Video Tracking Analysis of Contextual Fear Conditioning for the Screening of Anti-Alzheimer Drugs After Acute Administration in Tg2576 Mice

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ABSTRACT

Drug discovery and development demands highly standardized behavioral testing that should be up-grated to high-throughput screening open to cross-laboratory standardization. This is a challenging perspective for behavioral neuroscience. In this study, we present a video-tracking procedure to evaluate the performance of Tg2576 Alzheimer mice in the fear-conditioning test, in order to identify potential drugs to improve cognitive performance after acute administration. Fear memory was evaluated in plaque-free Tg2576 treated with vehicle, acute CHF5074 (10 or 30 or 100 mg/kg), a novel γ-secretase modulator, and acute DAPT (100 mg/kg), a γ-secretase inhibitor, 24 and 3 hours before behavioural testing. Both drugs belong to drugs aimed to target amyloidogenic hypothesis of Alzheimer disease. CHF5074, but not DAPT, shown to reverse contextual memory deficit after single administration.

Author Keywords

Contextual memory, γ-secretase modulators, β-amyloid.

Ethical Statement

Animal experiments were carried out according to the European Community Council Directives of 24 November 1986 (86/609/EEC) and approved by the Ethical Committee for Animal Experimentation of Bologna University with Italian Ministry of Health (authorization number BQ/mc/af35295-X/10). All experiments were designed to minimize the number of animals used as well as their discomfort.

INTRODUCTION

The effects of compounds interfering with γ-secretase, the enzymatic complex responsible of the formation of the β-amyloid (Aβ) peptide from amyloid precursor protein (APP), on plaque deposition in transgenic mouse models of Alzheimer's disease are known but scanty data are available on the effects of these drugs on memory performance in early age, when plaque deposition is still absent. We evaluated the effects of acute treatment with a novel γ-secretase modulator CHF5074, and acute DAPT, a γ-secretase inhibitor on contextual in plaque-free Tg2576 mice. Fear-conditioning test explores hippocampal and non-hippocampal memory, allowing testing of anti-Alzheimer drugs.

METHODS

The Tg2576 transgenic mouse carries a transgene coding for the 695-amino acid isoform of human APP derived from a large Swedish family with early-onset AD. The mouse expresses high concentrations of the mutant Aβ, develops significant amyloid plaques, and displays memory deficits. The colony is currently maintained by breeding hemizygous males with B6SJLF1 females. The non-transgenic colony control (001349-W) may be used as an experimental control. Five-month old heterozygous transgenic females and aged-matched transgene-negative littermates were used. Transgenic mice (n = 16-17 per treatment group) received two subcutaneous injections of vehicle, CHF5074 (10 or 30 or 100 mg/kg) or DAPT (100 mg/kg) 24 and 3 hours before behavioural testing (training session of the contextual fear conditioning test). Aged-matched wild-type animals (n = 26-27 per treatment group) received two subcutaneous injections of vehicle, CHF5074 (100 mg/kg) or DAPT (100 mg/kg) 24 and 3 hours before behavioural testing. Mice were trained and tested on 2 consecutive days (1). Training consisted of placing an animal in a chamber, illuminating

stimulus and house lights, and allowing exploration for 2 min (Ugo Basile Fear Conditioning, Calco, Varese, Italy). Afterward, an auditory cue [2 Hz] conditioned stimulus was presented for 15 sec A footshock [1.5 mAmp] was administered for the final 2 sec of the conditioned stimulus. This procedure was repeated and mice were removed from the chamber 30 sec later. Twenty-four hours after training, mice were returned to the same chambers in which training occurred (context), and freezing behavior was recorded by a computerized camera. At the end of the 5-min context test, mice were returned to their home cage. Approximately 1 h later, freezing was recorded in novel environment and in response to the cue. Freezing was expressed as a percentage of time in each portion of the test in which the animal remained immobile (at least 95% of his body for at least 500 msec). All phases of the test were recorded and immobility was detected by using the video tracking and analysis software ANY-maze (Stoelting Co., Wood Dale, IL). Data were analyzed with the appropriate model of analysis of variance (ANOVA) depending from the type of variable. Behavioral data were analyzed with two-way analysis of variance with "genotype" (wild-type and Tg2576) and "treatment" (vehicle, DAPT, CHF5074) as fixed factors and mouse as random factor. For balanced design (object recognition task), the ANOVA model included also "genotype by treatment" as fixed factor. Post hoc comparisons were directed only versus the transgenic control group (Tg2576-Vehicle) to reduce the loss of power due to multiple testing and were carried out with the Holm-Sidak's test. If needed, data were properly transformed (logarithmic or square root) to improve normality and homoscedasticity. Two-tailed *p* values were calculated. Calculations were done with the statistical software SigmaStat™ (Version 3.5, SPSS, Chicago, IL). Results were generally presented as mean ± standard error of mean (SEM).

RESULTS

Subcutaneous doses of 10, 30 and 100 mg/kg of CHF5074 were administered 24 and 3 hours before the training session. During Day 1, freezing in baseline conditions was similar in all groups. During Day 2, vehicle-treated Tg2576 mice had significantly lower freezing to the context than vehicle-treated wild-type mice (59.1 ± 3.9% vs 77.5 ± 2.6%, $p < 0.001$, Figure 1a). CHF5074 showed a bell-shaped dose-response curve with a non-significant increase of the contextual freezing with the 10 mg/kg dose (66.1 ± 3.6%, $p = 0.128$), a significant improvement with 30 mg/kg (71.5 ± 3.9%, $p = 0.008$) and no effects with the highest dose of 100 mg/kg (58.1 ± 4.6%) (Figure 1a). Compared to transgenic controls, DAPT 100 mg/kg had not effects on contextual freezing (55.6 ± 5.8%, Figure 1a). In wild-type mice, neither CHF5074 (100 mg/kg) nor DAPT (100 mg/kg) had effects on contextual freezing compared to vehicle-treated animals (78.1 ± 3.1% and 76.3 ± 3.0%, respectively, Figure 1a). There were no significant differences between treatment groups on freezing to sound.

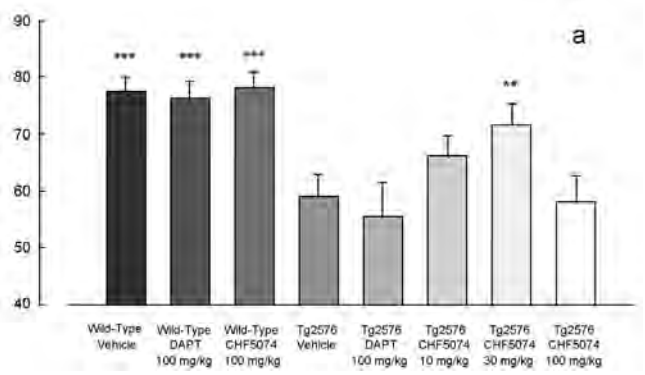

Figure 1. Acute CHF5074 treatment attenuates contextual memory deficit in young Tg2576 mice. Columns represent averages (± SEM) of the percent of time in which mice are immobile when put in the old context (upper panel) or to sound in a new environment (lower panel). Vehicle-treated Tg2576 mice showed a significant impairment of contextual memory compared to control wild-type mice treated with vehicle or CHF5074 (100 mg/kg, s.c.) or DAPT (100 mg/kg, s.c.). A medium dose of CHF5074 (30 mg/kg, s.c.) significantly attenuated contextual memory deficit in Tg2575 mice (upper panel). No significant group differences were observed on freezing behavior in the new environment after sound stimulus. *n* = 26-37 in wild-type mouse groups and n 16-17 in the Tg2576 mouse groups. **$p < 0.01$ and ***$p < 0.001$ vs. vehicle-treated Tg2576 mice.

DISCUSSION

CHF5074 is a new NSAID derivative, in which the anti-cyclooxygenase activity has been removed and anti-$A\beta_{42}$ secretory properties potentiated (Peretto et al., 2005). Chronic treatment with CHF5074 in the diet (375 ppm for 4 months) markedly reduced brain Aβ burden (-52% in the cortex; -77% in the hippocampus) (Imbimbo et al., 2007a) and completely reversed contextual memory deficit (Imbimbo et al., 2010). In the present study we found that CHF5074 improved memory after an acute treatment of young, plaque-free Tg2576 mice. The beneficial effects of acute and subchronic treatment with CHF5074 in young, plaque-free transgenic mice appear to be independent from an effect on soluble Aβ species, thus suggesting alternative hypotheses which require further investigations.

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