**Is Rivastigmine Safe as Pretreatment Against Nerve Agents Poisoning? A Pharmacological,
Physiological and Cognitive Assessment in Healthy Young Adult Volunteers**

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Key words: Rivastigmine, Cholinesterase inhibitors, Psychometric evaluation, Nerve agents,
Organophosphates

Abstract

Rivastigmine, a reversible cholinesterase inhibitor, approved as a remedy in Alzheimer's disease, was suggested as pretreatment against nerve agents poisoning. We evaluated the pharmacokinetic, pharmacodynamic, physiologic, cognitive and emotional effects of repeated rivastigmine in young healthy male adults, in a double blind, placebo controlled crossover trial. Three groups completed 3 treatment periods: 0, 1.5 and 3 mg twice a day, for a total of 5 intakes. Parameters monitored were: vital signs, ECG, laboratory tests, sialometry, visual accommodation, inspiratory peak flow, and cognitive function tests. Adverse reactions were mild. Peak blood levels and peak cholinesterase inhibition increased with repeated intakes, and high variability and non-linear pharmacokinetics were demonstrated. In addition, two cognitive functions were affected (perceptual speed and dynamic tracking). The complicated pharmacological profile and the high inter-personal variability limit the potential use of rivastigmine as pretreatment for war fighters and first responders.

Key words: Rivastigmine, Cholinesterase inhibitors, Psychometric evaluation, Nerve agents, Organophosphates

Introduction

The devastating effects of organophosphates (OP) nerve agents were recently demonstrated again in mass casualty events in Syria on August 2013 (Rosman et al, 2014). OP exposure results in the well-defined cholinergic crisis manifested as a dose dependent hyper-secretion, fasciculation, tremor, convulsions, coma, respiratory failure and death (Allon et al, 2011; Grauer et al, 2008; Markel et al, 2008; Munro, 1994; Rosman et al, 2014; Sidell et al, 2008).

Pyridostigmine is commonly used as a pretreatment against OP nerve agent toxicity (Sidell et al, 2008), followed by an antidotal mixture of anticholinergic drugs and an oxime, administered after nerve agent exposure. Pyridostigmine pretreatment by itself provides only minor protection against OP poisoning, mainly through enhancement of the protection provided by the antidotal injection (Sidell et al, 2008). Pyridostigmine does not readily penetrate the blood brain barrier (BBB), hence does not protect from OP-induced major CNS symptoms.

Rivastigmine is a reversible ChE inhibitor that penetrates the BBB (Weinstock, 1999). It is approved for the treatment of mild to moderate dementia of the Alzheimer's type (AD) and dementia related to Parkinson's disease (Corey-Bloom et al, 1998; Emre et al, 2004; Finkel, 2004; Rösler et al, 1998, 1999). Rivastigmine was also found effective in counteracting cognitive damage following traumatic brain injury and focal ischemia in both animal and human (Chen et al, 1998a, 1998b; Cummings, 2000; Giladi et al, 2003; McKeith et al, 2000; Moretti et al, 2001; Silver et al, 2009; Tanaka et al, 1995; Tsujimoto et al, 1993; Wesnes et al, 2002), and was tested for its effects against cognitive deterioration in schizophrenia and drug abuse (Ribeiz et al, 2010; Sofuoglu, 2010; Theunissen et al, 2014). In animal studies, pretreatment with central ChE inhibitors were repeatedly demonstrated to protect against OP nerve agents induced damage (Albuquerque et al, 2006; Alexandrova et al, 2014; Grunwald et al, 2002; Haug et al, 2007; Harris et al, 1991; Janowsky et al, 2004, 2005; Lallement et al, 2001; Philippens et al, 2000).

The potential use of rivastigmine as pretreatment against OP exposure requires a careful analysis of its effects on the general population. Most of the clinical studies with rivastigmine included elderly and AD patients; only few studies tested its effects on young healthy volunteers.

In six young healthy volunteers aged 18-40 that were administered a single dose of 3mg rivastigmine, inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in cerebrospinal fluid (CSF) was first observed 1.2 and 2hr, reached maximal effect of 39.4% and 9.7% inhibition, and lasted up to 8.4 and 3.6hr, respectively. Peripheral enzymes (RBC-AChE and Plasma-BuChE) were minimally inhibited (6-10%). No adverse effects were reported (Kennedy et al, 1999).

Tolerance and pharmacokinetics of rivastigmine (0.16- 4.6mg) were assessed in 80 volunteers in a placebo-controlled study. Up to 3 mg of rivastigmine was well tolerated, but 4 out of 7 subjects treated with 4.6 mg reported nausea, vomiting, headache or dizziness. No peripheral hypercholinergic effects were observed (Enz et al, 1991). In another study with 12 young healthy volunteers, 1.3 and 2mg induced an increase in the density of REM without affecting sleep quality (Holsboer-Trachler et al, 1993). Similar findings were obtained with healthy elderly volunteers (Schredl et al, 2000).

Repeated administration of rivastigmine was well tolerated in AD patients (Cutler et al, 1998). In another study with AD patients, repeated administration of 3mg induced 46% AChE inhibition and 77% BuChE inhibition in the CSF with 35% BuChE inhibition in plasma (Darreh-Shori et al, 2002). Chronic treatment with 3-4mg of rivastigmine for 3-12 months induced 20-30% inhibition of ChE, with CSF AChE inhibition slightly higher than that in plasma (Kaasinen et al, 2002). PET studies in AD patients following 3-5 months of rivastigmine treatment showed differential ChE inhibition of 27-37% in various cortical areas and demonstrate the high affinity of rivastigmine to central cholinergic sites (Hugo et al, 2008).

97 The repeated demonstration of the central effects of rivastigmine suggests likely alteration of
98 cognitive and emotional behaviors. If the drug is to be used as prophylactic treatment against OP
99 poisoning, the target population may consist of otherwise healthy people (e.g. first responders)
100 that may be adversely affected by these cholinergic alterations. Thus, a careful, objective
101 analysis of cognitive and physiological changes following single and repeated administration of
102 rivastigmine is required.

103 The objectives of the presented study were to assess the safety and tolerability of single and
104 repeated rivastigmine administration (1.5 and 3mg) in healthy young male volunteers, to
105 determine the pharmacokinetic and pharmacodynamic profile, and to assess physiological and
106 behavioral effects following, in correlation with plasma rivastigmine concentrations and whole
107 blood ChE inhibition in these subjects.

Methods

This was a single-center, double-blind, placebo-controlled, cross-over study, designed to assess the pharmacokinetic and pharmacodynamic profile and the physiological and behavioral effects following a single and repeated rivastigmine administration in healthy male volunteers. The institutional review boards of Tel-Aviv Medical Center and IDF Medical Corps approved the study. The study was registered in the NIH clinical trials registry (number CT00624663).

Subjects

19 healthy Caucasian males 18 to 40 years old were recruited by the Clinical Research Center at the Tel-Aviv Sorasky Medical Center. Four of the 19 volunteers were excluded before the initiation of dosing (3 developed flu-like symptoms and one retracted his consent). The remaining 15 subjects participated in the study. Sample size was determined by Power/Sample size calculator, based on the requirement of at least 20% differences in means of the behavioral tests performance (power=0.80, two-tailed, $\alpha=0.05$).

Inclusion criteria

No known history of significant neurological, renal, cardiovascular, respiratory, endocrinology, gastrointestinal, hematopoietic disease, neoplasm or any other clinically significant medical disorder; BMI (body mass index) range 18-29; non-smoking (by declaration) for a period of at least 6 months; and no history of drug or alcohol abuse.

Exclusion criteria

Known hypersensitivity to the drug or other carbamates; any history of or currently active cardiac, pulmonary, gastrointestinal, urinary or neurological disease or disorder; significant abnormalities in screening physical exam; abnormal clinical laboratory parameters or electrocardiogram (ECG) within 21 days of the start of the study; significant allergic response to other drugs; adherence (for whatever reason) to an abnormal diet during the 4 weeks prior to the study; recent significant change in body weight; use of any prescription or over-the-counter (OTC) medications, including vitamins and herbal or dietary supplements within 7 days prior to the first study dosing or during the study; history of blood donation in the 3 months preceding the first study dosing or intention to make blood donation during the study, or within the three months following the study completion; history of transfusion of blood or plasma derivatives in the 3 months preceding the first study dosing; participation in another clinical trial of a drug within 3 months prior first study dosing; Inability to communicate well with the investigators and medical staff (i.e., language problem, poor mental development or impaired cerebral performance); difficulty fasting or consuming the standard meals that will be provided; and any acute medical situation (e.g. acute infection) within 48 hours of study onset, which is considered of significance by the Principal Investigator.

Test Drug

Rivastigmine (Exelon, Novartis) capsules were purchased and their content was re-packaged by Concept for Pharmacy (Kfar Sava, Israel) in unmarked capsules for ingestion containing 1.5mg rivastigmine. Identical capsules containing the non-active excipients were also prepared and served as the placebo medication.

Regimens

Three rivastigmine regimens were evaluated: 0 mg (2 placebo capsules) BID, 1.5mg (1 rivastigmine capsule and 1 placebo capsule) BID, 3mg (2 rivastigmine capsules) BID. Rivastigmine was taken 1hr after meals. Each dosing regimen was repeated for 48hr for a total of five intakes (time 0, 12, 24, 36, 48h) starting at 8 am. The time from 1st to 5th intake of a regimen was termed a period, and each volunteer was tested in three successive periods with a washout time of at least one week between periods. Each volunteer was assigned all three regimens (period 1-3) randomly to eliminate possible order effect. Dose sequences were set using a random sequence generator.

Medical examinations

Full medical check-up was completed in all the volunteers prior to initiation of the study. This included comprehensive medical history, complete physical examination and laboratory tests (blood count, biochemistry, urinalysis, urine toxic screen, and serology for HIV, HBV and HCV).

Follow-up physical examination including vital signs and an ECG were performed 30min and 2 hours after each drug or placebo intake. A final physical examination was completed 24h after the last intake in each period. Follow-up laboratory tests were performed at the end of each period.

Physical complaints during the study were recorded using two parallel methods. The volunteers were instructed to report any adverse clinical event or complaint as they occurred, in addition to their responses to a structured questionnaire that included specific questions about subjective health effects (type, timing and severity) 3h after the 1st, 3rd and 5th drug or placebo intake and 24h after the 5th and last intake in each study period. Each complaint was further investigated and

180 its severity determined by the medical personnel. Medical intervention following a complaint
181 was recorded.

182 Physiological assessment of possible cholinergic effects was performed using the following tests:
183 saliva production, visual accommodation and acuteness, and exhalation peak flow.

184

185 Saliva production (sialometry)

186 The amount of secreted saliva was determined by placing a pair of cotton rolls on both sides of
187 the mandibular inner aspect of the lower jaw in the mouth for 6min. The cotton rolls were
188 removed with pincers and immediately placed in a sealed plastic bag. Each pair of rolls was
189 weighed with its bag before the test and afterwards. This measurement was completed before
190 each study period as a baseline and repeated 2h after the 1st and 5th intake and 24h after the 5th
191 intake in each period.

192

193 Visual accommodation and acuity

194 Visual accommodation test included the determination of the near and far point of
195 accommodation with a Clement Rod ruler. Visual acuity was tested by a Jaeger board, a common
196 test for optometric evaluation that its results are presented in Diopter units. The visual evaluation
197 was performed before each study period as a baseline and repeated 2h after the 1st and the 5th
198 intake and 24h after the 5th intake in each period.

199

200

201 Peak flow meter

202 This was used to monitor airflow and the degree of airway restriction. The volunteers were
203 instructed to blow through the device. The best of three readings was used as the recorded value.
204 Peak flow was measured before each study period as a baseline and repeated 2h after the 1st and
205 5th intake and 24h after the 5th intake in each period.

206

207 Blood samples

208 Blood samples for pharmacokinetic (PK) and pharmacodynamic (PD) profile of rivastigmine
209 were obtained in two batches adjacent to the 1st and 5th intake in each period. Each batch
210 included 9 samples; the first sample in each batch was drawn just before the intake (time 0) and
211 the rest taken 30, 60, 90min, 2, 4, 6, 8 and 12h after intake.

212

213 Plasma levels of rivastigmine (PK profile)

214 Whole blood samples of 4-6ml were collected into lithium-heparin test tubes. 10µl
215 physostigmine (0.01M) per 1ml of sampled blood was added immediately. Plasma was
216 separated, divided into two marked test tubes and stored in -80°C. The samples of each subject
217 were analyzed together. The samples were then defrosted in ice water and treated with
218 alkalization and extracted with a solvent. Levels of rivastigmine were evaluated through a gas
219 chromatograph – mass spectrometer device and determined by using an internal standard
220 (rivastigmine-d6). PK parameters including C_{max} (peak concentration), T_{max} (peak
221 concentration time), AUC_{t₀-t_∞} (area under the curve), CL/F (relative clearance) and VSS/F
222 (relative volume of distribution in steady state) were calculated for each batch of results using a
223 PK software (Thermo Scientific™ Kinetica, version 5.0, Philadelphia, PA, USA).

ChE activity (PD profile)

Triplicates of 50µl whole blood were diluted 1:25 in distilled water and stored in -80°C. ChE activity was determined according to the radiometric method of Johnson and Russell using [³H]acetylcholine (Perkin Elmer) (Johnson et al, 1975). All blood samples of each subject were analyzed at the same assay. ChE inhibition was calculated using the baseline activity before treatment.

Cognitive and emotional tests

Tests were selected based on their reported sensitivity to the central effects of cholinergic drugs like physostigmine, donepezil, scopolamine, atropine and caramiphen (Grön et al, 2005; Levy et al, 1996; McNair et al, 1981; Parrott, 1987, 1989; Seppälä and Visakorpi, 1983; Wesnes et al, 1987). Some of the tests were used for the assessment of the effect of rivastigmine in DLB (dementia with Lewy bodies) patients (Wesnes et al, 2002). The main characteristics of each test are presented in table 1.

Post-dose performance tests were conducted at approximately the same time of day as the baseline evaluation was carried out, corresponding to approximately 2h after the 1st intake.

Duration of the tests, including between-test rest, was approximately 40min. In total, performance was evaluated 4 times in each period: at baseline, ~2h after 1st and 5th intakes and 24h after 5th intake.

244 Table 1: Description of the cognitive performance tests

No.	Test	Function	Test Duration	Task Performance	Reliability*	Number of Parameters
1	Visual Scanning	Target detection and perceptual speed	5 min.	Visual scanning and target detection in a distractor- stimuli- background	r=0.98	10
2	Spatial Information Processing	Spatial integration and short term memory	3 min.	Histograms comparison in a delayed matching to sample	r=0.95	8
3	Time Wall	Time estimation	2 min.	Assessment of the point in which a moving object will arrive to destination	r=0.96	5
4	Differential RT	Psycho-motorics	6 min.	Forced choice serial reaction time	r=0.99	12
5	Sternberg Memory Search	Selective comparison and retrieval in short term memory	3-6 min.	Rehearsal and categorization of set of items	r=0.96	8
6	Unstable Tracking	Dynamic, motor response control	3 min.	Moving target tracking by joystick, in a loading task	r=0.97	3
7	Divided Attention	Time sharing of two tasks	2-3 min.	Concurrent performance of two tasks: Memory Search (6) and Tracking (7)	r=0.99	13
8	Profile of Mood States	Subjective feelings of motivation and emotion	2 min.	Ordinal scaling of 4 emotional, two motivational and one social factor.	r=0.99	10

245

246 *Test-to-pretest reliability: Correlation between the last 2 tests

247

248

Data Analysis

Electronically collected data were subjected to comprehensive descriptive and comparative analysis using SPSS® computer program (version 17, IBM, Armonk, NY, USA). Continuous endpoints were summarized using N, mean, minimum, maximum, standard deviation and CI (95%); categorical endpoints were restricted to frequency counts and percentages.

Comparative analysis of various measures was done by paired t-test, MANOVA, χ^2 Test and Fisher Exact Test as indicated. Post hoc comparisons were conducted according to the effects obtained (main effects or interactions) in the MANOVA. Relationships between performance tests and biochemical or physiological measures were determined by Pearson or Spearman correlations.

Pharmacokinetic and pharmacodynamic data were analyzed by repeated-measures, two-way ANOVA, followed by post hoc, simple main effects contrasts analysis for interactions and compared by paired t-test. Physiologic measures were analyzed by two-way ANOVA, followed by post hoc, simple main effects contrasts analysis for interactions, or Bonferroni test, as required. Behavioral scores of each test were separately analyzed by MANOVA for repeated measures, followed by post hoc, multiple comparisons of simple main effects contrasts analysis for interactions, or Bonferroni or Scheffe tests, as required.

Results

Of the 15 volunteers that started the study one was excluded due to noncompliance with the study regulations. Fourteen volunteers completed the study and were included in the final analysis. There were no serious adverse events during the study.

Symptoms

During all three study periods 11 participants reported a total of 69 symptoms. Fifty-five of them (79.7%) occurred during the medication periods (1.5 or 3mg). Gastrointestinal symptoms were most frequently reported (33, 47.8% of total complaints), mainly nausea (10, 14.5%) and bloating (9, 13%). Symptoms specific to heightened cholinergic effect were rarely reported. Reported symptoms and numbers of episodes are presented in Table 2.

Dizziness, nausea and bloating were related to medication intake while other symptoms developed relatively equal in both medication and placebo periods.

The severity of symptoms as subjectively defined by the participants was mostly mild (60, 87%); the rest were moderate. The objective medical evaluation performed for each complaint determined all of them as mild. Sixty-six (95.6%) symptoms were self-improved. In 3 cases an analgesic was administered to relief headache with full recovery.

The 69 symptoms clustered into 39 different clinical events, as some complaints developed together. These events occurred more frequently during medication periods (29, 74.4%) but were not significantly different from placebo (Fisher Exact Test, $p=0.15$). The symptoms during drug periods appeared mostly within 4h of intake. This was statistically significant for the 3mg group (28 out of 35, 80%, Fisher Exact Test: $p<0.0001$) but not for the 1.5mg group (11 out of 20, 55%, Fisher Exact Test: $p=0.053$).

292 Table 2: Symptoms following rivastigmine across all three periods

Symptom	Number of episodes			
	placebo	1.5 mg	3 mg	Total
Dizziness	2	3	7	12
Nausea	0	1	9	10
Bloating	0	5	4	9
Headache	3	2	2	7
Abdominal discomfort	2	2	2	6
Malaise	1	0	5	6
Diarrhea	4	2	0	6
Vomiting	0	1	1	2
Dry mouth	1	0	1	2
Throat discomfort	0	0	2	2
Pre syncope	0	2	0	2
Sweating	0	1	0	1
Ocular pain	1	0	0	1
Weakness	0	0	1	1
Nose bleed	0	0	1	1
Visual disturbance	0	1	0	1
Total	14	20	35	69

293

294 The majority of symptoms lasted less than 4h (44, 63.7% with placebo; 35, 63.6% without
 295 placebo). There was significant variability between participants in regard of symptoms (χ^2 Test,
 296 $p<0.0001$). While three volunteers did not complain at all, four others reported 67% of all
 297 symptoms (46 out of 69) and the majority of these were reported during drug periods (36 out of
 298 55).

299

There were no differences in the repeated physical examinations of the participants throughout the study. No clinically significant abnormalities were found in the routine laboratory tests and the ECG recordings before and after each study period.

Saliva production

Saliva production is summarized in Fig. 1. Salivation decreased after 1st placebo intake and returned to baseline 24h post last intake. In addition, a decrease in salivation was seen 24h after last intake of the 3mg dose. These observations were supported by a two-way ANOVA with repeated measures (dose x time) that showed a significant interaction between dose and day ($F(6/78)=2.78$, $p<0.017$). Further, post hoc analyses showed a significant decrease in saliva during placebo intake compared to baseline level, and at 24h compared to 2h post 3 mg dosing ($p<0.01$).

The changes seen in the amount of salivations were within normal limits and were not reported as adverse effects.

Figure 1: Saliva accumulation at baseline (day 0), 2hr after the 1st (day 1) and 5th (day 3) intakes of rivastigmine (0, 1.5, 3mg), and 24hr post last intake.

Peak flow measurements

There were no significant differences between placebo and drug periods. There was a significant increase in the peak flow over time over all groups from day 1 to 4, with no significant difference between groups. A two-way ANOVA (dose x dosing day) showed only a significant main effect of day ($F(3/39)=4.76$, $p<0.006$). This increase may reflect overall improvement in test performance.

Visual accommodation and acuity

No significant differences were found between groups, days or periods in both near and far points.

Pharmacokinetics (PK)

PK profiles were completed for 1st (day 1) and 5th intake (day 3). The means and standard errors of the drug plasma concentrations over time are summarized in Fig. 2.

Figure 2: Pharmacokinetic profile: Mean ($\pm$ SEM) of blood rivastigmine levels before 1st and 5th intakes of rivastigmine (0, 1.5 or 3mg) and at various time points afterward.

337 In both drug doses, the concentrations were undetectable within 12h after intakes indicating no
 338 accumulation in the blood between intakes. A repeated-measures, two-way ANOVA (dose x
 339 time), showed a significant interaction between dose and time ($F(38/494)=5.36$, $p<0.0001$). This
 340 was reflected in a higher peak plasma level after the 5th intake compared to 1st intake even
 341 though no accumulation in the blood was observed. This significant difference appeared 30min
 342 after drug administration and disappeared 6h following intake ($p<0.001$).

343 The calculated PK parameters are presented in Table 3. $AUC_{t_0-t_{\infty}}$ significantly increased in both
 344 doses regimens between the 1st and 5th intakes although no accumulation of the drug in the blood
 345 was observed (χ^2 Test, $p=0.02$). $AUC_{t_0-t_{\infty}}$ increased approximately three-fold when the dose was
 346 increased by 2-fold; statistical significance was calculated only for the first intake (χ^2 Test,
 347 $p=0.015$). Further analysis to explore differences in the PK parameters between intakes and
 348 doses was performed using paired t-test. Calculated peak concentrations (C_{max}) were
 349 significantly higher after 5th intake compared to 1st for the 1.5mg dose ($t(13)=3.3$, $p=0.005$) but
 350 not for the 3mg dose. There were no statistically significant differences between time to peak
 351 (t_{max}) between intakes. Calculated relative clearance (CL/F) was significantly reduced after the
 352 5th intake compared to 1st in both doses (1.5mg dose: $t(10)=3.37$, $p=0.007$; 3mg dose: $t(12)=3.54$,
 353 $p=0.04$). The relative clearance after 1.5mg first intakes was significantly higher compared to
 354 3mg ($t(10)=7.28$, $p<0.0001$). Calculated relative volumes of distribution in steady state (V_{ss}/F)
 355 were significantly reduced after 5th intake compared to 1st (1.5mg dose: $t(10)=2.38$, $p=0.038$;
 356 3mg dose: $t(12)$, $p=0.015$). The V_{ss}/F after the 1.5mg 1st intakes were significantly larger
 357 compared to 3mg ($t(10)=2.54$, $p=0.29$). The elimination rate constant (k_{el}) did not change
 358 significantly between doses and intakes, as both CL/F and V_{ss}/F have changed.

359 The data demonstrated high variability in both drug concentrations between subjects (3-5 folds),
 360 with non-linear kinetics even at the low doses tested here.

Table 3: Pharmacokinetic parameters

	1.5 mg, 1 st (mean±SE)	1.5 mg, 5 th (mean±SE)	3 mg, 1 st (mean±SE)	3 mg, 5 th (mean±SE)
C _{max} (ng/ml)	1.89±0.34	3.33±0.53	5.74±1.2	7.31±0.28
t _{max} (Hr)	2±0.28	1.5±0.29	2.5±0.3	2.1±0.3
AUC _{t0-t∞} (ng·Hr/ml)	3.84±0.45	8.37±1.6	14.45±2	23.28±3.1
CL/F (Liter/Hr)	389±44	277±52	217±60	158±19
V _{ss} /F (Liter)	1076±158	736±145	783±217	246±66

Pharmacodynamics (PD)

PD profiles after the 1st and 5th intakes based on mean cholinesterase inhibition values are illustrated in Fig. 3. Baseline enzyme activity was determined individually for each participant prior to each drug administration. Mean maximal enzyme inhibition after the 1st and 5th 1.5mg dose intake was 6.9%±2.7 and 10.7%±2.6, respectively. Mean maximal enzyme inhibition after the 1st and 5th 3mg dose intake was 20.2 %±3 and 27.9%±2.4 respectively. This difference was also noted in the number of subjects with blood ChE inhibition exceeding 20% following 3 mg dose: 8 out of 14 after the 1st intake and 14 out of 14 after the last intake. This increased effect cannot be explained by simple accumulation of drug over repeated dosing since in both doses the enzyme activity returned to baseline within 12h or sooner after each intake.

A repeated-measures, two-way ANOVA (dose x time) showed a significant interaction between dose and time ($F(19/247)=7.74$, $p<0.0001$). A significant difference in peak inhibition index between 1st and 5th administration was observed in both treatment doses ($p<0.001$). A significant difference in ChE inhibition between the two doses could be seen 3h following 1st administration, and as early as 1h after the 5th one ($p<0.01$).

Figure 3: Pharmacodynamic profile: Mean ($\pm$ SEM) of % ChE inhibition before 1st and 5th intakes of rivastigmine (0, 1.5 or 3mg) and at various time points afterward.

Peak inhibition times were 3h to 4h after intake without significant difference between doses and study days. Mean durations of inhibition over 20% after 1st and 5th 3mg dose intakes were 3.4h($\pm$ 0.7) and 4.7h($\pm$ 1), respectively. There was a constant time delay of 1.5 to 2hr between the times of peak drug plasma concentration (Fig 2) and the time of peak whole blood ChE inhibition (Fig 3).

Behavioral tests (Fig. 4)

Visual Scanning test – Perceptual Speed. The results of the scanning strategy index (correlation factor measure) are presented in Fig. 4A. The correlation factor of subjects in all three doses decreased significantly 2h following 1st intake compared to baseline (3mg: by 64%, $p<0.001$, 1.5mg: 36%, $p<0.05$, 0: 38%, $p<0.01$). Following placebo treatment the correlation factor remained low and returned slowly back to baseline towards the end of the experiment. However, in subjects treated with 3mg of rivastigmine the correlation returned to baseline level 2h following the 5th intake, and then decreased even further (84%) 24h post dosing. In subjects treated with 1.5mg, level of performance remained low 2h following the 5th intake, and decreased further (63%) 24h post dosing. Indeed, significant differences ($p<0.05$) in performance between both rivastigmine groups and placebo were found 2h following 5th intake and 24h post last intake.

This impairment in the strategy of scanning was associated with a decrease in correct responses and an increase in target-detection reaction time, although these differences were not statistically significant.

Tracking tests. These tests included control of dynamic, rapid and accurate manual responses.

The average tracking error (deviation from target), a measure of accuracy, received by manipulating the joystick is presented in Fig. 4B. The differences obtained in the manual responses between the different rivastigmine-groups and the placebo group, through time, were very similar to those obtained in the visual scanning test (strategy of scanning measure), although in this test only a tendency for statistical significance was found by the repeated-MANOVA, in the interaction between groups and time ($F(6/72)=1.89$, $p<0.095$). While minor changes were seen in the placebo group over time, an increase in tracking error was illustrated in both rivastigmine-groups (13-24%, for the 3mg and the 1.5mg groups, respectively) 2h following the 1st intake, compared to baseline. Two hours following the 5th intake the performance of both

groups returned toward baseline, and 24h post dosing, a further increase in tracking error was shown (25-21%), for the 3mg and 1.5mg rivastigmine-groups, respectively. No significant differences between groups were found in any other perceptual, cognitive or psychomotor tests evaluated.

Figure 4: Behavioral changes (mean±SEM) following rivastigmine (0, 1.5, 3mg) at baseline (base), 1st intake (day 1), 5th intake (day 3) and 24 hr post last intake. A: Strategy of visual scanning, presented as a Correlation Factor. B: Tracking error (deviation from target), a measure of accuracy. C: Fatigue scores (a motivational factor). *p<0.05-0.001 vs. same dose baseline. #p<0.05 vs. same time control intake.

Correlation was found between ChE inhibition levels and the visual scanning test scores following rivastigmine 3mg intakes (correlation coefficients 0.58-0.66, $p<0.03$). Significant correlation was also found between rivastigmine levels and the tracking test scores after rivastigmine 3mg (correlation coefficients 0.58-0.69, $p<0.04$).

Profile Of Mood States (POMS) questionnaire. No significant differences were found in the responses to this questionnaire. However, the results of the fatigue factor (one of the two motivational factors) should be noted. Fig. 4C shows a significant main effect of time ($F(3/36)=3/01$, $p<0.042$), with no differences between groups. Compared to baseline, there was a progressive increase in fatigue throughout the study which disappeared 24h after the last intake (a quadratic effect, $F(1/12)=4.49$, $p<0.05$). This result emphasized the decrease in motivational behavior associated with the design of this clinical trial.

Discussion

We evaluated the pharmacokinetic and pharmacodynamic profiles and the physiological and cognitive effects of single and repeated rivastigmine administration in young healthy male adults. The aim was to assess safety and tolerability of the medication if administered to operational personnel and first responders as a pre-treatment against nerve agents poisoning. Over all, there was substantial variability in the effects of the drug and complexity in its properties. These may limit the intended wide spread use of the medication as prophylactic treatment.

Rivastigmine intake resulted in dose-dependent, mild, self-limited adverse physiological reactions. Temporal appearance of the symptoms was related to the drug peak plasma level times and peak ChE inhibition times (t_{max}) but not to the concentrations and level of inhibition.

Although the adverse reactions were mild, the impact of these drug-related effects on operational performance of first responders and soldiers could not be ruled out.

Sialometry results can be parsimoniously explained by the summation of two opposing effects: decreased salivation during stress and increased salivation following ChE inhibition (Hugo et al, 2008; Khosravani et al, 2009). The stress associated with the study induced decreased salivation, as is evident in the placebo group, and this was counteracted by rivastigmine. The changes seen in the amount of salivations were within normal limits and were not reported as adverse effects.

The PK/PD profiles showed non-linear properties contrary to previous reports that indicated non-linearity only at higher doses (Enz et al, 1991; Kennedy et al, 1999; Holsboer-Trachler et al, 1993; Schredl et al, 2000). Both clearance and volume of distribution changed between doses and intakes. Rivastigmine is metabolized by its target enzyme in the liver and at other sites to the OH derivative NAP. Presumably the biotransformation of the drug reaches early saturation, thus passing from first order kinetics to zero order; clearance is then decreased progressively. The drug has a very large volume of distribution estimated in hundreds of liters. Upon repeated administration and/or increase in doses the volume decreases as observed in the study. One explanation for that is wide variability in binding and distribution to different compartments. It has been shown previously that rivastigmine PK profile in the cerebrospinal fluid (CSF) is not identical to the plasma (Cutler et al, 1998). The PK parameters were evaluated for five repeated doses (48h). Because non-linear kinetics was observed, the PK profile expected after a longer time period of administration is unclear. In addition, significant variability in the PK parameters was encountered among the study subjects. This complicates widespread operational implementation.

Non-linearity and great diversity was also observed in the ChE inhibition results correlating with the PK profiles. Following 1.5mg dose intake the ChE inhibition in whole blood did not exceed

20%, a level of inhibition regarded therapeutic for the intended indication (Cutler et al, 1998). ChE inhibition surpassed the 20% level only with 3mg doses. However, the duration of enzyme inhibition greater than 20% was short lived, usually around 3-4h for each intake or 6-8h per day. The medicated person is below the proposed therapeutic enzyme inhibition level for 16-18h per day. It is possible that the 20% inhibition mark is not relevant for rivastigmine as it was suggested in animal experiments only for the quaternary pyridostigmine that does not readily enter the brain. Nevertheless without any alternative pharmacodynamic target the drug in the tested doses may be inadequate for proper protection against poisoning.

Furthermore, both blood rivastigmine levels and ChE inhibition showed an unexpected increase in peak effects from 1st to 5th intakes. Simple additive effects cannot explain this increase over repeated intakes as both parameters returned to baseline levels within 12h following each intake. Since repeated administrations are expected in the intended use of the drug, these unexplained effects adds to the concerns regarding the use of low dose rivastigmine as pretreatment against OP exposure. In comparison, no similar increases in ChE inhibition or drug levels were observed after repeated intakes of pyridostigmine bromide, an approved pretreatment drug for nerve agent exposure (Marino et al, 1998; Seng et al, 2009). Potential mechanisms for this unique pharmacological profile are accumulation in extra-vascular compartments and rapid alteration in the capacity and extent of rivastigmine biotransformation and elimination. Further research is needed to confirm and evaluate these results.

The evaluation of the cognitive effects revealed two different functional impairments following rivastigmine treatment. The more profound negative effect encountered was on perceptual speed as observed in the visual search test. During the 3mg period the participants' performance declined by 26% to 72% (depended on the test time) compared to placebo. Another impaired function was the dynamic response as evaluated by the unstable tracking test. The average error in the test increased by 13% to 27% during the medication periods (depended on dose and test

time) compared to placebo. Both cognitive functions were markedly impaired 24h after the last drug administration, implying a long-lasting effect.

The impaired cognitive functions demonstrated in the study represent both ends of the data analysis process: the perception and the motor response. These qualities are extremely relevant to soldiers and field operators; they are fundamental for target identification and stick-operated shooting accuracy. Other central functions (e.g., verbal and spatial memory) were not impaired and motivation and emotions were also unaffected by the medication. These results correlate to previous studies that show centrally selective, long lasting effects of rivastigmine (Enz et al, 1991; Holsboer-Trachler et al, 1993; Schredl et al, 2000).

Adjusted drug regimens may produce different behavioral effects. Further advanced evaluation can be performed to better understand and characterize the cognitive effects of rivastigmine; the use of designated operational and military simulators is advised. Yet the reported study results cannot be disregarded, and operationally, possible detrimental influence cannot be excluded.

The study has several limitations. The number of participants was small; the sample size was calculated based on an estimated variability of the results that was found to be greater during the study. The participants were all young Caucasian adult males and the results cannot reliably be extrapolated to other populations. The cognitive and behavioral tests were performed in a laboratory setting. In addition, the fatigue factor in the POMS questionnaire emphasized the decrease in motivational behavior associated with the rigorous design of this clinical trial, which required lengthy repetition of computerized tests.

Conclusions

529 The unpredictable nature of the adverse effects, the non-linear pharmacological property, wide
530 variety between subjects, and the negative influence on some cognitive functions limit the
531 potential use of rivastigmine as pre-treatment against nerve agents poisoning in a high-
532 performance operational population.

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