The potential role of bioscavenger in the medical management of nerve-agent poisoned casualties

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**ABSTRACT**

The provision of effective Medical Countermeasures (MedCM) for all agents and routes of exposure is a strategic goal of defense research and development. In the case of military autoinjector-based therapies for nerve agent poisoning, current treatment effectiveness is limited by the oxime reactivator being effective against only certain agents, by rapid clearance times of the drugs and because the doses may not be optimal for treatment of severe poisoning. Prolonged poisoning by nerve agents entering the body through the skin is also challenging. Since casualty handling timelines have reduced significantly in recent years, it may be sufficient for first aid therapy to provide protection for only a few hours until further medical treatment is available. Therefore, the traditional evaluation of first aid therapy in animal models of survival at 24 h may not be appropriate. At various echelons of medical care, further therapeutic interventions are possible. The current basis for the medical management of nerve-agent poisoned casualties is derived mainly from clinical experience with pesticide poisoning. Adjunct therapy with a bioscavenger (such as human butyrylcholinesterase (huBChE)), could have utility as a delayed intervention by reducing the toxic load. It has previously been demonstrated that huBChE is an effective post-exposure therapy against percutaneous VX poisoning. It is recommended that the scope of animal models of nerve agent MedCM are extended to cover evaluation of both first aid MedCM over significantly reduced timescales, and subsequent supportive therapeutic and medical management strategies over longer timescales. In addition to bioscavengers, these strategies could include repeated combined and individual therapy drugs to alleviate symptoms, other classes of drugs or ventilatory support.

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1. Introduction

The Strategic Defence and Security Review of 2015 restated the continuing risks faced by the UK including “Chemical and biological attacks against the UK or its forces” which may become more likely and/or have a greater impact over the longer term [1]. Although the use of chemical weapons by state parties is now judged to be less likely than in the past, acquisition by non-state actors may be more likely [2]. The continuing use of chemical agents like sarin and chlorine in Syria [3] reinforces the requirement for effective therapies to counter such highly toxic materials [4,5].

Organophosphorus compounds include pesticides and nerve agents that act primarily by inhibiting the enzyme acetylcholinesterase (AChE, E.C. 3.1.1.7). The primary routes of exposure to these compounds are ingestion, inhalation and absorption through the skin. AChE inhibition by anticholinesterase agents in both the peripheral and the central nervous system leads to an excess of the neurotransmitter acetylcholine (ACh) in the synaptic cleft of cholinergic synapses. Muscles, glands and nerves can become overstimulated by excessive amounts of ACh, producing a range of
toxic effects and ultimately death by loss of respiratory function [6]. Nerve agents are divided into two main groups, G agents and V agents. G agents are more volatile, posing primarily an inhalation hazard, whereas V agents, typified by VX, are more persistent, less volatile and absorption through the skin represents a particularly hazardous exposure route [7]. Exposure to airborne vapour causes an acute cholinergic crisis that may develop within minutes of exposure to nerve agent, and the resultant respiratory failure may be fatal if not treated [8]. In contrast, percutaneous exposure to nerve agents results in a slower onset of poisoning and an extended exposure to toxic agent in the blood [9–12].

2. First aid medical countermeasures

Research into traditional nerve agent antidotal therapy has historically been focussed on the military and has generally been restricted to immediate self- and buddy-aid [13–24]. This is partly because many exposure scenarios have concentrated on acute (inhalation) poisoning with volatile agents. Standard post-exposure therapy for nerve agent toxicity consists of a muscarinic receptor antagonist, usually atropine, combined with an AChE reactivator (oxime), such as pralidoxime, obidoxime or HI-6. A benzodiazepine anticonvulsant (usually diazepam or avizafone, a water-soluble pro-drug which is converted in the body to diazepam) is the usual treatment for control of seizures [7]. The therapy may be supported by a pretreatment with a carbamate AChE inhibitor (pyridostigmine), the action of which is to shield a proportion of AChE from irreversible inhibition by nerve agent [25,26]. Military therapeutic approaches are based on autoinjection devices to enable immediate self- or “buddy”-aid in case of rapid development of signs of poisoning [27].

Atropine is a broad-spectrum therapeutic, acting to competitively displace ACh from muscarinic acetylcholine receptors. The usual autoinjector dose (2 mg) was chosen to be the maximum intramuscular dose compatible with military activities in the event of an autoinjector being used in the absence of nerve agent poisoning [28]. Similarly, the selection of the therapeutic dose of avizafone was heavily influenced by the desire to reduce sedative effects to a minimum following administration of one autoinjector.1

There is a synergistic relationship between atropine and avizafone, and it has been shown that the requirement for avizafone/diazepam can be reduced if the atropine dose is increased [29]. The oxime dose2 was calculated from an animal toxic dose and human metabolic properties to pralidoxime (Table 1). Effective concentrations of diazepam are also only maintained for a limited period [33].

The combination of these factors means that the issued autoinjector therapies have some shortcomings in that limited protection is afforded against agents such as tabun, where the inhibited AChE is resistant to reactivation by the oxime, and soman, where the inhibited AChE ages rapidly. Moreover, relatively rapid clearance of these first aid therapies limits their effectiveness against poisoning when agent exposure is by the percutaneous route [23,38,39]. In these circumstances, it is very likely that additional medical interventions will be needed to manage a nerve agent casualty.

3. Bioscavengers

Stoichiometric bioscavengers are protein molecules that can bind to nerve agents and prevent them from inhibiting central and tissue cholinesterase. These have been widely investigated as a novel, broad-spectrum nerve agent MedCM. For safety reasons, human enzymes have been the preferred candidates, particularly if the concept of use is administration as a pretreatment to healthy (unpoisoned) individuals [40]. It has been shown that the stoichiometric bioscavengers, human AChE and butyrylcholinesterase (BChE, E.C. 3.1.1.8), provided substantial protection when administered as a pretreatment against acute challenge by a range of nerve agents in various animal species; for reviews see Lenz et al. [41], and Nachon et al. [42]. There are, however, some disadvantages to the large-scale pretreatment of individuals with significant quantities of protein. These include cost, possible immunogenicity and the difficulties associated with pretreating at the appropriate time relative to anticipated exposure to toxic agent.

In the case of percutaneous nerve agent poisoning, agent is absorbed gradually from the site of contamination resulting in an extended period over which toxic levels of nerve agent are present in the body [9,12,43]. Although this means that there is potentially a longer opportunity in which to administer post-exposure therapy, it also presents a further challenge in that the therapy must provide a long lasting protection. Although percutaneous VX poisoning can be treated effectively with conventional MedCM, the survival benefit persists only as long as therapeutic levels of the drugs are maintained. For example, Joosen et al. [10] found that, in guinea-pigs, a single dose of atropine, obidoxime and diazepam, administered at the appearance of first signs of poisoning, extended the period to detrimental physiological decline and death for several hours. In contrast, repetitive administration of these drugs on the reappearance of signs remained effective as long as treatment was continued.

This indicates that repeated bolus application or, by inference, continuous infusion of MedCM may protect for long enough that a nerve agent-poisoned casualty could receive further treatment and decontamination as part of the casualty handling chain, but this approach is dependent on sufficient access to supplies of MedCM.

An alternative approach would be to use a therapy in which the protection afforded matched more effectively the time course of poisoning. HuBChE has a long residence time in the blood and better matches the toxicokinetics of a percutaneous nerve agent exposure than the other MedCM components [44] (Fig. 1), meaning that the circulating levels of a percutaneous nerve agent could be

### Table 1

Pharmacokinetic parameters of antidotal therapy drugs in humans.* parameter not stated; derived from graphical data in Ref. [36].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose &amp; route of administration</th>
<th>$t_{max}$</th>
<th>$t_{1/2}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>2 mg, i.m.</td>
<td>~0.3 h$^*$</td>
<td>2.34 h</td>
<td>[36]</td>
</tr>
<tr>
<td>Diazepam</td>
<td>avizafone 20 mg, i.m.</td>
<td>0.75 h</td>
<td>13.9 h</td>
<td>[33]</td>
</tr>
<tr>
<td>Pralidoxime</td>
<td>700 mg, i.m.</td>
<td>0.63 h</td>
<td>2.94 h</td>
<td>[34]</td>
</tr>
<tr>
<td>HI-6</td>
<td>500 mg, i.m.</td>
<td>0.69 h</td>
<td>1.15 h</td>
<td>[35]</td>
</tr>
</tbody>
</table>

$^*$The doses are 10 mg avizafone in the UK Autoject (Combopen®) Nerve Agent Antidote Lab, up to 3 autoinjectors as required, or separate autoinjectors each containing 10 mg diazepam in the US Convulsive Antidote, Nerve Agent (CANA) device.

$^2$500 mg of pralidoxime methane sulphonate in a UK Combopen® or 600 mg of pralidoxime chloride in the US Antidote Treatment Nerve Agent, Auto-Injector (ATNAA).

Following autoinjector administration, rapid absorption leads to $t_{max}$ within 15–60 min for all drug components (Table 1) [33,34]. Atropine and pralidoxime in particular are rapidly eliminated, so effective concentrations are maintained for a limited period only [35–37]. An alternative oxime, HI-6, which is in development as a replacement for pralidoxime in the UK MedCM, has similar pharmacokinetic properties to pralidoxime (Table 1). Effective concentrations of diazepam are also only maintained for a limited period [33].
reduced by the presence of huBChE over many hours. We have previously demonstrated the efficacy of huBChE as a post-exposure, pre-symptomatic therapy and, when in combination with conventional MedCM, as a post-exposure therapy when administered on signs of poisoning following percutaneous exposure to VX in guinea-pigs [44].

The protection afforded by one dose of conventional MedCM (atropine 17.4 mg kg⁻¹, avizafone 3.14 mg kg⁻¹ and HI-6 27.9 mg kg⁻¹) in combination with huBChE (14,980 U kg⁻¹ i.m.), administered on observable signs of cholinergic crisis following percutaneous VX challenge, was 2.24 at 24 h (Price et al., unpublished, Table 2). In contrast, without huBChE, the same MedCM administered as three divided doses, on the appearance of signs of poisoning and subsequently on worsening signs over a total duration of 6–9 h, were unable to protect guinea-pigs to 24 h against 2 × LD₉₀ of VX [45] and protected only 46% of animals to 24 h against 1.2 × LD₉₀ VX (Rice and Price, unpublished observations). These studies demonstrate that bioscavenger-based approaches may have utility for treatment of percutaneous poisoning in post-exposure settings. Early treatment may reduce the requirement for conventional MedCM drugs and the prolonged period of protection may reduce the requirement for additional intensive medical intervention and monitoring.

4. Military timescales for the medical management of nerve agent-poisoned casualties

Within the military environment it is necessary to consider the management of casualties in relation to the military medical chain and in respect to the logistical burden of supplying treatment options at the various echelons. Recent experiences in military theatres such as Afghanistan and Iraq have led to continuous improvement in survival of combat casualties, as a consequence of reduced timelines for evacuation coupled with further-forward deployment of advanced life-saving technology and techniques [47,48]. Penn-Barwell et al. attribute the improvement in outcome to multiple factors, notably “a system that adopts an “end-to-end” approach, blurring the boundaries between point of wounding treatment, prehospital en route care, receiving field hospital management, and in-flight care during repatriation to continuing care in the National Health Service” [47]. Although this statement is derived from experience with conventional battlefield injuries, a large fraction of which were catastrophic trauma cases in which haemorrhage was a major cause of death [49], it is likely to be equally applicable to chemical casualties.

The recognition that shortened casualty evacuation times and the provision of more advanced medical care closer to the point of injury has improved outcomes for survival also has implications for the performance requirements for first aid MedCM against chemical agents. It suggests that these MedCM (self- or buddy-administered autoinjectors) only need to be effective for the time between the recognised “trigger to treat” and reaching further medical support (Fig. 2). As with all first aid, the purpose of this initial drug therapy is to stabilise the casualty until more definitive care can be given, the range and sophistication of which increases through the echelons.

Treatment protocols for casualties who require continued supportive therapy and monitoring following 3 autoinjectors are generally based on clinical experience from organophosphorus pesticide poisoning. The World Health Organisation (WHO) has estimated an excess of 250,000 deaths annually from pesticide poisoning worldwide, and a body of research exists on the treatment [50]. Similarly, treatments used in the rare cases of human nerve agent poisoning in, for example, the Iran-Iraq conflict in 1983–1988 [51] and the Matsumoto and Tokyo sarin attacks in 1994 [52–54], can inform recommendations [55], although the link between treatment and clinical outcome is often not reported clearly.

Atropine is regarded as the mainstay of treatment for OP poisoning. In several clinical case reports the administration of atropine is clearly associated with a reduction of cholinergic signs and a favourable outcome [56]. Non-controlled (i.e. all patients received atropine) clinical studies of OP-poisoned patients have consistently described beneficial effects of giving atropine, however there has been controversy concerning the optimal dose of atropine [56,57]. In the Iran-Iraq war, atropine was almost exclusively used as the supportive therapy for nerve agent-poisoned casualties [51]. The atropine was tittered to effect, based on respiratory status and pulse rate. In some cases of severe poisoning doses of 20–200 mg were used [7]. Although the efficacy of alternative antimuscarinic or anticholinomimetic agents (benactyzine, scopolamine, gacyclidine and huperzine) has been demonstrated in animal models of nerve agent poisoning, none have been evaluated by high quality randomised clinical trials in humans [22,58–61]. It has been suggested that benactyzine may be more suitable for military personnel operating in high temperature environments than atropine [55] because the inhibition of sweating is less than that of atropine. Benactyzine is also more lipid-soluble than atropine which may enhance CNS effects such as seizure termination.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>LD₉₀ µg kg⁻¹</th>
<th>95% confidence interval</th>
<th>Slope estimate</th>
<th>Protection ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>613</td>
<td>551–675</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>huBChE + atropine + avizafone + HI-6</td>
<td>1371</td>
<td>Indeterminate</td>
<td>49.23</td>
<td>2.24</td>
</tr>
</tbody>
</table>

LD₉₀ values determined in guinea-pigs following percutaneous VX challenge. Saline control or therapy was administered i.m. on observable signs of systemic cholinergic poisoning (0.5–9 h, dose-dependent). No other medical intervention was given. Price et al., unpublished.
For OP pesticide poisoning, the WHO has published a recommendation to use oximes to treat all symptomatic patients who need atropine; however, the effectiveness of oximes in human OP pesticide poisoning has been widely debated and further evidence is needed to support dosing strategies. As is the case for atropine, there are very limited reports of the use of oximes in victims of nerve agent poisoning. Reports of the Matsu-moto incident in Japan do not discuss the use of oximes; however,
pralidoxime methiodide (PAM) was used in the Tokyo incident and was reported to be effective [53]. For the Iranian tabun and sarin casualties, use of the limited supplies of obidoxime was reserved for patients in critical condition and was restricted to emergency units and field hospitals. Although no data on clinical outcomes are provided, it is implied that the treatment was successful: ‘reaction was generally immediate and complete’ [51].

Benzodiazepines such as diazepam are the recommended anticonvulsants for patients presenting with seizures and other CNS symptoms. Although controlled studies in humans are lacking, there is sufficient evidence for efficacy that it would in fact be unethical to conduct a trial comparing a benzodiazepine with a placebo [67]. Diazepam was used to treat victims of both the Matsumoto and Tokyo sarin attacks, and was reported to control convulsions and seizures [53]. Foroutan reported using diazepam as an anticonvulsant as well as a muscle relaxant in the Iranian casualties, but the incidence of seizures was not described [51].

Consideration should be given to the use of a bioscavenger such as huBChE in higher echelon care by intravenous administration by medically-trained personnel. For a number of reasons, deployment at advanced medical care stations is more realistic than wider availability for self-or buddy-aid. Although a lyophilised preparation is in development, present formulations require maintenance of cold-chain storage to ensure stability of this protein. The high cost of plasma-derived huBChE may also limit its availability [42] and therefore targeted use in the medical management of these casualties would enable more effective use of this limited resource. An indication for the use of bioscavenger in medical management of nerve agent poisoned casualties may be prolonged reliance on conventional pharmacological medical management, which may be indicative of percutaneous poisoning. In such cases bioscavenger administration may reduce the circulating toxic load of nerve agent in the body and reduce reliance on pharmacological therapy. A similar concept of use has recently been discussed by Lockridge [68], who reviewed three cases of organophosphorus pesticide poisoning in which purified human plasma BChE was used.

Contaminated casualties may pose a risk to responders, so hazard management will routinely include external and wound decontamination. The requirement to decontaminate will depend on the physical properties and persistence of the agent; removal of clothing may be all that is required following vapour exposure to non-persistent agent. In cases of percutaneous poisoning effective decontamination would improve the clinical outcome of the nerve agent casualty by limiting the dose received [69], but the detection and decontamination of very small amounts of highly toxic material will be challenging. Decontamination of liquid agent may involve dry or wet decontamination. Dry decontamination relies on adsorption of liquid contaminant by powders (e.g. Fuller’s Earth, M291 skin decontamination kit). Bleach solution (0.5% aqueous sodium hypochlorite), reactive decontamination products (e.g. RSDL) or copious amounts of water can be used for wet decontamination [69,70]. Depending on the particular operation, the decontamination of the casualty could occur before or after initial evacuation, but should not interrupt or delay medical treatment [71]. There may be a requirement to carry out life-saving interventions before any decontamination takes place.

5. Animal models of first aid and medical management

The guinea-pig is widely used as a small animal model for proof-of-principle MedCM studies to OP poisoning. Non-human primates or pigs are considered appropriate models for pivotal efficacy studies [20,26,72–76]. Each species has advantages and disadvantages that need to be considered in the interpretation of results and extrapolation to man [20,77–79]. Animal studies of nerve agent MedCM efficacy have conventionally used survival at 24 h as an end-point [16,17,59,80,81]. In these studies, treatment provided good protection from acute nerve agent poisoning, but in some groups mortality increased sharply between 4 h and 24 h. An earlier end-point which is based on likely casualty processing times (e.g. 4–6 h) would be more appropriate for identifying effective first aid MedCM and would be less likely to exclude useful treatments that have a short duration of effectiveness. Assessment of the integration of first aid therapy with supportive medical care has not been undertaken. Pigs have been used in experimental treatment studies for injury caused by other toxic chemicals [82–87], but less extensively in nerve agent therapy evaluation [88–90]. There are research studies in pigs and clinical trials information for treatment strategies following organophosphate pesticide poisoning. Typically these studies and clinical cases involve ingestion of the OP and therefore result in a delayed onset of signs and symptoms of poisoning. This is similar to that observed following percutaneous nerve agent poisoning [50,91–93]. The adaptation of these pig models to assess both first aid and continued medical management could be used to further investigate the utility of huBChE as a delayed, post-exposure therapeutic intervention. Other therapeutic strategies that could be explored in the model include, for example, novel reactivators (oximes) [54,55], anticonvulsants [59,66], antinicotinics [57,58], GABA- or glutamate antagonists [59–101] and neuroprotectants [102].

6. Conclusions

It is likely that following any exposure to nerve agents in a military environment a combination of first aid and further medical intervention would be required; this is particularly relevant in cases of nerve agent absorbed by the percutaneous route. Research into medical countermeasures has previously focussed primarily on first aid interventions for nerve agent poisoning. We suggest that there is a requirement to extend the scope of current therapeutic interventions for nerve agent poisoning in animal models to encompass continued management of the “casualty” through the medical chain. This has implications for the animal models in use, and also the time points for both intervention and assessment of efficacy. The timescales for evaluation of first aid therapy should reflect the realistic duration over which therapy is expected to be effective. Subsequent therapeutic interventions should reflect the capabilities available at the various levels of medical care. It is proposed that bioscavengers such as huBChE may have utility in the medical management of nerve agent-poisoned casualties. This is primarily as an adjunct to other drug-based, or other therapeutic interventions. In particular, the use of bioscavengers has particular relevance to the treatment of percutaneous nerve agent poisoning.

Acknowledgements

We are grateful to Surgeon Commander Steven Bland and Dr Stevan Emmett for comments on the UK Armed Services protocols. HuBChE was supplied for research use by USAMRDC, under an Equipment & Materials Transfer Memorandum of Understanding. This work was funded by the UK Ministry of Defence. Crown Copyright © [2016] Published by Elsevier Ireland Ltd. This is an open access article under the Open Government Licence (OGL) (http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/).

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Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.cbi.2016.04.038.
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