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Slow Repetitive Nerve Stimulation in Patients with Acute Organophosphorus Poisoning after Clinical Recovery

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Abstract

Background: Prolonged inhibition of acetylcholine esterase may lead to the intermediate syndrome. Neuromuscular junction (NMJ) dysfunction has been shown with repetitive nerve stimulation (RNS). Subclinical NMJ dysfunction may also occur. We aimed to examine the NMJ function following acute organophosphorus (OP) poisoning by using exercise modified slow RNS.
Methods: A cohort study was conducted with matched controls. Patients with acute OP poisoning were enrolled. NMJ function, muscle power and tendon reflexes were assessed at discharge and six weeks after exposure. NMJ function was assessed with exercise modified supramaximal slow RNS of the median nerve.
Results: There were 68 patients and 71 controls. Mean (SD) age of patients and controls were 32 (12) and 33 (12) years. In some particular amplitude, the decrement response was statistically significant. They were decrement response at rest, at fourth amplitude (95% CI: -0.2 to -2.7) and two minutes post-exercise at fourth and fifth amplitudes (95% CI: -0.8 to -5, -1 to -5 respectively) in the second assessment compared to controls, decrement response at rest at fourth and fifth amplitudes (95% CI: -4 to -0.5, -3.9 to -0.01 respectively) and two minutes post-exercise at fourth amplitude (95% CI: -5 to -0.8) in the second assessment compared to the first assessment. Patients in the first assessment and controls showed more than 8% decrement response either to the second, fourth or fifth stimuli in seven and five occasions respectively.
Conclusion: There was no significant neuromuscular junction dysfunction assessed by exercise modified slow repetitive stimulation following acute exposure to OP. Since, NMJ dysfunctions are likely to occur following OP poisoning, other electrodiagnostic modalities such as SF-EMG are probably more efficient to assess these abnormalities.

Keywords: Organophosphorus compounds; Poisoning; Electrodiagnosis; Neuromuscular junction; Exercise modified slow repetitive stimulation

INTRODUCTION

Self-poisoning with organophosphorous (OP) compounds is a serious health problem especially in agricultural areas of developing countries (1,2). OP compounds bind to the esteratic site on the acetylcholine esterase (AChE) molecule, phosphorylate the enzyme, and inhibit its action (3). Binding between the esteratic site on the enzyme and the phosphorus atom is stable and lasts hours or weeks to break off, depending on the compound involved. Studies have shown that a phenomenon of enzyme aging occurs which involves cleavage of a free radical from the inhibited enzyme, making it resistant to reactivation (3). Hence, the direct result is accumulation of excess acetylcholine (ACh) at the cholinergic nerve endings all over the body. Following inhibition, the enzyme recovers at about one percent per day (3). Restoration of AChE levels occurs by spontaneous or induced reactivation of the enzyme and by new enzyme synthesis (3).

Accumulated ACh at nerve endings initially stimulates exhaustion of cholinergic synapses and eventually leads to, neuromuscular junction (NMJ) dysfunction (3). The excess ACh stimulates presynaptic nicotinic and muscarinic receptors and post-synaptic ACh receptors, leading to influx of Ca^{2+} (4). In animal models, this caused muscle necrosis (5,6).

Since patients with OP ingestion complain of muscle weakness even with normal muscle power after clinical recovery, we aimed to study subclinical NMJ dysfunction in OP poisoned patients after clinical recovery.

METHODS

A prospective cohort study was conducted at a general hospital and a teaching hospital in the Southern province of Sri Lanka after receiving approval of the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. The study was conducted according to the Declaration of Helsinki and subjects gave their informed consent.

In this study, 71 patients with acute OP poisoning with 71 age and gender matched controls were enrolled. Controls were selected from volunteers who did not have a history of acute pesticide exposure. The age of controls was matched to ±3 years of the OP poisoned patients. Subjects with neuropathies, diabetes mellitus or history of head injury or who were on long term medication were excluded from the study.

NMJ function, muscle power and tendon reflexes were at

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clinical recovery and six weeks post-exposure.

Definition of clinical recovery was when patients did not require atropine to abolish cholinergic symptoms. Exercise modified slow repetitive supramaximal stimulation was used to assess NMJ function on the median nerve of the dominant upper limb. Neuropack S1(Nihon Kohden, Tokyo, Japan) was used for repetitive nerve stimulation (RNS). RNS was carried out by administrating 10 stimulations at a rate of 3Hz with a filter setting of 5-20 kHz. Forearm and hand were fixed by straps to restrict movements and to ensure that contraction of the abductor pollicis brevis was isometric. Recording electrodes were placed over the abducter pollicis brevis, while the reference electrode was pasted over the proximal phalanx of the thumb and the ground electrode was attached between the recording electrode and the stimulating probe (7). Laboratory temperature was maintained at 25^{OC} during the test.

The nerve was stimulated at wrist in 3 occasions: (A) at rest, the first train, (B) immediately after 30 seconds maximal isometric exercise, the second train, and (C) 2 minutes after the exercise, the third train (8). Participants were asked to abduct the thumb against fixed resistance for the isometric exercise (8). The post-exercise facilitation and the post-exercise exhaustion were calculated after the isometric exercise (7,8).

The decrement responses were quantified by calculating the percentage change in amplitude as follows (9):

- (A) First train;
  - percentage change in amplitude between the second and the first action potential of the first train of stimuli ((A1-A2)/A1 × 100)
  - percentage change in amplitude between the fourth and the first action potential of the first train of stimuli ((A1-A4)/A1 × 100)
  - percentage change in amplitude between the fifth and the first action potential of the first train of stimuli ((A1-A5)/A1 × 100)
- (B) Second train;
  - percentage change in amplitude between the second and the first action potential of the second train of stimuli ((B1-B2)/B1 × 100)
  - percentage change in amplitude between the fourth and the first action potential of the second train of stimuli ((B1-B4)/B1 × 100)
  - percentage change in amplitude between the fifth and the first action potential of the second train of stimuli ((B1-B5)/B1 × 100)
- (C) Third train;
  - percentage change in amplitude between the second and the first action potential of the third train of stimuli ((C1-C2)/C1 × 100)
  - percentage change in amplitude between the fourth and the first action potential of the third train of stimuli ((C1-C4)/C1 × 100)
  - percentage change in amplitude between the fifth and the first action potential of the third train of stimuli ((C1-C5)/C1 × 100)

Up to 8% decline in compound muscle action potential (CMAP) amplitude with RNS was considered as normal (9).

The post-exercise facilitation was assessed by calculating ratio of the amplitude of the first action potential in the second train to the amplitude of the first action potential of the first train (B1/A1) (7,8), and the post-exercise exhaustion was evaluated by calculating the ratio of the amplitude of the first action potential of the third train to the amplitude of the first action potential of the first train (C1/A1) (8).

Muscle power was assessed in flexors and extensors of the wrist, biceps, triceps, dorsiflexors and plantar flexors of the feet and toes, flexors and extensors of the knee, flexors and extensors of the hip, abductors and adductors of the thigh. The muscle power was graded according to the Medical Research Council scale for grading muscle function from 0 to 5 scores (10).

Tendon reflexes were assessed in knee, ankle, triceps, biceps and supinator. The tendon reflexes were graded from 0 to 4 (0-absent, 1-present (as a normal ankle jerk), 2-brisk (as a normal knee jerk), 3-very brisk, 4-clonus) (10).

Statistical analysis was done by using GraphPad Prism software version 4 (GraphPad Software Inc, California, USA). Normal distribution of the data was evaluated with Kolmogorov-Smirnov test. The paired T-test was used to compare the results of the first and the second assessment and the unpaired T-test was used to compare the results of patients and controls. Results of the analysis were reported with 95% confidence interval.

RESULTS

General Findings

A total of 71 patients were enrolled in this study. Three patients could not tolerate the test. The number of matched controls was 71. Demographic data of the patients and controls are shown in table 1.

Table 1. Demographic data of patient and control group

Descriptive data	Patients (n, 68)	Controls (n, 71)
Age (years)*	32 (12)	33 (12)
Gender (male/female)	50/18	51/20
BMI (Kg/m2)*	21.9 (4.4)	22.0 (6.8)

*Values are shown with mean (SD)

Slow repetitive nerve stimulation

Table 2 shows decrement response at rest, immediately after isometric exercise, two minutes post-exercise, post-exercise facilitation and post-exercise exhaustion. Statistically significant difference of decrement response was observed at rest (A4) and two minutes post-exercise (C4, C5) in the second assessment compared to controls (95% CI; -0.2 to -2.7, -0.8 to -5, -1 to -5 respectively). The decrement response was significant at rest (A4, A5) and two minutes post-exercise (C4) in the second assessment compared to the first assessment (95% CI; -4 to -0.5, -3.9 to -0.01, -5 to -0.8 respectively).

Seven amplitudes of patients in the first assessment and five amplitudes of controls showed more than 8% decrement response either to the second, fourth or fifth stimuli (Table 3).

Muscle power

In the first assessment muscle power was five in all

Table 2. Decrement response, post-exercise facilitation and post-exercise exhaustion of patients and controls

Parameter	Controls	Patients		Controls vs 1st assessment		Controls vs 2nd assessment		1st assessment vs 2nd assessment	
	Mean % (SE)	1st assessment Mean % (SE)	2nd assessment Mean % (SE)	Mean difference (controls – patients)	95% CI	Mean difference (controls – patients)	95% CI	Mean difference (1st 2nd assessment)	95% CI
Decrement at rest (A2)	-0.5 (0.3)	-1.4 (0.5)	-0.9 (0.3)	0.9	1.9 to -0.2	0.4	1.2 to -0.5	-0.7	-1.8 to 0.3
Decrement at rest (A4)	-0.6 (0.4)	-1.5 (0.5)	0.8 (0.5)	0.9	2 to -0.4	-1.5	-0.2 to -2.7*	-2.3	-4 to -0.5*
Decrement at rest (A5)	-0.5 (0.5)	-1.0 (0.5)	0.8 (0.5)	0.6	2 to -0.9	-1.3	0.05 to -2.7	-1.9	-3.9 to -0.01*
Decrement at immediately after maximal isometric exercise (B2)	-0.9 (0.5)	-1.2 (0.5)	-0.3 (0.2)	0.3	1.6 to -1	-0.6	0.6 to -1.8	-1.2	-2.4 to 0.05
Decrement at immediately after maximal isometric exercise (B4)	-0.5 (0.5)	-0.2 (0.5)	0.4 (0.5)	-0.3	1.2 to -1.7	-0.9	0.5 to -2.3	-0.7	-2.6 to 1.2
Decrement at immediately after maximal isometric exercise (B5)	-0.8 (0.5)	-0.2 (0.5)	0.6 (0.6)	-0.7	0.9 to -2	-1.5	0.2 to -3	-0.8	-2.9 to 1.3
Decrement 2 min after maximal isometric exercise (C2)	-1.6 (0.4)	-1.6 (0.4)	-1.2 (0.7)	0.03	1.2 to -1	-0.4	1.1 to -1.9	-0.5	-2.3 to 1.3
Decrement 2 min after maximal isometric exercise (C4)	-1.3 (0.5)	-1.2 (0.4)	1.6 (1.0)	-0.2	1 to -1.4	-2.9	-0.8 to -5*	-3.0	-5 to -0.8*
Decrement 2 min after maximal isometric exercise (C5)	-1.6 (0.5)	-0.8 (0.5)	1.5 (1.0)	-0.7	0.8 to -2	-3.1	-1 to -5*	-2.4	-5 to 0.3
Post exercise facilitation (B1/A1)	1.05 (0.04)	0.9 (0.03)	1.0 (0.03)	0.06	0.2 to -0.06	0.03	0.1 to -0.08	0.02	-0.09 to 0.1
Post exercise exhaustion (C1/A1)	1.01 (0.04)	1.0 (0.05)	1.0 (0.04)	0.02	0.1 to -0.1	0.04	0.2 to -0.08	0.02	-0.1 to 0.2

* Statistically significant values

Table 3. Number of participants who showed more than 8% decrement considering the second, fourth or fifth stimuli

Stimulus	Patients (n, 68)			Controls (n, 71)		
	During rest	Immediately after the isometric exercise	Two minutes after the isometric exercise	During rest	Immediately after the isometric exercise	Two minutes after the isometric exercise
Second	0	0	0	0	0	0
Fourth	0	2	1	0	2	0
Fifth	0	2	2	2	1	0

muscle groups examined in 60 patients. Ten patients had muscle power of four in at least one of the muscles. One patient had muscle power of either three or two in the muscles examined. In the second assessment, the muscle power was found to be five in all patients. All controls had muscle power of five.

Tendon reflexes

Sixty-seven patients showed normal tendon reflexes. One patient showed absent knee jerk. Three patients showed very brisk reflexes in at least one of the tendons. The tendon reflexes were normal in the second assessment of patients and controls.

DISCUSSION

In this study, we found no detectable NMJ dysfunction after clinical recovery following acute OP poisoning assessed with exercise modified slow RNS. The gold standard electrophysiological tool to examine the neuromuscular junction function is single-fiber electromyography (SF-EMG) (9,11,12). However, applying RNS was considered to be an alternative method. In this study, few patients had RNS abnormalities in the first assessment and this was not different from controls. Six weeks after the exposure, RNS results showed significant decrement responses. Hence, RNS does not appear to be a useful method to assess early NMJ dysfunctions.

NMJ dysfunction following OP poisoning has been demonstrated with fast repetitive nerve stimulation before clinical recovery (13), but there is no evidence whether these changes persist over long-term. Notwithstanding, in a study which the effects of occupational exposure to OP on neuromuscular function were investigated, no detectable neuromuscular disturbances with RNS and with SF-EMG were found (14). Though, the frequency of neuromuscular transmission abnormalities detected with SF-EMG was shown to be significantly higher than those detected with RNS (14,15).

Decrement response is more recognizable in fast repetitive stimulation than the slow repetitive stimulation. The current study concentrated only on the slow repetitive stimulation with exercise modification to magnify the function of NMJ. However, it was previously shown that this method may not be sensitive enough to detect subtle abnormalities (16).

The amplitude of CMAP may increase during high-frequency nerve stimulation or after voluntary activation of muscle (9). The measurement of CMAP amplitude in fast repetitive stimulation is not recommended since amplitude of the CMAP during high frequency stimulation may demonstrate inconsistent results (9). Therefore, we measured CMAP amplitude immediately and two minutes after the isometric muscle exercise. Even with isometric muscle exercise, this study could not demonstrate significant decrements in patients compared to controls. Previous studies on neuromuscular synapse function in organophosphate exposed workers also did not show significant decrement response compared to controls (7,8).

With each depolarization, Ca^{2+} releases into the periterminal space (9). If another depolarization occurs, high Ca^{2+} concentration leads to increase in released ACh quanta. If neuromuscular transmission is impaired, this greater ACh release briefly improves synaptic transmission which produces facilitation (9). Marked facilitation is characteristic of presynaptic blockade. Nevertheless, facilitation can occasionally occur with postsynaptic problems (9). Although it is recommended to calculate the changes in CMAP size between the first and the fourth or the fifth response of a train of stimuli (9), we took the second response as well, since Maselli et.al and Jayawardana et.al observed the maximum decrement at the second stimulus (13,17). Similarly, our findings showed maximum decrement either at the second or third stimulus.

The majority of cases in this study showed increment pattern progressively over the train. Consequently, the mean percentage decrements illustrated in table 2 are negative values. This pattern is described as pseudoincrement and was shown to be a normal variant (18).

Even though a decrement response over 8% was observed in few occasions, both in patients and controls in this study, these decrements were not confirmed. False positive results of RNS is a possible incident since such examinations are technically difficult and artifacts resulting from electrode placement, movements, changes of skin resistance and intramuscular temperature cannot be entirely controlled (11).

The current study showed impairment of muscle power in both proximal and distal muscles at one week after exposure to OP compounds in a few patients. Proximal muscle weakness has been observed in previous studies, including a case of muscle power impairment and exaggerated tendon reflexes with absent knee jerk following acute methamidophos (an OP insecticide) poisoning which was reported by Senanayake et al. (19). Correspondingly, weakness of proximal limb muscles (shoulder abduction

and hip flexion) with normal strength in the distal muscles was reported in patients who developed intermediate syndrome following acute OP exposure (20). Moreover, they showed that tendon reflexes were decreased in most patients (9/10) with intermediate syndrome, while the other patient had exaggerated tendon reflexes (20). Likewise, in the present study, most patients had normal tendon reflexes while one patient showed absent knee jerk and some patients showed exaggerated tendon reflexes which may be due to persistent cholinergic effects.

LIMITATIONS

The assessment tool of the current study was exercise modified slow RNS. Slow frequency RNS study is not a high sensitive test to evaluate NMJ disorders. Hence, we tried to increase the sensitivity of test by keeping on RNS study after 30 seconds and two minutes of full isometric contraction. However, the results might change, if RNS examinations were continued three, four and five minutes following full isometric contraction. SF-EMG and fast RNS were not performed as SF-EMG is not available in most clinics and fast RNS is painful and may not be tolerable by subjects. If SF-EMG or fast RNS were performed, we were probably able to gather positive results.

CONCLUSION

There was no significant NMJ dysfunction assessed with exercise modified slow repetitive stimulation following acute exposure to OP. Since, NMJ dysfunctions are likely to occur following OP poisoning, other electrodiagnostic modalities such as SF-EMG are probably more efficient to assess these abnormalities

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