with hard work, and in only a few studies could this factor be controlled (Stephens et al., 1995). In other cases exposure was associated with apprehension and fear (Maizlish et al., 1987). In several studies there is good reason to believe that some individual exposures may have been higher than 'low level'. Of the better designed epidemiological studies, those of Maizlish et al. (1987) and Richter et al. (1992) showed only acute (on exposure) effects; that of Stephens et al. (1995) only a small (but significant) effect; that of Fiedler et al. (1997) mostly negative effects, and that of Ames et al. (1995) showed no effect. Where positive effects were seen, these were only large enough to be subjectively apparent in the cases of current exposure.

4. Toxic mechanisms

Any persisting effects of exposures that were high enough to cause frank poisoning but not high enough to cause effects mediated by trauma, excitotoxicity or hypoxia, might involve a lasting down regulation of receptors secondary to cholinesterase inhibition. In addition there is good (in vitro) evidence of a non-anticholinesterase mediated action of some organophosphorus pesticides and carbamates on muscarinic, nicotinic, glutamatergic and GABAergic synapses (Camara et al., 1997). However, the relevance of these findings to the clinical or even the experimental animal situation is far from clear, especially since methamidophos, which does not act directly on receptors, still produces the full range of acute, intermediate, and delayed polyneuropathic syndromes in man (Camara et al., 1997).

It is most unlikely that the cholinergic syndrome would in itself be of direct significance in low level chronic exposure, since there is good evidence that tolerance can develop to the acute cholinergic effects of cholinesterase inhibitors (Schwab and Murphy, 1981; Hudson et al., 1986; Richardson, 1995). This leads to diminishing cholinergic signs during continued exposure, due to a combination of a reactive down-regulation of cholinergic receptors and functional adaptation to increased cholinergic tone. The intermediate syndrome may relate, at least in part, to muscle end plate necrosis secondary to excitotoxicity and calcium overload. There is good evidence that anticholinesterase agents, and carbamates in particular, can produce reversible damage to the neuromuscular junction at doses lower than those associated with severe toxicity (Hudson et al., 1986), and this may contribute to the mild motor dysfunction occasionally described clinically (Kaplan et al., 1993). Some reviewers have not distinguished clearly between such effects and classical OPIDPN (Jamal, 1997). Any analogous central excitotoxicity would be expected to prove less readily reversible, although the brain should also be less vulnerable since most synapses do not have the very high safety factor for transmission seen at the end plate, and inhibitory systems would balance hyper-excitation. There is however,

little evidence to suggest that this adaptation process can occur at sub-symptomatic dose levels.

For peripheral neuropathy, the primary biological target (for OPIDPN) is a protein known as neuropathy target esterase (NTE). This is a protein which, within the nervous system, is specifically localized to neurons (Glynn et al., 1998). Its biological function, other than as a target for neuropathic organophosphates, is unknown but NTE mutations are known to affect brain development in *Drosophila* (Lush et al., 1998). Many studies have shown that the nature of the toxicity (i.e. acute cholinergic or OPIDPN) produced by an agent can be predicted by its relative ability to interact either with acetylcholinesterase (AChE) or with NTE (Lotti and Johnson, 1978). Neuropathic potential is determined by this specific interaction with NTE plus general pharmacokinetic factors and the capacity for re-growth and repair. A number of agents which require a high dose to react with NTE have produced OPIDPN in persons who have been enabled to survive severe cholinergic intoxication by intensive care therapy. No current pesticides produce OPIDPN at dose levels which fail to produce severe acute cholinergic signs and, where less severe OPIDPN has been produced at threshold doses, it has proved to be reversible.

A mechanistic explanation of possible longer-term neurobehavioural consequences, either of mild poisoning or of low level exposures, is largely lacking at present, but several partially characterized brain proteins (Richards et al., 1998) show covalent binding with organophosphates at such doses. When compared with the known primary target in brain, AChE, some of these proteins show a k_a 3.7 times higher using diazoxon, and six times higher using dichlorvos. They were however, unaffected by two other potent cholinesterase inhibitors. Such interactions do not necessarily indicate an adverse effect, but one of the proteins is an *N*-acyl peptide hydrolase, which is inhibitable in vivo by a dose of dichlorvos low enough to cause no cholinergic signs. This may be linked to the cognitive enhancement seen in rats at low doses of metrifonate, an effect which may be unrelated to AChE inhibition (van der Staay et al., 1996). Cognitive enhancement appears to be the lowest dose effect conclusively demonstrable for some cholinesterase inhibitors on the brain.

5. Conclusions

There is good evidence that cholinesterase inhibitors cause three syndromes of poisoning in man and in experimental animals: acute pharmacological toxicity; intermediate syndrome; and delayed polyneuropathy. Acute poisoning is reasonably well defined in terms of nature and threshold, although low level effects shade into the range of non-specific symptoms. In man there is reasonable evidence for persisting after-effects of severe intoxication, but it is not yet clear if this is a specific effect or a general consequence of intoxication trauma. Intermediate syndrome is only seen as a complication of severe intoxication, and delayed neuropathy is only produced after severe intoxication with a limited range of agents.

There is some evidence from neuropsychological and electromyographic tests for a small short-term (up to 1 month or so) consequence of just acutely asymptomatic exposures to organophosphorus pesticides. As regards longer lasting effects, some well designed studies have failed to show any, and others have found subtle changes. Unfortunately difficulty in quantifying low levels of exposure limits the power of such studies, but the lack of lasting effects in studies where positive but reversible effects have been found is the main reason for doubting reports of long-term effects. Clinical studies suggest that therapeutic uses of specific cholinesterase inhibitors are without long-term hazard. There are however, protein targets present in brain which are known to be very sensitive to some anticholinesterases which may represent a target for low-level effects (either adverse or beneficial). The functions of these protein targets are not yet known.

Concerns about major adverse health effects of low level exposure to anticholinesterases in general seem entirely unwarranted on the basis of currently available data, but this is at present insufficient to rule out the possibility of subtle, agent-specific effects.

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