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Effects of systemic Thalidomide and intracerebroventricular Etanercept and Infliximab administration in a Streptozotocin induced dementia model in rats

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ABSTRACT

Tumor necrosis factor-alpha (TNF-α) upregulation enhances amyloid β (Aβ) induced neurotoxicity in Alzheimer's disease (AD). Intracerebroventricular streptozotocin (STZ) administration causes pathological changes and cognitive deficits similar to those seen in AD by causing impairment of brain glucose and energy metabolism. Recent reports indicate a protective role of Thalidomide, Etanercept, and Infliximab, all of which have anti-TNF-α activity, against cognitive and neuropathological changes in experimental and clinical studies. We aimed to investigate the protective effects of Thalidomide, Etanercept, and Infliximab in a rat model of intracerebroventricular STZ-induced dementia. Sprague–Dawley rats (250–300 g) were separated to sham ($n = 6$) and STZ ($n = 24$) groups. The STZ group was divided into four groups (STZ, STZ-thalidomide, STZ-etanercept, and STZ-infliximab). Morris's water maze (MWM) and passive avoidance (PA) tests were performed. At the end of the third week, brain tissues were obtained. Histopathological analysis, immunohistochemistry, and electron microscopic examinations were done. The improvement performance of the STZ group was significantly reduced in the MWM test ($p < 0.001$). Compared with the STZ, STZ-thalidomide, STZ-etanercept, and STZ-infliximab groups had significantly better performance ($p < 0.001$, <0.05 and <0.05, respectively) in the MWM test. STZ administration caused a significant decrease in the mean escape latency in PA reflex ($p < 0.001$). Thalidomide, Etanercept, and Infliximab were associated with better PA reflexes compared to the STZ group ($p < 0.001$ for all). Morphological and immunohistochemical results showed increased neurodegenerative changes compared to sham group. Our findings are in line with the findings reported in the literature and encourage further studies with TNF-α antagonists, in particular Thalidomide.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and causes an increasing healthcare cost burden (Hebert et al., 2013). The neuropathological features of AD include amyloid beta (Aβ) plaques (P), neurofibrillary tangles (NFTs, including tau protein phosphorylation [TPP]), insulin resistance, and inflammation (Heneka et al., 2010). Neuropathological characteristics of insulin resistance include impaired neuronal survival, energy production, gene expression and plasticity (de la Monte and Tong, 2014). Insulin resistance is reported to enhance both TPP and Aβ accumulation and toxicity and also to result in microvessel injury (de la Monte and Tong, 2014). Inflammation has drawn significant interest as a key factor in AD pathogenesis, and upregulation of tumor necrosis

Abbreviations: aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; Aβ, amyloid beta; BBB, blood–brain barrier; CNS, central nervous system; DAB, diaminobenzidine; H&E, hematoxylin and eosin; ICV, intracerebroventricular; ILT, initial latency time; IP, intraperitoneal; LT, latency time; MWM, Morris water maze; NTFs, neurofibrillary tangles; P, plaques; PBS, phosphate-buffered saline; RLT, retention latency time; STZ, streptozotocin; STZ-E, STZ-etanercept; STZ-I, STZ-infliximab; STZ-T, STZ-thalidomide; TNF-α, tumor necrosis factor-alpha; TPP, tau protein phosphorylation.

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factor-alpha (TNF-α) may precipitate $A\beta_{1-40}$ induced neurotoxicity (Alkam et al., 2008).

When used as an intracerebroventricular (ICV) injection, streptozotocin (STZ) causes prolonged impairment of brain glucose and energy metabolism and is widely used to mimic an AD-like condition characterized with cognitive deficits, TPP and neurofibrillary degeneration (Blokland and Jolles, 1993; Rodrigues et al., 2010).

Drugs with anti-TNF-α activity (Infliximab, Etanercept and Thalidomide) have been used in animal models and cases with dementia (Alkam et al., 2008; Ryu and McLarnon, 2008; Shi et al., 2011a; Tobinick et al., 2006; Tobinick and Gross, 2008; Tweedie et al., 2007). Infliximab and Etanercept are monoclonal antibodies against TNF-α. Thalidomide has anti-angiogenic effects and inhibitory effects on TNF-α, IL-6, IL-10, IL-12, and NF-κB. Infliximab and Etanercept cannot cross the blood–brain barrier (BBB) limiting their systemic use in inflammatory conditions of the central nervous system (CNS) (Tweedie et al., 2007). However, Thalidomide can readily cross the BBB (Ryu and McLarnon, 2008). As far as we are aware, the effects of Thalidomide, Etanercept, and Infliximab against learning and memory deficits and neuropathological changes have not been comparatively tested in ICV-STZ induced dementia.

We aimed to investigate the protective effects of intraperitoneal (IP) Thalidomide and ICV Etanercept and Infliximab in an ICV-STZ induced dementia rat model.

Materials and methods

Animals

Male Sprague–Dawley rats (250–300 g) were housed at room temperature (24–27 °C) and relative air humidity 60–65% with a 12-h light/12-h dark cycle. The animals were procured from the laboratory animal care division of Marmara University. Experiments were performed according to international ethical standards and approved by the Ethics Committee of the Marmara University, Animal Care and Use Committee.

Experimental groups

The rats were divided into sham ($n = 6$) and STZ ($n = 24$) groups. Sham group was given bilateral ICV 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), 20 μl on each side. This procedure was repeated on day 3. Rats in the STZ group underwent bilateral ICV injection of STZ (3 mg/kg, Sigma–Aldrich, St. Louis, MO, USA) (Rodrigues et al., 2007). The STZ group was divided into four groups, STZ, STZ-thalidomide (STZ-T), STZ-etanercept (STZ-E), and STZ-infliximab (STZ-I). STZ-T group received IP 50 mg/kg/day Thalidomide beginning 1 h prior to STZ injection, until day 21 (Rodrigues et al., 2007). Thalidomide (Sigma–Aldrich, St. Louis, MO, USA) was prepared in 0.5% carboxymethylcellulose on each day. STZ-E group received 0.3 mg/kg ICV Etanercept (Enbrel, Amgen, Thousand Oaks, CA, USA). STZ-I group received 5 mg/kg ICV Infliximab (Remicade, Centocor, Inc., County Cork, Ireland). From day 17 of ICV administration of STZ and CSF, all groups were subjected to trials on a Morris water maze (WM) and passive avoidance (PA). On day 21, all of the rats were sacrificed to obtain their brains.

ICV injection of STZ

Rats were anesthetized with 70 mg/kg of IP ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey). STZ was injected into bilateral lateral ventricles using the coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm ventral from the surface of the brain. The volume of the injection was constant at 20 μl for each side; the variation in the amount of drug was negligible because the rats had similar weights. The same dose of STZ was repeated 48 h afterwards. STZ was dissolved in aCSF and a solution of 22.5 mg/ml was prepared before each injection. In the sham group, aCSF was injected on the same days as for the STZ group as previously reported (Sharma and Gupta, 2001).

Learning and memory tests

Passive avoidance

A step through PA apparatus was used to assess memory retention deficit on days 17 and 18 as previously described (Elcioglu et al., 2013; Nakahara et al., 1988). The initial latency time (ILT) for the rat to enter the dark chamber was recorded. Rats with an ILT >60 s were excluded from further experiments. Twenty-four hours later, the retention latency time (RLT) was measured in the same way, and the RLT was recorded to a maximum of 300 s.

Morris water maze

The MWM is used to test spatial learning and memory in rats (Elcioglu et al., 2013; Morris, 1984). Yellow non-toxic watercolor paint was added to opacify the water. The tank was divided into four quadrants. During the test, a movable escape platform made of transparent Plexiglass was located below the water surface in the center of one of the quadrants. The top of the platform was 1 cm below the surface of the water in its raised position, so that the rat could easily climb onto it to escape from the water. The rats were given up to 120 s to find the hidden platform and were allowed to stay on it for 30 s. Rats failing to find the platform were gently guided on the platform only in the first trial (acquisition trial). The animals were given a daily session of 5 trials per day for 3 days. During a session, latency time (LT) to reach the platform was recorded for each rat in each trial and mean LT was calculated. A significant decrease in LT in subsequent sessions (retention) from the first session was considered as successful learning. During or after training is complete, the experimenter conducts a probe trial in which the escape platform is removed from the pool and the animal is allowed to swim for 120 s. The time spent in the target quadrant (i.e., the previous location of the platform) was recorded as the exploration time.

Histopathological studies

Brains from different groups ($n = 6$) were perfused as described previously (Ahmad et al., 2005). Briefly, 3 weeks after the surgery rats were anesthetized using IP sodium pentothal (Penthal-sodium, IE Ulugay, Istanbul, Turkey), and perfused transcardially with 10 ml of saline solution followed by 150 ml of phosphate-buffered fixative (2% paraformaldehyde, 2.5% glutaraldehyde), for at least 20 min. Immediately after perfusion, the brains were removed and further fixation was done in 2% glutaraldehyde–paraformaldehyde buffer for 24 h. After post-fixation, the tissue was dehydrated and embedded in paraffin wax and was cut with a microtome (Leica RM 2255) into 10 μm thick coronal sections on electrostatically charged slides. Routine histological examinations were carried out with Hematoxylin and Eosin (H&E) staining for general evaluation of the tissue and Bielschowsky stains for visualization of nerve fibers, neurofibrillary tangles and senile plaques those are seen in Alzheimer's disease (Yamamoto and Hirano, 1986). It is possible to use the Bielschowsky impregnation stain can be applied on paraffin sections (Bancroft and Gamble, 2008).

Semi-quantitative histological evaluation was scored blindly. Frontal, parietal and temporal regions in neocortex and CA1, CA2 and CA3 regions of hippocampus were examined in sham and treatment groups. NFTs and P were counted in 5 random fields (×100) in the neocortex and hippocampus after Bielschowsky staining, and