Impact of paraoxon followed by acetylcholinesterase reactivator HI-6 on gastric myoelectric activity in experimental pigs

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Abstract

OBJECTIVES: Organophosphorus compounds represent nerve agents, pesticides and several industrial compounds. Treatment after exposure to organophosphates involves the use of parasympatolytics, acetylcholinesterase (AChE) reactivators/modulators and anticonvulsive drugs. Wider clinical use of several AChE reactivators/modulators might be limited because of possible side effects, including gastrointestinal toxicity. In this study we evaluated the effect of paraoxon and an AChE reactivator (HI-6) on the gastric myoelectric activity in experimental pigs.
METHODS: Six female experimental pigs (mean weight 33 kg) entered the study. Intramuscular paraoxon (1.5 g) was administrated after the baseline gastric electrogastrography (EGG) recording, followed by HI-6 dimethansulphonate (1.5 g i.m.) 10 min. later. A further ten 15-minute-interval EGG recordings were performed. Running spectral analysis was used for the elemental evaluation of the EGG. The results were expressed as dominant frequency of slow waves at all intervals of EGG recordings. EGG power analysis was performed in all animals.
RESULTS: Paraoxon induced a non-significant decrease of dominant frequency (2.8±0.6 vs. 2.6±0.5 cycles per min.; p=0.092). Subsequent administration of HI-6 normalised dominant frequency to basal values and increased it significantly within the subsequent 30 minutes (3.0±0.4; p<0.001). Paraoxon administration did not influence the power (within a 10-minute exposure). However, the amplitudes increased significantly 90 minutes after administration of HI-6 (819±109 vs. 5054±732 μV^2; p<0.001).
CONCLUSIONS: AChE reactivator HI-6 blocked the gastric effect of paraoxon significantly. Subsequent myoelectric changes in the dominant frequency and power were executed by HI-6. The effect of paraoxon was non-significant.

ORIGINAL ARTICLE

Abbreviations:

AChE	- acetylcholinesterase
EGG	- electrogastrography
HI-6	- oxime (AChE reactivator)
DMS	- dimethansulphonate
i.v.	- intravenous
i.m.	- intramuscular
HPLC	- high-performance liquid chromatography
TLC	- thin layer chromatography
NMR	- nuclear mass resonance
C_{max}	- maximum drug concentration in blood

INTRODUCTION

Organophosphorus compounds represent a wide and heterogeneous group, containing pesticides, nerve agents, industrial compounds and others. Their biological activity of inhibiting acetylcholinesterase (AChE) ranks them as life endangering agents (Bajgar 2004, 2010; Bajgar *et al.* 2007; Kassa *et al.* 2012). The necessary treatment after exposure to organophosphates involves the use of parasympatolytics (anticholinergics), AChE reactivators (oximes), anticonvulsive drugs (e.g. benzodiazepines) and general treatment of metabolic dysbalance (Kassa *et al.* 2008b; Soukup *et al.* 2008; Bajgar *et al.* 2009; Bajgar 2010). Thus the reactivators of AChE are essential compounds in the treatment of cases of organophosphate intoxication (Bajgar *et al.* 2007; Kuca *et al.* 2009). Wider clinical use of several AChE reactivators/modulators might be limited because of possible side effects, including gastrointestinal toxicity.

Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical activity (Chen *et al.* 1994; Parkmann *et al.* 2003; Koch & Stern 2004). Our group has demonstrated that EGG is also reliable and feasible in experimental pigs (Varayil *et al.* 2009; Kvetina *et al.* 2010; Tacheci *et al.* 2011). Porcine EGG is fully comparable with that recorded in healthy humans (Tacheci *et al.* 2013). Pigs can be used in various preclinical experiments (Kvetina *et al.* 2008; Kopacova *et al.* 2010; Kunes *et al.* 2010; Tacheci *et al.* 2010; Bures *et al.* 2011a,b) as an omnivorous representative due to their relatively very similar gastrointestinal functions compared to humans (Kararli 1995; Suenderhauf & Parrott 2013).

The aim of this study was to evaluate the effect of paraoxon and an AChE reactivator (HI-6) on the gastric myoelectric activity in experimental pigs.

MATERIAL AND METHODS

Animals

Six experimental mature female pigs (*Sus scrofa f. domestica*, hybrids of Czech White and Landrace breeds; 3–4 months old, weighing 31–36.5 kg, mean 33, median 32.5 kg) were included into the study. Animals were fed twice a day (standard assorted food A1) and were allowed free access to water.

Experimental design

All EGG recordings were performed under general anaesthesia in the morning after 24 hours of fasting. Intramuscular injections of ketamine (20 mg/kg; Narkamon, Spofa, Prague, Czech Republic) and azaperone (2.2 mg/kg; Stresnil, Janssen Animal Health, Saunderton, UK) were used as an introduction to anaesthesia in all animals. A 15-minute baseline EGG recording was started 20 minutes after this intramuscular injection. Thiopental (Thiopental Valeant, Valeant Czech Pharma s.r.o., Prague, Czech Republic; i.v. infusion 200 mg/hr) was used for subsequent maintenance of general anaesthesia.

Intramuscular paraoxon (1.5 g) was administrated after the baseline EGG recording was completed, followed by HI-6 (1.5 g i.m.) 10 min. later. Further ten 15-minute-interval EGG recordings were performed. Paraoxon was obtained from Sigma-Aldrich (St. Louis, MO, USA). Oxime HI-6 DMS (dimethansulphonate) was synthesized in laboratories at Department of Toxicology, Faculty of Military Health Sciences University of Defence in Hradec Králové, using a process described previously (Kuca *et al.* 2008). The identification (TLC and NMR) (Jun *et al.* 2008; Kuca *et al.* 2008) of HI-6 DMS and its purity (99% HPLC) was also checked (Jun *et al.* 2010). Two vital signs were monitored, we used pulse oximetry to secure the experiment and heart rate to assess possible systemic circulatory effects of paraoxon and HI-6 DMS.

Electrogastrography

All animals were lying in a right lateral position during EGG recording. The epigastric area was shaved first and the skin was gently sandpapered afterwards. Six active self-adhesive electrodes were placed on the upper part of the abdomen, the 7th electrode (basal) was placed left of the middle sternum. A special abdominal belt (respiratory sensor) was used to identify possible artefacts due to breathing and body movements.

Surface cutaneous EGG was recorded using an Electrogastrography Stand Alone System (MMS – Medical Measurement Systems B.V., Enschede, the Netherlands).

MMS software (version 8.19) was used to assess EGG recordings. Running spectral analysis (based on Fourier transform) was used for the elemental evaluation of the EGG. The results were expressed as dominant frequency of slow waves at all intervals of EGG recordings. EGG power analysis (areas of amplitudes) was accomplished afterwards in all animals.

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, Protocol Number 14/12 (2012). 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).

Statistical analysis

Data were statistically treated by means of descriptive statistics, non-paired t-test, Mann-Whitney rank sum test and Pearson product moment correlation using the SigmaStat software (Version 3.1, Jandel Corp., Erkrath, Germany).

RESULTS

Dominant frequency

Paraoxon induced a non-significant decrease of dominant frequency within a 10-minute interval (mean 2.8±0.6 vs. 2.6±0.5 cycles per min.; p=0.092). Subsequent administration of HI-6 DMS returned dominant frequency back to basal values and increased it significantly within the subsequent 30 minutes (3.0±0.4; p<0.001), see Table 1 for details. Further changes during total 150-minute EGG recording were not statistically significant.

Analysis of amplitudes

Paraoxon administration did not influence the EGG power (within a 10-minute exposure). However, the amplitudes increased significantly 90 minutes after the HI-6 DMS administration (819±1092 vs. 5054±7322 μV^2; p<0.001), see Table 2 for details. Amplitudes subsequently decreased to mean 875 (confidence interval of mean 247, median 546) at 135 minutes and finally slightly increased to mean 2497 μV^2 (confidence interval of mean 1107, median 523) at 150 minutes.

Vital signs

There was a non-significant increase in heart rate after HI-6 DMS administration (p=0.151). The power of the performed test (0.184) was below the desired power of 0.800. Oxygen saturation remained within the normal range during the whole experiment in all animals.

DISCUSSION

The purpose of our project was to study possible effects of paraoxon and an AChE reactivator HI-6 DMS on electrogastrography in experimental pigs. To the best of our knowledge, there are no previously published data on the impact of organophosphates and/or AChE reactivators on gastric myoelectric activity in an experimental setting.

Paraoxon is an active metabolite of parathion, one of the most potent AChE-inhibiting pesticides available (Worek *et al.* 2008; Herkert *et al.* 2012). Paraoxon toxicokinetics in pigs was studied mostly after percutaneous absorption (Qiao *et al.* 1994). The bioavaibility of HI-6 salts after intramuscular administration is comparable with intravenous administration. According to previously published in vivo data, HI-6 is relatively rapidly released from muscle depot, effective plasma concentration is achieved in a few minutes after i. m. application (Zdarova Karasova *et al.* 2013, *in press*). The maximal concentration may be found in the time interval 10–30 min. after administration of HI-6 in experimental pigs. In subsequent time intervals there is a typical rapid elimination via the kidneys (our unpublished data).

Several studies were published on the interaction of paraoxon and HI-6 in pigs (Worek *et al.* 2008, 2011). HI-6 appears extremely safe and effective against paraoxon poisoning (Lundy *et al.* 2005, 2006). The maximum concentration of HI-6 in the plasma (C_{max}) after a single intramuscular injection in pigs was reached after 13±3 min. (Zdarova Karasova *et al.* 2013). We decided

Tab. 1. Electrogastrography. Running spectral analysis based on Fourier transform (dominant frequency in cycles per minute).

Parameter	Mean	C.I. of Mean	Median	25%–75% Quartiles
Rest	2.8	0.12	2.8	2.3 3.1
Paraoxon	2.6	0.11	2.3	2.1 3.1
HI-6	2.8	0.12	2.7	2.3 3.1
HI-6_30	3.0	0.09	3.1	2.8 3.3
HI-6_90	3.1	0.11	3.3	3.1 3.3

Rest – a baseline 15-minute EGG recording
Paraoxon – a 10-minute recording following i.m. paraoxon administration
HI-6 – a 15-minute recording following i.m. HI-6 administration
HI-6_30 – a 15-minute recording 30 minutes after HI-6 administration
HI-6_90 – a 15-minute EGG recording 90 minutes after HI-6 administration
C.I. – confidence interval

Tab. 2. Electrogastrography. Power analysis (amplitudes in μV^2).

Parameter	Mean	C.I. of Mean	Median	25%–75% Quartiles
Rest	819	229.0	308	195 870
Paraoxon	645	162.0	316	202 905
HI-6	678	192.7	357	239 860
HI-6_30	2316	830.9	579	215 1732
HI-6_90	5055	1533.6	1450	465 6590

Rest – a baseline 15-minute EGG recording
Paraoxon – a 10-minute recording following i.m. paraoxon administration
HI-6 – a 15-minute recording following i.m. HI-6 administration
HI-6_30 – a 15-minute recording 30 minutes after HI-6 administration
HI-6_90 – a 15-minute EGG recording 90 minutes after HI-6 administration
C.I. – confidence interval

on a shorter period between paraoxon and HI-6 administration. The dose of paraoxon was rather high in our study and it might induce non-reversible cholinergic effect and thus threaten the survival of experimental animals.

In humans, cholinergic stimulation increases gastric slow wave frequency (Koch & Stern 2004). Similar regulation occurs in the murine stomach. Acetylcholine and carbachol greatly accelerated gastric pacemaker frequency in the murine model (Kim *et al.* 2003). We found that paraoxon, a potent AChE inhibitor, induced only a non-significant decrease of dominant frequency within an initial 10-minute interval, subsequently soon blocked by HI-6. Although the toxic effect of paraoxon after intramuscular administration is rather rapid (Kassa *et al.* 2008a) the 10-minute interval to HI-6 administration which we decided on was not long enough to enable full paraoxon-induced AChE inhibition. This assumption is supported by another part of our study. This is the effect of HI-6 expressed 30 minutes and longer after its i.m. administration. After the latency of a few tens of minutes, increased activity of AChE might decrease available acetylcholine and the parasympatholytic effect prevails.

Surprisingly, there were no significant changes in heart rate in our study after paraoxon and subsequent HI-6 administration. On the other hand, in the study by Lundy *et al.* (2005), HI-6 DMS failed to produce dramatic changes in any of the cardiovascular or respiratory parameters measured. There were no clinically significant alterations in minute ventilation, end tidal CO2, heart rate, mean arterial pressure, arterial O2 saturation or body temperature. The lack of effect of 500 mg of HI-6 dichloride in pigs of this type has been previously demonstrated (Gorransson-Nyberg *et al.* 1995). This and the previous study appearing to be in excellent agreement shows however that the intravenous dose of 1899 mg DMS (when the plasma HI-6 concentration reached 761 μg/mL) resulted in a reduction of approximately 20% in arterial pressure of fairly short duration presumably as a result of the weak ganglionic blocking properties of HI-6 (Lundy & Tremblay 1979). No significant effects on biochemistry, haematology, electrolytes or blood gasses were observed following HI-6 dichloride or DMS at any dose in the presence or absence of atropine.

Very large doses of HI-6 which in baboons produced large plasma concentrations are well tolerated judging from both physical signs and cardiovascular or respiratory responses and lack of clinically significant alterations in clinical chemistry parameters (Amitai *et al.* 1995). This is important with respect to the determination of an effective human dose. The dose of HI-6 DMS used in our study was two times higher than is currently supplied in the available autoinjector multipen HAD (CHemProtect, a.s.). Various experimental studies also clearly show that doses of HI-6 which were demonstrated to be protective against large nerve agent doses (5xLD50) in primates, were in the range of 50 mg/kg (Hamilton & Lundy 1989; van Helden *et al.* 1992) or even much higher in some cases (Amitai *et al.* 1995). It is also clear that there are extreme differences in the dose of oximes required to treat different nerve agents.

We are fully aware that our initial results must be interpreted with caution. Especially the explanation of possible mechanism of HI-6-induced changes in dominant frequency and EGG power is difficult and further studies are needed to clarify this issue.

In conclusion, AChE reactivator HI-6 blocked the gastric effect of paraoxon significantly. Subsequent myoelectric changes in the dominant frequency and EGG power were executed by HI-6. The effect of paraoxon was non-significant.

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Conflicts of interest

The authors disclose no conflicts.

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