Chemico-Biological Interactions 296 (2018) 34–42

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Efficacy of atropine sulfate/obidoxime chloride co-formulation against sarin exposure in guinea pigs

Marloes J.A. Joosen[a,*], Steven D. Klaassen[a], Elwin Verheij[a], Tomas van Groningen[a], Alex S. Cornelissen[a], Mario H. Skiadopoulos[b], Laura Cochrane[b], Jeff D. Shearer[b]

[a] TNO Defense, Security and Safety, CBRN Protection, Lange Kleiweg 137, 2288, GJ, Rijswijk, the Netherlands
[b] Emergent BioSolutions, Emergent Prod. Dev. Gaithersburg, 300 Professional Drive, Gaithersburg, MD, 20879, USA

ARTICLE INFO

Keywords:
Sarin
Nerve agent
Obidoxime
Atropine sulfate
EEG
Autoinjector

ABSTRACT

The efficacy and pharmacokinetics of the aqueous co-formulation contents of the Trobigard™ (atropine sulfate, obidoxime chloride) auto-injector were evaluated in a sarin exposed guinea pig model. Two subcutaneous (sc) sarin challenge doses were evaluated in guinea pigs instrumented with brain and heart electrodes for electroencephalogram (EEG) and electrocardiogram (ECG). Sarin challenge doses were chosen to reflect exposure subclasses with sublethal (moderate to severe clinical signs) and lethal consequences. The level of protection of intramuscular human equivalent doses of the co-formulation was defined by (1) the mitigation of signs and symptoms at a sublethal level and (2) the increase of survival time at the supralethal sarin dose levels.

Pharmacokinetics of both atropine sulfate and obidoxime were proportional at 1 and 3 human equivalent doses, and only a small increase in heart rate was observed briefly as a side effect.

At both sarin challenge doses, 54 μg/kg and 84 μg/kg, the co-formulation treatment was effective against sarin-induced effects. Survival rates were improved at both sarin challenge levels, whereas clinical signs and changes in EEG activity could not in all cases be effectively mitigated, in particular at the supralethal sarin challenge dose level. Reactivation of sarin inhibited cholinesterase was observed in blood, and higher brain cholinesterase activity levels were associated with a better clinical condition of the co-formulation treated animals.

Although the results cannot be directly extrapolated to the human situation, pharmacokinetics and the effects over time related to plasma levels of therapeutics in a freely moving guinea pig could aid translational models and possibly improve prediction of efficacy in humans.

1. Introduction

Nerve agents such as sarin covalently bind to the active site of acetylcholinesterase, a highly efficient and crucial enzyme in cholinergic neurotransmission. Following binding of neve agent to the active site; acetylcholine hydrolysis consequently becomes impossible, leading to cholinergic overstimulation at both nicotinic and muscarinergic synapses in the peripheral and central nervous system [1]. Nerve agents act via this critical enzyme target, yielding a very steep probit slope for the LD_{50} in the majority of animal species [2]. Once saturated, the most effective treatment is by reactivation of acetylcholinesterase with an oxime such as obidoxime. In addition, adequate atropinisation is required to interfere with muscarinergic effects. When used in combination with atropine, obidoxime is effective against sarin and VX and is the most effective of the currently fielded oximes against organophosphate (OP) pesticides [3]. Immediate intravenous (i.v.) or intramuscular (i.m.) injection with atropine and oximes is recommended in case of suspect organophosphorous nerve agent exposure [4]. In a clinical setting, up to 50 mg of atropine and 1250 mg of obidoxime may be administered in adults over 24 h depending on the severity of the poisoning. Autoinjector devices have been developed to yield rapid intramuscular access for nerve agent medical countermeasures in military settings, and depending on severity of signs, administration of 1–3 injectors containing atropine and an oxime (220 mg for obidoxime chloride) is generally recommended. In certain scenarios or medical management doctrines, diazepam or midazolam is added as an anticonvulsant to culminate seizure development [1,5].

Historically the level of protection by a nerve agent countermeasure has been defined as “protective ratio”, reflecting the LD_{50} with treatment/LD_{50} in naïve animals. Such an approach does not provide

* Corresponding author.
E-mail address: Marloes.joosen@tno.nl (M.J.A. Joosen).

https://doi.org/10.1016/j.cbi.2018.09.004
Received 21 June 2018; Received in revised form 10 August 2018; Accepted 11 September 2018
Available online 12 September 2018
0009-2797/

information about the condition of an animal following treatment. The goal of autoinjector administered treatment is to increase survival of a victim, and to maintain the victim's own capability to reach advanced medical help. In the latter case, “the level of protection” could be defined by the normalization of physiological parameters, which is the consequence of adequate acetylcholinesterase reactivation and atropinisation, induced by the drug levels following i.m. injection [6]. Clinical effects of sarin exposures can range from mild to moderate and lethal consequences. Historically, a broad range of subcutaneous (s.c.) sarin LD_{50} values has been reported, from 30 μg/kg up to 50 μg/kg in guinea pigs [2]. It must be noted that the time to death is not linearly correlated to the dose of sarin or OP nerve agent in guinea pigs due to the steep probit slope. The general scenarios are that animals dosed above the LD_{50} die rapidly, within 15–30 min [7], or in certain cases they may survive for 24 h in a convulsive/moribund status. Such doses are in the range of 50–56 μg/kg, approximating a reported LD_{85} without treatment by Wilhelm *et al.* [8].

The present study was conducted to evaluate the efficacy of an atropine sulfate and obidoxime chloride co-formulation, representing the contents of a Trobigard™ (atropine sulfate, obidoxime chloride) autoinjector against sublethal and supralethal sarin exposure levels. To that end, 54 μg/kg sarin s.c., an approximate LD_{85} was used, inducing severe clinical signs and lethality. Additionally, a supralethal dose of 84 μg/kg sarin s.c. was used, leading to rapid and complete blood and brain cholinesterase inhibition and 100% lethality within 10 min to 4 h. Using these different dose characteristics allows assessment of medical countermeasure effectiveness against exposures with different severity. In parallel to 24 h survival outcome, video monitoring of clinical signs, measurement of neurological and cardiovascular physiological parameters were included in the study to provide a more complete evaluation of the therapeutic efficacy of the Trobigard autoinjector coformulation. Cholinesterase activity in red blood cells and brain, as well as, plasma atropine and obidoxime pharmacokinetics were measured to evaluate the association of these parameters with outcome. The results obtained from the present study providing pharmacokinetics and the effects over time related to plasma levels of therapeutics in a small freely moving guinea pig could aid translation and possibly improve prediction of efficacy in humans.

2. Materials and methods

2.1. Animals

Male Dunkin –Hartley albino guinea pigs were obtained from an accredited supplier (Envigo, Horst, The Netherlands), with a starting weight of approximately 350–400 g. Prior to the experiments the guinea pigs were housed with two animals per cage (Macrolon type IV), and allowed to acclimatize to standard conditions for one week. Room temperature was kept at 19–22 °C, relative humidity was maintained at 55–65% and lights were on from 7 a.m. to 7 p.m. Fresh tap water and standard guinea pig diet (Teklad global diet 2040, Envigo, Horst, The Netherlands) were available *ad libitum*. All experiments were carried out according to the EU Legislation for testing on experimental animals (EU Directive 2010/63/EU), and in line with International Guiding Principles for Biomedical Research Involving Animals. Unnecessary suffering was minimized in all cases.

2.2. Chemicals

Sarin (GB nerve agent, CAS 107-44-8) was obtained from the TNO stocks (purity > 99%). During each experimental day, sarin injection solutions were prepared freshly in PBS. Coformulation solutions for dosing consisting of 1 mg/mL atropine sulfate and 110 mg/mL obidoxime in a aqueous formulation were obtained from Trobigard autoinjectors (Emergent BioSolutions, Gaithersburg MD, USA). The contents of the Trobigard autoinjector were dispensed into a glass vial to allow withdrawal into a syringe for the coformulation dose administration to the test subjects. Solutions were stored dark at RT for a maximum of 48 h and diluted with water if required.

Table 1
Study design.

Group	Sarin Dose (S.C.)	Treatment Formulation	Treatment (Dosage mg/kg***) Atropine Sulfate/ Obidoxime	No of guinea pigs
1	54 μg/kg*	Vehicle	–	8
2	54 μg/kg*	Coformulation	0.4/44	8
3	84 μg/kg**	Vehicle	–	5
4	84 μg/kg**	Coformulation	0.4/44	8
5	0	Coformulation	0.4/44	7
6	0	Coformulation	0.13/14.6	9
TOTAL				45

*: ~1.8 LD50; **: ~2.8 LD50; *** 0.4 mL/kg.

2.3. Study design

The study consisted of two phases: an evaluation phase, in which the efficacy of a three autoinjector human equivalent dose against a lethal and (sub)lethal dose sarin was evaluated, and a pharmacokinetic/pharmacodynamic experiment for measuring effects of a single and triple autoinjector equivalent in unchallenged guinea pigs. An overview of the experimental groups is provided in Table 1. Each animal experiment was performed according to a similar protocol, consisting of acclimatization, preparatory surgery, recovery and animal exposure, observation and biochemical workup.

2.3.1. Animal preparation

For all groups, animal preparation was similar and as follows. Four days before challenge, animals were anaesthetized with isoflurane (IsoFlo,100% isoflurane, Abbott) in oxygen, and equipped with an indwelling jugular vein catheter (4 cm; 5–380040 Brainlink), cortical EEG electrodes (unilateral screws on dura mater, +1 and −7 mm from bregma) and ECG electrode leads (Lead II configuration; right collar bone and the left second rib). The cannula and leads were exteriorized at the skull level and attached leads and EEG screws were attached to a connector. The cannula was locked with 500 IU heparin in glycerol and capped, and fixed with the head stage, to the skull with dental cement (GC Fuji-Plus). Animals received 5 mg/kg carprofen s.c. before and 24 h after surgery, and diluted Borgal antibiotic once before surgery (final dose Trimethoprim 4 mg/kg and sulfadoxine 20 mg/kg s.c.).

2.3.2. Challenge, treatment and observation

On the day of the experiment, animals were placed in macrolon Type III cages, and a TL11M2-F40-EET transmitter body (Data Science international) was attached to the connector on the skull. Physiological data, EEG and ECG, was collected using Ponemah Software (DataScience international) at 500 and 1000 Hz respectively. In parallel, video recordings of the animals were obtained throughout the experiment. Animals were exposed according to the study design outlined in Table 1. Baseline values were obtained for at least 30 min, during which pre-exposure blood samples were obtained, after which animals were challenged s.c with either a supralethal (~2.8 LD50, 84 μg/kg) or a lower dose level (~1.8 LD50, 54 μg/kg) of sarin (diluted in PBS prior to injection, 1 mL/kg). One minute after sarin, animals were. treated i.m with 0.4 mg/kg atropine sulfate and 0.44 mg/kg and obidoxime dichloride, equivalent to 3 Trobigard autoinjector human doses (3Eqv) assuming a 2 mL/70 kg human autoinjector dose and 4.625 Body Surface Area (BSA) guinea pig to human correction factor according to Reagan-Shaw et al. [9]. Animals dosed for the pharmacokinetic study (groups 5 and 6) were only injected i.m with 3 or 1 autoinjector equivalents (Table 1). Following treatment administration,