ORIGINAL ARTICLE

HI-6 oxime (an acetylcholinesterase reactivator): blood plasma pharmacokinetics and organ distribution in experimental pigs

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Abstract

OBJECTIVES: Oxime HI-6 DMS (dimethanesulfonate) is an asymmetric bispyridinium aldoxime and essential acetylcholinesterase (AChE) reactivator. The high effectiveness is due to its wide spectrum of therapeutic activity against different structures of nerve agents. Aim of this study was to compare plasma time profiles and tissue distribution (to delimitation of potential toxicity risks) after its intramuscular (i.m.) and intragastric (i.g.) administration to experimental pigs.
METHODS: The study entered female Landrace pigs (Sus scrofa f. domestica), 4–5 months old animals, 29±3.2 kg of body weight. Before the HI-6 DMS administration (i.m. injection or i.g. using a gastric tube), vena auricularis was cannulated (under general anaesthesia) for collection of blood samples. The tissue distribution study was carried out at expected t-max. Concentrations of HI-6 DMS in blood plasma and other tissue samples were detected by means of HPLC method.
RESULTS: Fast absorption after i.m. administration, relatively slow absorption and no even elimination after i.g. administration were found. Tissue distribution showed low accumulation in the liver, but a higher content in the kidneys and high concentrations in the brain and gastrointestinal wall.
CONCLUSIONS: Plasma time profiles after i.g. administration has a prolonged pharmacokinetics. Tissue distribution study showed potential side effects to the stomach due to a higher accumulation of HI-6 in this tissue after i.g. administration but not after a standard i.m. administration. Higher content of HI-6 in the kidneys after i.m. administration suggests the main way of the oxime elimination.

Abbreviations:

HI-6	- Oxime (Acetylcholinesterase reactivator)
DMS	- dimethanesulfonate
AChE	- acetylcholinesterase
i.g.	- intragastric
i.m.	- intramuscular
Cmax	- maximum of drug concentration in blood
Tmax	- time when Cmax occurred
TNMR	- nuclear mass resonance
HPLC	- high performance liquid chromatography
UV/VIS	- ultraviolet/visible
AUC	- area under the curve
PyAls	- pyridinium aldoximes

INTRODUCTION

HI-6 dimethanesulfonate (DMS) is a salt of the oxime HI-6 used in the treatment of nerve-agent poisoning. It is known to be the best re-activator component of inactivated acetylcholinesterase (AChE) after soman, sarin and cyclosarin poisoning (Bogan *et al.* 2012). Oxime HI-6 is still a promising molecule to be more effective than the commonly-used oximes (pralidoxime and obidoxime) and has a relatively low toxicity compared with the other oximes (Clement *et al.* 1995).

Oximes are typically applied intramuscularly (i.m.) mainly because of their physicochemical properties. On the other hand, the dosing of oxime is limited by its solubility, and the i.m. administration of a higher volume is painful.

HI-6 DMS is very well defined by many studies in rats and guinea-pigs (Karasova *et al.* 2010a; 2010b; 2011, 2013a; Zemek *et al.* 2013) but complete pharmacokinetic and toxicological data in large experimental species are still missing. We used the pig in many experimental studies (Kvetina *et al.* 2008; Kopacova *et al.* 2010; Kunes *et al.* 2010; Tacheci *et al.* 2010; Bures *et al.* 2011a,b) because it is a representative of large (non-rodent) experimental species also due to its relatively very similar biochemical and physiological (including gastrointestinal) functions compared to humans (Karalri, 1995; Suenderhauf *et al.* 2013).

The present study follows our previous experiments determinating the pharmacokinetics of HI-6 in experimental pigs (Karasova *et al.* 2013b). The part of these experiments were also aimed to the evaluation of potential adverse effects of oximes to the gastrointestinal tract (GIT) because of oximes directly impact the cholinergic system leading to hyperactivation of cholinergic system and thus also important changes of myoelectric activity of GIT (Bures *et al.* 2013). The main aim of this work was to describe the pharmacokinetic profiles after HI-6 DMS intragastric (i.g.) and intramuscular (i.m.) administration to experimental pigs, and to delimitate potential risk by evaluation of its tissue distribution.

MATERIAL AND METHODS

Chemicals

Oxime HI-6 DMS, 1-({[4'-(aminocarbonyl)pyridinium]methoxy}methyl)-2-((hydroxyimino)methyl)pyridinium dimethanesulfonate, CAS 1 44252-71-1, was synthesized in our laboratory and its structural parameters and purity was confirmed using NMR and chromatographic analysis (Jun *et al.* 2008, 2010; Kuca *et al.* 2008). All other drugs and chemicals of analytical grade were obtained commercially and used without further purification.

Animals

The study was done on female Landrace pigs, *Sus scrofa f. domestica*, average body of weight 29±3.2 kg (pharmacokinetics – six animals for i.g. and five animals for i.m. administration; distribution study – three animals for each route of administration). Animals were housed indoors in the animal facility (temperature 18±2 °C, humidity 55±5%). The animals received standard granulated diet for pigs and were allowed tap water *ad libitum*.

Experiments were started after 14 days of pigs acclimatization. Animals were anaesthetised intramuscularly with a single dose of ketamine 30 mg/kg (Narkamon, Spofa, Czech Republic), azaperone 2 mg/kg (Stresnil, Janssen Pharmaceutica, Belgium) in mixture. Subsequently, the animals were placed on supine position, intubated and anaesthetised by 0.5% isoflurane. Venous access (for blood samples collection) was established by inserting an intravenous catheter (B-Braun, Germany) into vena auricularis.

The oxime HI-6 DMS (a single dose of 1 500 mg diluted in 10 mL sterile water) was administered to pigs i.m. or i.g. (by a gastric tube). Such a high doses applied were chosen due to evaluating its potential side effect on the GIT (data not shown). Blood samples (5 mL) were collected at regular time intervals: 20, 40, 60, 90, 120, 180 min after HI-6 administration into heparinized tubes (Sarstedt, Li-He tube). Plasma was prepared by centrifugation (3 000 *g*, 10 min, 4 °C). Tissue samples (for distribution study) were collected at Tmax (30min for i.m. and 3 h for i.g. administration) determined by our previous experiments (not published data). All biological samples were frozen at –80 °C prior to analysis.

Ethics

The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence, Faculty of Military Health Services, Hradec Králové, Czech Republic. Animals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe 2009).

Analysis of HI-6 DMS

Determination of HI-6 concentrations in tissue samples and in blood plasma were done using validated high-

performance liquid chromatography (HPLC) method with UV detection. Detailed description of method used and separation conditions were described previously (Karasova *et al.* 2013b).

Sample preparation for HPLC analysis

Blood plasma samples (200 µL) were mixed with 50 µL trichloroacetic acid in order to precipitate proteins. The samples were spun at 21 000 *g* at 4 °C for 15 minutes (M 240R, Hettich, Germany), and the supernatant was used for HPLC analysis. Other tissue samples were prior preparation homogenized.

A calibration curve for determination of oxime concentration was established using plasma samples spiked with oxime HI-6 (1.25; 2.50; 5.00; 10.00; 20.00; and 40.00 µg/mL samples, in triplicate). The retention time of oxime HI-6 was ~ 6.8 min. Data analysis were evaluated using program Prism4 (Graph Pad Software, USA).

Pharmacokinetics

Noncompartmental analysis was performed using the Kinetica software, version 4.0 (InnaPhase Corporation, Thermo Fisher Scientific Inc. Waltham, MA, USA). Maximum concentration (Cmax) and the time to the maximum concentration (Tmax) were determined directly from the observed data. The area under the plasma concentration-time curve from zero up to the sampling interval of 180 min (AUC0–180 min) was calculated by a combination of the linear (from 0 to 60 min) and log-linear trapezoidal methods (from 60 to 180 min). The area under the mean plasma concentration-time curve from zero up to infinity (AUCtotal) was determined as the sum of the AUC0–180min and of the extrapolated part of the area i.e. the ratio of the concentration predicted at the time interval of 180 min and the terminal rate constant λz. The λz was estimated using linear regression of the logarithmically transformed concentrations against the time. The half-life was calculated as follows: t1/2= ln(2)/λz. Statistical analysis was performed using GraphPad Prism, version 5.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Plasma time profile after i.m. administration exhibits a standard pharmacokinetic curve (Figure 1). The very fast invasive part of plasmatic curve (Tmax 38) and relatively slow elimination from blood (elimination half-life 106 min). Plasma time profile after i.g. administration is prolonged (Tmax 57 min) without gradual decreasing of blood concentrations in the eliminating phase (Figure 2). Due to this fact, some of parameters (clearance, half-life and apparent volume of distribution) cannot be determined. The summary of the main pharmacokinetic parameters are shown in Table 1. Figures 3 and 4 show the concentrations in individual tissues. The high concentrations in kidney indicate the

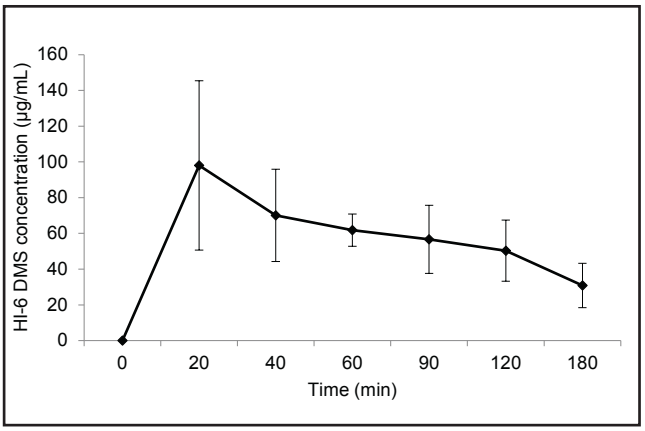

Fig. 1. Plasma time profile of HI-6 DMS after intramuscular administration to pigs. Each point in the time curves represents the mean with S.E.M (n=5).

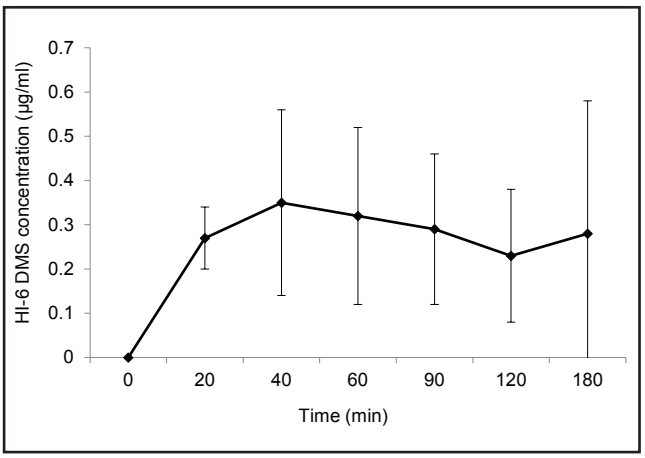

Fig. 2. Plasma time profile of HI-6 DMS after intragastric administration to pigs. Each point in the time curves represents the mean with S.E.M (n=6).

Tab. 1. The main pharmacokinetic parameters of HI-6 calculated from plasma concentrations after a single dose of i.m. and i.g. administration of HI-6 DMS (1.5 g).

Parameter	HI-6 DMS (i.m.)	HI-6 DMS (i.g.)
C_{max} (µg/ml)	106±37	0.42±0.10
T_{max} (min)	38±9	57±25
AUC_{total} (min.mg/l)	15060±17091709	–
AUC_{0-180} (min.mg/l)	10020±902	48±11
λ_z (1/min)	0.007±0.001	–
Half-life (min)	106±19	–
Clearance (ml/min/kg)	105±11	–
V_z (l/kg)	152±2	–

All values are means ± S.E.M. (i.g. n=6; i.m. n=5). C_{max} = maximum plasma concentration of HI-6, T_{max} = time to reach C_{max}, AUCtotal= area under the concentration-time curve of plasma HI-6 from zero up to infinity, AUC_{0-180} area under the concentration-time curve of plasma HI-6 in the time segment, λ_z = terminal rate constant, Half-life = the time required for the concentration of the HI-6 to reach half of its original value, V_z = apparent volume of distribution.

main route of elimination of HI-6 from body. Higher concentrations were also found in the heart on the other hand the accumulation of HI-6 is very low in the liver. Lower brain concentrations were found in comparison to the hypophysis (pituitary gland).

DISCUSSION

In the previous experimental studies, our research team focused on determining the effects of various type xenobiotics (including side effects) on myoelectrical activity of stomach in pigs (Bures 2011a; 2011b; 2013; Kunes 2010; Kvetina 2008; Tacheci 2010). This paper presents partial results (pharmacokinetics and tissue distribution) of currently carried out experiments evaluating potential side effects of acetylcholinesterase reactivators on gastrointestinal tract. Besides testing of newly synthesized reactivators (K203 or K027) we are also studying HI-6 as standard oxime. The doses of HI-6 DMS administered were three times higher than therapeutically standardly use in practice (500 mg in autoinjector for intramuscular delivery). Such high doses were chosen due to evaluating of their potential side effects on gastrointestinal tract and to defining of toxicological risk.

The oxime HI-6 is still the most effective among commonly used oximes; nevertheless, it is a weak reactivator of tabun-inhibited AChE (Kuca *et al.* 2009; Lundy *et al.* 2006). Therefore the new structural analogues of monopyridinium or bispyridinium oximes to increase the effectiveness of antidotal treatment of acute poisonings with nerve agents are developed. The therapeutic effectiveness of all oximes is based on their bioavailability and fast absorption after administration (Jokanovic *et al.* 2009). The main therapeutic target is AChE in the central and peripheral nervous system and neuromuscular junctions. The main reason why oximes are applied i.m. is lower number of biological barrier needed to cross in the way to reach blood circulation. Other non-invasive routes of administration are still considered as non-effective (Voicu *et al.* 2010a,b) because limited ability oximes to cross the biological membranes (Karasova *et al.* 2013c). This fact was also confirmed in this present study where the maximal blood concentrations after intragastric administration are two orders of magnitude lower (0.42 vs 106 μg/mL) in comparison to intramuscular HI-6 delivery.

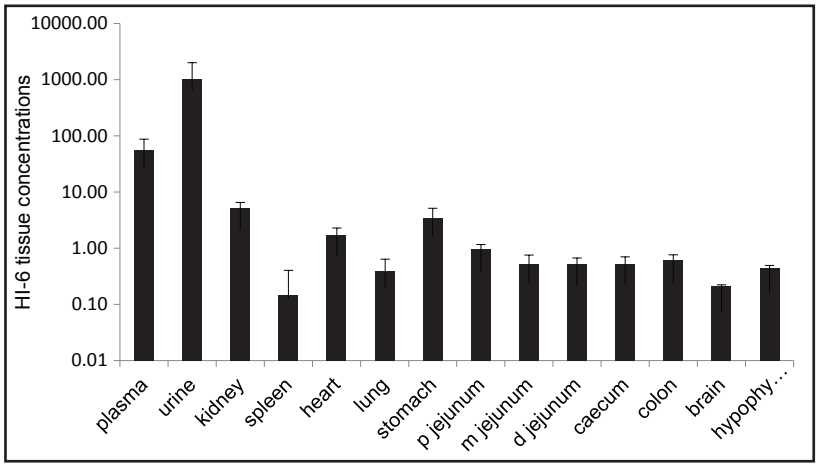

Fig. 3. HI-6 tissue concentrations (μg/mL) 30 min after single dose of HI-6 DMS intramuscular administration. Each column represents the mean with S.E.M (n = 3).(p jejunum – proximal part; m jejunum – middle part; d jejunum-distal part).

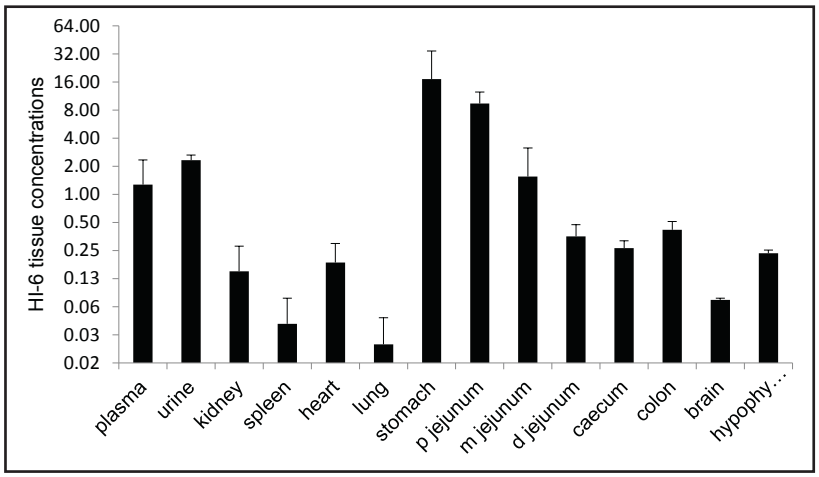

Fig. 4. HI-6 tissue concentrations (μg/mL) 3h after single dose of HI-6 DMS intragastric administration. Each column represents the mean with S.E.M. (n = 3). (p jejunum – proximal part; m jejunum – middle part; d jejunum-distal part).

Generally, pyridinium aldoximes (PyAls) are polar organic compounds with large negative lipophilicity (logP) values (Kalász *et al.* 2014). An attempts to improve the antidote efficacy, some of PyAls, such as K027, (Kuca *et al.* 2003), K048 (Kuca *et al.* 2004), K074 (Kuca *et al.* 2005) or K 203 (Musilek *et al.* 2007) were synthesized. A novel direction in the development of antidotes deals with the introduction of non-quaternary organic compounds. The absence of any quaternary group gives them logP higher than that of pyridinium aldoximes. Non-quaternary reactivators follow different rules than quaternary reactivators when penetrating into the brain.

Important role in reactivation potency of oximes play pH, degree of its ionization at the AChE active site (Worek *et al.* 2011) and also its structural state (Mercey *et al.* 2012).

Other factor influenced efficacy of oximes in the body is their elimination. In the present study, the relatively high levels of HI-6 in the kidneys were found. It was also presented in previous

paper (Karasova *et al.* 2013a). Another important factor is their low plasma protein binding. It was determined that oximes are in very low level binded to the human serum albumin in vitro studies. HI-6, obidoxime, and trimedoxime, which are standardly used in the military, exhibited pharmacologically insignificant binding of 1%, 7%, 6%, respectively. K127 (4%) and K027 (5%) (Zemek *et al.* 2013).

According to presented results, HI-6 DMS is well released from muscle depot. Its maximal blood concentration was found 38 min after i.m. administration. Tissue distribution of oxime after i.m. and after i.g. administration showed the higher concentrations in the kidneys and urine (suggesting elimination pathway). Interestingly, the high levels of the oxime were found in the particular tissues of gastrointestinal tract after both route of administration (after i.m. – comparable with levels in kidneys; after i.g. – one order of magnitude higher than in kidneys and blood plasma). Lower concentration levels in the hypophysis compared to the brain suggests the existence of a functional blood brain barrier. In this study we present pharmacokinetics and tissue distribution of HI-6 DMS after its high dose administration, because the primary objective of the experiments was to define the possible side effects on the gastrointestinal tract (not presented in this paper).

In conclusion, HI-6 DMS quickly reach the systemic circulation after intramuscular administration. Enterally administered oxime diplays a non-classical pharmacokinetic profile and prolonged elimination phase (although high doses were supplied). This could be caused by limitation in transport across biological membranes as mentioned above. Potential side effect after administration of high dose of oxime may be expected in gastrointestinal tract, heart and kidney.

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