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Featured Article

Established amyloid-β pathology is unaffected by chronic treatment with the selective serotonin reuptake inhibitor paroxetine

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Abstract

Introduction: Treatment with selective serotonin reuptake inhibitors has been suggested to mitigate amyloid-β (Aβ) pathology in Alzheimer's disease, in addition to an antidepressant mechanism of action.
Methods: We investigated whether chronic treatment with paroxetine, a selective serotonin reuptake inhibitor, mitigates Aβ pathology in plaque-bearing double-transgenic amyloid precursor protein $(APP)_{swe}$/presenilin 1 $(PS1)_{\Delta E9}$ mutants. In addition, we addressed whether serotonin depletion affects Aβ pathology. Treatments were assessed by measurement of serotonin transporter occupancy and high-performance liquid chromatography. The effect of paroxetine on Aβ pathology was evaluated by stereological plaque load estimation and $A\beta_{42}/A\beta_{40}$ ratio by enzyme-linked immunosorbent assay.
Results: Contrary to our hypothesis, paroxetine therapy did not mitigate Aβ pathology, and depletion of brain serotonin did not exacerbate Aβ pathology. However, chronic paroxetine therapy increased mortality in $APP_{swe}/PS1_{\Delta E9}$ transgenic mice.
Discussion: Our results question the ability of selective serotonin reuptake inhibitor therapy to ameliorate established Aβ pathology. The severe adverse effect of paroxetine may discourage its use for disease-modifying purposes in Alzheimer's disease.

Keywords: Alzheimer's disease; Cerebral amyloidosis; Neocortex; Monoamine; Serotonin; Selective serotonin reuptake inhibitor; SERT occupancy; [^{3}H]DASB; Autoradiography; Transgenic mouse model; 5,7-dihydroxytryptamine; Stereology

1. Introduction

The serotonergic system degenerates in Alzheimer's disease (AD) along with the cholinergic and noradrenergic systems [1–3]. In patients with AD, the level of serotonin (5-hydroxytryptamine [5-HT]) is significantly reduced in several cortical regions, in particular in the frontal and temporal cortex [4,5]. This pathological change has been suggested to contribute to the depressive symptoms that frequently precede the cognitive decline in patients with AD [6–8]. It is still being disputed whether antidepressive treatment of patients with AD with selective serotonin reuptake inhibitors (SSRIs) impacts on the decline of cognition in AD [9–12] or the conversion from mild cognitive impairment to AD [13]. In AD, the density of specific cortical

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5-HT receptors correlates positively to amyloid β (Aβ) pathology and negatively to cognitive performance [14], with a reduced density of the 5-HT_4R and 5-HT_6R being observed in mild cognitive impairment [15–17]. Evidence that SSRI treatment impacts on Aβ accumulation comes from positron emission tomography studies, showing reduced uptake of Pittsburgh compound B, a proposed marker of Aβ pathology, in the brains of individuals with a history of SSRI therapy, including citalopram and fluoxetine [18]. In addition, acute, successive administration of citalopram (30 mg) to healthy individuals with 2 hours interval is reported to diminish cerebrospinal fluid levels of Aβ [19].

Interestingly, studies in transgenic mouse models of AD have suggested that SSRI treatment has major impact on Aβ pathology [18–20]. In the $APP_{swe}/PS1_{\Delta E9}$ (amyloid precursor protein [APP]/presenilin 1 [PS1]) transgenic mouse, which is a well-established model of Aβ pathology [21], 4 months treatment with citalopram (8 mg/kg/day *per os*) from 3 months of age results in ∼50% lower Aβ plaque load in the neocortex at 7 months of age [18]. In 6-month-old APP/PS1 mice, 4 weeks of treatment with citalopram (10 mg/kg/day *i.p.*) impairs initial Aβ plaque formation and growth [18]. Five months treatment with paroxetine (5 mg/kg/day *i.p.*) from 5 months of age results in lower Aβ levels at 10 months of age in the hippocampus of 3 × Triple-Tg mice [20], a model of combined Aβ and tau pathology. In addition, 5-week treatment with fluoxetine (10 mg/kg/day *i.p.*) is suggested to reduce Aβ load in 18-month-old APP/PS1 mice [22], at an age when these mice show a reduction in 5-HT in the neocortex [23,24]. Finally, infusion of 5-HT into the hippocampus of APP/PS1 mice has been reported to reduce interstitial levels of Aβ [18], by mechanisms involving stimulation of the 5-HT_4R and 5-HT_6R and increased processing of APP via the nonamyloidogenic route [25,26].

Importantly, there are still no preclinical studies, where plaque-bearing APP/PS1 mice have been chronically treated with doses of SSRI, resulting in ≈80% occupancy of the serotonin transporter (SERT), which is considered therapeutic for the treatment of depression [27]. Therefore, we tested the hypothesis that chronic treatment of plaque-bearing 9-month-old APP/PS1 mice for 9 months with paroxetine (5 or 10 mg/kg/day *per os*) would mitigate Aβ pathology and improve behavior (locomotion, social memory). We also tested the hypothesis that a neurotoxin-induced loss of cortical 5-HT at 9 months of age would impact on Aβ pathology at 12 months. The results of this study question the proposed beneficial effect of SSRI therapy and 5-HT on Aβ pathology in mice with manifest Aβ pathology.

2. Methods

2.1. Paroxetine treatment and neurotoxin injection

Paroxetine: $APP_{swe}/PS1_{\Delta E9}$ (APP/PS1) and littermate wild-type (Wt) mice [21] were bred on a B6C3 background in house or at Taconic A/S, Denmark [28]. Paroxetine (Seroxat oral solution 2 mg/mL, GSKline) was administered in the drinking water in a dose of 10 mg/kg/day or 5 mg/kg/day (Table 1) to 9-month-old male APP/PS1 and Wt mice. APP/PS1 and Wt controls received normal drinking water. Treatment efficacy was assessed by autoradiographic measurement of the SERT occupancy using [^{3}H]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile, as detailed in Supplementary Methods.

Neurotoxin: To destroy 5-HT fibers and neurons, 5,7-dihydroxytryptophan (5,7-DHT) was stereotaxically injected into the lateral ventricles of 9-month-old male APP/PS1 and Wt mice (Table 1), as described elsewhere [31], but injecting a total dose of 80 μg 5,7-DHT. Mice with unsuccessful lesions, as determined by the measurement of 5-HT in neocortical tissues, were excluded from statistical analysis (Danish Veterinary & Food Administration: J.no. 2007/562-50, J.no. 2012-15-2935-00,023, J.no. 2012-DY-2934-00,008).

Tissue processing: See Supplementary Methods.

2.2. Behavior

The behavior of 18-month-old mice was assessed using the open field, the elevated plus maze, and the social

Table 1
Study design

Experimental group	Treatment/intervention	Genotype		Treatment period (months)	Behavioral analysis
		Wt (*n*)	APP/PS1 (*n*)		
Nine months paroxetine treatment	Vehicle	20	14	9–18 months	Open field, elevated plus maze, social interaction, social memory* Y-maze†
	Paroxetine 10 mg/kg/day	15	5		
	Vehicle	15	7	9–18 months	Open field, elevated plus maze, social interaction, social memory Y-maze‡
	Paroxetine 5 mg/kg/day	11	7		
Three months paroxetine treatment	Vehicle	6	10	9–12 months	Not done
	Paroxetine 10 mg/kg/day	6	7		
Three months 5,7-DHT-induced loss of 5-HT	Sham	6	6	9–12 months	Open field, social interaction, social memory Y-maze‡
	5,7-DHT	6	6		

Abbreviations: 5,7-DHT, 5,7-dihydroxytryptophan; APP, amyloid precursor protein; 5-HT, 5-hydroxytryptamine; PS1, presenilin 1.
*Results reported in Olesen et al. [29].
†Results reported in Olesen et al. [30].
‡Unpublished results.