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Research report

The selective positive allosteric M1 muscarinic receptor modulator PQCA attenuates learning and memory deficits in the Tg2576 Alzheimer's disease mouse model

Vanita Puri*, Xiaohai Wang, Joshua D. Vardigan, Scott D. Kuduk, Jason M. Uslaner

Merck Research Laboratories, West Point, PA, USA

HIGHLIGHTS

- Shown previously, PQCA attenuates scopolamine deficits in various cognition tests.
- Here we characterized the effects of PQCA in a Tg2576 mouse model.
- PQCA dose-dependently improved recognition memory in this mouse model.
- Co-administration of PQCA and donepezil produced additive/synergistic effects.

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ABSTRACT

We have recently shown that the M1 muscarinic receptor positive allosteric modulator, PQCA, improves cognitive performance in rodents and non-human primates administered the muscarinic receptor antagonist scopolamine. The purpose of the present experiments was to characterize the effects of PQCA in a model more relevant to the disease pathology of Alzheimer's disease. Tg2576 transgenic mice that have elevated Aβ were tested in the novel object recognition task to characterize recognition memory as a function of age and treatment with the PQCA. The effects of PQCA were compared to the acetylcholinesterase inhibitor donepezil, the standard of care for Alzheimer's disease. In addition, the effect of co-administering PQCA and donepezil was evaluated. Aged Tg2576 mice demonstrated a deficit in recognition memory that was significantly attenuated by PQCA. The positive control donepezil also reversed the deficit. Furthermore, doses of PQCA and donepezil that were inactive on their own were found to improve recognition memory when given together. These studies suggest that M1 muscarinic receptor positive allosteric modulation can ameliorate memory deficits in disease relevant models of Alzheimer's disease. These data, combined with our previous findings demonstrating PQCA improves scopolamine-induced cognitive deficits in both rodents and non-human primates, suggest that M1 positive allosteric modulators have therapeutic potential for the treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized in part by loss of cholinergic neurons and accumulation of insoluble protein deposits of β-amyloid [1–4]. Currently available treatments for AD improve symptoms primarily by increasing cholinergic transmission through inhibition of acetylcholinesterase (AChE), the enzyme responsible for metabolizing acetylcholine. However, AChE inhibitors, such as donepezil, exhibit limited efficacy and unwanted GI side effects [5–7], which is thought to be due to the high expression of AChE and muscarinic M2 and M3 receptors in the periphery [8,9]. In addition, the tolerated doses of the standard of care produce only partial improvement in memory while still accompanied by severe GI side effects leading to discontinuation of the medicine in the patients. In order to improve efficacy and reduce side-effect liability, we have identified muscarinic M1 receptor positive allosteric modulators (PAMs), which selectively enhance M1 receptor activity [10]. Importantly, M1 receptors are highly concentrated in brain regions relevant to AD but less so in the periphery [8,11].

Previous reports demonstrating the precognitive effects of M1 activators have used models in which cholinergic function is impaired with scopolamine, a non-selective muscarinic receptor

* Corresponding author at: Merck & Co., Inc. WP44E-200, 770 Sumneytown Pike, PO Box 4, West Point, PA 19486, Tel.: +1 215 652 3886; fax: +1 215 993 6240.
E-mail address: vanita_puri@merck.com (V. Puri).

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antagonist. For example, Ma et al. (2009) demonstrated that the M1 PAM BQCA attenuates a scopolamine-induced impairment in contextual fear conditioning in mouse [12], and Chambon et al. (2011) demonstrated that BQCA attenuates a scopolamine-induced deficit in spontaneous alternation in rat [13]. Uslaner et al. (2013) reported that the more potent M1 PAM PQCA attenuates scopolamine-induced impairment in novel object recognition in rat and object retrieval and self-ordered spatial search in rhesus and cynomolgus macaque, respectively [14]. Importantly, Vardigan et al. (2014) showed that the M1 PAM PQCA had a greater therapeutic window with respect to GI effects in rhesus macaque as compared to the current standard of care, donepezil, suggesting that an M1 PAM might produce less side-effects than the standard of care and/or might have greater efficacy because side-effects will not restrict dose to the same degree [7].

Although the results described above in which scopolamine was used as a model of cholinergic dysfunction are relevant, it is important to recognize that AD is marked by elevated Aβ plaques and Tau hyperphosphorylation, and tangle, as well as dysfunction in other neurotransmitter systems beyond acetylcholine. It is therefore important to examine the effects of putative AD treatments in models exhibiting these other characteristics. For example, Tg2576 mice overexpress human amyloid precursor protein (APP) with Swedish mutation that predisposes some humans to AD. Such a model provides an alternative approach to study the pathophysiological events in AD and to characterize novel therapeutics [14,15]. These mice show rapid increase in beta-amyloid (Aβ) plaques from 9–12 months and behavioral deficits around the time of plaque accumulation [14,16,17].

In order to further characterize the therapeutic utility of an M1 PAM as a treatment for AD, here we examined the influence of PQCA on a cognition deficit in Tg2576 mice. Utilizing the novel object recognition (NOR) test we first examined age-related performance in Tg2576 mice at ages pre- (3–4 months old) and post- (9–12 months) plaque accumulation. Following this initial characterization, we compared the effects of donepezil and PQCA alone and in combination on performance.

2. Materials and methods

2.1. Animals

All experimental protocols described in this study were approved by the Merck and Co., Inc. Institutional Animal Care and Use Committee and conducted in accordance with the Guide for Care and Use of Laboratory Animals. Female Tg2576 mice (Taconic Farms) weighing 15–40 g, 3–6 months and 9–12 months old and age-matched C57BL/6 mice (Taconic farms) were housed 4/cage under reverse 12 h dark: light cycle (white light on at 6 pm). Animals were housed in rooms in which temperature and relative humidity were maintained at 21 ± 2 °C and 45–55% respectively. Food and water were available ad libitum. All animals were monitored daily for signs of distress, and any health concerns were brought to the attention of the institutional veterinarian.

2.2. Apparatus

The testing arena consisted of a vinyl, non-transparent cylinder approximately 19″ in diameter and 14″ in height. Objects used in the test sessions were custom fabricated plastic object consisting of basic geometric shapes (white cone and yellow sphere) similar in overall size (approximately 1.7″ H × 2.1″ Dia.). Activity of the animals was video recorded and scored using computerized visual tracking equipment and software (Cleversys).

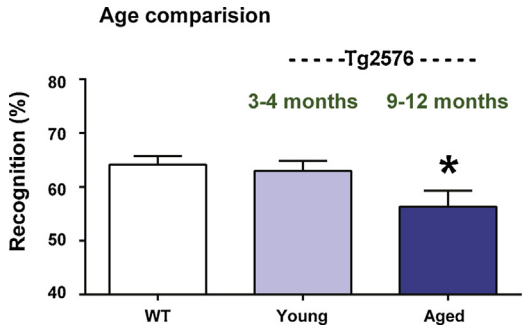

Fig. 1. Recognition memory as a function of age and genotype. Aged Tg2576 mice demonstrated lower levels of novel object recognition compared to age-matched WT mice or younger Tg2576 mice. * indicates significantly different ($p < 0.05$) than the age-matched WT group. $N = 15–20$ in each group.

2.3. Novel object recognition in mice

One hour prior to testing, animals were brought to the testing room and habituated. Following habituation to the room, each mouse was placed in the testing arena for 10 min, with two symmetrically positioned identical objects (defined as E1). Exploration of an object was defined as the mice having its nose pointed toward the object and its nose being <2 cm from the object. The amount of time exploring each object was recorded. 24 h later, the mouse was again placed in the testing arena for 5 min (defined as E2) with one identical object it had been exposed to during E1 (familiar) and one novel object. The time spent exploring the familiar and novel object was recorded. The tendency of rodents to preferentially explore the novel object in the retention trial is considered evidence for recognition memory. Secondary measures that were recorded included the time spent exploring the objects during E1 and E2 and the total distance traveled during E1 and E2. Prior to testing, the arena was cleaned with 70% isopropyl alcohol.

2.4. Compounds

Thirty minutes prior to E1 animals were given vehicle (0.9% saline), donepezil (Sequoia Research Products, 0.1, 0.3, 1 and 3 mg/kg; i.p.), PQCA (0.1, 1 and 10 mg/kg; i.p.) or vehicle (5% beta cyclodextrin).

2.5. Statistical analysis

Data are expressed as mean ± SEM. The data were analyzed with one way analysis of variance followed by Fisher's LSD post hoc tests for multiple comparisons.

3. Results

3.1. Tg2576 mice express an age-dependent impairment in novel object recognition.

Fig. 1 shows the influence of age on novel object recognition in Tg2576 mice. No significant difference was found on recognition between wild type (WT) mice at 3–4 months and 9–12 months, so these data were combined. ANOVA revealed a main effect of group ($F_{2,181} = 3.44$, $p = 0.034$). Relative to the WT, there was a significant decrease in recognition memory in the 9–12 month old Tg2576 mice ($p = 0.014$). In contrast, no significant impairment in recognition memory was observed in young (3–4 month) Tg2576 mice ($p = 0.644$).