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All-trans retinoic acid rescues memory deficits and neuropathological changes in mouse model of streptozotocin-induced dementia of Alzheimer's type

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ABSTRACT

Recent studies have revealed that aberrant vitamin A signaling may lead to memory deficits in rodents. Present study investigates the potential of all-trans-retinoic acid (ATRA) an agonist at retinoid acid family of receptors, in cognitive dysfunctions associated with experimental dementia. Streptozotocin (STZ) [3 mg/kg, intracerebroventricularly (i.c.v)] was administered on alternate days (day 1 and day 3) to induce dementia in Swiss albino mice. STZ mice were administered ATRA (10 mg/kg; 20 mg/kg, p.o.) for a total of 19 days following second i.c.v injection of STZ [day 4 to day 22]. Morris water maze (MWM) test was performed on days 19, 20, 21, 22 and 23 to assess learning and memory of the animals. Following MWM test, the animals were sacrificed for biochemical and histopathological studies. Extent of oxidative stress was measured by estimating the levels of brain reduced glutathione (GSH) and thiobarbituric acid reactive species (TBARS). Brain acetylcholinestrase (AChE) activity and serum cholesterol levels were also estimated. The brain level of myeloperoxidase (MPO) was measured as a marker of inflammation. STZ produced a marked decline in MWM performance of the animals, reflecting impairment of learning and memory. STZ treated mice showed marked accentuation of AChE activity, TBARS and MPO levels along with fall in GSH level. Further the stained micrographs of STZ-treated mice indicated pathological changes, severe neutrophilic infiltration and amyloid deposition. ATRA treatment significantly attenuated STZ-induced memory deficits, biochemical and histopathological alterations. The findings demonstrate that the memory restorative ability of ATRA may be attributed to its anti-cholinesterase, anti-oxidative and anti-inflammatory potential.

1. Introduction

Alzheimer's disease is the most common form of neurodegenerative diseases associated with dementia in elderly. The pathogenesis of AD is multifactorial, including deposition extracellular neuritic plaques of β-amyloid (Aβ), intracellular neurofibrillary tangles containing hyperphosphorylated tau, inflammation, oxidative stress and aberrant cholesterol metabolism (Yamasaki et al., 2012). With modest benefits achieved from the targeted therapeutics, we suggest that treating the disease from multiple angles may prove more effective. Retinoic acid (RA), the active metabolite of vitamin A, has been known to modulate essential physiological as well as pathological processes including vision, reproduction, proliferation, differentiation and apoptosis (Germain, 2006; Ziouzenkova and Plutzky, 2008). Vitamin A deficiency is most common in children resulting in blindness and is associated with 200,000 deaths per year (Chiang et al., 1998). It has been strongly implicated in the pathogenesis of obesity, diabetes and cardiovascular diseases (Bonet et al., 2003; Villarroya et al., 2004). Retinoic acid has been reported to regulate the expression of certain genes through its action on nuclear receptors: retinoic acid receptors (RAR) and retinoid X receptors (RXR) (Germain, 2006; Mangelsdorf and Evans, 1995). Many of the therapeutic benefits of RA, including cancer chemoprevention and treatment of dermatological disorders are mediated through RAR receptors (Swift et al., 2008; Tsai et al., 2009). Components of the retinoid metabolic pathway have been identified in the adult brain tissues, including the cortex, amygdala, hypothalamus and striatum, indicating that they may contribute to specific functions in CNS (Lee et al., 2009). Reports have suggested that deprivation of vitamin A produces Aβ accumulation and loss of hippocampal long term potentiation in rodents (Cocco et al., 2002; Ding et al., 2008). Metabolites of retinoic acid have been also known to control fundamental processes including neurite outgrowth (Muley et al., 2008) and neurotransmitter release (Carta et al., 2006). Studies have revealed that ATRA induces the expression of manganese superoxide dismutase (MnSOD2) gene in neuroblastoma cells, thereby reducing the oxidative

Abbreviations: Aβ, β-amyloid; ABCA-1, ATP-binding cassette transporter A-1; ABCG-1, ATP-binding cassette transporter G-1; AβPP, amyloid β protein precursor; ACSF, artificial cerebrospinal fluid; AChE, acetylcholine esterase; AD, Alzheimer's disease; ATRA, all-trans retinoic acid; BSA, bovine serum albumin; CSF, cerebrospinal fluid; DMSO, dimethyl sulfoxide; DTNB, 5,5-dithiobis (2-nitrobenzoic acid); ELT, escape latency time; GSH, reduced glutathione; HE, hematoxylin and eosin; i.c.v, intracerebroventricular; MWM, Morris water maze; MnSOD2, manganese superoxide dismutase; MPO, myeloperoxidase; RAR, retinoic acid receptor; RXR, retinoid X receptor; STZ, streptozotocin; TBARS, thiobarbituric acid reactive species; TSTQ, time spent in target quadrant.

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damage, which is an important pathological factor in AD (Kiningham et al., 2008). Reports also demonstrate that, impairment of spatial learning and memory and the depression of synaptic plasticity that occurs in RAR or RXR mutated rodents were reversed by the administration of retinoic acid (Ding et al., 2008; Goodman, 2006). Furthermore, clinical evidence also show that the serum concentrations of vitamins A, C, E and β-carotene were significantly reduced in the AD patients than in controls (Grant, 1991). Importantly, some reports have also suggested defective retinoid transport and function in the AD brain, signifying that increasing the availability of RA in brain may prevent or reduce Aβ-associated neurodegeneration (Goodman, 2006; Tafti and Ghyselinck, 2007). However, to date, there have been no conclusive experimental evidence explaining the protective potential of all-trans retinoic acid (ATRA) in animal models of dementia. In the present study, we examined the effect of ATRA, a highly lipophilic and biologically active isoform of retinoic acid, on the neurodegenerative pathology and memory deficits induced by streptozotocin in mice.

2. Materials and methods

2.1. Experimental animals

Swiss albino mice (20–25 g) of either sex (procured from Hisar agriculture university, Hisar, India) were employed in the present study and were housed in the departmental animal house with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd. Mumbai, India). They were exposed to 12 h light and 12 h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. The experiments were performed between 9.30 and 17.30 h in semi sound proof laboratory conditions. The experimental protocol was duly approved by the institutional animal ethical committee and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 107/1999/CPCSEA).

2.2. Drugs and reagents

All drugs were freshly prepared before use. All-trans retinoic acid was procured as gift sample from Taizhou Tianrui Pharmaceuticals Co. Ltd., China and was dissolved in dimethyl sulfoxide (0.05% v/v DMSO). Streptozotocin and 1,1,3,3 tetra-methoxy propane were purchased from Sigma Aldrich, USA. 5,5′-Dithiobis (2-nitro benzoic acid) DTNB, bovine serum albumin (BSA), and reduced glutathione (GSH) standard were purchased from Sisco Research Laboratories Pvt Ltd., Mumbai, India. Thiobarbituric acid was purchased from Loba Chemie, Mumbai, India. Streptozotocin was dissolved in freshly prepared artificial cerebrospinal fluid (ACSF) (147 mM NaCl; 2.9 mM KCl; 1.6 mM $MgCl_2$; 1.7 mM $CaCl_2$; and 2.2 mM dextrose). Standard cholesterol estimation kit (Monozyme India Limited, Secunderabad) was used to estimate total serum cholesterol level.

2.3. Laboratory models

2.3.1. Intracerebroventricular (i.c.v) streptozotocin (STZ)-induced dementia

Mice were anesthetized with anesthetic ether (Sharma et al., 2008). A polypropylene tube was placed round a hypodermic needle of 0.4 mm external diameter exposing about 3 mm at the tip, and was attached to a 10 μl Hamilton microliter syringe (Top Syringe, Mumbai, India), which was inserted perpendicularly through the skull (not more than 3 mm) into the brain of mouse. The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into right and left ventricle on alternate days. Two doses of STZ (3 mg/kg, i.c.v, 10 μl each) were administered bilaterally on days 1 and 3. The STZ concentration was adjusted to deliver 10 μl per injection. The injection was made in two locations due to the difficulty of administering 10 μl to a single site. To ascertain that the drug was administered exactly into the cerebral ventricles, some mice (20%) were injected with 5 μl of diluted potent blue dye and their brains were examined macroscopically after sectioning. STZ was dissolved in artificial CSF (25 mg ml^{-1}) solution which was made freshly just before the injection. Control mice were administered ACSF via i.c.v injection in a similar manner.

2.3.2. Morris water maze (MWM) test

Morris water maze test was employed to assess learning and memory of the animals (Morris, 1984). MWM is a swimming based model where the animal learns to escape on to a hidden platform. It consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1 °C). The water was made opaque with white colored non-toxic dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 s to locate submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was guided gently onto platform and allowed to remain there for 20 s. Day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. Animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4) remained constant throughout the training period.

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

On fifth day, platform was removed and each mouse was allowed to explore the pool for 120 s. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as index of retrieval or memory. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study.

2.4. Biochemical parameters

2.4.1. Collection of samples

Animals were sacrificed by cervical dislocation, brains were removed and homogenized in phosphate buffer (pH = 7.4). The homogenates were than centrifuged at 3000 rpm for 15 min. The supernatant of homogenates were used for biochemical estimations as per the methods described below. Blood sample was collected by retro-orbital puncture just before sacrificing the animal. The blood was then kept at room temperature for 30 min after which it was centrifuged at 4000 rpm for 15 min to separate serum. Serum was used to estimate the level of serum total cholesterol, while additional brain samples were preserved in 4% neutral formalin for histopathological examination.

2.4.2. Estimation of brain acetyl cholinesterase (AChE) activity

The whole brain AChE activity was measured by the method of Ellman et al. (1961) with slight modifications (Voss and Sachsse,