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Memory enhancement by Tamoxifen on amyloidosis mouse model

Deepika Pandey [a], Sugato Banerjee [a], Mahua Basu [b], Nibha Mishra [a,*]

[a] Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra 835215, Ranchi, India
[b] St. Xavier's College 30, Mother Teresa Sarani, Kolkata 700016, India

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ABSTRACT

Tamoxifen (TMX) is a selective estrogen receptor modulator (SERM) used in the treatment of breast cancer. Earlier studies show its neuroprotection via regulating apoptosis, microglial functions, and synaptic plasticity. TMX also showed memory enhancement in ovariectomized mice, and protection from amyloid induced damage in hippocampal cell line. These reports encouraged us to explore the role of TMX in relevance to Alzheimer's disease (AD). We report here, the effect of TMX treatment a) on memory, and b) levels of neurotransmitters (acetylcholine (ACh) and dopamine (DA)) in breeding-retired-female mice injected with beta amyloid$_{1-42}$ ($A\beta_{1-42}$). Mice were treated with TMX (10 mg/kg, i.p.) for 15 days. In Morris water maze test, the TMX treated mice escape latency decreased during training trials. They also spent longer time in the platform quadrant on probe trial, compared to controls. In Passive avoidance test, TMX treated mice avoided stepping on the shock chamber. This suggests that TMX protects memory from Aβ induced toxicity. In frontal cortex, ACh was moderately increased, with TMX treatment. In striatum, dopamine was significantly increased, 3,4-dihydroxyphenylacetic acid (DOPAC) level and DOPAC/DA ratio was decreased post TMX treatment. Therefore, TMX enhances spatial and contextual memory by reducing dopamine metabolism and increasing ACh level in $A\beta_{1-42}$ injected-breeding-retired-female mice.

Introduction

Tamoxifen (TMX) is a selective estrogen receptor modulator (SERM), used in the treatment of hormone-dependent breast cancer. Potential of TMX in brain disorders has been reported over a decade. Many studies reported its neuroprotective property by boosting anti-apoptotic factors, suppressing excitotoxicity, and attenuating microglial response, in brain injuries (Kuo et al., 2012; Liu et al., 2010; Tsai et al., 2014). TMX also protected from beta amyloid induced toxicity in hippocampal cell culture (Gursoy et al., 2002). Recently, Velazquez-Zamora et al., reported that TMX improved prefrontal associated allocentric memory and increased layer III pyramidal neuronal dendritic density. (Velazquez-Zamora et al., 2012; Zabihi et al., 2014). However, human studies showed mixed results. Newhouse and group reported that TMX treatment could revert anti-cholinergic mediated memory loss. The effect was more prominent in APO e4+ (apolipoprotein E4) genotype women, compared to APO e4-women (Newhouse et al., 2013). Thus, neuroprotective and cognition enhancing effects of TMX prompted us to explore its effect in relevance to Alzheimer's disease (AD).

AD is one of the most common causes of dementia. It is characterized by deposition of β-amyloid (Aβ) plaques, neurofibrillary tangles, and degeneration of cholinergic neurons (Wenk, 2003), in the cerebral cortex and hippocampus. We investigated the effect of TMX on spatial learning using Morris water maze and contextual learning with passive avoidance test, subsequently we estimated acetylcholine and dopamine levels in frontal cortex and striatum respectively. We used breeding-retired-female mice, a model of postmenopausal condition and aging. These mice were then injected with $A\beta_{1-42}$, to induce pathology similar to amyloidosis (Soto et al., 1998).

Materials and methods

Animals

All animals were maintained in our animal facility under standard housing conditions, temperature (24–27 °C), humidity (40–50%) with 12 h light–dark cycles, free access to food and water. We have used female-breeding-retarded Swiss albino (12–13 months) mice. The experiments were approved by Institutional animal ethics committee of CPCSEA (Reg. no.: 621/02/ac/CPCSEA).

$A\beta_{1-42}$ icv injection

$A\beta_{1-42}$ peptide was dissolved in DMSO to obtain a 2.3 mM stock solution. The stock solution was diluted with phosphate buffer saline (PBS) to final DMSO concentration (0.002%), and divided into 1 mg/mL aliquots. For fibrillization, it was incubated at 37 °C for 5 days. Mice were anesthetized by intraperitoneal injection of

* Corresponding author.
E-mail address: nmishra@bitmesra.ac.in (N. Mishra).

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xylazine:ketamine (10:100 mg/kg, i.p.). Intracerebroventricullar injection was performed as described previously (Mishra et al., 2013), briefly 5 μL of 1 mg/mL solution was injected to bregma at the injection rate of 0.2 μL/s. The injection location was verified by injecting same volume of methylene blue. Sham mice were similarly injected with PBS. Aβ injected mice were treated for 15 days with either saline or TMX, 10 mg/kg, i.p. Higher dose was selected to obtain both neuroprotection and memory enhancement as per previous studies (Baksi et al., 1985; Zhang et al., 2005).

Memory performance tests

Morris water maze tests had been adapted from previously described protocol (Vorhees and Williams, 2006). We used a circular tank (120 cm) with a platform (10 cm^2 diameter) submerged (0.5 cm) under water (20–22 °C). Four trials per day (from different directions randomized everyday) were performed, for four days. Each trial was carried out by placing mouse in water, facing the wall. On first day, if they could not find platform within 60 s they were placed on platform for another 60 s, after drying they were returned to the warm cage. Inter-trial-interval was 10 min. Time required to escape to the platform (escape latency) was recorded using tracking software (Ethovision system Noldus, Wageningen, Netherlands). On fifth day, after 24 h probe trial (without platform) was done to evaluate memory consolidation. The mice were allowed to swim for 60 s; time spent in the platform quadrant was recorded.

Passive avoidance test is based on fear conditioned learning behavior. The passive avoidance apparatus was a two compartment shuttle chambers, one illuminated compartment and other dark with shock generator. In acquisition trials (2 days), mice were allowed to explore apparatus for 5 mins. Once they are inside the dark chamber with all four paws; they were subjected to electric shock (1 mA, 2 s duration). On third day in the retention trial, they were placed in the illuminated compartment. Their latency to enter darker compartment (step through latency) was recorded automatically.

Determination of DA and ACh level

We quantified dopamine (DA) and acetylcholine (ACh) level in striatum and frontal cortex. After 15 days of treatment, Aβ injected mice were decapitated and the head was snap frozen. The brain was dissected; frontal cortex and striatum were isolated. Striatum was homogenized in 600 μL of ice cold solution of 0.4 M perchloric acid (PCA) containing 0.4 mM sodium metabisulfite and Na_2EDTA. The resulting homogenate was centrifuged at 12,000 g for 20 min at 4 °C. Supernatant was filtered through 0.45 μm cellulose membranes. The mobile phase was methanol: buffer (7.5: 92.5, v/v), it was composed of 0.07 M sodium acetate, 0.04 M citric acid, 130 μM EDTA, and 230 μM sodium octane sulfonate. HPLC (Waters HPLC systems, Milford, MA, USA) analysis was done at 35 °C, pH 4.5 at flow rate 1 mL/min, equipped with electrochemical detector.

Frontal cortex was homogenized in 200 μL ice cold solution of 0.1 M PCA containing 100 μM ethylhomocholine centrifuged at 12,000 g for 15 min at 4 °C supernatant, filtered through 0.45 μm filter. The mobile phase was a 0.07 M sodium phosphate buffer containing 0.2 mM Na_2EDTA, 0.65 mM tetramethylammonium chloride, and 0.3 mM sodium octanesulfonic acid adjusted to pH 8.4 at 35 °C. HPLC analysis was carried out at 35 °C, flow rate 1 mL/min equipped with an electrochemical detector and enzyme reactor (having immobilized AChE and choline oxidase). Protein estimation was done by Lowry method.

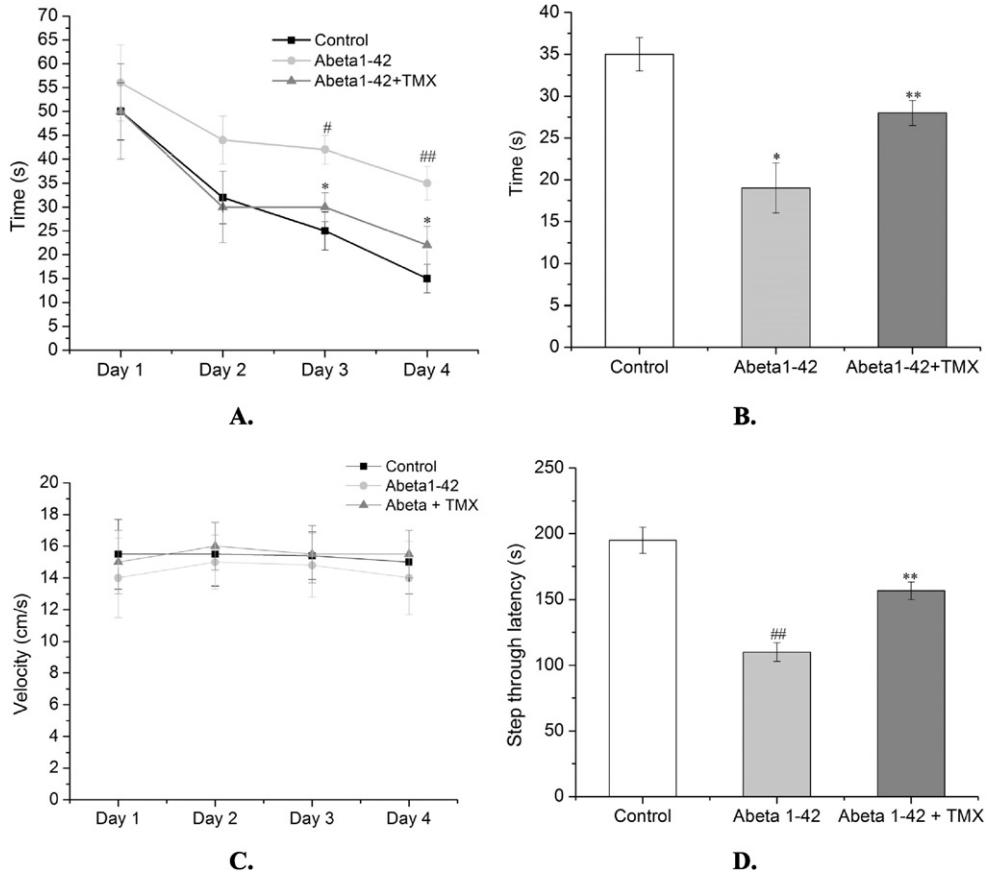

Fig. 1. Effect of TMX on spatial memory: A. Escape latency in MWM, B. Time spent in platform quadrant in MWM, C. Swimming velocity in MWM, D. Step through latency to dark-shock chamber in Passive avoidance apparatus. Aβ injected group was compared with sham control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. TMX treated group is compared with Aβ injected group, $^{*}p < 0.05$, $^{**}p < 0.01$. Values are expressed as mean ± SEM, n = 6 ± 1.