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REVIEW ARTICLE

Possible role for anisodamine in organophosphate poisoning

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In cases of organophosphate poisoning, patients are treated with a combination of antidotes. In addition to these poison-directed antidotes, patients may require extra oxygen and artificial ventilation; other modalities may also be needed due to the wide range of toxic effects. Anisodamine is a belladonna alkaloid, and like other drugs from this family is non subtype-selective muscarinic, and a nicotinic cholinoceptor antagonist, which has been employed in traditional Chinese medicine. As a muscarinic antagonist, it displays similar pharmacological effects to atropine and scopolamine. However, anisodamine is not only less potent than atropine and scopolamine but also less toxic. Current *in vitro* and animal model studies have demonstrated that anisodamine has protective effects in a variety of diseases. Organophosphate poisoning involves not only the central and peripheral nervous systems, but also the cardiac and respiratory systems, as well as activation of inflammatory processes and oxidative stress. Therefore, the anticholinergic and additional activities of anisodamine appear to be relevant and justify its consideration as an addition to the existing remedies. However, more research is needed, as at present data on the role of anisodamine in the management of organophosphate poisoning are limited. Here, we review the beneficial effects of anisodamine on processes relevant to organophosphate poisoning.

Abbreviations

α7nAChR, α7 nicotinic ACh receptor; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; mAChRs, muscarinic ACh receptors; nAChRs, nicotinic ACh receptors; OP, organophosphate; PMVEC, pulmonary microvascular endothelial cells; QNB, 3-quinuclidine-benzylate; Th1/2, T-helper cells 1 and 2; TSST-1/2, toxic shock syndrome toxin type-1/2

Tables of Links

TARGETS	
GPCRs[a]	**Ligand-gated ion channels**[b]
α_1-adrenoceptor	α7 nicotinic ACh receptor (α7nAChR)
M_1 receptor	**Enzymes**[c]
M_2 receptor	AChE
M_3 receptor	

LIGANDS	
ACh	LPS
Atropine	Methylprednisolone
Dexamethasone	Neostigmine
IFNγ	Pralidoxime
IL-1β	QNB
IL-4	Rivastigmine
IL-10	Scopolamine
IL-17	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c]Alexander *et al.*, 2015a,b,c).

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Introduction

In cases of organophosphate (OP) poisoning, it is accepted that patients should be treated with a combination of an anti-cholinergic drug, usually atropine, an oxime as a cholinesterase reactivator, and if the patient develops convulsions and seizures, a benzodiazepine such as diazepam or midazolam is added (Eddleston *et al.*, 2006, 2008; Rosman *et al.*, 2014). Together with the abovementioned drugs, it is important to use supplemental oxygen and ventilate the casualty if needed (Markel *et al.*, 2008; Sawyer *et al.*, 2012). However, too high a dose of atropine may lead to excessive anticholinergic toxicity, which may even be fatal (Eddleston *et al.*, 2004; Sidell *et al.*, 2008; Wang *et al.*, 2014b).

Anisodamine is a belladonna alkaloid, and like other drugs from this family is a non-subtype-selective muscarinic, and also a nicotinic cholinoceptor antagonist (Zhao *et al.*, 1993). It has been employed in traditional Chinese medicine for many ailments, mainly to improve the microcirculation in states of shock, and also in organophosphate poisoning (Poupko *et al.*, 2007; Wang *et al.*, 2014a, b). However, the clinical toxicological information available on anisodamine in western countries is limited. As a muscarinic antagonist, it displays similar pharmacological effects to atropine and scopolamine, including inhibition of secretion (e.g. salivation, diaphoresis and respiratory secretions), bronchodilation, inhibition of gastrointestinal motility and changes in cardiovascular function (Anon. 1975; Zang *et al.*, 1987; Guo *et al.*, 1992a; Pan and Han, 2000; Wang *et al.*, 2003a; Pan and Han, 2004). Several studies have shown that anisodamine is at least one order of magnitude less potent than atropine and scopolamine (Anon. 1975; Zang *et al.*, 1987; Guo *et al.*, 1992a; Pan and Han, 2000; Wang *et al.*, 2003a; Poupko *et al.*, 2007). Relatively little clinical information is available on its human toxicity. Based on animal studies, it seems to be less toxic than atropine and scopolamine (Poupko *et al.*, 2007). The usual treatment dose in humans is 10 $mg \cdot kg^{-1}$, yet doses as high as 500 $mg \cdot kg^{-1} \cdot day^{-1}$ did not produce any serious adverse effects (Li *et al.*, 1999). The lower potency of anisodamine and the finding that it is less likely to impair learning and memory when compared with atropine are probably related to its decreased lipid solubility and limited blood–brain barrier permeability (Zhang, 2002). Current *in vitro* and animal model studies have demonstrated that anisodamine has beneficial effects in a variety of disease conditions of different aetiologies and in various organs. These include cardiac arrhythmias (Chen and Gu, 1988; Yang *et al.*, 1991), acute lung injury (Zhang *et al.*, 2005), asthma (Xu *et al.*, 2011), arthritis (Zhou *et al.*, 2014) and septic shock (Zhao *et al.*, 2011). It was found to be protective at cellular, organ system and animal levels in inflammation (Liu *et al.*, 2009; Xu *et al.*, 2011; You *et al.*, 2014), oxidative stress (Luo *et al.*, 1992; Esberg and Ren, 2004; Liu *et al.*, 2013), coagulopathy (Xiu *et al.*, 1982; Ruan *et al.*, 2001, 2002) and molecular signalling pathways (Norby and Ren, 2002; Xing *et al.*, 2015).

OP poisoning, either by pesticides or chemical warfare nerve agents, is characterized by a complex toxidrome involving more than one body system – mainly the central and peripheral nervous systems and the cardiorespiratory system (Markel *et al.*, 2008; Sidell *et al.*, 2008; Hulse *et al.*, 2014). In fact, regulation of all organ systems involves ACh and its receptors (Wessler and Kirkpatrick, 2008). Furthermore, the pathophysiology of OP poisoning involves, in addition to cholinergic effects, inflammatory processes and oxidative stress in the affected organs (Collombet, 2011; Banks and Lein, 2012). In this manuscript, the effects of anisodamine on these processes will be reviewed in order to present the broad spectrum of potential beneficial effects relevant to the management of OP poisoning, starting with the studies on the use of anisodamine in the management of OP poisoning in humans and in counteracting the toxicity of related pharmacological agents in animals.

Anisodamine as an antidote in OP poisoning

Anisodamine has long been considered as an antidote in OP poisoning in China and Tibet (Poupko *et al.*, 2007; Wang *et al.*, 2014a, b). As a muscarinic antagonist, it has similar effects to those of atropine and scopolamine, including reducing salivation, lacrimation and sweat, diminishing gastrointestinal motility, mydriasis, bronchoconstriction and cardiovascular changes characteristic of the toxicity of muscarinic agonists (Poupko *et al.*, 2007; Wang *et al.*, 2014a, b). It is less potent than scopolamine at inducing central effects (Poupko *et al.*, 2007; Zhang *et al.*, 2008, 2009), and in septic shock, it was reported to show fewer adverse effects than atropine (Li *et al.*, 1999). In a neuropsychopharmacological study in mice, anisodamine did not cause spatial cognitive deficits and even improved cognition at repeated high doses, based on Morris water maze test, and did not depress LTP recordings in rats, while scopolamine has detrimental effects on both activities (Zhang *et al.*, 2008). This difference in *in vivo* effects was attributed by the authors to the poor blood–brain barrier permeability of anisodamine compared with scopolamine, with estimated octanol–water partition coefficient (log*P*) values of 0.25 and 0.76, respectively (Zhang *et al.*, 2008). These differences could also be attributed in part to its lower binding affinity to brain mAChRs compared to scopolamine; this was measured in mouse brain homogenates by competitive displacement of [^{3}H]-quinuclidinyl benzilate (QNB), showing IC_{50} values of 72.0 ± 1.7 and 0.18 ± 0.02 μM for anisodamine and scopolamine, respectively (Zhang *et al.*, 2008). This is important in case of blood–brain barrier dysfunction, which was shown to occur in patients with severe CNS toxicity due to nerve agents or OP pesticides (Abdel-Rahman *et al.*, 2002; Song *et al.*, 2004; Testylier *et al.*, 2007).

The potential of anisodamine in ameliorating systemic cholinergic toxicity is evident from studies in a mouse model of cognitive impairment, where a cholinergic deficiency was induced by a high dose of scopolamine to mimic human Alzheimer's disease (Wang *et al.*, 2003a; Zhang *et al.*, 2009). In an earlier study, this cognitive impairment could be overcome by administration of the muscarinic agonist pilocarpine (20 or 40 $mg \cdot kg^{-1}$ i.p. twice daily), but this was accompanied by typical muscarinic side effects of salivation, enhanced bowel movement and bradycardia. However, these were ameliorated by 20 $mg \cdot kg^{-1}$ anisodamine, with similar cognitive improvements (Wang *et al.*, 2003a).

The same research group showed later that administration of anisodamine to mice injected with scopolamine and treated with the AChE inhibitor rivastigmine counteracted

the muscarinic and neuromuscular nicotinic-mediated adverse effects of rivastigmine, which included hypersalivation, hyperperistalsis and muscle cramps, while facilitating cognitive amelioration (Zhang *et al.*, 2009). This may indicate a potential role for the combination of both drugs as a prophylactic treatment against OP poisoning, nerve agents in particular. We have previously shown that rivastigmine can act as a prophylactic drug in nerve agent poisoning in animal models. However, in a human safety study (phase I), we observed that it had adverse effects, which would prevent its use as a single compound for such an indication (Lavon *et al.*, 2015). The results presented here indicate that the combination of anisodamine and rivastigmine could serve as a future prophylactic mixture. This is yet to be studied.

As a cholinergic antagonist, anisodamine can inhibit cholinergic hyperexcitability, reduce hypersecretion, stimulate the respiratory centre, relax the spasm of smooth muscle, accelerate the heart rate and abolish other muscarinic symptoms (Wang *et al.*, 2014a). Wang *et al.* (2014a) described a patient with severe OP poisoning. She was admitted in a moderate clinical condition, and despite receiving the accepted treatment protocol, within 4 h from admission, her clinical condition had deteriorated, and she was intubated and ventilated. Twenty-two hours after being poisoned, she received a total of 960 mg atropine i.v., 3.25 g pralidoxime i.v., and her serum AChE activity had changed from 59% on admission to 72% of the normal activity level, but she still needed mechanical ventilation. At this stage, treatment with anisodamine was initiated. Four hours later, 26 h after being poisoned, a total of 480 mg anisodamine and 0.5 g pralidoxime was given i.v., AChE activity was restored to 75% of normal range, and her blood gases returned to normal. On the following day, she received four more doses of anisodamine, 20 mg each. Her clinical condition gradually improved, 5 days post exposure she was extubated, and on day 9, she was released from hospital. Anisodamine was given for 3 days. This case report shows that anisodamine had a better clinical outcome than high and repeated doses of atropine (Wang *et al.*, 2014a).

The same group of researchers performed a retrospective analysis of 64 OP-poisoned patients (Wang *et al.*, 2014b). All of them received the routine, accepted antidote treatment, consisting of repeated atropine and pralidoxime doses, yet in all, atropinization was not achieved after 12 h, following high doses of atropine. Thirty-six patients continued with the original atropine treatment protocol, while 28 started an anisodamine treatment protocol at this stage. In the atropine group, time to atropinization was 29 ± 7.0 h, compared with 24.3 ± 4.3 h in the anisodamine group ($P < 0.05$). Hospital stay in the anisodamine group was 5.3 ± 2.5 days compared with 6.9 ± 2.3 days in the atropine-only group ($P < 0.05$). This study indicates that anisodamine can shorten the time to atropinization and reduce the hospital stay of OP-poisoned patients. The time to reach complete atropinization is critical, as a delay in atropinization may increase the likelyhood of the establishment of irreversible damage. Furthermore, there are still concerns regarding the toxicity of the administration of high doses of atropine (Eddleston *et al.*, 2004). A comparison of existing atropine treatment protocols in OP poisoning has shown that the protocols are poorly evidence-based, highly variable between published sources, and most of them are too slow to achieve the desired atropinization dose in time (Eddleston *et al.*, 2004). A follow-up study has shown some change towards faster dosing protocols, but the variability still exists (Connors *et al.*, 2014), which may be one of the reasons why atropinization is difficult to attain in some severely poisoned patients (Kaur *et al.*, 2014; Iyer *et al.*, 2015). Recent reviews covering novel and alternative ways for treating OP poisoning (Kaur *et al.*, 2014; Iyer *et al.*, 2015) have supported the Wang *et al.* (2014a,b) proposal that anisodamine can be used as a safe adjunct or substitute to atropine, for cases where atropinization is difficult to achieve.

Anti-oxidant and membrane effects of anisodamine

Anisodamine is known to possess an anti-oxidant action and can protect against free radical-induced cellular damage. It has been shown to interact and disrupt liposomes similar to the cell's membrane lipid bilayer, by increasing their fluidity (Hwang *et al.*, 1983). Anisodamine was found to interact with membrane acidic phospholipids, which play an important role in neural cells. This may explain its ability to protect nerve cells (Wang *et al.*, 1993). Luo *et al.* (1992) have shown that anisodamine exerted a protective effect in cultured bovine pulmonary endothelial cells from injury induced by oxygen free radicals (Luo *et al.*, 1992). The authors noted that there was no evidence of any scavenging effects of anisodamine and, therefore, these findings also support the hypothesis that anisodamine is a membrane stabilizing compound (Luo *et al.*, 1992). In a swine model of cardiac arrest, Liu *et al.* (2013) found that anisodamine-treated animals had significantly lower plasma levels of malondialdehyde, elevated levels of superoxide dismutase and ATP and lower levels of mitochondrial ROS, when compared with controls and an adrenaline-treated group (Liu *et al.*, 2013). Mitochondrial injury, observed using transmission electron microscopy, was milder than in the other two groups. They concluded that anisodamine alleviates mitochondrial oxidative-induced injury in the myocardium by regulating the metabolism of ROS.

These properties of anisodamine, together with its anticholinergic effects, may add to its value as a therapy for OP poisoning, as an antioxidant treatment has already been shown to be beneficial in such cases (Elsinghorst *et al.*, 2013).

Role of anisodamine in the regulation of immune and inflammatory responses

There is growing evidence that the cholinergic anti-inflammatory pathway has an important role in regulating both immune and inflammatory responses. Activation of mAChRs and nAChRs stimulate and inhibit the immune and inflammatory responses, respectively (Razani-Boroujerdi *et al.*, 2008). The cholinergic anti-inflammatory pathway denotes the interaction between the nervous and the immune systems, specifically between the efferent vagus nerve and peripheral α7 nicotinic ACh receptors (α7nAChRs) expressed on monocytes and macrophages (Pavlov *et al.*, 2003; Rosas-Ballina and Tracey, 2009). Through vagal release of ACh, the

nervous system can significantly inhibit the ability of macrophages to release TNF-α, thus attenuating the systemic inflammatory responses (Wang *et al.*, 2003b; Rosas-Ballina and Tracey, 2009). In the CNS, signalling through α7nAChRs in microglia has an anti-inflammatory effect, similar to the effect found in macrophages (Shytle *et al.*, 2004). As a muscarinic receptor antagonist, anisodamine may lead to increased ACh-mediated activation of α7nAChR, together with muscarinic receptor inhibition, which in turn activates the cholinergic anti-inflammatory pathway and controls cytokine production (Liu *et al.*, 2009; Zhao *et al.*, 2011). Anisodamine was shown to significantly reduce TNF-α and IL-1β production in both *in vitro* and *in vivo* models of LPS-induced inflammation and shock (Liu *et al.*, 2009). The proposed mechanism was increased activation of α7nAChR by endogenous ACh, diverted from the anisodamine-inhibited muscarinic AChR (Liu *et al.*, 2009). A later study from this group suggested that anisodamine acts synergistically with IL-10 to up-regulate the expression of α7nAChR (Li *et al.*, 2011). However, as not all of the effects of LPS were abolished by a selective α7nAChR antagonist, other mechanisms besides the cholinergic anti-inflammatory pathway, such as antagonism of adrenoceptors by large doses, could not be ruled out (Liu *et al.*, 2009). Moreover, the ability of atropine to protect mice from LPS-induced lethality was found to be independent of IL-10 (Fuentes *et al.*, 2008), thus pointing to more than one mechanism of protection by cholinolytics. The combined treatment of anisodamine with the peripheral reversible cholinesterase inhibitor neostigmine was more effective than each drug alone in protecting rats from LPS lethality and in reducting blood levels of TNF-α and IL-1β and from haemorrhagic shock in beagle dogs (Sun *et al.*, 2012). This combination allowed both drugs to be used at a lower dose, so reducing the adverse effects of neostigmine, and was also found to be protective in other models of severe or debilitating disease involving inflammation, such as obstructive jaundice, collagen-induced arthritis and ischaemic stroke (Li *et al.*, 2014; Zhou *et al.*, 2014; Qian *et al.*, 2015).

Anisodamine was found to protect animals from toxic shock syndrome toxin-1 (TSST-1) induced by *Staphylococcus aureus* (Nakagawa *et al.*, 2005) and from Shiga toxin type 2 (Zhang *et al.*, 2001). In both studies, the protective effect involved the down-regulation of pro-inflammatory cytokines like TNF-α, and in the case of TSST-1, the effect was mediated by inhibition of NF-κB activation (Nakagawa *et al.*, 2005).

In a model of brief forebrain ischaemia–reperfusion in gerbils, a condition involving inflammation, treatment with anisodamine resulted in a large number of viable neurons in the CA1 subfield in the hippocampus, compared with the untreated group (Chen and Zeng, 2000). The researchers also performed the open-field test as a sensitive tool for the assessment of memory deficits resulting from hippocampal cell loss. Anisodamine reduced the open-field habituation impairment in the ischaemic animals. Moreover, anisodamine appeared to inhibit the production of hydroxyl radicals (Chen and Zeng, 2000). Inflammation and oxidative stress are hallmarks in OP-induced CNS pathology (Collombet, 2011; Banks and Lein, 2012; Iyer *et al.*, 2015). The anti-inflammatory, antioxidant and cell-membrane membrane-protective properties of anisodamine are consistent with its neuroprotective role in this case.

Pulmonary effects

The most imminent threat in OP poisoning results from pulmonary insufficiency. This is the result of hypersecretion, respiratory muscle paralysis and bronchoconstriction (Eddleston *et al.*, 2006; Markel *et al.*, 2008; Sidell *et al.*, 2008; Hulse *et al.*, 2014; Rosman *et al.*, 2014). Moreover, damage to the respiratory centres of the brain adds to the respiratory compromise, as it is well known that severe brain injury, including in cases of OP poisoning, is often associated with pulmonary insufficiency (Eddleston e*t al.*, 2006; Carey *et al.*, 2013). The immediate local pulmonary effects of OPs – bronchoconstriction and hypersecretion – are reversed by atropine and scopolamine (Nambiar *et al.*, 2007; Perkins *et al.*, 2011a,b), indicating that a local excess of ACh due to inhibition of cholinesterases is involved in this effect (Graham *et al.*, 2006). Similarly, in chronic respiratory diseases like asthma and chronic obstructive pulmonary disease, respiratory symptoms are mediated by ACh (Gosens *et al.*, 2006) and modulated both experimentally and clinically by anticholinergic drugs (Moulton and Fryer, 2011).

As a non-selective muscarinic antagonist, anisodamine was found to alleviate acute lung injury (ALI) induced by a variety of causes in animal models. Both clinical and experimental lung injuries can be induced by direct insults like aspiration, pneumonia or inhalation of toxic gases, or indirectly by trauma, sepsis and systemic inflammatory response in general (Perl *et al.*, 2011; Matthay *et al.*, 2012). The effect of anisodamine in clinical secondary acute lung injury was demonstrated by Huang *et al.* (2002), who showed that patients with brain damage treated with anisodamine in addition to the standard therapy had improved pulmonary function and increased alveolar and arterial oxygenation, when compared with patients receiving standard care alone (Huang *et al.*, 2002). The authors linked this effect to anisodamine-induced inhibition of TNF-α and IL-6, which are regarded as mediators of such an injury.

The LPS of gram-negative bacteria can lead to ALI through both direct and indirect effects (Menezes *et al.*, 2005; Perl *et al.*, 2011). This includes direct injury to endothelial cells and indirect injury due to activation of inflammatory cells. The injured tissue and activated inflammatory cells release pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-17, specifically IL-17A and IL-17F, leading to additional leukocyte recruitment and damage in the inflamed airways and lungs (Ferretti *et al.*, 2003; Miyamoto *et al.*, 2003; Yang *et al.*, 2008; Prause *et al.*, 2009). The involvement of the cholinergic system in LPS-induced lung injury was demonstrated directly by Xu *et al.* (2012). They showed that pulmonary dysfunction and inflammatory responses in mice treated intratracheally with LPS could be attenuated by either α7nAChR agonist or the non-subtype selective muscarinic antagonist atropine or the specific M_3 receptor antagonist 4-DAMP but not specific M_1 or M_2 receptor agonists, thus showing the role of the M_3 subtype in induction of lung injury in this model. Activation of pulmonary microvascular endothelial cells (PMVECs) is considered to be an early step in the inflammation process of ALI (Wu *et al.*, 2008). You *et al.* (2014) performed an *in vitro* study in rat PMVECs and an *in vivo* study in a rat model of LPS-induced ALI to test whether anisodamine has a modulatory effect and further to see if it

involves IL-17 expression. They showed that anisodamine reduced lung injury and the expression of IL-17A and IL-17F in PMVECs and in rats following exposure to LPS. Furthermore, the reduction in lung injury and IL-17A/IL-17F production in LPS-treated animals were comparable with that of the known anti-inflammatory drug methylprednisolone. Studies with anti-inflammatory agents showed promising results in preclinical models and small-scale clinical studies but did not provide conclusive evidence of efficacy in larger clinical trials, prompting further research into this important strategy (Matthay *et al*., 2012). Clinical studies with methylprednisolone showed improvement in clinical parameters but no conclusive evidence of reduced mortality (Meduri *et al*., 1998, 2002, 2007). The results of You *et al*. (2014) indicate a possible role for anisodamine in the treatment of sepsis-induced ALI, prompting additional research to investigate this. Anisodamine was found to be protective in other animal models of indirect ALI. In a canine cardiopulmonary bypass model, anisodamine reduced lung injury by significantly reducing lung water content, pulmonary granulocyte sequestration and oxygen free-radical release compared with untreated controls (Gu *et al*., 1991). Anisodamine also had a protective effect in lungs in a rabbit lung injury model following exposure to oleic acid (Deng *et al*., 1989).

The protective effect of anisodamine in injury mediated by inhalation of lung-damaging toxic industrial chemicals was demonstrated after inhalation of the toxic industrial compound perfluoroisobutylene (PFIB), an electrophilic toxic compound similar to phosgene but with higher potency (Zhang *et al*., 2005). Lung injury by this compound results from both direct and indirect mechanisms. The direct injury is probably due to PFIB's electrophilic attack on nucleophilic groups of amino acids in the alveolar membranes (Zhang *et al*., 2005) and an impaired lung microcirculation contributes to the development of ALI. The major indirect effects result from neutrophil sequestration and accumulation, key factors involved in ALI in general (Grommes and Soehnlein, 2011; Perl *et al*., 2011; Matthay *et al*., 2012; Williams and Chambers, 2014) and together with other factors contribute to the injury evoked by PFIB and the resulting ALI (Zhang *et al*., 2005). Zhang *et al*. have shown that in a rat model of PFIB-induced ALI, high doses of anisodamine (30 $mg \cdot kg^{-1}$) attenuated pulmonary oedema (decreased wet lung/body weight ratio, dry lung/body ration and total protein concentration) (Zhang *et al*., 2005). Similar effects and also a reduction in neutrophil recruitment, oxidative stress, ultrastructural changes in alveolar epithelial cells and decreased mortality, were observed in PFIB-exposed mice treated with the more potent cholinolytic agent QNB (Zhang *et al*., 2005), suggesting a role for cholinolytic agents, including anisodamine, in the management of chemically-induced lung injury.

In a mouse model of ovalbumin-induced asthma, anisodamine was shown to significantly suppress the peribronchial and perivascular infiltration and accumulation of eosinophils in the airways, similar to the effects of atropine and dexamethasone (Xu *et al*., 2011). This indicates the probable involvement of muscarinic receptors in airway inflammation following eosinophil infiltration. This model of airway reactivity includes an increase in the contractile responses to ACh. As with dexamethasone, this increase was significantly inhibited by anisodamine. Anisodamine also attenuated airway epithelial hyperplasia and thickening of the basement membrane. Because anisodamine prevented bronchial contractile responses only following ovalbumin challenge, this may indicate that ACh affects inflamed airway smooth muscle. ACh is known to promote the release of cytokines from epithelial and smooth muscle cells during inflammation, which in turn attract inflammatory cells to the airways and induce remodelling of the tissue (Gosens *et al*., 2006; Xu *et al*., 2011; Kolahian and Gosens, 2012). In this study, anisodamine had a similar effect as atropine in that it attenuated eosinophilic airway inflammation and affected the balance of Th1/Th2 lymphocytes, suppressing the Th2-mediated immune response and enhancing the Th1-mediated immune response (down-regulation of Th2-associated IL-4 and up-regulation of Th1-associated IFNγ, respectively) (Xu *et al*., 2011). This suggests that ACh is involved in these immune inflammatory responses, as also shown by Wen *et al*. (2006). More published data indicate that the cholinergic system has a role in immune responses and inflammation and because muscarinic receptors are involved in the regulation of Th1/Th2 (Wen *et al*., 2006; Razani-Boroujerdi *et al*., 2008; Qian *et al*., 2011; Hwang *et al*., 2013), anisodamine and other muscarinic antagonists may act as important modulators of inflammation through their influence on non-neural cholinergic systems. The immune-modulatory effects of anisodamine might be mediated by affecting the balance of muscarinic and nicotinic receptor activation.

The inhibition of inflammatory processes and resulting amelioration of lung injury by α7nAChR agonists was shown in several models of ALI, demonstrating the role of cholinergic signalling through this receptor in the control of pulmonary inflammation (Wu *et al*., 2014). Taken together, these recent findings and the beneficial effects of anisodamine through α7nAChR in models of septic shock endorse the potential of anisodamine in the therapy of acute injury and chronic pulmonary diseases.

Another alternative mechanism is that by inhibiting the M_2 inhibitory ACh receptors on cholinergic nerve terminals, anisodamine contributes to the release of ACh in the cholinergic anti-inflammatory pathway (Rosas-Ballina and Tracey, 2009). A reduction in M_2 receptors has been shown to contribute to a hypercholinergic immunosuppressive state, both in the brain and in the periphery (Allon *et al*., 2011).

Early in acute respiratory distress syndrome (ARDS), activated neutrophils are sequestered in the lungs and produce ROS and other products leading to lung tissue damage (Grommes and Soehnlein, 2011; Williams and Chambers, 2014). This is an important mechanism in the initiation of ARDS, and anisodamine may help in preventing any further deterioration. Anisodamine seems to possess both anti-oxidative and superoxide scavenging activities and was used in both experimental and clinical cases of ARDS (Yu *et al*., 1985; Luo *et al*., 1987, 1992). As mentioned earlier, anisodamine protects tissues from oxygen free-radical-induced injury probably through its membrane-stabilizing properties (Luo *et al*., 1992).

In an early study on systemic and pulmonary circulations in rabbits, anisodamine was found to dilate both pulmonary and systemic vessels, even though the effect was defined as relatively weak (Fan *et al*., 1986).

Cardiovascular effects

Current evidence shows that anisodamine depresses cardiac conduction and also has a dose-dependent anti-arrhythmic effect (Chen and Gu, 1988; Yang *et al.*, 1991). Anisodamine inhibited cardiomyocyte contractility and the transient rise in intracellular Ca^{2+} concentration and also depressed cardiomyocyte contraction invoked by the muscarinic agonist carbachol (Norby and Ren, 2002). Anisodamine inhibited the uptake of Ca^{2+} by the skeletal muscle sarcoplasmic reticulum Ca^{2+} ATPase, possibly by inducing a structural modification of the transmembrane domain of the protein, which is the site of Ca^{2+} binding (Pang and Chen, 2004). This finding may provide an explanation for the modulating effect of anisodamine on cardiac function (Poupko *et al.*, 2007). Arrhythmias may occur as delayed effects of OP poisoning, and these may be fatal (Allon *et al.*, 2005; Bar-Meir *et al.*, 2007). In rats surviving sarin poisoning (exposure to ~LCt_{50}), an acute and temporary QT prolongation was observed initially, followed by a long-lasting increased sensitivity to adrenaline-induced arrhythmias (Allon *et al.*, 2005, 2011). Anisodamine was shown to decrease the adrenaline-induced ventricular tachycardia in rabbits (Chen and Gu, 1988; Poupko *et al.*, 2007). These findings warrant further studies on the role of anisodamine in controlling OP-related arrhythmias.

Other direct protective effects of anisodamine on the heart stem from its antioxidant properties. Protection of the myocardium and myocardial mitochondria from oxidative damage in ischaemia–reperfusion was discussed above (Liu *et al.*, 2013). Anisodamine attenuated the inhibition of cardiomyocyte contractility mediated by oxidative stress, invoked by the peroxide radical donor pyrogallol (Esberg and Ren, 2004). The protective effect may not result from oxygen radical scavenging but from alleviation of the oxidative stress-induced Ca^{2+} overload by a direct effect on the sarcoplasmic reticulum (Esberg and Ren, 2004). In another study, anisodamine reduced cardiomyocyte apoptosis in a rat model of ischaemia–reperfusion (Xing *et al.*, 2015).

Anisodamine improves blood flow in the microcirculation through various mechanisms, including (i) evoking vasodilatation by antagonizing ACh-induced prejunctional inhibition of adrenergic transmission, antagonizing ACh-induced endothelium-dependent relaxation and α_1-adrenoceptor blockade (Varma and Yue, 1986; Guo *et al.*, 1992b, c, 1993); (ii) both direct and indirect effects on the heart (increased contractility and increased venous return and preload) (Yao *et al.*, 1984); and (iii) an anti-thrombotic and fibrinolytic effects, in which it inhibits platelet aggregation both *in vitro*, *in vivo* and in humans. The exact mechanism is still unclear but seems to include inhibition of thromboxane synthesis, inhibiting endothelial cell activation, exerting anti-proteolytic activity and suppressing TNF-α-induced tissue factor, which is needed for thrombin activity (Xiu *et al.*, 1982; Su *et al.*, 1984; Xiao *et al.*, 1988; Ruan *et al.*, 2001; Zhang *et al.*, 2001; Ruan *et al.*, 2002). This may also explain its efficacy in cases of shock, coronary artery disease, thrombophlebitis and disseminated intravascular coagulation.

Conclusions

Taken together, it seems that anisodamine may exert its positive effects in multiple pathways, leading to an improvement in the clinical condition of OP-poisoned patients faster than atropine. Although the number of publications on the role of anisodamine in the management of OP poisoning is small, the evidence from other research and clinical areas point strongly to its potential role in the management of OP poisoning. These include, in addition to cholinolytic activity *per se*, anti-inflammatory, antioxidant, antiarrhythmic, anticoagulant and haemodynamic beneficial activities. Of these, the most prominent studies cited are in the alleviation of acute lung injury and cardiovascular effects, being the most serious and life-threatening complications of peripheral OP toxicity, and requiring special attention. Also noted is the lower toxicity of anisodamine compared with that of atropine, and this may be important in cases when atropine is inefficient or contraindicated. The efficacy of anisodamine in the treatment of OP poisoning, either alone or in combination with other treatment modalities, should be further evaluated, both in the experimental and the clinical settings. Basic research on the mechanism of action of anisodamine with reference to other naturally derived and synthetic cholinolytic drugs is essential to evaluate and better define its role in the management of OP poisoning. These should include additional pharmacokinetic and receptor binding studies to identify the specific receptors and pathways involved in its pharmacological effects, identification of cholinergic and non-cholinergic mechanisms in neural, pulmonary and cardiovascular effects of anisodamine and their integrative assessment by physiologically based pharmacokinetic/pharmacodynamic (PB/PK/PD) modelling.

Conflict of interest

The authors declare no conflicts of interest.

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