SHORT REPORT

Pathophysiological studies of neuromuscular function in subacute organophosphate poisoning induced by phosmet

J L Good, R K Khurana, R F Mayer, W M Cintra, E X Albuquerque

Abstract
A 51 year old man developed progressive cranial and proximal muscle weakness, hyperreflexia and mental change. The disorder progressed over 9 days following the fifth weekly spraying with the organophosphate (OP) insecticide, phosmet, with limited symptoms of acute toxicity. Marked decremental responses of 50–80% on slow and fast rates of stimulation were improved to 15% by edrophonium or neostigmine. Intracellular recordings at the endplate region of intercostal muscle revealed small miniature endplate potentials (mepps), reduced mean acetylcholine sensitivity and normal membrane potentials. Electron microscopy revealed degeneration and regeneration of the endplates. This study demonstrates that OP poisoning due to phosmet can produce a subacute postsynaptic neuromuscular syndrome without marked symptoms of acute toxicity.

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Organophosphate (OP) insecticide poisoning in humans can produce: 1) acute peripheral and central cholinergic block; 2) an intermediate syndrome with weakness, and 3) a delayed distal polyneuropathy. The acute syndrome that occurs 12 to 72 hours after exposure to OP involves inhibition of acetylcholinesterase (AChE) by the OP and direct actions at the synapse involving a variety of chemosensitive receptor ion channels. Central and peripheral cholinergic signs occur. The neuromuscular block to repetitive nerve stimulation shows decremental responses that are increased by edrophonium. The delayed neuropathic syndrome occurs 1–3 weeks after exposure to the OP and probably results from inhibition of the neurotoxic esterase. A distal axonopathy usually occurs with sensory and motor signs. More recently, an intermediate syndrome has been described in OP poisoning that occurs 1–4 days after the acute poisoning. The patients reported developed progressive weakness that was often severe and involved cranial, respiratory and proximal limb muscles which persisted for up to 18 days. Three of the patients had signs suggesting CNS dysfunction. Electromyographic studies suggested a neuromuscular junction (NMJ) defect of the postsynaptic type, but the exact mechanism of the dysfunction was not determined.

We describe the pathophysiological changes in a patient who presented with a subacute progressive neuromuscular syndrome without marked symptoms of acute toxicity after multiple OP insecticide sprayings. The defect in this neuromuscular syndrome occurred at the endplate-receptor sites, is similar to the intermediate syndrome previously reported, and differs from the acute and delayed syndromes. These results are important in the diagnosis and management of patients exposed to organophosphates. A preliminary report of this study has been published in abstract form.

Case report
A 51 year old man was admitted to the hospital 5 days after the onset of diplopia, light headedness and a staggering gait. The patient developed these symptoms 18 hours after the fifth weekly exposure to a liquid spray insecticide containing an organophosphate compound, phosmet, (phosphorodiothioic acid S-[(1,3-di-hydro-1,3-dioxo-2H-isouindol-2-y) methyl],0,0-dimethylester). He described his face and hands becoming wet from the insecticide and he did not bathe until the following morning. Over the next few days, he noted progressive unsteadiness of gait, dysphagia, change in voice tone and excessive diaphoresis. Five days after exposure, he noted jaw weakness and droopy eyelids.

The patient’s general examination was normal. Profuse oral secretions, bilateral ptosis, and marked facial diaphoresis were noted. He was alert, mentation was normal, and speech was dysarthric. Extraocular movements were limited in all directions with the exception of downgaze. Pupils were 3 mm and reactive to light. There was moderate symmetrical weakness of facial, jaw, tongue and neck muscles. There was mild weakness of distal muscles and marked weakness of proximal muscles, especially antigravity muscles that fatigued. The tendon jerks were brisk throughout. Bilateral Babinski responses were present. Sensory examination was normal.

The patient was admitted with the presumed
diagnosis of acute OP poisoning. Atropine (2 mg) and pralidoxime chloride (2-PAM, 1 gm) infusions were started. Within several minutes of 2-PAM infusion, the patient had increased systemic and ventilatory weakness requiring intubation. After the patient was stabilised on a ventilator, 3 additional doses of 2-PAM were given without side effects over the next 24 hours. Atropine (1·0 mg initially and thereafter 0·5 mg) was continued 4 times daily for 6 days. Paralysis was maximum on day 9. At this time, he was observed to have visual hallucinations, disorientation, and myoclonic jerks of his extremities. These signs persisted for approximately 10 days during which time the EEC showed diffuse slow wave activity.

The patient remained unchanged for the following 18 days, and supportive care continued. By day 28, there was improvement in strength of neck flexion, shoulder and anti-gravity muscles. By day 44, he no longer required mechanical ventilation or tube feedings. Babinski responses were absent. Examination on day 64 (time of the muscle biopsy) showed that he had mild fatiguable proximal and antigravity muscle weakness in the upper and lower limbs. Facial and oropharyngeal movements were near normal. There was mild subjective diplopia on extreme lateral gaze. Neuromuscular examination was normal by 5 months.

Laboratory studies revealed normal routine blood and urine studies, creatine kinase, EKG and chest radiographs. MRI of the brain was normal. CSF was normal except for a total protein of 75 mg%. On admission red blood cell cholinesterase level was within normal limits (6110 U/L); and follow up levels on days 16 and 23 were 5420 and 4180 respectively and at 10 months (5114 U/L) remained normal (normal range 6666-3590). Studies to rule out other causes of neuromuscular disease were normal. ANA and acetylcholine receptor antibody titres were negative.

Methods

Nerve conduction and repetitive stimulation-neuromuscular studies were performed in peripheral nerves of upper and lower extremities using established techniques.\(^\text{14}^\)\(^\text{15}\) Recordings were performed in the intensive care unit and care was taken to keep the patient warm (rectal temperature of 37°C) during the procedure. Recordings were obtained at 9 to 95 days after exposure to phosmet.

Repetitive stimulation was performed at rates of 2, 5, 10, 20, and 50 Hz for 9 responses in the median and peroneal nerves. Recordings of the median nerve were made before and after 10 mg of intravenous edrophonium at days 11 to 47. Recordings were made 5 to 40 minutes after 1·5 mg of intramuscular neostigmine on day 12. Measurements of the motor amplitudes were peak-to-peak and percentage change (decrement) was calculated for the fourth and ninth responses compared with the initial one.

Concentric needle and single fibre (SF EMG) electromyography and fibre densities were performed in the extensor digitorum communis and first dorsal interosseus muscles using established techniques.\(^\text{14}\) SF EMG recordings were obtained when the patient was improving and able to cooperate, 3–4 months after exposure.

A diagnostic intercostal muscle biopsy was obtained 2 months after exposure because of persistent muscle weakness with informed consent by the patient. Muscle was removed from the right sixth intercostal space in the anterior axillary line using standardised anaesthesia and surgical technique. The muscle was immediately placed in oxygenated Krebs-Ringer solution and prepared for intracellular recordings and ultrastructure as previously described.\(^\text{15}\) Conventional microelectrode techniques were used for recording from human intercostal muscle. High resistance pipettes (approximately 150 MΩ) filled with 2·5 M acetylcholine (ACh) were used for microiontophoretic application of ACh at the neuromuscular junctions. Results were compared statistically (Student t test) with control data that we have obtained from normal adult human intercostal muscle biopsied during thoracotomies for cardiopulmonary disease. The muscle fibres were fixed in ice-cold phosphate buffered 2·5% glutaraldehyde for thin sectioning and electron microscopic examination of the endplate regions.\(^\text{15}\)\(^\text{16}\)

Results

Clinical electrophysiology

Initial recordings were performed 9 days after exposure at which time the weakness was maximal. Electromyography at this time did not show any spontaneous activity such as fasciculations or fibrillation potentials. In median, ulnar and peroneal nerves, distal motor responses were mildly reduced in amplitude and prolonged in latency. Motor conduction velocities were normal. No nerve conduction block on proximal or distal stimulation or repetitive motor responses were observed following single supramaximal shocks. Antidiromic motor F responses were normal in amplitude and latency. Serial studies were performed in the median nerve-theneral muscles and these results are shown in table 1.

### Table 1 Nerve conduction and repetitive stimulation studies in median nerve after phosmet exposure

<table>
<thead>
<tr>
<th>Time</th>
<th>CMAP mV</th>
<th>DL MS</th>
<th>MCV m/s</th>
<th>% Decrement at 5 Hz</th>
<th>Before</th>
<th>After Tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>9-0</td>
<td>5-0</td>
<td>54</td>
<td>48</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>11</td>
<td>11-1</td>
<td>5-0</td>
<td>54</td>
<td>55</td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>13</td>
<td>13-0</td>
<td>5-0</td>
<td>54</td>
<td>58</td>
<td>46</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>19-0</td>
<td>4-7</td>
<td>56</td>
<td>42</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>19-0</td>
<td>4-7</td>
<td>56</td>
<td>39</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>47</td>
<td>22-0</td>
<td>4-2</td>
<td>62</td>
<td>16</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>95</td>
<td>19-0</td>
<td>3-9</td>
<td>55</td>
<td>+6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Normal subjects

Mean (SD) 17-0 (4-8) 3-5 (0-5) 55 (4) 0 (10) 0 (10) 0 (10) 0 (10)

**CMAP**—Compound muscle action potential amplitude in millivolts, mV, from thenar muscles.

**DL**—Distal motor latency in milliseconds, ms, from wrist to thenar muscles.

**MCV**—Maximal motor conduction velocity in meters per second, m/s, between elbow and wrist.

**R**—Motor responses 4th or 9th on repetitive nerve stimulation.
Repetitive stimulation at slow rates (2–10 Hz) in the median and peroneal nerves produced marked decremental responses (44–50%) at the fourth response with some recovery by the last response (figure 1). At fast rates of stimulation (50 Hz) marked decrement (80%) was observed without recovery. Serial recordings were performed in the median nerve and the results are shown in table 1. Response to intravenous edrophonium chloride was studied from days 11 to 47 (table 1) and showed a marked decrease in the decrement (58–42% reduced to 33–7%) at slow and fast rates of stimulation. The decrease in decrement was associated with transient increase in strength on muscle testing. The beneficial effect of edrophonium persisted throughout the 6 week period of neuromuscular weakness. Similar beneficial effects were obtained with intramuscular neostigmine that reduced the decremental response of 58% (4th response at 65 Hz) to 10% at 35 minutes after the injection.

Three months after exposure when the patient had mild proximal muscle weakness and fatiguability, no decremental responses were observed. However, SF EMG revealed increased variability in the interpotential intervals (jitter) in all 20 potential pairs examined.

Table 2. Endplate recordings in intercostal muscles in patient with phosmet exposure and normal subjects

<table>
<thead>
<tr>
<th>Recordings</th>
<th>Patient</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane</td>
<td>–75 (2-73)</td>
<td>–76 (2-1)</td>
</tr>
<tr>
<td>Potential, mV</td>
<td>45*</td>
<td>30</td>
</tr>
<tr>
<td>MEP, mV</td>
<td>0-36 (0-07)*</td>
<td>0-83 (0-22)</td>
</tr>
<tr>
<td>MEPP, mV</td>
<td>40:481*</td>
<td>31:435</td>
</tr>
<tr>
<td>Frequency, sec–1</td>
<td>40:581</td>
<td>30:870</td>
</tr>
<tr>
<td>ACh sensitivity</td>
<td>1182 (216)*</td>
<td>3730 (102)</td>
</tr>
<tr>
<td>mV/nC</td>
<td>11</td>
<td>20</td>
</tr>
</tbody>
</table>

Mean (SE)
*Number of fibres studied
**Number of fibres/number of potentials counted
*Significance p < 0.01 from normals
MEPP—miniature endplate potential
ACh—Acetylcholine

No impulse blocking was observed. Fibre densities were normal. No spontaneous fasciculations or denervation potentials were observed.

Endplate physiology

Intracellular recordings at the endplate region of intercostal muscle fibres revealed that the resting membrane potentials were similar to controls (table 2). Spontaneous miniature endplate potential (mepp) frequency was reduced (p < 0.01) compared with controls. Mean mepp amplitude was reduced (p < 0.01) compared with control values. The shape of the mepps was slightly altered as the potentials were prolonged by 20%. As many of the mepps were small (< 0.5 mV), it is possible that some were not observed above the noise level of the recording system accounting for the reduced frequency.

Microiontophoretic application of ACh to the endplate region revealed decreased and marked, mean (SD), variability of the ACh sensitivity in different fibres [2730 (533) mV/nC to 448 (128)]. Mean ACh sensitivity was reduced significantly (p < 0.01) compared with controls (table 2). Falling phase of the ACh potential was prolonged by 20%.

Ultrastructure

Endplate regions at low magnification showed that the subjunctional myofibrils appeared normal without evidence of a myopathy. Neuromuscular junctions contained numerous synaptic vesicles, mitochondria and synaptic clefts with well established basal lamina. The endplate soleplasm contained an increase of smooth endoplasmic reticulum, ribosomes, polysomes and invaginated nuclei compared with controls suggesting high metabolic and regenerative activity (figure 2). Some endplates showed subsynaptic vesiculation and phagocytic lysosomal activity suggesting degeneration. At higher magnification some of the secondary synaptic clefts were widened and filled with debris and junctional folds were simplified suggesting endplate damage.

Discussion

The patient described in this report had a subacute progressive neuromuscular syndrome associated with CNS dysfunction following the fifth weekly spraying with phosmet, an OP insecticide. Paralysis became maximum by 9 days, continued for 30 days, but some weakness persisted for 4 months. CNS signs of visual hallucinations, disorientation and myoclonic jerks occurred at the peak of the toxicity and most likely reflected central cholinergic dysfunction. These signs cleared rapidly without residual effects. Limited symptoms and signs of acute OP toxicity were noted. No myopathy or delayed peripheral neuropathy occurred. The neuromuscular dys-function was associated with CNS involvement which was observed also in patients with the intermediate syndrome following exposure to OP insecticides. Acute and delayed CNS cholinergic dysfunction is well recognised in...
Endplate studies in OP poisoning

experimental animals exposed to OP.\textsuperscript{2, 9}
Although the paralysis in our patient did not occur after overt cholinergic toxicity, progressed over a longer period (9 days rather than 4) and persisted longer (30 days rather than 18) than those reported,\textsuperscript{10} it is likely that OP toxicity due to phosmet can produce a delayed progressive neuromuscular syndrome in humans. In this syndrome the endplate physiological studies clearly document postsynaptic neuromuscular dysfunction. This is characterised by: mildly reduced CMAP amplitudes, decremental responses reversed by edrophonium and neostigmine, small mepps and reduced junctional ACh sensitivity. These results support those of the previous report in which limited electromyographic studies suggested a postsynaptic defect.\textsuperscript{10} Decremental responses at slow rates of stimulation observed in this patient but not in those reported in the intermediate syndrome\textsuperscript{10} may reflect greater neuromuscular blockade, desensitisation and loss of ACh receptors. This may account for the prolonged duration of the syndrome. This subtype OP postsynaptic neuromuscular block can be differentiated electrophysiologically from acute OP cholinergic block that results in spontaneous repetitive firing of muscle action potentials and decremental-incremental responses.\textsuperscript{17} In acute OP toxicity, edrophonium increased the decremental responses,\textsuperscript{8} while in subacute toxicity it reduced the decremental responses toward normal. Edrophonium is recommended as a test in OP toxicity to document the acute cholinergic block. The acute syndrome may benefit from therapy with pralidoxime chloride, an AChE reactivator that may have varied actions,\textsuperscript{8} but it should not be used in the subacute syndrome with postsynaptic dysfunction, since it may increase the neuromuscular block and weakness.

The endplate physiological findings in this patient with subacute neuromuscular weakness are similar to those reported in myasthenia gravis that have shown loss of functional postsynaptic ACh receptors.\textsuperscript{13, 19} The mechanism of receptor loss in subacute phosmet toxicity is unknown but is likely to result from endplate degeneration as shown on the ultrastructural studies. The physiological findings of mildly prolonged distal motor latency and reduced mepp frequency and the pathological finding of endplate damage suggest that nerve terminals may be affected by OP toxicity. Experimental studies with chronic AChE inhibitor toxicity in rats have shown similar physiological and morphological changes of the neuromuscular junctions.\textsuperscript{20, 21} Postsynaptic dysfunction was greater and persisted longer than presynaptic changes, was prevented by chronic nerve section and was therefore possibly due to the prolonged increased activity of synaptic ACh. Prolonged action of ACh at the NMJ has been reported to produce a myopathy that is mediated by calcium activation of a protease and requires intact ACh receptors.\textsuperscript{22}

A myopathy was not observed in our patient and this may reflect the endplate degeneration with loss of receptors. Prolonged action of synaptic ACh accounts for the acute OP syndrome, and it is possible that it may induce the subacute neuromuscular syndrome by prolonged receptor desensitisation and influx of calcium that damages the NMJ. The endplate damage may be enhanced also by the direct effects of AChE inhibitors that act as AChR agonists and channel blockers.\textsuperscript{23} It is possible that similar changes may occur at CNS cholinergic junctions. In addition, unpublished data from our laboratory suggest that organophosphates cause release of excitatory and inhibitory transmitters in the CNS.\textsuperscript{24}

Our patient was exposed to phosmet, an insecticide recommended for the control of fruit tree pests.\textsuperscript{25} It is less toxic as an AChE inhibitor than other insecticides such as paraaxon.\textsuperscript{26} Phosmet has very low water solubility and is lipid soluble.\textsuperscript{26} It is possible that repeated weekly exposure to a lipid soluble OP, like phosmet, spray by inhalation and skin contact resulted in the subacute progressive neuromuscular and CNS syndrome in our patient. There are no other reports of subacute toxicity due to phosmet but fenthion, which also has high lipid solubility, produced 4 of the 10 reported cases of the intermediate syndrome.\textsuperscript{10} Proposed mechanisms by which some of the OP may produce subacute effects range from higher lipid solubility to delayed conversion to a more active metabolite.\textsuperscript{27} Although it is possible that the insecticide which the patient used contained a toxic product of phosmet, there are no reports of other toxic substances in the compound.\textsuperscript{25}
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