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Pharmacokinetic profile and quantitation of protection against soman poisoning by the antinicotinic compound MB327 in the guinea-pig

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HIGHLIGHTS

- The bispyridinium compound MB327 protects guinea-pigs from soman poisoning.
- Mode of action is not reliant on reactivation of aged inhibited acetylcholinesterase.
- First syntheses of d6-MB327 diiodide and dimethanesulfonate salts.
- Used as internal standards for mass spectrometric quantitation of MB327 in guinea-pig plasma.

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ABSTRACT

Current organophosphorus nerve agent medical countermeasures do not directly address the nicotinic effects of poisoning. A series of antinicotinic bispyridinium compounds has been synthesized in our laboratory and screened in vitro. Their actions can include open-channel block at the nicotinic receptor which may contribute to their efficacy. The current lead compound from these studies, MB327 1,1′-(propane-1,3-diyl)bis(4-*tert*-butylpyridinium) as either the diiodide (I_2) or dimethanesulfonate (DMS) has been examined in vivo for efficacy against nerve agent poisoning. MB327 I_2 (0–113 mg kg^{-1}) or the oxime HI-6 DMS (0–100 mg kg^{-1}), in combination with atropine and avizafone (each at 3 mg kg^{-1}) was administered to guinea-pigs 1 min following soman poisoning. Treatment increased the LD_{50} of soman in a dose-dependent manner. The increase was statistically significant ($p < 0.01$) at the 33.9 mg kg^{-1} (MB327) or 30 mg kg^{-1} (HI-6) dose with a comparable degree of protection obtained for both compounds. Following administration of 10 mg kg^{-1} (i.m.), MB327 DMS reached plasma C_{max} of 22 μM at 12 min with an elimination $t_{1/2}$ of 22 min. In an adverse effect study, in the absence of nerve agent poisoning, a dose of 100 mg kg^{-1} or higher of MB327 DMS was lethal to the guinea-pigs. A lower dose of MB327 DMS (30 mg kg^{-1}) caused flaccid paralysis accompanied by respiratory impairment. Respiration normalised by 30 min, although the animals remained incapacitated to 4 h. MB327 or related compounds may be of utility in treatment of nerve agent poisoning as a component of therapy with atropine, anticonvulsant and oxime, or alternatively as an infusion under medical supervision.

1. Introduction

Medical countermeasures (MedCMs) to nerve agent poisoning do not currently contain a component which directly counteracts the effect of elevated acetylcholine at nicotinic synapses. While oximes alleviate this by restoring acetylcholinesterase (AChE) function at these sites (Timperley et al., 2011), there are limitations to the efficacy of oximes for AChE inhibited by some nerve agents such as tabun (Heilbronn and Tolagen, 1965) or where rapid 'ageing' of the inhibited enzyme occurs such as with the AChE-soman adduct (Fleisher et al., 1965). A series of bispyridinium compounds has been synthesized and screened for ion-channel blocking properties at the nicotinic receptor (Timperley et al., 2005). In vitro studies have demonstrated that one of these compounds, MB327, restores function in nerve agent-poisoned guinea-pig, rat and human muscle preparations (Seeger et al.,

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2012; Turner et al., 2011). Related compounds also bind to the human alpha 7 nicotinic acetylcholine receptor and the nicotinic receptors from electric eel *Torpedo californica* (Niessen et al., 2012a, 2011). MB327 binds to the human M_5 muscarinic acetylcholine receptor (Niessen et al., 2012b) and causes a relaxation of rat jejunum smooth muscle (Königer et al., 2013).

MB327 (Fig. 1) has been assessed in vivo in mouse and guinea-pig models of nerve agent poisoning. In atropine-pretreated mice, MB327 (referred to as P62) provided some protection, reducing an LD_{95} dose of soman to an LD_{50} (Schoene et al., 1976). In guinea-pigs, MB327 DMS, in combination with physostigmine salicylate and hyoscine hydrobromide, protected 4/8 of animals against a $5 \times LD_{50}$ challenge of sarin and 5/8 against $5 \times LD_{50}$ of tabun. In comparison, HI-6 protected 8/8 and 1/8 animals, respectively (Turner et al., 2011). In the same model, protection against soman provided by MB327 DMS was 6/8, and by HI-6, 7/8 (Timperley et al., 2012). This broadly similar efficacy across nerve agents with different oxime sensitivities and ageing rates suggests MB327 and related bispyridinium compounds may provide a broad-spectrum adjunct to conventional therapy for nerve agent poisoning. However, these previous studies did not use the additional therapy components used in current MedCMs (antimuscarinic and anticonvulsant) (Timperley and Tattersall, 2015). An assessment was therefore conducted to determine if MB327 was beneficial when administered in combination with the currently-fielded UK MedCM combination of atropine sulfate and avizafone hydrochloride. A comparison was also made to a combination treatment comprised of atropine sulfate, avizafone and the oxime HI-6. Soman was selected as the challenge agent for these studies as soman-inhibited AChE ages rapidly rendering it resistant to oxime reactivation. Poisoning by soman thus poses one of the more difficult challenges for MedCM.

Both MB327 I_2 and MB327 DMS were studied. Initially, the former was available in greater quantities than the latter, which is synthesised from the diiodide by ion exchange (Timperley et al., 2012). The decision was made to use MB327 I_2 for efficacy studies, adjusting its concentration to account for the different molecular masses of MB327 I_2 and MB327 DMS, and to save the latter for the pharmacokinetic (PK) studies. The dimethanesulfonate salt is preferable for PK studies due to its greater water solubility. Syntheses of comparable salts with a d_6-deuterated linker and their use as internal standards for mass spectrometric quantitation of MB327 I_2 and MB327 DMS in guinea-pig plasma are also described.

2. Methods

2.1. Animals

All animal work was carried out under the terms and conditions of a Project Licence (PPL 30/2864) granted by the UK Home Office under the Animals (Scientific Procedures) Act, 1986. Male HsdDhl:DH Dunkin–Hartley guinea-pigs were supplied by Harlan Interfauna (Hillcrest, UK). Bodyweight on arrival was 233 ± 9 g (n = 76; mean ± SD) for the efficacy study, 245 ± 6.4 g (n = 30; mean ± SD) for the adverse events study and 334 ± 31 g (n = 7; mean ± SD) for the PK study. The animals were allowed to acclimatize for at least 4 days prior to study, and were kept in standardised conditions to meet UK Home Office guidelines (room temperature 21 °C, humidity 50%). The lights were on from 06:00 to 18:00 h. Daily body weight and temperature recordings were monitored throughout the experiment as a measure of health and wellbeing.

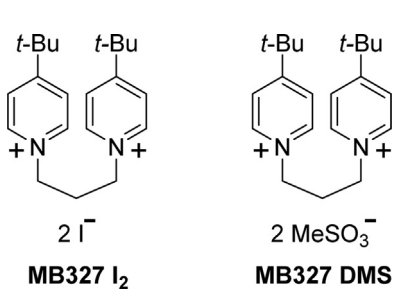

Fig. 1. Antinicotinic bispyridinium compounds.

2.2. Chemicals and drugs

Atropine sulfate was supplied by Sigma Ltd. (Dorset, UK), avizafone by Roche Ltd. (Welwyn Garden City, UK), HI-6 by Phoenix Chemicals Ltd. (Bromborough, UK) and soman by chemists working in the UK Single Small Scale Facility, Dstl Porton Down (>95% pure, diluted in isopropanol). d_6-1,3-Dibromopropane and other reagents were from Aldrich Ltd. (Dorset, UK) and were used as received. Thin layer chromatography (TLC) was performed on MK6F silica gel 60 Å plates (Whatman, USA) with detection by iodine vapour. Nuclear magnetic resonance (NMR) data were collected at 9.4 T using a Bruker Avance III spectrometer equipped with a 5 mm BBFO + probehead.

2.3. Deuterated standards

Undeuterated MB327 I_2 and MB327 DMS were prepared according to a published procedure (Timperley et al., 2012). Deuterated analogues were synthesized by Finkelstein reaction of d_6-1,3-dibromopropane with an excess of sodium iodide in acetone (Fig. 2). The d_6-1,3-diiodopropane (1) obtained was bis-quaternized by two molar equivalents of 4-*tert*-butylpyridine in boiling nitromethane to give d_6-MB327 I_2. Treatment of this with a suspension of silver(I) methanesulfonate in methanol provided d_6-MB327 DMS.

2.3.1. d_6-1,3-Diiodopropane (1)

A solution of d_6-1,3-dibromopropane (5.0 g, 24 mmol) in acetone (15 ml) was added dropwise over 20 min to a stirred solution of sodium iodide (15.0 g, 100 mmol) in acetone (55 ml) in a 250-ml round-bottomed flask. The addition caused precipitation of sodium bromide. Stirring was continued for 12 h. The sodium bromide was filtered off and rinsed thoroughly with acetone. The solvent was removed from the filtrate to leave sludge. This was partitioned between dichloromethane (55 ml) and water (30 ml) and the aqueous layer extracted with dichloromethane (2 × 30 ml). The organic layers were combined and dried ($MgSO_4$), the drying agent filtered off, and the solvent removed from the filtrate to leave a mobile yellow liquid (8.56 g). Fractionation of this using a micro-distillation apparatus gave the title compound as a pale orange mobile liquid (6.38 g, 88%). Bp 61 °C/1 mmHg. Analysis by gas chromatography–mass spectrometry showed that it was 99% pure (the 1% impurity was identified as the mono-substituted product $Br(CD_2)_3I$).

2.3.2. 1,1′-(d_6-Propane-1,3-diyl)bis(4-tert-butylpyridinium) diiodide (d_6-MB327 I_2)

d_6-1,3-Diiodopropane (6.04 g, 20 mmol) was added dropwise to a stirred solution of 4-*tert*-butylpyridine (8.10 g, 60 mmol) in nitromethane (30 ml) in a 100-ml round-bottomed flask. The mixture was heated for 18.25 h. TLC analysis of a sample versus the starting materials, eluting with 1:2 hexane–acetone, was performed. None of the d_6-1,3-diiodopropane was seen upon developing the plates with iodine vapour; a main spot at retention factor (R_f) 0.05 (desired product), a fainter one at R_f 0.15 (mono-substitution product), and a spot corresponding to the excess of 4-

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