Intravenous application of HI-6 salts (dichloride and dimethansulphonate) in pigs: comparison with pharmacokinetics profile after intramuscular administration

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Abstract

OBJECTIVES: Oxime HI-6 is an acetylcholinesterase reactivator therapeutically efficient against nerve agents. Because of their physico-chemical properties, oximes are typically applied intramuscularly (i.m.). This route of administration has also some disadvantages, and alternative strategies ought to be examined. We evaluated the pharmacokinetic profiles of two HI-6 salts after their intravenous (i.v.) administration, and compare the results with the known pharmacokinetics after i.m. administration.

METHODS: Pigs were administered with HI-6 salts (i.v), either HI-6 dichloride (10.71 mg/kg) or molar equivalent HI-6 dimethansulphonate (13.59 mg/kg). Doses of the HI-6 salts corresponded with a standard HI-6 dichloride dose in one autoinjector (500 mg) and were recalculated for one kilogram of body weight.

RESULTS: The main pharmacokinetic parameters are comparable after i.v. and i.m. HI-6 administration. The compared pharmacokinetic parameters were half-life, terminal rate constant, mean residence time of the molecule in the body, clearance, and the apparent volume in the terminal phase. The bioavailability after i.m. administration was comparable with that of i.v.; these results suggest that the oxime is well released from the muscle depot. Significant differences were found in parameters Cmax and Tmax which are important in cases of emergency when rapidity and bioavailability are paramount for the success of treatment.

CONCLUSIONS: I.v. administration should solve the problem of rapid clearance. Infusion or bolus administration may be considered as a logical subsequent step in oxime treatment strategy. The main advantage is in maintenance of an effective therapeutic plasma concentration, a more easily achievable effective therapeutic concentration, and fewer local adverse reactions.
INTRODUCTION

Oxime HI-6 is a promising acetylcholinesterase (AChE; EC 3.1.1.7) reactivator. The high effectiveness is due to its wide spectrum of therapeutic activity against different structures of nerve agents. Oxime HI-6 is considered to be more effective than the commonly-used oximes (pralidoxime and obidoxime); relatively good reactivation potency was found in the treatment of VX, Russian VX, sarin, cyclosarin and also soman intoxication (Karasova et al. 2010; Lundy et al. 2011). The other advantage is its relatively low toxicity compared with the traditional oximes (Clement et al. 1995).

Oximes are typically applied intramuscularly (i.m.) mainly because of their physicochemical properties. HI-6 dichloride is supplied to military personnel in wet/dry autoinjectors as the first self-aid (Lundy et al. 2005). The most important factor that critically influences oxime therapeutic effectiveness is its rapid bioavailability in plasma and tissue (Jokanovic 2009); thus for example, application via the gastrointestinal tract is not considered as adequate.

According to previously published *in vivo* data, oxime HI-6 is relatively rapidly released from the muscle depot, and an effective plasma concentration is achieved in a few minutes after i.m. application (Karasova et al. 2013a). The maximal concentration is achieved in the time interval 20–30 min after administration. Subsequently, there is typically rapid elimination via the kidney. However, this route of application has also some disadvantages. The dose of oxime is limited by its solubility, and the application of a higher volume into the muscle is painful. The other problem relates to the solubility of oxime HI-6 in the autoinjector chamber. The time interval for autoinjector application is relatively short: the oxime HI-6 has to be dissolved in the injection vehicle in a very short time frame. Undissolved crystals may block the injection needle and lead to the reduction of total dose (Thiermann et al. 1998; Lundy et al. 2005). In the case of severely poisoned patients repeated autoinjector application has been recommended. However, intramuscular administration is invasive, and its repetition can be painful (Lamson et al. 2011). Thus new strategies should be examined.

The main aim of our work was to describe the pharmacokinetic profiles of two HI-6 salts, the dichloride and dimethansulphonate (DMS), after i.v. administration, and to compare these results with the known pharmacokinetics profiles for i.m. administration. The applied doses were derived from the standard autoinjector dose (i.m.), and recalculated to correspond with the weight of the experimental animals.

MATERIAL AND METHODS

**Chemicals**

Salts of oxime HI-6 were synthesized in our laboratory by the process previously described. The structural parameters and purity of HI-6 oxime were confirmed using TLC, NMR and HPLC analysis. (Jun et al. 2008, 2010; Kuca et al. 2008). Purity was 99%. Other chemicals (analytical reagent grade) were purchased from standard commercial sources (Merck, Darmstadt, Germany and Sigma-Aldrich, Steinheim, Germany). Double-distilled and deionized water was used for mobile phase preparation.

**Instrumentation**

All analyses were performed on 1260 Infinity series Agilent liquid chromatograph (Palo Alto, CA, USA), composed of degasser, quaternary pump, light-tight autosampler unit set, thermostated column compartment and UV/VIS detector. The maximum HI-6 absorption is 310 nm. Agilent ChemStation software (Palo Alto, CA, USA) was used for analysis of results.

**Separation conditions for HI-6 salts in plasma and brain samples**

Analytical column LiChrospher® 60, 250×4.6 (5 μm) was used for analysis with installed guard column (4×4 RP-select B; Merck, Darmstadt, Germany). The mobile phase composition was 80:20 (v/v) purified water/acetonitrile, with aqueous component 3 mmol/l octane sulphonic acid and 1 mmol/l tetramethylammonium chloride, pH=1.8. The flow rate of the mobile phase was 1.4 ml/min. All chromatograms were obtained at conditioned temperature (30°C) (Karasova et al. 2012).

**Animal treatment**

The use of animals in this study was under the supervision of the Ethics Committee (Faculty of Military Health Sciences in Hradec Kralove, University of Defence in Brno, Czech Republic). The presented study was performed on juvenile female Landrace pigs, *Sus scrofa domestica* (VEMAS Inc., Zamberk, Czech Republic). Animals were housed indoors at the vivarium (temperature 18±2°C, humidity 55±5%), and under standard 12h light/dark cycles. The animals received standard laboratory diet A1 (VEMAS Inc., Zamberk, Czech Republic) and were allowed tap water *ad libitum*.

The animals were divided into two groups (n=3) and labeled by ear tags. The average body weight was approximately 22±2 kg. Experiments were conducted after 14 days of acclimatization. All animals were
premedicated (i.m.) with ketamine 30 mg/kg (Narketamon, Spofa, Czech Republic) in combination with azaperone 2 mg/kg (Stresnil, Janssen Pharmaceutica, Belgium) and atropine 0.05 mg/kg (Atropin Biotika A.U.V., Slovak Republic). Subsequently, the animals were placed in the dorsal recumbent position on an operating table, intubated with an ET 6.0–6.5 and anaesthetized by isoflurane inhalation (at concentration 2–0.5%). Venous access was established by inserting a 16 gauge i.v. catheter (Cavafix Cetro, B-Braun, Germany) into vena jugularis externa. Catheter outlet was via subcutaneous tunnel behind the ear, and fixed to the skin tissue (Lundy et al. 2005; Karasova et al. 2013a).

After anesthesia stabilization, the oxime HI-6 dichloride (i.v.; 10.71 mg/kg, prepared in situ using 0.9% saline) or the oxime DMS salt (i.v.; 13.59 mg/kg, prepared in situ using 0.9% saline) was administered. Doses applied i.v. correspond with doses applied i.m. Blood samples (500 μl) were serially removed at regular time intervals: 1, 3, 5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, 180 and 240 min after i.v. administration, and were drawn into heparinized tubes. Plasma was prepared by centrifugation (1 600 g, 10 min, 4 °C, Universal 320R, Hettich, Germany) and frozen at −80 °C prior to analysis (one week).

Sample preparation for HPLC analysis

200 μl plasma samples 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 used for HPLC analysis.

Calibration

A calibration curve 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. The amounts of oxime HI-6 in each sample were converted to concentration by interpolation of the calibration curve using the data analysis and statistical software program Prism4 (Graph Pad Software, USA).

Pharmacokinetics modeling

Pharmacokinetics modeling was performed using Kinetics software, version 4.0 (InnaPhase Corporation, Thermo Fisher Scientific Inc. Waltham, MA, USA). Population parameter values were estimated using the measured plasma concentrations of the animals and then the animal’s pharmacokinetic parameters were obtained according to the maximum a posteriori Bayesian fitting method. Moreover, a standard non-compartmental approach was used to determine $C_{\text{max}}$ and $T_{\text{max}}$ from the experimentally assayed concentrations. The area under the plasma concentration-time curve (AUC) total or in the time interval was calculated by the linear trapezoidal method.

RESULTS

The absorption curves of both HI-6 salts after i.v. administration were measured and compared to examine the possibility that the DMS salt might have better pharmacokinetic profile than the dichloride salt. In addition, the achieved i.v. results were compared with the known pharmacokinetics profile after i.m. administration of the same doses of both HI-6 salts.

Figure 2 shows the plasma HI-6 concentrations measured following i.v. injection of 10.71 mg/kg of HI-6 dichloride compared with values obtained after similar administration of the molar equivalent of HI-6 DMS (13.59 mg/kg). The pharmacokinetic constants calculated for i.v. administration are detailed in Table 1. In the same table the statistically important constants of the i.v. and i.m. pharmacokinetic profiles are also compared.

Many of the main pharmacokinetic parameters are comparable between the i.v. and i.m. routes of administration. No significant differences between HI-6 salts were found. The bioavailability after i.m. administration was comparable with that of i.v. Significant differences were found in parameters $C_{\text{max}}$ and $T_{\text{max}}$.

DISCUSSION

The therapeutic effectiveness of all oximes is based on their bioavailability and fast absorption after administration (Jokanovic et al. 2009). Although they are quickly released from the muscle depot into the vascular system and plasma (Karasova et al. 2013a), the subsequent transport to the point of action is limited by biological barrier (Karasova et al. 2013b). The main therapeutic target is AChE in the central and peripheral nervous system and neuromuscular junctions. Their lower biological barrier to permeation is the main reason why oximes are applied i.m. Other non-invasive routes of administration are still considered as non-effective (Voicu et al. 2010a,b).

Fig. 1. Calibration curve of HI-6 for chromatographic analysis

$$y = 22.07x + 0.2643$$

$R^2 = 0.9995$
The ionization of oximes at physiological pH also plays a crucial role in their distribution process. The oxime ionized state explains the pharmacodynamic effect (reactivation potency) at the AChE active site (Worek et al. 2011). On the other hand, the passive transport through the biological barriers is strictly limited by this structural state (Mercey et al. 2012).

The other factor that influences oxime efficacy is the oxime elimination process. Oximes are rapidly eliminated from plasma via the kidneys. This fast elimination would be explained by their low barrier permeation, such that much of the oximes stay in plasma (Karasova et al. 2013a). A second important factor is their low plasma protein binding. The fast elimination that leads to reduction of plasma oxime levels should be considered as a major reason for failure of therapy.

From the pharmacokinetics point of view, the main focus of investigation should be on simplifying
the application routine, reducing adverse effects, and improving oxime bioavailability (Kušić et al. 1991; Clement et al. 1995). In recent years, intravenous formulation for HI-6 administration has been discussed. Intravenous administration may be preferred over an i.m. application in some case of emergency (Pawar et al. 2006).

If we compare important pharmacokinetic parameters after i.v. and i.m. administration, many of them are comparable. Also, no significant differences between the HI-6 salts were found. Comparable pharmacokinetic parameters were half-life, terminal rate constant, mean residence time of the molecule in the body, clearance, and apparent volume in the terminal phase. The bioavailability after i.m. administration was comparable to that of i.v.; these results suggest that oxime is well released from muscle depot. As we expected, significant differences were found in classical parameters such as Cmax and Tmax. Both these parameters are important in the case of emergency when rapidity and bioavailability is critical for the success of treatment (Yanagisawa et al. 2006).

Intravenous application should also solve the problem of rapid clearance. Infusion application may be considered as a logical subsequent step in oxime treatment strategy. The main reason is the maintenance of an effective therapeutic plasma concentration, more easily achievable effective therapeutic concentration, and fewer local adverse reactions. Especially for in-hospital use, it is preferable that the oxime is delivered independently from other treatments in order to allow the physician to titrate the dose of oxime and atropine individually, according to the casualty’s condition.

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