Toxicology in Vitro 35 (2016) 11–16

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Bispyridinium non-oximes: An evaluation of cardiac effects in isolated hearts and smooth muscle relaxing effects in jejunum

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ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 10 May 2016
Accepted 11 May 2016
Available online 13 May 2016

Keywords:
Bispyridinium non-oximes
Cardiac
Isolated heart
Oximes
Smooth muscle function

ABSTRACT

Bispyridinium non-oximes seem to be promising candidates for the generic treatment of nerve agent poisoning as they interact with nicotinic and muscarinic acetylcholine receptors. The lead compound MB327 showed therapeutic effectiveness in vitro and in vivo but was toxic at higher doses. In the present study, the effect of various bispyridinium non-oximes on isolated heart and small intestine function was investigated. Bispyridinium non-oximes and oximes were tested in at least seven different concentrations in rat jejunum preparations pre-treated with carbachol. All bispyridinium non-oximes showed classical dose response curves with MB327 being the most effective (EC_{50} = 6.6 μM) and MB782 being slightly less effective (EC_{50} = 10.4 μM). Neither the bispyridinium non-oximes nor the oximes showed cardiotoxic effects in the isolated Langendorff heart. The tested bispyridinium compounds showed no direct cardiac effect but had variable smooth muscle relaxing effects. Further in vivo studies are required to get more insight into potential toxic mechanisms of these promising nerve agent antidotes.

1. Introduction

Poisoning by organophosphorus (OP) pesticides in suicidal intention or by accident leads up to 300,000 deaths worldwide every year, primarily in southeast Asia (Gunnell et al., 2007). In addition, the recent homicidal use of the OP nerve agent sarin in Syria 2013, exerting a substantial higher toxicity than OP pesticides resulted in an enormous number of poisoned civilians and in thousands of deaths (Rosman et al., 2014). The toxic mechanism of OP compounds is mediated by the inhibition of the pivotal enzyme acetylcholinesterase (AChE) with subsequent accumulation of acetylcholine (ACh) in the synaptic cleft. This ACh-overflow finally leads to cholinergic crisis and death by central and peripheral respiratory failure (Marrs, 1993; Thiermann et al., 2013). Currently, a pre-treatment is only feasible in a military environment, e.g. with the reversible AChE inhibitor pyridostigmine (Lundy, 1999). However, its use is discussed controversially and a relation to the Gulf War syndrome has been postulated (Binns, 2008). Current standard post-exposure therapy consists of an enzyme reactivator, mostly obidoxime or pralidoxime (2-PAM) in combination with atropine (Eyer, 2003). Oximes restore the AChE function by removing the phosphyl group bound to the active site of the enzyme (Worek and Thiermann, 2013) and atropine acts as competitive muscarinic receptor antagonist. Unfortunately, 60 years of research for a multipotent broad spectrum oxime covering all OPs were not successful (Eyer and Worek, 2007; Worek et al., 2004; Worek and Thiermann, 2013).

An alternative therapeutic approach counteracts the toxic effects of OP compounds at nicotinic receptors. Previous work with the bispyridinium non-oxime SAD128 and the bispyridinium oxime HI-6 indicate interaction of such compounds with nicotinic and muscarinic receptors (Alkondon et al., 1988; Alkondon and Albuquerque, 1989; Clement, 1981; Kuhnen-Clausen, 1972; Lundy and Tremblay, 1979). In fact, SAD-128 showed a therapeutic effect in soman-poisoned mice and HI-6 could restore OP-blocked neurotransmission in isolated rat diaphragms without reactivating inhibited AChE (Clement, 1981; Oldiges and Schoene, 1970; Schoene et al., 1976; van Helden et al., 1991). A variety of bispyridinium non-oximes were able to partially restore soman-blocked neurotransmission in isolated guinea pig diaphragms (Tattersall, 1993). MB327 (Table 1) is at present the most promising compound. It restored neurotransmission in soman-blocked rat, guinea pig and human respiratory muscles and showed therapeutic effectiveness in nerve agent-poisoned guinea pigs in vivo (Price et al., 2015; Seeger et al., 2012; Turner et al., 2011). However, a recent guinea pig study demonstrated a toxic effect at high MB327 doses (Price et al., 2015). This prompted us to investigate potential effects of MB327 and related bispyridinium compounds in isolated rat heart and jejunum models.

2. Materials and methods

2.1. Chemicals

Obidoxime was supplied by Merck KG (Darmstadt, Germany) and HI-6 dichloride monohydrate was kindly donated by Dr. Clement (Defence Research Establishment Suffield, Ralston, Alberta, Canada).

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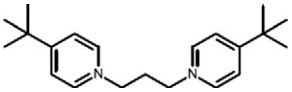
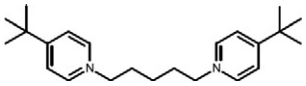
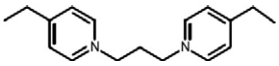
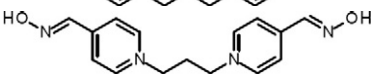
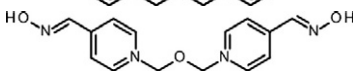
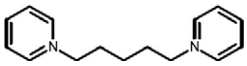
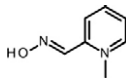
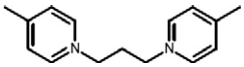
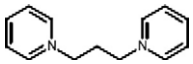
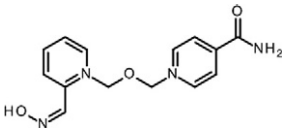
http://dx.doi.org/10.1016/j.tiv.2016.05.005
0887-2333/

Table 1
Smooth muscle relaxing effects and chemical structure of different compounds. The EC_{50} is given as mean and the corresponding confidence interval is presented (n ≥ 10 segments of different parts of the jejunum of at least three rats per concentration).

Compound	Structure	EC_{50} (μM)	Confidence interval (μM)
MB327		6.6	6.3–7.0
MB782		10.4	9.7–11.2
MB454		35.4	32.7–38.2
TMB-4		35.8	34.4–37.2
obidoxime		126.3	118.7–134.3
MB442		232.2	209.1–257.8
2-PAM		261.8	246.0–278.7
MB414		263.8	240.9–289.0
MB408		491.9	456.5–430.0
HI-6		963.0	910.6–1018.0

Carbachol, pyridine-2-aldoxime-methochloride (2-PAM) and TMB-4 were purchased from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany) and NaCl, $CaCl_2$, $NaHCO_3$, NaH_2PO_4, Glucose and KCl were delivered by Carl Roth GmbH + Co. KG (Karlsruhe, Germany). MB327, MB 408, MB 414, MB442, MB454 and MB782 were kindly supplied by Dr. C. M. Timperley (DSTL, Porton Down, UK; see Table 1). All tested compounds showed 98% purity based on the analysis of LC–MS, $^1H/^{13}C$ NMR.

2.2. Rat heart preparation

All experiments were in accordance with the German Animal Welfare Act of 18 May 2006 (BGBl. I S. 1206, 1313) and the European Parliament and Council Directive of 22 September 2010 (2010/63/EU).

Male Wistar rats (300 ± 50 g; Charles River, Sulzfeld, Germany) were held in small groups of 6 animals with a 12/12 h light-dark cycle, standard laboratory diet (Altromin, Lage, Germany) and water ad libitum. The rats were given an at least 7 day adapting phase. At the time of the experiment the animals did not show any signs of disorder or disease.

After the heart was dissected it was immediately placed in ice cold buffer and fixed at a modified Langendorff apparatus (Bell et al., 2011; Langendorff, 1895; Skrzypiec-Spring et al., 2007; Sutherland and Hearse, 2000). The heart was perfused via the aorta and coronary arteries with gassed (95% O_2 and 5% CO_2), modified Krebs Henseleit buffer (NaCl 118.0 mM, $NaHCO_3$ 24.88 mM, glucose 5.55 mM, KCl 4.5 mM, sodium pyruvate 2.0 mM, $MgSO_4$ 1.66 mM, $CaCl_2$ 1.6 mM, KH_2PO_4 1.2 mM; pH = 7.4, T = 38.0 °C) at constant pressure (60 mm Hg). The Langendorff heart setup consisted of a gassed buffer reservoir (Radnoti, Monrovia, CA, USA) with a downstream pump (IPC High Precision Multichannel Dispenser, Ismatec, IDEX, Wertheim, Germany), which could either work with constant pressure or constant flow (Fig. 1). The buffer was then pumped in a smaller non-gassed reservoir with a bubble-trap, after which an inline flow probe (ME3PXN,

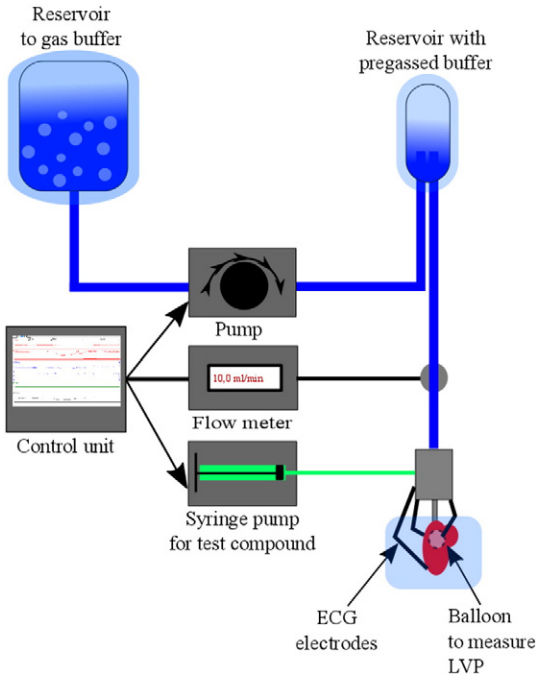


Fig. 1. Experimental setup of the isolated heart model with feedback mechanism for the buffer pump and the substance pump. The pump transported buffer from a gas-flushed reservoir to a smaller reservoir with a bubble trap (not shown). Then the flow probe measured the actual buffer flow through the aorta and coronary arteries and the flow meter transmitted either a positive or a negative feedback to the buffer and substance pumps. The heart and reservoirs were water-jacketed (39 °C) to maintain a constant temperature.