

Fresh Red Blood Transfusion as a Successful Erythrocyte Cholinesterase Supplement in Organophosphate Poisoning

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Abstract

Despite improvements to standard treatments (atropine, oxime) and intensive care management, the mortality associated with organophosphate (OP) poisoning has not substantially decreased. In this study, we evaluated the role of packed red blood cells (RBCs) transfusion in acute OP poisoning. Patients diagnosed with OP poisoning were included in this prospective study, and then were transfused with packed RBCs stored less than 10 days or 10 to 35 days. Cholinesterase (ChE) level in blood, atropine usage and durations were recorded. We found both shorter- and longer-storage RBCs (200~400 ml) significantly increased AChE level in blood, improved ChE recovery, and reduced the usage and shortened the duration of atropine and followed clinical recovery. Shorter-storage RBCs had better effect than longer-storage (longer storage) ones. Due to erythrocyte cholinesterase supplement, packed RBCs might be used as an alternative approach in patients with OP poisoning, especially at the early stages.

Keywords: Organophosphate; Acetylcholinesterase; Butyrylcholinesterase; Atropine; Oximes

Introduction

Organophosphorus (OP) compounds are chemical substances that come up with a significant toxicological threat. Several pesticides, fungicides, rodenticides were made of OPs, such as parathion, malathion, dimethoate, trichlorfon, dichlorvos, that were commonly used in Asia. In acute and chronic forms, OPs poisoning prevail in industry and agriculture [1]. Due to the availability and toxicity, OPs poisoning happen regularly in accidental intoxication and suicides. Actually acute self-poisoning with organophosphate (OP) pesticides is common in rural Asia, and causes hundreds of thousands of deaths each year [2,3].

OP penetrates via the respiratory tract, alimentary tract and dermal integuments. OPs inhibit cholinesterase, and lead to the increase and accumulation of endogenous acetylcholine concentration, which then affect muscarinic ACh receptors functions mostly in central nervous system, heart muscle, bronchi, alimentary tract and nicotinic ACh receptors in muscular lamina. Thus they exert direct toxic influence on the central nervous system, even could cause death due to pulmonary edema, cerebral edema, respiratory paralysis [4]. OPs affect cholinesterase of all types irreversibly, including acetylcholinesterase (AChE), and pseudocholinesterase (PChE, or butyrylcholinesterase, BChE) [5]. Patients with severe acute poisoning may also lead to delayed sudden death. Some types of organic phosphorus poisoning can occur in 8 to 14 days after the poisoning of delayed neuropathy. Standard therapy includes resuscitation, antidote administration, gastric lavage and/or activated charcoal and supportive care [6].

Despite the use of antidote and intensive care, the high mortality with OPs poisoning still calls for new alternative treatments [7]. Red blood cells (RBCs) transfusion as a mail supply of AChE has been posed as an alternative therapy, but few studies have investigated it, and need to be clarified. Recently we found RBCs transfusion, especially the shorter-storage ones as a supplement of active AChE, could promote ChE restoration, help to improve clinical symptoms.

Materials and Methods

Patients who were diagnosed as OP intoxication in our emergency medicine clinic between Jan 1st, 2014 and Jan 1st, 2016, were included in this study. A standard patient data, demographic information, ChE levels, were recorded.

Diagnosis of OP poisoning was based on information taken either from the patient or their family about the agent involved in the exposure. We confirmed the diagnosis of OP poisoning by measuring plasma cholinesterase (ChE) levels [8]. ChE levels were determined using an Olympus U2700 spectrophotometric chemistry analyzer (Beckman Coulter, Tokyo, Japan). The inclusion criteria contains (1) OP intoxication (exposure history/information), and (2) serum ChE activity less than 2000 KU/L (normal 4000~11000 KU/L).

Standard toxication treatment protocol was applied to all patients. As details, gastric lavage was performed with 2000 ml of 0.9% saline; 0.9% saline and 5% dextrose infusion was started at 2000 ml/m² per day. Intravenous (IV) atropine was administered with started at 1 mg/kg per day; 2 g IV single dose pralidoxime was administered. The patients were admitted to the ICU based on the severity of the clinical signs and symptoms.

As to the RBCs transfusion group, 200 ml~400 ml packed RBCs were transfused during the 3 hours after toxication; 200 ml~400 ml packed RBCs were transfused at 10 hours after toxication if still not atropinization or with still low ChE. All the packed RBCs were administrated during 72 hrs. As for the shorter-storage RBCs refers to storage less than 10 days (including 10 days), while the longer-storage RBCs refers to storage more than 10 days, but less than 35 days (absolutely qualified RBCs).

Level of statistical significance was taken as $P < 0.05$ for all tests.

Results

To evaluate the role of packed red blood cells (RBCs) transfusion in acute OP poisoning, 60 patients with acute OP poisoning were included in this study. Shorter-storage RBCs (less than 10 days) transfusion were applied to 20 patients, longer-storage RBCs (more than 10 days, but less than 35 days) transfusion were applied to 17 patients, and 23 patients did not received transfusion. All the patients had orally OP intake history either for accident or suicide. 28 patients were poisoned with dimethoate, 12 with methamidophos, 9 with parathion, 6 with malathion, 3 with trichlorfon, and 2 with dichlorvos.

Since OPs main toxicity is ChE inhibition, the duration of ChE recover to normal level is critical of the therapeutic effect. As shown in Table 1, the duration time for patient's blood ChE recovery to 70% (2, 800 KU/L) and 90% of normal level (3, 600 KU/L) were compared, both shorter-storage and longer-storage RBCs transfusion had a significant reduction, while shorter-storage RBCs had even better effects (Table 1).

	n	Time for ChE recover to 2800 KU/L (hr, $x \pm s$)	Time for ChE recover to 3600 KU/L (hr, $x \pm s$)
Shorter storage RBCs transfusion	20	46.8 \pm 15.6*	103.4 \pm 7.8*
Longer storage RBCs transfusion	17	53.4 \pm 12.2#	112.5 \pm 8.8#
No RBCs transfusion	23	67.2 \pm 14.9	132.3 \pm 10.9

Table 1: Effect of RBCs transfusion on duration of ChE recovery. * $P < 0.01$, # $P < 0.05$.

Shorter-storage RBCs transfusion and longer-storage RBCs transfusion shorten the time for ChE recover to 2, 800 KU/L from 67.2 \pm 14.9 hours to 46.8 \pm 15.6 hours and 53.4 \pm 12.2 hours respectively. Shorter-storage RBCs transfusion and longer-storage RBCs transfusion shorten the time for ChE recover to 3, 600 KU/L from 132.3 \pm 10.9 hours to 103.4 \pm 7.8 hours and 112.5 \pm 8.8 hours respectively.

In order to follow AChE elevation effects by RBCs transfusion, the blood AChE levels before and after RBCs transfusion 6 hours were detected. In the shorter-storage RBCs transfusion group, the level of blood AChE after transfusion was significantly increased (Figure 1). This significant increase was due to the fresh RBCs transfusion. However, in the longer-storage RBCs transfusion group, the level of blood AChE was not increased significantly (Figure 2), might due to the reduction of AChE during storage. These results suggest that fresh RBCs transfusion significantly improve ChE recovery.

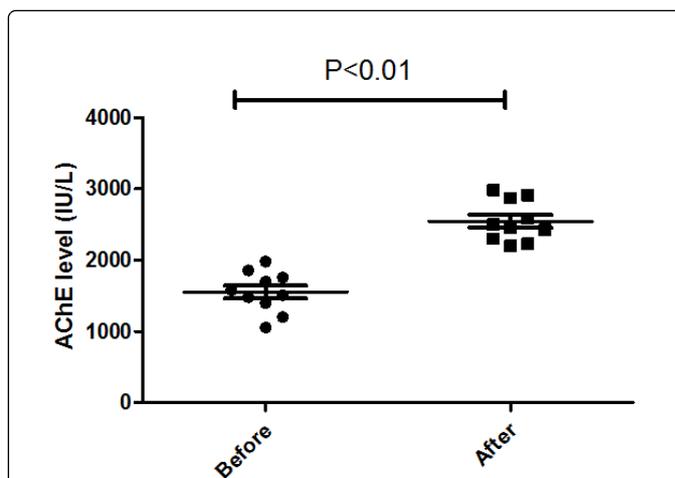


Figure 1: AChE levels of the patients before and after shorter-storage RBCs transfusion. Blood AChE level was detected before and 6 hours after shorter-storage RBCs transfusion respectively. Results were plotted and performed by student *t* test.

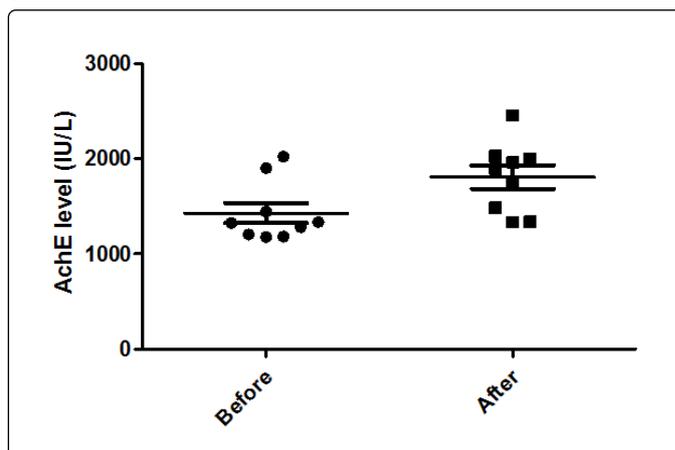


Figure 2: AChE levels of the patients before and after longer-storage RBCs transfusion. Blood AChE level was detected before and 6 hours after longer-storage RBCs transfusion respectively. Results were plotted and performed by student *t* test.

Atropine usage and durations reflect the severity and progression of the poisoning. We next examined whether RBCs transfusion also reduced the amount and duration of atropine usage. As shown in Table 2, shorter-storage and longer-storage RBCs transfusion significantly reduced the total amount of atropine usage from 685.4 \pm 68.4 mg to 423.8 \pm 52.8 and 498.2 \pm 55.4 mg respectively. Shorter-storage and longer-storage RBCs transfusion also reduced the duration of atropine from 7.2 \pm 0.57 days to 6.3 \pm 0.48 days and 6.6 \pm 0.63 days respectively. These results suggest that RBCs transfusion significantly reduces the amount and duration of atropine usage in OP patients.

	n	Amount of atropine requirement (mg)	Duration of atropine requirement (days)
Shorter storage RBCs transfusion	20	423.8 \pm 52.8#	6.3 \pm 0.48#

Longer storage RBCs transfusion	17	498.2 ± 55.4 [#]	6.6 ± 0.63
No RBCs transfusion	23	685.4 ± 68.4	7.2 ± 0.57

Table 2: Effect of RBCs transfusion on total usage and duration of atropine requirement. [#]P<0.05.

Discussion

In the present study, we found that RBCs transfusion significantly improved ChE recovery and reduced the amount and duration of atropine usage. OP intoxications are potentially fatal poisonings which result from both accidental and purposeful intake. These poisoning could be acute or chronic, with high risk of remote consequences [9]. OP compounds are very strong inhibitors for carboxylic ester hydrolases. As a result of ChE inhibition, acetylcholine accumulates, leads to paralysis of acetylcholine receptors due to continuous stimulation, then, muscarnic, nicotinic, and central nervous system symptoms occur [10].

The two types of cholinesterase are AChE and BChE. Among the whole blood ChE activity, AChE from erythrocytes accounts for 60% to 80%, and BChE from plasma accounts for 20% to 40%. AChE is primarily concentrated in the blood on red blood cell membranes, neuromuscular junctions, and other neural synapses. BChE is produced in the liver and found primarily in blood plasma. AChE has a higher specificity to Ach than BChE. After combined by free OPs in the circulation, ChEs both the AChE and BChE will be metabolized, so as to prevent severe damage to central nervous system. In severe poisoning, it needs about four weeks for enzyme recovery. If the low enzyme activity status lasts long, could cause 'rebound' or uintermediate syndrome, and might lead to paralysis of respiratory muscle, that increase the mortality rate.

In present study, we found shorter-storage RBCs transfusion (200~400 ml) on the basis of standard therapy could increase cholinesterase activity, reduce the usage and shortened the duration of atropine significantly, the longer-storage RBCs transfusion had similar but lower improvement effect. These results suggest that RBCs transfusion could improve clinical therapeutic effects on OPs intoxication patients. Red blood transfusion may deliver additional erythrocyte cholinesterase, which could be the potential target substrate for OP. Increasing the activity of AChE over 30% leads to normal neuromuscular transmission and better wearing conditions, and then could improve the overall outcome [11]. In addition, transfusion of red blood cell could not only substitute circulating cholinesterases, but could also bind with OP compounds to prevent them enter central nervous system and muscle. These results help to present additional therapeutic options in the early stage of intoxication in patients with reduced cholinesterase, especially when atropine or specific antidote treatments were ineffective.

It has been reported that RBCs AchE activity remains constant before day 7, and showed significant reduction at day 45 [12]. Although the storage methods have slight different, such we keep 35 days in China, the constant AchE activity during storage was observed [13]. Here we found shorter-storage packed RBCs transfusion improved blood cholinesterase activity and clinical symptoms in OP intoxication patients, however longer-storage packed RBCs showed lower effects. These results suggest that in the emergency, both shorter-

storage and longer-storage qualified RBCs could be used to supply AchE, leading to improvement.

Previous studies have reported whole blood transfusion [9] or fresh frozen plasma transfusion [14] could restore the enzymatic function by its biscavenging effect. However, packed RBCs transfusion has advantages over whole blood transfusion, since the total volume administrated into patients is less that could avoid risks of overloading and fever or allergy by substances in serum. Some studies tried to use human ChE substitute's administration [15], however, due to the high costs and large quantity needed, these substitutes could not be used in human now.

In this study, we found application of RBCs could substitute cholinesterases in OP poisoning, help to restoration of enzymatic function and bind with OP compounds. RBC-AChE could function as a natural bioscavenger that binds OP compounds stoichiometrically and inactivates them effectively. Therefore red blood cell transfusion both shorter-storage and longer-storage could be a good alternative approach.

Conclusions

Based on our results, early blood transfusion can effectively reduce the extent of toxic symptoms and prevent further progression, especially when oximes are not available. However, further evaluation needs to be examined.

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