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FORMULATION DEVELOPMENT AND EVALUATION OF DIFFERENT FORMULATIONS FOR NERVE GAS POISONING

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ABSTRACT

Organophosphorous (OP) nerve agent poisoning can be treated by muscarinic antagonist i.e. atropine sulphate and reactivator of the inhibited acetylcholinesterase (AChE) i.e. pralidoxime chloride or obidoxime chloride. Obidoxime chloride is being considered more effective against various nerve agents like tabun, sarin and VX. Excipients and storage temperature are important measures to assure safety of parenteral formulations. Type of rubber materials, preservatives and temperature can affect the AChE reactivation potency of the obidoxime chloride and atropine sulphate. In the present study *in-vitro* reactivation of sarin-inhibited electric eel AChE by obidoxime chloride and atropine sulphate combination were evaluated with different preservatives, with or without phenol and with different polymer septums. In the presence of methyl paraben or benzyl alcohol the average reactivation of AChE by obidoxime chloride was more than 95% in room temperature with the different septum materials, whereas at 50°C the reactivation was lesser than room temperature. Obidoxime chloride in different combinations of preservatives and septums stored at 25°C showed good reactivation as compared to obidoxime chloride stored at higher temperatures (40 and 50°C). The highest reactivation potency was achieved in the combination of methyl paraben and benzyl alcohol.

KEYWORDS *Obidoxime chloride, atropine sulphate, autoinjectors, nerve agents, acetyl cholinesterase.*

1. INTRODUCTION

Nerve agents are highly toxic organophosphorous compounds (OP), chemically related to some insecticides. They can be used as a chemical warfare agent or by terrorist groups (Nagao et al., 1997; Morita et al., 1995; Macllwain 1993; Nozaki et al., 1995; Clement 1994). The four most common nerve agents are tabun, sarin, soman and VX and huge quantities of these are stockpiled and are being destroyed as per the Chemical Weapon Convention (Sidell, 1997). All over the world OP poisoning in the form of insecticides is a leading cause of loss of life (Eddleston et al., 2006; Namba 1971). Inhibition of AChE is an important toxic action of OP compounds (Silman et al., 2005; Scott 2007). This causes the accumulation of acetylcholine (ACh) at nerve endings resulting in prolonged and intensified action on the effector site (Kassa et al., 2006; Eyer 2003; Wiener et al., 2004; Worek et al., 2002). The standard treatment of nerve agent poisoning includes a muscarinic antagonist e.g. atropine sulphate and a reactivator of inhibited AChE by an oxime. Currently recommended oximes are pralidoxime chloride (2-PAM; 2[{hydroxyiminomethyl]-1-methylpyridinium chloride) and obidoxime chloride [{(1, 1'-oxybis-methylene) bis (4-hydroxyimino) methyl} pyridinium dichloride]. The treatment of OP poisoning must begin within minutes and the antidotes have to be administered with the autoinjectors by deep intramuscular route. Both the drugs atropine sulphate and the oxime are required for an effective treatment (Spohrer et al., 1994; Vijayaraghavan et al., 2007). The recommended dose of atropine sulphate is 2 mg and that of pralidoxime chloride is 600 mg and obidoxime chloride is 220 mg. Though, autoinjectors are available separately for atropine sulphate and for oxime (Spohrer et al., 1994), the drugs can also be combined in the same cartridge unit. Since the dose of pralidoxime chloride is more than that of obidoxime, the former in presence of atropine sulphate becomes highly concentrated. Obidoxime chloride is a stronger reactivator of OP inhibited AChE than the pralidoxime chloride. Obidoxime chloride alone or in combination with atropine sulphate is more potent and effective in antidotal therapy against OP poisoning as compared to pralidoxime chloride (Johnson et al., 2000). At the same time it is also less toxic compared to other oximes. A reusable autoinjector has been designed for combination of atropine sulphate with obidoxime chloride in which once the drug's shelf life expires, the drug cartridge can be replaced with fresh cartridges. Also the same autoinjector device can be reused several times with fresh cartridge, in case of mass casualty management. The present study is aimed to evaluate the in-vitro reactivation of sarin inhibited electric eel AChE by obidoxime chloride in the presence of atropine sulphate in autoinjector cartridges with different preservatives and

septum materials, stored at different temperature i.e. 25°C (room temperature), 40°C and 50°C.

2. MATERIALS AND METHODS

2.1 Chemicals: Sarin (isopropyl methyl phosphono-fluoridate) was synthesised in the Synthetic Chemistry Division and was found to be more than 99% pure by gas chromatographic analysis. Extreme care was taken during the synthesis and storage of sarin, as per approved guidelines of the Institute. Atropine sulphate (>99% pure) was purchased from Merck (Germany). Obidoxime chloride was also synthesised in the Synthetic Chemistry Division according to the reported method and its purity was checked by TLC and was found to be more than 99% pure. Electric eel AChE (ec.3.1.17), 5, 5'-dithiobis 2-nitro benzoic acid (DTNB), acetyl thiocholine iodide methyl-4-hydroxy benzoate (99%) and benzyl alcohol were purchased from Sigma-Aldrich (USA). General chemicals were obtained from Merck (India).

2.2 Preparation of autoinjector cartridges with different preservatives and back-septums

One hundred milligrams of atropine sulphate and 11 grams of obidoxime chloride were weighed accurately and transferred into a 100-ml volumetric flask and brought to volume with sterile water for injection. The atropine sulphate-obidoxime chloride cartridges were prepared with different exicipients (phenol 2% w/v, methyl paraben 0.1% w/v, benzyl alcohol 0.2% w/v). The solutions were filtered through 0.2µm membrane filter (Millipore Corp., Mass, USA) and dispensed in autoinjector drug cartridge. 2.2 ml volume of the above solution was added to each glass cartridge. A convoluted needle was placed inside the glass cartridge. Bromobutyl, neoprene and silicone back septums were used in cartridges while for font seal bromobutyl cap were used (hermedically sealed). The cartridges were sealed with aluminium cap using hand operated crimping device. The drug solution preparation and filling the cartridge was done aseptically in laminar hood and batches of cartridges were stored at 25°C (room temperature), 40^{o}C and 50^{o}C in stability chambers as per the international conference of harmonization guidelines (ICH guideline). The cartridges were stored for one month and then assayed for obidoxime chloride (Radha Sharma et al., 2009; Radha Sharma et al., 2010).

2.3 Reactivation of sarin inhibited electric eel acetylcholinesterase (AChE) by obidoxime chloride: For inhibition of AChE, five units of electric eel AChE (EC.3.1.1.7) were taken in 1.0 ml phosphate buffer (0.1 M; pH 7.6). This was incubated for 10 minutes at 37°C with 2.0

x 10^{-8} M sarin to obtain an inhibition of the enzyme activity in the range of 95-98%. To determine control enzyme activity, similar preparation was made without the addition of sarin. AChE activity was determined using the method of Ellman (Ellman et al., 1961). The enzyme activity was measured by change in absorbance per minute for 4 minutes at 410 nm in Specord-200 UV-Vis spectrophotometer. Percent reactivation was calculated considering control enzyme activity as 100% in presence of obidoxime chloride. Spontaneous reactivation of inhibited AChE was also assayed using same protocol. The reactivation mixture contained enzyme and sarin but no oxime. All assays were carried out in triplicate.

2.4 Statistical analysis

The data are represented as mean ± S.E. They were analysed by one way analysis of variance followed by Student-Newman-Keuls multiple comparison test. A probability of 0.05 and less is taken as statistically significant. Sigma Stat (SPSS. Inc., USA) was used for statistical analysis.

3. RESULTS

In the present study, sarin inhibited the enzyme more than 98%. Table 1 representsents the reactivation efficacy of sarin-inhibited AChE by obidoxime chloride in the presence of various excipients and septum materials after one month of storage at different temperatures. In the presence of methyl paraben or benzyl alcohol the average reactivation of AChE by obidoxime chloride was more than 95% in room temperature with the different back-septum materials, whereas at 50°C the reactivation was less than 95%. But, in the presence of phenol there was a significant degradation both with methyl paraben as well as with benzyl alcohol at all the conditions studied. The reactivation was lesser than 70% in the presence of phenol and the degradation appears to be an abrupt change. There was no significant difference between methyl paraben and benzyl alcohol alone with various septum materials at all the temperatures studied (Radha Sharma et al., 2015).

Table 1- Reactivation efficacy of sarin-inhibited acetylcholinesterase by obidoxime chloride in the presence of selected excipient and septum materials after 1 month of storage.

Excipients	Backseptum materials	Temperature		
		25°C	40°C	50°C
Methyl Paraben	Silicon	96.4±0.24	95.4±0.1	95.1±0.05
Benzyl Alcohol		95.8±0.4	94.7±0.02	94.5±0.02
Methyl Paraben + Phenol		67.7±0.05[a]	66.8±0.05[a]	67.7±0.05[a]

Benzyl Alcohol + Phenol		71.0±0.04[a]	69.1±0.05[a]	68.5±0.05[a]
Methyl Paraben	Bromobutyl	100.9±0.4	97.0±0.17	96.4±0.2
Benzyl Alcohol		101.9±0.28	95.5±0.1	95.6±0.05
Methyl Paraben + Phenol		63.2±0.17[a]	62.7±0.2[a]	64.0±0.05[a]
Benzyl Alcohol + Phenol		76.9±0.05[a]	67.0±0.05 [a]	68.4±0.05[a]
Methyl Paraben	Neoprene	97.6±0.05	95.3±0.1	94.2±0.1
Benzyl Alcohol		93.3±0.05	92.7±0.1	91.3±0.2
Methyl Paraben + Phenol		65.2±0.1[a]	64.6±0.05[a]	65.8±0.05[a]
Benzyl Alcohol + Phenol		65.3±0.1[a]	64.4±0.1[a]	63.8±0.02[a]

Each value represent mean ± SEM, n=3;

a Statistically significant from the corresponding group without phenol.

4. DISCUSSIONS

As in this date Chemical Weapon Convention is signed and ratified by 188 countries and the stockpiled nerve gases are being destroyed. The use of chemical weapon during war may be a less likely, but terrorist organizations may use it, as was demonstrated by the use of sarin gas in Tokyo subway. Therefore preparedness for mass casualty management has to be kept ready always. The preferred antidote for nerve gas is atropine sulphate and an oxime like pralidoxime chloride or obidoxime chloride. They have to be administered by autoinjector in field conditions. So far, currently available autoinjectors consists of atropine sulphate and pralidoxime chloride or obidoxime chloride in separate autoinjectors and are advised to be administered one after other. Both atropine sulphate and the oxime are required, and it practiced for nerve gas management. This takes more time and cause discomfort. If both atropine sulphate and pralidoxime chloride are combined it would be more concentrated. Hence atropine sulphate and obidoxime chloride combination is preferred. The recommended concentration of atropine sulphate, pralidoxime chloride and obidoxime chloride is 1 mg/ml, 300 mg/ml and 110 mg/ml (11%; 0.306 M) respectively. This combination is stable for a minimum of 2 years at room temperature since the combination is stable for one month at 50^{o}C (Lachman, 1986). Atropine sulphate and obidoxime chloride combination was used in this study, and a variety of materials are used in making the cartridges *viz.*, bromobutyl or silicon back septum, needle, borosilicate glass cartridge, silicon oil polishing of backseptums and also the excipients like methyl paraben or benzyl alcohol as stabilizers. Reports are available that atropine sulphate is very stable compound and expected to be stable up to 5 years in room temperature (McEvoy 2002). Reusable auto-injector is beneficial as the device can be used for multiple injections by replacing the drug cartridge during mass casualty management. The present study proves that a combination of atropine sulphate and obidoxime chloride can be used with either one of the stabilizer viz., methyl paraben or

benzyl alcohol and the septum materials like bromobutyl or silicon do not interfere in the stability.

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