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TOXICOKINETICS AND METABOLISM

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Organophosphate neuropathy due to methamidophos: biochemical and neurophysiological markers

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Abstract Neuropathy target esterase (NTE), the putative target enzyme for organophosphate induced delayed polyneuropathy (OPIDP), can be measured in lymphocytes but has rarely been assessed in acute human poisoning. Serum autoantibodies to nervous system proteins develop in hens poisoned with neuropathic insecticides and also have not been studied after human poisoning. Serial lymphocyte NTE (LNTE) was measured in a 16-year-old boy after acute poisoning with methamidophos for evaluation as a predictor of subsequent neuropathy. The profiles of serum autoantibodies to neurofilament triplet proteins, myelin basic protein, and glial fibrillary acidic protein were measured in order to characterize changes occurring as a result of OPIDP. Clinical neuropathy characterized by steppage gate and profound lower extremity weakness, decreased grip and pinch strength, and decreased ulnar and absent tibial compound muscle action potentials developed 2 weeks following poisoning. Sensory examination and nerve conduction studies were normal. On day 3 following poisoning LNTE was depressed (77% compared with subsequent baseline enzyme activity). Marked increases in serum immunoglobulin G (IgG) autoantibodies to glial fibrillary acidic protein and to neurofilament 200 were observed after the development of OPIDP. We conclude that inhibition of lymphocyte NTE is predictive of subsequent OPIDP. Serum autoantibody titers to nervous system proteins may be useful markers of neuropathy.

Key words Methamidophos · Peripheral nervous system diseases · Neurofilament proteins · Glial fibrillary acidic protein · Lymphocytes

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Introduction

Organophosphate insecticides, which are widely used in agriculture throughout the world, are a frequent cause of acute poisoning as a result of inhibition of neuronyl acetylcholinesterase (Gallo and Lawryk 1991; Wesseling et al. 1997). Some organophosphorus compounds can cause an organophosphate induced delayed polyneuropathy (OPIDP). These include chlorpyrifos, dichlorvos, O-ethyl O-4-nitrophenyl phenylphosphorothioate (EPN), leptophos, methamidophos, mipafox, omethoate, parathion, triortho-cresyl phosphate (TOCP), trichlorforn, and trichlornat (Lotti 1992). Typically, neuropathy appears two or more weeks following acute poisoning, usually after recovery and discharge from hospital, and is characterized pathologically by a dying-back ax-

onopathy. In recent years the insecticide methamidophos (O,S-dimethyl phosphorothioamidate) has been the most frequently reported cause of OPIDP (Senanayake and Johnson 1982; Zheng 1990; McConnell et al. 1994).

Although the mechanism for OPIDP has not been clearly elucidated, a putative target enzyme, neuropathy target esterase (NTE), may be involved in the development of this neuropathy (Lotti 1992). A threshold inhibition and aging of 70–80% of enzyme activity in brain is necessary to produce neuropathy in experimental hens (Johnson 1982). Some higher O-alkyl homologues of methamidophos readily cause OPIDP; however, methamidophos causes neuropathic inhibition of NTE in hen brain and the subsequent development of OPIDP in atropine protected survivors only at doses which are eight times the unprotected 50% lethal dose (LD_{50}) resulting from acetylcholinesterase inhibition (Johnson et al. 1991). The relative inhibition of acetylcholinesterase and of NTE by the two stereoisomers of methamidophos (which is a racemic mixture) and the role of each isomer in the development of neuropathy is an area of active research (Lotti et al. 1995; Johnson et al. 1989, 1991; Vilanova et al. 1987).

NTE activity may be measured in circulating lymphocytes and platelets, as well as in the central and peripheral nerves (Maroni and Bleecker 1986). Therefore, it has been suggested (Lotti et al. 1984) and there is limited human evidence (Lotti et al. 1986; Moretto et al. 1994) that lymphocyte NTE (LNTE) activity may predict the development of OPIDP. The threshold inhibition of enzyme activity (in brain or in lymphocytes) associated with neuropathy in humans is not known.

Autoantibodies to neurofilament triplet proteins (NFs), which derive primarily from the axon, to glial fibrillary acidic protein (GFAP) in astrocytes, and to myelin basic protein (MBP) from the myelin sheath have been detected in serum of hens which developed neuropathy after a single dose of phenyl saligenin phosphate, a congener of TOCP (El Fawal et al. 1994). This assay, an enzyme-linked immunosorbent assay (ELISA), capitalizes on the 'immunoprivileged' status of the nervous system provided by the blood-brain and blood-nerve barriers. Cytoskeletal elements (e.g. NFs, GFAP) released from injured cells are perceived as foreign by the immune system, producing antibodies to these autoantigens.

The following case report provides further evidence that LNTE inhibition predicts organophosphate induced delayed polyneuropathy in patients poisoned with methamidophos. It is the first time that serum autoantibodies to nervous system tissue have been examined as markers of neuropathic effect in a patient who subsequently developed OPIDP.

Materials and methods

A patient poisoned with methamidophos was evaluated. Nerve conduction studies of ulnar sensory and sural nerves using orthodromic stimulation and of ulnar and tibial motor nerves were conducted using a TD 20 Mk1 (Teca Instruments, Pleasantville, Calif.) and standard techniques (Sethi and Thompson 1989). Quantitative vibrotactile threshold was measured using a factory calibrated Vibratron II (Sensortek, Clinton, N.J.), and upper extremity grip and pinch strength were assessed using a Jamar dynanometer (Asimow Engineering, Los Angeles, Calif.) and a pinch gauge (B and L Engineering, Santa Fe Springs, Calif.), according to standard protocols (Gerr et al. 1990; Mathiowetz et al. 1985) . Twenty-five milliliters of blood were obtained by venipuncture, heparinized, and transported in plastic tubes at room temperature for analysis within 90 min of LNTE according to the method described by Maroni and Bleecker (1986). Erythrocyte and plasma cholinesterase were measured using a modified Ellman assay (McConnell et al. 1992). Serum from 5 ml of blood was frozen at −70 °C and subsequently thawed for measurement of autoantibodies to neurofilament triplet proteins (NF 68, NF 160, and NF 200), and to GFAP and MBP, as described previously (El-Fawal et al. 1996). Gas chromatographic analysis with nitrogen/phosphorus specific detection identified a sample of the insecticide concentrate provided by the patient's family to be methamidophos.

Results

A 16-year-old hispanic male was brought to hospital shortly after intentionally ingesting approximately 100 ml of an unidentified insecticide. On admission he was reported to be unconscious, tachypneic, and to have diffuse rhonchi, miosis, and prominent fasciculations of pectoral and abdominal musculature, a clinical presentation consistent with poisoning with a cholinesterase inhibiting organophosphate insecticide. Fluid from gastric lavage reportedly smelled of pesticide. The patient regained consciousness and his lung fields cleared during the administration of 18 mg of intravenous atropine sulfate over the first 1.5 h after admission. He was treated for recurrent sialorrhea, blurred vision, generalized weakness, fasciculations, nausea and vomiting with a total of 91 mg of intravenous atropine sulfate and 1 gram of pralidoxime during his 7-day hospitalization.

The patient was discharged 8 days after admission. At that time, neurological examination was normal. However, pinch strength was reduced (Table 1), reflecting

Table 1 Vibrotactile threshold, pinch and grip strength studies before and after development of clinical neuropathy

	Number of days after poisoning			Normal range
	7	24	52	
Vibrotactile threshold[a]				
Right index finger	0.29	0.18	−0.19	<0.81
Right first toe	1.85	0.71	−0.16	<1.31
Key pinch strength[b]	14	7	9	>21 lbs
Palmar pinch strength[b]	18	9	7	>18 lbs
Grip strength[b]	64	52	52	>91 lbs

[a] Results are in log microns of peak vibration amplitude. Normal values are based on 90% confidence intervals derived from Gerr (1990)

[b] Normal values are based on 90% confidence intervals derived from Mathiowetz et al. (1985)

either residual cholinergic depolarization blockade at the motor endplate as a result of acute poisoning and cholinesterase inhibition, or early neuropathy. Quantitative vibrotactile threshold was mildly elevated in the lower extremity. Approximately 5 days later, the patient began to experience pain in the gluteal region, thighs and calves. One day later he first noticed weakness of his lower extremities and had difficulty walking, then weakness of grip. On examination he had bilateral foot drop and a steppage gait, weakness of wrist extension and flexion and of abductor pollicis brevis, and there was marked weakness of the interossei of the hands. He had weakness, more marked distally, of iliopsoas, quadriceps, and extensor and flexor muscles of the feet. He was unable to dorsiflex his feet against gravity, to stand on his toes or heels, or to do deep knee bends without assistance. Deep tendon reflexes were normal in the upper extremities. The knee reflex was hyperactive without clonus, and the ankle reflex was absent. The sensory examination was normal. Objective measurements of grip and pinch strength 7 weeks after poisoning demonstrated considerable deterioration from the discharge examination (Table 1). Quantitative vibrotactile threshold on subsequent measurements was normal. Nerve conduction studies demonstrated reduced amplitude of ulnar nerve distal compound muscle action potential, and tibial motor nerve responses could not be elicited (Table 2). Sensory nerve conduction studies were normal.

Cholinesterase activity approximately 24 h after ingestion, but before treatment with the cholinesterase reactivator, pralidoxime, was low in erythrocytes and in plasma (Table 3), a characteristic finding during acute poisoning. Activity of LNTE, measured approximately 3.5 days following poisoning, also was low. LNTE was within the normal range, but erythrocyte cholinesterase activity was still mildly depressed 7 weeks after poisoning (Table 3). Autoantibody isotypes immunoglobulin M (IgM) and IgG against the NF triplet proteins, GFAP (IgG only), and MBP were detected in the serum collected 3.5 days after poisoning and 52 days after poisoning, when neuropathy was well established. There was a marked increase in IgG to GFAP and to NF 200 during this period. There was a moderate decrease in IgM to GFAP and of both isotypes to NF 68.

Table 3 Laboratory studies before and after development of clinical neuropathy (*NTE* Neuropathy target esterase · *NF* 68 Neurofilament 68 · *GFAP* glial fibrillary acidic protein · *MBP* myelin basic protein)

	Number of days after poisoning			Normal range
	1	3	52	
Erythrocyte cholinesterase	2.7		24.2	>26 IU/g hemoglobin[a]
Plasma cholinesterase	0.4		2.6	>1.13 IU/ml[a]
Lymphocyte NTE		3.1	13.3	>9.1 nmol/min per mg protein[b]
Autoantibodies (ng/ml serum)[c]				
NF 68				
IgM		253	160	
IgG		206	112	
NF 160				
IgM		0.12	0.13	
IgG		0.58	0.61	
NF 200				
IgM		0.48	1.00	
IgG		0.31	13.29	
GFAP				
IgM		100	34	
IgG		1.21	13	
MBP				
IgM		0.93	2.13	
IgG		0.00	0.46	

[a] Measured using Testmate OP field kit (EQM Research, Cincinnati, Ohio); normal values are derived from 90% confidence interval for unexposed local population
[b] Normal values are based on 90% confidence interval derived by Maroni and Bleecker (1986)
[c] Normally not present in serum

Discussion

Clinical features of this case and the severe cholinesterase inhibition requiring treatment with atropine, and the subsequent development of a motor neuropathy, were compatible with acute poisoning due to ingestion of methamidophos, which was identified by analysis of a sample of the pesticide ingested. LNTE and serum autoantibodies to nervous system proteins are potentially useful early markers of disease. In hens 70–80% inhibition of brain NTE activity is necessary for neuropathy to result (Johnson 1982). In this case early measurement of LNTE, which was 77% inhibited compared with a later baseline measurement, was predictive of subsequent neuropathy. These results also are consistent with a preliminary report of the one other case of methamidophos neuropathy for whom LNTE was measured (and found to be >90% inhibited) after acute poisoning (Moretto et al. 1994). In that report LNTE continued to be inhibited 4 days after poisoning but had recovered by

Table 2 Nerve conduction studies 52 days after acute poisoning (NCV nerve conduction velocity). Values *in parentheses* indicate the normal range

	Distal latency in ms (normal)	Distal amplitude in μV (normal)	Proximal amplitude in μV (normal)	NCV in m/s (normal)
Nerve:				
Ulnar motor	2.60 (<3.1)	3222 (>5000)	2927 (≥5000)	61.0 (≥50)
Tibial motor	Absent			
Ulnar sensorial	1.40 (≤3.2)	25 (≥10)	–	67.4 (≥54)
Sural	1.80 (≤4.2)	19 (≥4)	–	75.0 (≥42)

9 days. Human LNTE data also were available for a single case of chlorpyrifos neuropathy, for whom a prolonged cholinergic syndrome continued for 17 days, and LNTE was 60% inhibited at the time of first measurement 30 days after poisoning (Lotti et al. 1986). In a fatal poisoning with chlorpyrifos and other pesticides, LNTE inhibition correlated with peripheral nerve NTE (Osterloh et al. 1983).

In the hen model the inhibition of LNTE is similar to that for the biologically relevant NTE in the brain 24 h after dosing with TOCP, leptophos, or di-*n*-butyl diclorvos. However, by 48 h LNTE inhibition was much less than, and poorly correlated with, inhibition of brain NTE (Schwab and Richardson 1986). Therefore, for LNTE to be useful clinically, further experimental work is required to identify differences (possibly pesticide specific) in the correlation of LNTE with NTE in brain at different times after poisoning. In addition, serial measurements of LNTE in patients poisoned with neuropathic insecticides would help elucidate the time for reactivation of LNTE in humans, which also may be pesticide specific, and the threshold inhibition required to produce neuropathy. Although mild or subclinical chronic neuropathy may be common among workers previously poisoned with methamidophos (McConnell et al. 1994), screening of LNTE in workers exposed to methamidophos (but not poisoned) is unlikely to be useful in predicting neuropathy. Cholinesterase is so much more sensitive to methamidophos than is LNTE that asymptomatic workers are unlikely to have significant inhibition of LNTE (Johnson et al. 1991).

The presence of autoantibodies to NFs, GFAP, and MBP is consistent with controlled studies in hens exposed to OPIDP-inducing phenyl saligenin phosphate (El-Fawal et al. 1994). The presence of IgM autoantibodies to GFAP in serum early after poisoning and the subsequent decline in IgM and increase in IgG autoantibodies between the first and second examinations is consistent with the pattern which would be expected after an acute central nervous system insult. This isotype switching between the two autoantibody examinations is suggestive of a T lymphocyte dependent immune response (Roitt 1987). GFAP is the major intermediate filament of astrocytes, with very little expressed normally in Schwann cells (Mancardi et al. 1991). GFAP was the only antigen studied in this individual, which is not also present in the peripheral nervous system, and the pattern of response is consistent with central nervous system involvement in OPIDP. Because normally there are no circulating antibodies to nervous system proteins and the development of IgG is a late response to antigen exposure, the early high titer of IgG autoantibodies to GFAP suggests a prior history of central nervous system injury. This patient reported a prior history of poisoning (not requiring medical attention), which was compatible with cholinesterase inhibiting pesticides associated in previous studies with neurobehavioral deficits (Rosenstock et al. 1991).

The pattern of other observed autoantibody response to poisoning is less clear. In the case of NF 68, which is present both in the central and peripheral nervous system, there were declines in IgM and IgG titers. A decline in IgM after antigen challenge normally occurs as IgG becomes the predominant isotype. The decline in anti-NF 68 IgG may indicate that autoantibodies, if present, were primarily directed against phosphorylated proteins or that the binding of additionally released autoantigen with circulating immunoglobulins was masking their detection (Tizard 1992; Cheng 1998). Numerous experimental studies have demonstrated that there is rapid and persistent phosphorylation of nervous system cytoskeletal proteins after single exposures to organophosphates, but assays for autoantibodies to phosphorylated proteins are not available (Lapadula et al. 1991, 1992). The predominance of anti-NF 68 titers (compared to low titers to MBP, for example) is consistent with the axonopathic pathophysiology of OPIDP and with the larger quantities in the axon of NF 68 than of the other neurofilament triplet proteins. The presence of IgG autoantibodies to nervous system structural proteins (NF 68, for example) early following exposure also may indicate some prior nervous system insult, as the development of autoantibodies is not specific to organophosphate induced injury (El-Fawal 1996). Myelin degeneration may have occurred secondary to axonal injury (Davis and Richardson 1980).

Methamidophos is an insecticide in widespread use in agriculture, both in the developed and in developing countries. Substituting other insecticides which do not cause neuropathy could prevent cases such as that reported in this paper. In the meantime, clinicians should be alert to the possibility of neuropathy following acute cholinergic poisoning with methamidophos. A recent study in hens demonstrated that treatment with calcium channel blockers prior to or immediately after dosing with neuropathic organophosphates may prevent the development of neuropathy (El Fawal et al. 1990). The potential for preventive treatment adds urgency to the development of a better understanding of the pathophysiology of this disease, including the validation in clinical series of the time course of LNTE recovery and the identification of the threshold inhibition of LNTE for the development of OPIDP in humans. Further study of serum autoantibodies to nervous system tissue in the epidemiological and clinical evaluation of patients with exposures to organophosphates is necessary to determine whether there are consistent patterns of antibody response associated with OPIDP.

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