Phase II study of magnesium sulfate in acute organophosphate pesticide poisoning

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Background. Acute organophosphorus (OP) poisoning is relatively common and a major cause of death from poisoning in developing countries. Magnesium has been shown to be of benefit in animal models. Methods. We conducted a phase II study of bolus doses of (MgSO4) in 50 patients with acute organophosphate poisoning. Patients eligible for inclusion had ingested OP and had cholinergic symptoms consistent with moderate or severe poisoning. All patients received standard care of atropinization titrated to control muscarinic symptoms and pralidoxime. The trial was run in 4 sequential groups of patients. Participants in each group received a different total dose of MgSO4 (20%) administered as intermittent bolus doses infused over 10–15 min or placebo. There was one control patient for every 4 patients who received MgSO4. Group A (16 patients) received a total of 4 gm MgSO4 as a single bolus, group B (8 patients) received 8 gm (in two 4 gm doses q4H), group C (8 patients) received 12 gm (in three 4 gm doses q4H) group D (8 patients) received 16 gm (in four 4 gm doses q4H) and control (10 patients) received placebo. Patients were closely monitored for any adverse reaction like significant clinical neuromuscular disturbance and respiratory depression. Results. No adverse reactions to magnesium were observed. The 24 hour urinary magnesium concentration were 74.18 mg/dl and control (118.06 mg/dl) and there was one control patient for every 4 patients who received MgSO4. Group A (16 patients) received a total of 4 gm MgSO4 as a single bolus, group B (8 patients) received 8 gm (in two 4 gm doses q4H), group C (8 patients) received 12 gm (in three 4 gm doses q4H) group D (8 patients) received 16 gm (in four 4 gm doses q4H) and control (10 patients) received placebo. Patients were closely monitored for any adverse reaction like significant clinical neuromuscular disturbance and respiratory depression. Results. No adverse reactions to magnesium were observed. The 24 hour urinary magnesium concentration were 74.18 mg/dl and control (118.06 mg/dl) (p = 0.019), while it was much lower than the 80% of the intravenous magnesium load. Six patients died in control group compared to 3 in 4 gm, 2 in 8 gm and 1 in 12 gm group. There was no mortality in 16 gm group. Conclusion. Magnesium was well tolerated in this study. Larger studies are required to examine for efficacy.

Keywords Other; Respiratory support; Metabolic Organophosphate; Magnesium

Introduction

Pesticides particularly organophosphate compounds are commonly used as agricultural insecticides worldwide. Their easy availability makes them a popular method of self harm particularly in the rural areas of Bangladesh. The case fatality for self poisoning in the developing world is commonly 10–20% but for particular pesticides, it may be as high as 50–70%.1 Although the exact data are not available in Bangladesh, hospital-based studies suggest that it is the commonest poisoning in Bangladesh with nearly half of the admissions to the emergency with poisoning being due to organophosphate (OP).2

The recommended therapy for OP poisoning is the intravenous administration of atropine and pralidoxime. Atropine, a competitive inhibitor of acetylcholine counteracts the muscarinic effects of OP poisoning. Pralidoxime is used to reactivate cholinesterase enzymes but is ineffective once the enzyme has become irreversibly bound to organophosphate.3 The use of these standard drugs has failed to reduce the mortality and morbidity in different hospitals in Bangladesh.4

Administration of magnesium to animals poisoned with organophosphorus pesticides (OP) has been shown to improve outcome.5 The mechanism of action of magnesium remains elusive, it is considered to attenuate the toxicity of OP compounds by reducing acetylcholine release from pre-synaptic nerve terminal.6

The use of magnesium in acute OP poisoning in humans has been reported in two studies. In the first study in 1998, Singh et al reported in four patients that magnesium decreased the observed effects of OP poisoning on the electroencephalogram.7 More recently in 2004, a very small randomized study on 45 patients by Pajoumand et al reported that magnesium decreased mortality (0/11 patients died with magnesium versus 5/34 control patients).8 This study was small and there are numerous parts of the methodology that are incompletely described. The potential side effects of using magnesium includes respiratory depression and peripheral vasodilatation resulting in sudden drop of blood pressure.
Thus we sought to investigate the safety and clinical value of magnesium sulfate in the treatment of acute OP pesticide poisoning by means of a phase II clinical trial.

Patients and methods

This was an open label, phase II dose escalation trial of bolus doses of magnesium sulfate in patients with known organophosphate poisoning. The study was conducted in one adult Medicine unit of Dhaka Medical College Hospital, Dhaka, Bangladesh from July 2006 to June 2007. This ward admits patients over the age of 12 years. The protocol was reviewed and approved by the Ethical Review Committee of Bangladesh Medical Research Council. Written informed consent was obtained from patients or their close relatives.

All patients were assessed at the time of admission. Only patients with definite history of OP ingestion and typical cholinergic syndrome were considered as OP poisoning. "Cholinergic syndrome" was defined by the presence of muscarinic symptoms with or without nicotinic symptoms and mental status changes. Patients whose GCS score at presentation was 8 were considered severe poisoning. The acute cholinergic crisis is characterized by miosis, bradycardia, bronchorrhea, bronchoconstriction, emesis, lacrimation, salivation, hypotension, fasciculations, muscle weakness, altered level of consciousness, respiratory failure and seizures.

Eligible patients included men and women, aged 12–60 years with symptomatic acute OP poisoning and had not received any specific antidote or mechanical ventilator support before admission. Patients were excluded from the study if there was history or suspicion of ingestion of other drugs concomitantly or the patient was suffering from other systemic illness like cardiac and renal insufficiency or was admitted 24 hours after ingestion.

During the study period, out of 90 OP poisoned patients, 50 were prospectively enrolled. The causes of exclusion are described in Fig. 1. Patients were randomised following initial atropinization. Four consecutive patients received MgSO4 with a dose defined by the study protocol then every fifth patient was enrolled as a control and received standard care. As the randomization was sequential it produced a quasi-random allocation of 40 cases and 10 controls.

This was a sequential incremental dose escalation study which created 4 groups of patients who received different total daily doses of MgSO4 (4, 8, 12 or 16 grams) (Fig. 1). The study medication 20% MgSO4.7H2O, was prepared from a locally available formulation and administered as intermittent bolus doses (4 gm every 4 hour) infused intravenously over 10–15 min. MgSO4 was given only during the first 24 hours after admission. Control patients received a volume of normal saline equivalent to the volume of magnesium given at each dosing strata.

Patients were assessed for evidence of magnesium toxicity. Specifically prior to the injection of MgSO4 the patient was examined for: pulse, blood pressure, presence of deep tendon reflexes, respiration rate > 16/minute and urine output > 100 ml since the preceding injection. In addition,
1 g of calcium gluconate was made available as an antidote for symptomatic hypermagnesaemia.

Blood samples were taken before and 24 hours after the first intervention (MgSO₄). Twenty four hour urine was collected starting after the first MgSO₄ dose. The concentration of magnesium was measured using Sapphire 120 auto analyzer (Ireland).

All patients received a standard treatment protocol comprising gastric lavage, intravenous atropine and pralidoxime. Atropine was started 1.8 – 3 mg bolus thereafter comprising gastric lavage, intravenous atropine and pralidoxime (Ireland).

after the period of early cholinergic syndrome. Other biochemical results were within the normal range except in serum biochemical (Na, K, Cl, HCO₃, urea, and creatinine) results even after escalated magnesium doses. In fact, all biochemical results were within the normal range except in the very severe cases with organ failure.

The blood magnesium concentration did not vary significantly with different dosages amongst magnesium-treated patients (ANOVA, P = 0.617). The mean (SD) serum concentration of magnesium before any intervention was 2.63 ± 0.20 mg/dl. The difference between pre and 24 hours post-magnesium treatment was also not significant (2.78 ± 0.3 mg/dl and 2.63 ± 0.12 mg/dl) even after 16 gm magnesium (Table 2). Urinary magnesium concentration was significantly higher in patients who received 16 gm MgSO₄ (234.74 ± 74.18 mg/dl) than that in patients in the control group (118.06 ± 30.76 mg/}

**Table 1.** Clinical features and initial atropine load (or baseline characteristics) among different groups of organophosphate-poisoned patients with or without magnesium sulfate treatment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 10)</th>
<th>Group-A (4 gm) (n = 16)</th>
<th>Group-B (8 gm) (n = 8)</th>
<th>Group-C (12 gm) (n = 8)</th>
<th>Group-D (16 gm) (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe (≤ 8) vs. Moderate poisoning (≥ 8)</td>
<td>5/5</td>
<td>2/14</td>
<td>1/7</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Age (median) years (IQR)</td>
<td>20 (18 – 25)</td>
<td>22 (18.50 – 26.75)</td>
<td>28 (21 – 45)</td>
<td>20 (16 – 28)</td>
<td>30 (19.75 – 38.25)</td>
</tr>
<tr>
<td>Weight (median) kg (IQR)</td>
<td>50 (46.25 – 60)</td>
<td>47.50 (45–53.75)</td>
<td>50 (50 – 62.50)</td>
<td>50 (45–60)</td>
<td>57.50 (37.50–65)</td>
</tr>
<tr>
<td>Pulse Rate (median)/min (IQR)</td>
<td>100 (88.50–111.50)</td>
<td>96 (48.25–120)</td>
<td>72 (64–107.50)</td>
<td>80 (72–100)</td>
<td>82 (75–107)</td>
</tr>
<tr>
<td>Resp. Rate (median)/min (IQR)</td>
<td>15 (10.5–21.5)</td>
<td>21 (16.5–24)</td>
<td>20 (19–24)</td>
<td>16 (8–18)</td>
<td>16 (11.5–18)</td>
</tr>
<tr>
<td>temp (IQR)</td>
<td>98 (98–98.75)</td>
<td>98 (98–98.75)</td>
<td>98 (98–99)</td>
<td>98 (98–99)</td>
<td>98 (98–98.25)</td>
</tr>
<tr>
<td>Mean arterial pressure (median) mmHg (IQR)</td>
<td>85 (74.10–92.50)</td>
<td>83.33 (75.83–92.50)</td>
<td>93.33 (80–103.33)</td>
<td>93.33 (73.33–96.67)</td>
<td>85 (75.84–87.50)</td>
</tr>
<tr>
<td>Ingestion-admission interval (hours) (median) (IQR)</td>
<td>3.5 (2.25–5.57)</td>
<td>4 (4–8)</td>
<td>18 (1.65–19)</td>
<td>5 (2–10)</td>
<td>3.5 (1.58–4.5)</td>
</tr>
<tr>
<td>Initial Atropine load (median) mg (IQR)</td>
<td>28.89 (4.89–45.54)</td>
<td>8.55 (5.30–27.90)</td>
<td>7.12 (3.62–15.36)</td>
<td>12.18 (5.12–28.62)</td>
<td>6.68 (3.15–19.89)</td>
</tr>
</tbody>
</table>

IQR = Interquartile range, Kruskal–Wallis test done for among group comparisons.

Statistical analysis was performed using statistical package Stata 10. Simple logistic regression analysis was done to find out the odds of dying. Comparisons were carried out using Student’s test, ANOVA and chi-square test in case of magnesium concentration. Wilcoxon rank sum test and Kruskal–Wallis test was done for between/among group comparison of atropine dose. P value < 0.05 was considered significant.

**Results**

The median age of the treatment group (26 male and 14 female) was 22 (IQR 18–29.5) years and the control group (6 male and 4 female) was 20 (IQR 18–26) years. The OP ingested was identified in 26 cases either from the brought samples or by showing the specimen to the patients. Malathion was the commonest being identified in 8 (30.8%) cases, Chlorpyrifos in 6 (23%) cases and Fenthion in 5 (19.2%) cases. The common clinical findings at the time of admission among different groups were not significantly different (Table 1).

Magnesium appeared to be well tolerated in all dosing strata. No immediate adverse effects of hypotension or diminished knee reflex were observed following magnesium sulfate administration. No patient received calcium gluconate. There were no statistically significant differences in the serum biochemical (Na, K, Cl, HCO₃, urea, and creatinine) results even after escalated magnesium doses. In fact, all biochemical results were within the normal range except in the very severe cases with organ failure.

The blood magnesium concentration did not vary significantly with different dosages amongst magnesium-treated patients (ANOVA, P = 0.617). The mean (SD) serum concentration of magnesium before any intervention was 2.63 ± 0.20 mg/dl. The difference between pre and 24 hours post-magnesium treatment was also not significant (2.78 ± 0.3 mg/dl and 2.63 ± 0.12 mg/dl) even after 16 gm magnesium (Table 2). Urinary magnesium concentration was significantly higher in patients who received 16 gm MgSO₄ (234.74 ± 74.18 mg/dl) than that in patients in the control group (118.06 ± 30.76 mg/
Table 2. Doses of magnesium sulfate and their outcome variables (n).

<table>
<thead>
<tr>
<th>Group/Mg Doses</th>
<th>No of patient crisis (%</th>
<th>Median of subsequent post atropine-loading infusion doses (IQR)</th>
<th>Median serum Mg concentration before intervention (mg/dl (SD))</th>
<th>Mean serum Mg concentration 24-hour after intervention (mg/dl (SD))</th>
<th>Death (%)</th>
<th>Intubation (%)</th>
<th>Requirement (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7 (70%)</td>
<td>0.88 (0.8 to 2.3)</td>
<td>127 mg (60 to 757)</td>
<td>0.19 (6)</td>
<td>2.56 (2)</td>
<td>11.84 (11.5)</td>
</tr>
<tr>
<td>Group-A 4gm</td>
<td>16</td>
<td>3 (19%)</td>
<td>1.9 (0.8 to 4.3)</td>
<td>159 mg (118 to 440)</td>
<td>3.1 (12)</td>
<td>2.5 (2)</td>
<td>15.23 (10)</td>
</tr>
<tr>
<td>Group-B 8gm</td>
<td>8</td>
<td>2 (25%)</td>
<td>2.25 (0.6 to 5.3)</td>
<td>126 mg (48 to 338)</td>
<td>1.3 (12)</td>
<td>2.58 (2)</td>
<td>14.82 (7)</td>
</tr>
<tr>
<td>Group-C 12gm</td>
<td>8</td>
<td>2 (25%)</td>
<td>2.25 (0.6 to 5.3)</td>
<td>153 mg (87 to 367)</td>
<td>1.3 (12)</td>
<td>2.58 (2)</td>
<td>14.82 (7)</td>
</tr>
<tr>
<td>Group-D 16gm</td>
<td>8</td>
<td>1 (13%)</td>
<td>76 mg (36 to 65)</td>
<td>76 mg (36 to 65)</td>
<td>1 (13%)</td>
<td>2.78 (2)</td>
<td>118.04 (6)</td>
</tr>
</tbody>
</table>

N = Number of patient, SD = Standard deviation, IQR = Interquartile range, Kruskal-Wallis test for atropine load, ANOVA and t test for between/among magnesium concentration in different groups.

Twelve patients died, 9 of these patients were classified as severe on admission and 5 were in the control group (Table 2). The cause of death in the three patients presenting with moderate severity was septicemia (control), acute renal failure (Group A), IMS and sudden respiratory arrest (group B). The risk ratio for death for patients who received any magnesium was of 0.25 (95%CI: 0.10 to 0.61), however there was no difference in patients with severe toxicity. In moderate toxicity the risk ratio for death was 0.35 (95%CI: 0.04 to 3.31).

Only seven patients were provided mechanical ventilator support due to lack of facilities. Two patients each in group-B, (GCS-5,13) and group-C, (GCS 5, 6) developed IMS (2–7 days). Among them only one in group-C (GCS-5) survived; requiring 20 days ventilator support. No one developed IMS in group-D (Table 2).

The loading dose of atropine required was higher in the control group than the magnesium groups, reflecting more severe patients in this group. There was no statistical difference in the median total atropine doses required (KW test, P-0.342) or in the subsequent post atropine-loading infusion doses (KW test, P-0.185) (Table 2).

Discussion

The causes of the high case fatality in OP poisoning are multi-factorial and includes; the high toxicity of locally available poisons, delayed presentation to hospital, the scarcity of health care professionals compared with the large numbers of patients, the lack of facilities including ICU care, antidotes, and trained personnel for the management of pesticide-poisoned patients.16,17

The primary mechanism of organophosphate toxicity is inhibition of acetylcholinesterase enzyme leading to an increase in the neurotransmitter acetylcholine and subsequent overstimulation of central and peripheral synapses.18,19 Inhibition and reactivation of acetylcholine esterase are largely dependent on the nature, relative concentration and the affinity of the OP compounds towards the enzyme. Variability of interaction causes ageing by depletion of one or more alkyl groups attached to the bound phosphate. While the use of atropine to antagonize muscarinic effects is standard care, the role of oximes is not well defined as efficacy or safety of oximes in these settings is not established.20 Oximes are relatively expensive and ineffective once acetylcholinesterase has aged. Thus alternative or adjunctive treatments that may alter acetylcholine release or protect the neuromuscular junction need to be explored.21

Magnesium inhibits acetylcholine release probably through blocking calcium channel.8 It has several attractive additional therapeutic properties including muscle relaxation, which could control spasms, and cardiovascular effects...
Magnesium is inexpensive and familiar to doctors as it is widely used in eclampsia with a standard intravenous dose of 4 gm infused over 20–30 minutes followed by an infusion of 1 gm/hour for 24 hours. An alternative protocol for eclampsia is a loading dose of 10 gm intramuscularly followed by 5 gm magnesium sulfate every 4 hours.

In this study magnesium was given at a total dose of 4 gm, 8 gm, 12 gm and 16 gm in different groups of patients and were monitored for safety to exclude any adverse reactions such as significant clinical neuromuscular disturbance or respiratory depression. Outcome measures for effect on organophosphate toxicity included respiratory failure, death and total amount of atropine required.

One of the objectives of the present study was to determine the possible adverse effects associated with high magnesium infusion along with achievement of a specific therapeutic concentration. The most frequently reported adverse effects of magnesium are due to cardiovascular effects of calcium-channel blockade leading to low blood pressure from vasodilatation and a slow heart rate. Blood pressure is usually lowered most after 8 to 10 hours of continuous infusion when the serum concentration is high. It may also cause weakness and loss of deep tendon reflexes.

In this study patients were monitored closely with vital signs taken at least hourly and recorded on a modified atropine observation chart, no adverse effect was documented in any group receiving different doses of magnesium sulfate. We used intermittent bolus doses and no patient was found to develop hypotension. Antero-lateral ST-T wave changes in 4 gm group may have been ischaemic or myocarditis or alternatively be related to sympathetic and parasympathetic over-activity, hypoxemia, acidosis or electrolyte derangements.

There have been limited available data about magnesium pharmacokinetics. Magnesium distributes mostly into bone, skeletal muscle, and blood cells; less than 1% is in the extracellular fluid compartment. Serum magnesium half life is only 4–5 hours (t1/2 = 4.6–5.2 h) and kidney accounts for all magnesium elimination. Bolus infusion of the first dose followed by continuous infusion is widely accepted to maintain constant high concentration of serum magnesium concentration. Termination of infusion after a loading dose of 4 gm and use of intravenous intermittent bolus doses may cause fluctuation of serum magnesium concentration. Our capacity to measure repeated magnesium was limited by funding. Serum magnesium concentration, before and after MgSO4 infusion, did not vary significantly both within and between the groups. This reflects the relatively short half-life of magnesium and the time of sampling in our study and is consistent with previous research. Although widely used the normal range of plasma magnesium (1.8–2.4 mg/dl) does not exclude magnesium deficiency. Serum magnesium concentration data, in most circumstances, are sparse, unbalanced, and unstructured. Hence, plasma magnesium may not reflect the true total magnesium content in the body. It is helpful to evaluate the amount of magnesium excreted in the urine following an infusion of magnesium. Patient with magnesium deficiency excrete less than 80% of intravenous magnesium load in the urine within 24 hours. In our study median magnesium concentration from 24-hour urine collections was 176.43 mg/dl (IQR 122.84 – 236 mg/dl), assuming normal urine volumes this is likely to be less than 80% of the administered magnesium load for most patients, even for 4 gm of magnesium load.

Our study did not show a significant difference in atropine requirement because the atropine dose was protocol driven and infusion was largely determined by the initial loading dose. Larger sample size and longer duration of administration of MgSO4 would be needed to explore this as an outcome. GCS is an important predictor of severity in acute OP poisoning. Traditionally artificial ventilation is indicated when GCS ≤ 8 in order to maintain respiratory patency. In this study limited resources did not allow us to provide ventilator support to all patients whose GCS ≤ 8. This may influence the study outcome. In group-D, one needed intubation for management of early respiratory failure but no one developed IMS; 2 patients in each group-B and group-C developed IMS and needed ventilation. IMS developed after 24 hours when acute crisis was resolved on the other hand magnesium was given with in first 24 hours that was rapidly excreted with urine and plasma label not rise more then 3.6 mg/dl in any group.

Limitations

A major limitation in this study is the unblinded quasi random allocation of sequential patients, this could potentially lead to a selection bias to consent and select a patient with lower clinical toxicity to the trial if two patients had presented at the same time. The sequential allocation of patients did not allow for stratification for severity or for type of organophosphate. A further potential limitation is that we did not have the capacity to confirm exposure by analysis and identification of the specific organophosphate or by inhibition of cholinesterase. However cohort studies in similar settings showed laboratory confirmation of agrochemicals ingestion in 95% of cases. The study could not exclude coin ingestions as contributing to symptoms such as decreased GCS which was used as a marker of severity, previous studies on the predictive value of GCS had not excluded coingestion of alcohol.

As a phase 2 study, it was not powered for efficacy and these results can not be generalised. The apparent differences in mortality of 60% (6/10) in control group to 15% (6/40) in MgSO4 group should not be over interpreted as this could be explained by the higher proportion of severe toxicity seen in the control patients. Arguably the lower doses of MgSO4 may have reduced the likelihood of benefit if there is a significant dose response. The limited critical care capacity would reduce the ability to demonstrate benefit. Secondary outcomes such as atropine doses can be affected by clinical practice however this was minimized by using a standard protocol with defined endpoints for dose escalation and reduction. As the atropine dose is protocol driven and infusion largely determined by the initial loading dose a larger sample size and longer duration of administration of MgSO4 would be needed to explore this as an outcome. Based on serum concentrations there was no evidence of accumulation of magnesium at 24 hours. Funding
limited our ability to do more frequent magnesium concentrations. We were unable to calculate total excretion as the volume of the 24 urine collection was not recorded although the assay was done on a pooled sample.

Conclusion

No adverse effects could be attributed to intermittent bolus injections of magnesium doses (up to 16 grams). Larger controlled studies should be performed to determine the efficacy of magnesium sulfate in OP poisoning.

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Declaration of interest

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