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Hyaluronidase: Its effects on HI-6 dichloride and dimethanesulphonate pharmacokinetic profile in pigs

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HIGHLIGHTS

- New promising strategy after oxime HI-6 i.m. application was described.
- Previously published data (Toxicology) about HI-6 DMS salt were confirmed.
- Better pharmacokinetics profile was found in combination with hyaluronidase.
- Hyaluronidase is able to increase tissue permeability and therapy effectiveness.

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ABSTRACT

Pigs were administered intramuscularly molar equivalents of HI-6 salts (HI-6 dichloride 10.71 mg/kg and HI-6 DMS 13.59 mg/kg) either with or without hyaluronidase (60 U/kg). Hyaluronidase is supposed to increase tissue permeability and diminishes discomfort caused by the intramuscular injection. Doses of HI-6 salts corresponded with standard HI-6 dichloride dose in one autoinjector (500 mg) and were recalculated for 1 kg of body weight.

According to the results, both HI-6 salts applied in combination with hyaluronidase had increased tissue absorption and improved pharmacokinetic profile. The C_{max} was significantly higher in case of HI-6 DMS plus hyaluronidase (29.6 ± 2.98 μg/ml) administration increase compared to HI-6 DMS (23.8 ± 3.04 μg/ml) and HI-6 dichloride (19.0 ± 0.93 μg/ml); both without hyaluronidase. Bioavailability calculated as AUC_{total} (HI-6 DMS with hyaluronidase, 4119 ± 647 min μg/ml) was also significantly higher compared to HI-6 DMS (2259 ± 329 min μg/ml) and HI-6 dichloride (1969 ± 254 min μg/ml); both without hyaluronidase.

The results suggest that administration of HI-6 salt with higher solubility is the first step in the improvement of application strategy, but use some substances with spreading effect (hyaluronidase) may also leads to better absorption and better bioavailability. Improved bioavailability could to go hand in hand with increased effectiveness of therapy without the need of multiple autoinjector applications.

1. Introduction

Oxime HI-6 is one of the promising acetylcholinesterase (AChE; EC 3.1.1.7) reactivators due to its wide spectrum of therapeutic activity against organophosphate nerve agents. According to previously published *in vitro* and *in vivo* data it is more effective than traditionally used oximes such as pralidoxime or obidoxime. HI-6 is considered particularly effective in the treatment of soman, cyclosarin, VX and Russian VX poisoning (Lundy et al., 2011; Karasova et al., 2010a,b). On the other hand HI-6 is not as effective in case of tabun and pesticide poisoning (Musilek et al., 2011).

Oximes are typically applied intramuscularly mainly because to their physico-chemical parameters. HI-6 dichloride is supplied to military personnel in wet/dry autoinjectors as the first self-aid (Lundy et al., 2005). Although HI-6 dichloride appears to be relatively safe for human use as data form preclinical (toxicity studies) and human trials suggest; it is still not licensed for civilian use in case of a terroristic attack (Krummer et al., 2002).

The most important factors that strictly influence therapeutic effectiveness of oximes are rapid absorption of the oxime from muscular depot and the oxime blood concentration (bioavailability of oxime). Both of these parameters are dependent on the amount of

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oxime in the autoinjector chamber (usually 500 mg of HI-6 dichloride). This dose is limited by the solubility of HI-6 in the small volume of dissolving agent available in the autoinjector (Thiermann et al., 1998). Since the application time interval is relatively short, the HI-6 has to dissolve in a very short time frame. Undissolved crystals may block the injection needle and lead to the reduction of total dose (Lundy et al., 2005). The lower HI-6 dichloride solubility was previously solved with the introduction of new dimethanesulfonate (DMS) salt, which increased HI-6 solubility especially under lower temperatures (Kuca et al., 2007). In case of severely poisoned patients repeated autoinjector application was recommended. However, intramuscular administration is invasive and its repetition can be painful (Lamson et al., 2011). The main interest should be to focus on simplifying the application routine and on the reduction of adverse effects.

According to previously published results have both HI-6 salts comparable/identical mechanism of action and also therapeutic effectiveness (Bogan et al., 2012). DMS salt also appears to be exceptionally safe and could be recommended for human use (Lundy et al., 2005). The higher solubility of HI-6 DMS may be also demonstrated on the change of the pharmacokinetic profile especially on C_{max} and time interval when it was achieved. In the presented study we attempt to establish a relationship between both salts with respect to crucial pharmacokinetic parameters (C_{max} and bioavailability). Furthermore, we investigated changes in pharmacokinetic profiles after application of HI-6 salts in combination with enzyme hyaluronidase (hyaluronoglucosaminidase; EC 3.2.1.35).

Hyaluronidase depolymerizes the mucopolysaccharide hyaluronic acid (component of the tissue cement) and increases membrane permeability. It is used in standard therapy for some time now (El-Safory et al., 2010). Its application renders to spreading effect in injected compartment (muscle in case of AChE reactivators) (Dunn et al., 2010). The faster and better absorption of oxime from the muscular depot may lead to the increase in therapeutic effectiveness.

The main aim of our work was to compare pharmacokinetic profiles of both salts after i.m. application. Moreover, we evaluated changes in pharmacokinetic profiles after application of HI-6 salts in combination with hyaluronidase. The applied doses were derived from standard autoinjector dose and recalculated to correspond with the weight of experimental animals. These results should closely demonstrate real pharmacokinetic profile of oxime HI-6 after i.m. administration of standard dose.

2. Methods

2.1. Chemicals

Both salts of oxime HI-6 were synthesized in our laboratory. The purity of oxime HI-6 (99%) was confirmed by TLC and NMR (Kuca et al., 2008; Jun et al., 2008). Hyaluronidase from bovine testes (Finepharm S.A., Jelenia Góra, Poland) with specific activity 12,000 IU/mg was a gift from Contipro Group s.r.o. (Dolni Dobrouc, Czech Republic). 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.

2.2. 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 results analysis.

2.3. HPLC determination of HI-6 in plasma

Analytical column LiChrospher® 60,250 × 4.6 (5 μm) was used for analysis with installed guard column (4 × 4 RP-select B; Merck, Damstadt, Germany). The mobile phase composition was 80:20 (v/v) purified water/acetonitrile; aqueous component (3 mmol/l 1-octanesulfonic acid and 1 mmol/l tetramethylamonium chloride, pH = 2.2). The flow rate of the mobile phase was 1.4 ml/min. All chromatograms were obtained under 30 °C temperature (Karasova et al., 2012).

2.4. Animal treatment

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

Animals were divided into four 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.) by ketamine 30 mg/kg (Narkamon, 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, 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 (in 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 ear and fixed to the skin tissue.

2.5. Injection of HI-6 salts or HI-6 salts with hyaluronidase in pigs

After the anesthesia stabilization the oxime HI-6 dichloride (i.m.; 10.71 mg/kg, prepared *in situ* using 0.9% saline) and DMS salt (i.m.; 13.59 mg/kg, prepared *in situ* using 0.9% saline) were applied. Both salts were also administered in the same doses in combination with hyaluronidase (i.m.; 60 U/kg). Control blood samples were drawn into heparinized tubes from the arterial catheter and additional samples were drawn at various time intervals up to 4 h following HI-6 or HI-6/hyaluronidase applications.

Blood samples (800 μl each) were drawn into heparinized tubes at regular time intervals: 0, 1, 3, 5, 10, 20, 30, 40, 60, 90, 120, 180 and 240 min after i.m. application. Plasma was prepared by centrifugation (1600 × *g*, 10 min, 4 °C, Universal 320R, Hettich, Germany) and frozen at −80 °C prior to analysis (1 week).

2.6. Sample preparation for HPLC analysis

100 μl plasma samples were mixed with 25 μl trichloroacetic acid in order to precipitate proteins (in triplicates). The samples were spun at 21,000 × *g* at 4 °C for 15 min (M 240R, Hettich, Germany). The supernatant was used for HPLC analysis.

2.7. Calibration

A calibration curve was established using plasma samples spiked with oxime HI-6 (0.94, 1.90, 3.75, 7.50, 15.00 and 30.00 μg/mL samples, in triplicates). The calibration points were recalculated as body of HI-6 molecule without salts. The retention time of oxime HI-6 was ~5.7 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 GraphPad Prism 4 (Graph Pad Software, USA).

2.8. Pharmacokinetic analysis and statistical methods

Standard noncompartmental analysis was performed using the Kinetica software, version 4.0 (InnaPhase Corporation, Thermo Fisher Scientific Inc., Waltham, MA, USA). Maximum concentration (C_{max}) and the time to the maximum concentration (T_{max}) were determined directly from the observed data. The area under the mean plasma concentration–time curve from zero up to the last sampling interval of 240 min ($AUC_{0-240\,min}$) was calculated by a combination of the linear (from 0 to 90 min) and log-linear trapezoidal methods (from 90 to 240 min). The area under the mean plasma concentration–time curve from zero up to infinity (AUC_{total}) was determined as the sum of the $AUC_{0-240\,min}$ and of the extrapolated part, i.e., the ratio of the concentration predicted at the time interval of 240 min and the terminal rate constant λ_z. The λ_z was estimated using the linear regression of the log transformed concentrations at 90, 120, 180 and 240 min plotted against time. The half-life was calculated as follows: $t_{1/2} = \ln(2)/\lambda_z$. Statistical analysis was performed using GraphPad Prism, version 5.0 (GraphPad Software, San Diego, California, USA). The pharmacokinetic characteristics of HI-6 observed after administration of both salts alone and in combination with hyaluronidase (i.e., in four groups of animals) were compared using one-way analysis of variance. When a significant effect was found, the Tukey's multiple comparison test was used to compare the means between the respective groups. For all statistical procedures, the P value < 0.05 was taken as significant. Kruskal–Wallis test was used to evaluate T_{max}.