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Physiological and neurobehavioral effects of cholinesterase inhibition in healthy adults ☆

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HIGHLIGHTS

- Tested acute neurobehavioral effects of cholinesterase inhibitors in healthy adults
- Hourly assessments of RBC cholinesterase, reaction time, and declarative memory
- Unlike donepezil, huperzine A and galantamine selectively inhibited AChE.
- Despite AChE inhibition, neurobehavioral performance neither improved nor diminished.
- Maintenance of neurocognitive function under reduced cholinergic tone is discussed.

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ABSTRACT

Introduction: Based on common pharmacodynamic mechanisms, recent efforts to develop second generation alternatives for organophosphate (OP) prophylaxis have expanded to include cholinesterase (ChE) inhibiting compounds traditionally approved for use in the treatment of Alzheimer's disease (AD). The primary purpose of this study was to determine the extent to which low-dose huperzine A, galantamine, or donepezil selectively inhibited acetylcholinesterase (AChE) versus butyrylcholinesterase (BChE) activity in healthy adults and whether such inhibition impacted neurobehavioral performance.
Methods: In addition to hourly red blood cell cholinesterase sampling, neurobehavioral function was assessed before and after a single oral dose of huperzine A (100 or 200 μg), galantamine (4 or 8 mg), donepezil (2.5 or 5 mg), or placebo (n = 12 subjects per drug/dose).
Results: Compared to placebo, both dosages of huperzine A and galantamine inhibited circulating AChE but not BChE. With the exception of huperzine A (200 μg), which maintained declarative recall performance across sessions, compounds did not improve neurobehavioral performance. Some aspects of neurobehavioral performance correlated with AChE activity, although associations may have reflected time of day effects.
Discussion: Although huperzine A and galantamine significantly inhibited AChE (and likely increased central acetylcholine levels), neither compound improved neurobehavioral performance. The latter was likely due to ceiling effects in this young, healthy test population. Under conditions of reduced cholinergic activity (e.g., Alzheimer's disease), AChE inhibition (and corresponding maintenance of cholinergic tone) could potentially maintain/augment some aspects of neurobehavioral function.

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1. Introduction

Organophosphorus (OP) compounds, a class of chemical warfare nerve agents (CWNAs) which includes soman, sarin, and VX [1], act as potent, irreversible inhibitors of the peripheral and central nervous system (CNS) AChE and BChE enzymes that hydrolyze (and thus remove) acetylcholine (ACh). Accumulation of ACh in the peripheral and CNS causes hypersalivation, lacrimation, respiratory distress, muscle fasciculation, and *status epilepticus* [2].

Currently, FDA approved prophylaxis against OP poisoning (specifically the OP soman) consists of pyridostigmine bromide (PB) tablets

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taken over a two-week period [3]. PB is a reversible ChE inhibitor that protects peripheral [red blood cell (RBC)] AChE from CWNA phosphorylation by temporarily sequestering (shielding) the active site of the enzyme [4]. While OP agents are eliminated from the body, PB-inhibited AChE spontaneously reactivates, thereby restoring AChE activity. However, because PB does not penetrate the blood brain barrier (BBB), it does not directly protect central AChE, and thus does not prevent neuropathological states resulting from OP exposure, such as *status epilepticus* [2,5]. In addition, PB inhibits BChE, diminishing its ability to act as a scavenger for CWNAs.

Based on common pharmacodynamic mechanisms, recent efforts to develop second generation alternatives to PB for OP prophylaxis have expanded to include other cholinesterase-inhibiting compounds (e.g., donepezil, galantamine, and Huperzine A) which are typically indicated for use in the treatment of symptoms associated with AD. Donepezil, galantamine, and Huperzine A are specific and selective inhibitors of AChE and thus, preserve BChE's OP scavenger capacity [6,7]. In contrast to PB, all three compounds cross the BBB, potentially protecting CNS AChE from OP but also potentially enhance neurobehavioral performance via increased cholinergic tone [8,9]. Donepezil and galantamine are FDA approved for treatment of the behavioral and cognitive impairments seen in Alzheimer's disease (which is characterized by loss of central cholinergic neurons [10]), and huperzine A currently is undergoing Phase II clinical trials. Pharmacokinetic profiles for huperzine A and galantamine are similar (through the oral route), with approximate time to peak concentration of 80 min [11], whereas donepezil's time to peak concentration is 180–300 min [12].

The primary objectives of the current investigation were to determine the extent to which huperzine A, galantamine, and donepezil inhibit AChE versus BChE and impact neurobehavioral performance. Rivastigmine also is FDA-approved for the treatment of Alzheimer's disease and also penetrates the CNS. However, because rivastigmine (like PB) is non-selective and inhibits BChE (abolishing its OP scavenger capacity), it was not of interest as a potential OP prophylactic.

2. Method

This study was approved by the Walter Reed Army Institute of Research Institutional Review Board and the United States Army Medical Research and Materiel Command Human Research Protections Office and was performed in accordance with the ethical standards put forth in the 1964 Declaration of Helsinki. All study activities were performed at the Walter Reed Army Institute of Research (Silver Spring, Maryland).

2.1. Subjects

Subjects were 84 healthy non-smoking men ($n = 37$; mean age = 25.85 years, range = 18–38 years) and non-pregnant, non-lactating women ($n = 47$; mean age = 23.67 years, range = 18–37 years) who responded to advertisements posted at local military institutions and universities. Informed consent was obtained and included an explanation of all procedures and possible drug side-effects. Subjects were screened for past and current physical, psychiatric, sleep, and other health problems known to impact neurobehavioral performance. All subjects reported total daily sleep time of approximately 6–9 h, daily caffeine consumption of less than 400 mg, and screened negative (via urine sample) for commonly used drugs of abuse. Subjects were compensated for successful study completion. One additional volunteer (female, age 22.62 years) elected to withdraw 90 min after galantamine 8 mg administration. This volunteer reported experiencing moderate nausea and dizziness. Her data were not included in analyses.

Number of subjects per group (12) was selected based on power calculations conducted on data from previous studies of stimulants [13] and sleep-inducing compounds in healthy adults [14]. A calculated estimate of power as a function of sample size indicated that a total N of 84 subjects (12 per group) would yield a power above 0.95 [input = 7 groups, $a = 0.05$, number of measurements = 10, correlation among repeated measures = 0.5, estimated effect size = 0.4].

2.2. Drugs

Huperzine A was provided by Biomedisyn Corporation (Woodbridge, Connecticut). Donepezil was purchased from Eisai Inc. (Woodcliff Lake, New Jersey). Galantamine was purchased from Janssen Ortho, LLC (Gurabo, Puerto Rico). Placebo consisted of calcium phosphate dehydrate purchased from Josef Rettenmaier and Sohne Pharmaceuticals, LP (Patterson, New York). Following a single oral administration in humans, huperzine A, donepezil, and galantamine reach maximum AChE inhibition in 1.3, 3–4, and 1 h respectively, and display AChE inhibition half-lives of 4.8, 70, and 7 h, respectively [15].

Doses were selected (a) based on clinical guidelines for lowest starting doses and (b) to minimize side-effects. Huperzine A doses correspond to one-fourth (100 μg) and one-half (200 μg) of the maximum single dose administered in a Phase IIa FDA clinical trial with Alzheimer's disease (AD) patients [12]. Galantamine doses correspond to the lowest recommended starting dose (4 mg) and most common starting dose (8 mg) for AD patients [15]. Donepezil doses correspond to one half (2.5 mg) or full value (5 mg) of the lowest recommended starting dose for AD patients [15,16]. All doses were packaged in identical opaque capsules to maintain the double-blind, in addition, the dose ranges were designed to encompass the 20-30% inhibition of RBC AChE observed with the pre-treatment dose of PB in humans [3].

2.3. Cholinesterase sample collection and assay

Whole blood samples were collected from the outer edge of the palmar surface of the finger using a high flow lancet (blade depth = 2.0 mm, width = 1.5 mm) according to the manufacturer's recommended procedure (Becton Dickinson & Company, Franklin Lakes, New Jersey). Each sample was collected in separate EDTA-treated 100 μl capillary tubes to prevent clotting (StatSampler; Iris Sample Processing, Westwood, Maine). Blood samples were stored in a −80 °C freezer until assayed.

AChE and BChE activities were simultaneously determined in whole blood samples, according to previously described methods [4]. Briefly, the reaction indicator 4,4′-dithiodipyridine (DTP) was used as the ultraviolet colorimetric indicator to minimize hemoglobin interference and yield a high signal-to-noise ratio. Three thiocholine substrates (acetyl-, butyryl-, and propionyl-thiocholine) provided simultaneous redundant and independent measurement of red blood cell AChE and BChE activities using a 96-well microtiter plate spectrophotometer. Use of unprocessed whole blood obviated the need for centrifugation and use of specific ChE inhibitors. The assay yielded AChE and BChE peripheral blood activities (units/mL).

2.4. Study design and procedures

The study design consisted of one between-subjects factor for study compound/dose (seven drug/dose groups indicated above) and one within-subjects (repeated) factor for test session (one pre-drug session, and up to nine post-drug sessions, depending on neurobehavioral task).

Table 1 lists the timeline of study activities. Subjects reported to the laboratory at 1800 h on day 1, at which time they were familiarized with cholinesterase sample collection procedures and neurobehavioral tasks. Subjects remained in the laboratory overnight and were allowed 8 h undisturbed time in bed (2300 h day 1–0700 h day 2) housed in individual bedrooms. Non-caffeinated beverages and food were provided for breakfast and lunch (at approximately 0800 h and 1200 h, respectively). Water was allowed ad libitum.

Subjects were quasi-randomly assigned to drug group such that all subjects participating at the same time (up to four) were administered