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MINOCYCLINE DECREASES ACETYLCHOLINESTRASE ACTIVITY IN INTRA-CEREBROVENTRICULAR STREPTOZOTOCIN INFUSED RATS

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ABSTRACT

Alzheimer's disease, a synonym for life threatening dementias is characterized by oxidative stress and neuroinflammation induced neuronal loss, impaired energy metabolism, and cholinergic deficit leading to severe cognitive impairments and other abnormal neuropsychiatric changes. Cholinergic hypothesis is the most accepted theory explaining pathology of AD and Acetylcholinestrase inhibitors are the main stay of AD therapy. In the present study, the effect of Minocycline, a tetracycline derivative, was investigated against intracerebroventricular streptozotocin induced cholinergic deficits. Intracerebroventricular administration of streptozotocin (3mg/kg) bilaterally on day 1 and 3 was able to produce significant cholinergic deficits as evidenced by increase in level of acetylcholinestrase while chronic treatment with Minocycline (10, 20 and 40mg/kg, i. p.) for 21 days significantly decreased it. The results of the present study support the candidature of Minocycline in learning and memory disorders resembling dementia of Alzheimer's type.

INTRODUCTION: The United Nations population projections estimate that 370 million people will be older than 80 years by 2050 and the associated increase in patients with Alzheimer's disease will pose a substantial socio-economic burden [1]. Alzheimer's disease (AD) is a slow progressive neurodegenerative disorder, clinically characterized by a noticeable cognitive decline defined by a loss of memory and learning ability, together with a reduced ability to perform basic activities of daily living and a diverse array of neuropsychiatric symptoms such as apathy, verbal and physical agitation, irritability, anxiety, depression, delusions and hallucinations [2]. One of the most fundamental and consistent features of AD is the severe degeneration of cholinergic neurons projecting from basal forebrain to cortical and hippocampal areas [3, 4]. A 90% loss of basal forebrain cholinergic neurons has been found in AD patients [3, 5].

Streptozotocin (2- deoxy- 2- (3- methyl- 3- nitrosoureido)- D- glucopyranose, STZ) is an antibiotic derived from the soil bacteria Streptomyces achromogenes [6] and is oftenly used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β cells. Besides its antibiotic and diabetogenic properties, STZ is genotoxic in a variety of assays, including microbial mutagenesis, unscheduled DNA synthesis, micronucleus, chromosomal abberations and sister chromatid exchanges. While parenteral injection of STZ induces diabetes, by damaging pancreatic beta cells possibly through the generation of ROS[7] intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats leads to long-term and progressive deficits in learning, memory, and cognitive performance that is similar to Alzheimer's disease [8]. Thus ICV STZ produce neuropathological and biochemical alterations similar to those observed in sporadic Alzheimer's disease and therefore considered to be a valid experimental model to study early pathophysiological changes in Alzheimer's disease [9]. Minocycline, the most lipid soluble and most active tetracycline antibiotic has been in use for over 30 years to treat pneumonia and acne vulgaris, infections of the skin, genital, and urinary systems [10] and rheumatoid arthritis [11, 12].

Minocycline exert biological effects independent of their antimicrobial properties [13]. These include anti-inflammatory activities such as inhibition of iNOS [14], up regulation of interleukin 10 etc. [15]. Minocycline effectively crosses the blood-brain barrier due to its small (495 d) and lipophilic nature [16] and has been shown to exert neuroprotective effects distinct from its bacteriostatic activity in animal models of cerebral ischemia [17] Parkinson's and Huntington's disease [18]. Based on several encouraging evidences of Minocycline in neurodegenerative disorders, the present study was designed to investigate the effect of Minocycline in i. c. v. streptozotocin induced cognitive and cholinergic deficits in rats.

MATERIAL AND METHODS:

Animals and Experimental Groups: The experiments were carried out in adult (7-8 months old) male Wistar rats (230-250 g) obtained from animal house of Onkar College of Pharmacy, Sajuma, Punjab, (India). All animals were housed in rodent cages in the animal room where the temperature was maintained approximately at 24-25^{o}C and relative humidity of 60-65%) with 12 hours dark-light cycle (lights on 06.00 - 18.00 h).The food in the form of dry pallets and water were made available ad libitum. All behavioral experiments were carried out between 10 AM and 4 PM. The protocol was reviewed and approved by the Institutional Animal Ethics Committee and the animal experiments were carried out in accordance with

the Indian National Science Academy Guidelines for use and care of animals. After adapting to the new environment for at least 7 days, animals were divided into seven groups and each group comprised of 10 animals.

Group 1: Sham Operated (SH) (Sham-operated rats wherein the surgery was performed minus drilling of holes and placement of the cannula).

Group 2: (SH+ aCSF) artificial cerebrospinal fluid (aCSF) was infused i. c. v. in a volume of 10 µl in each ventricle on day 1 and 3.

Group 3: STZ Control (STZ + normal saline, as vehicle for MIN)

(Rats were infused with i. c. v. streptozotocin (3mg/kg) dissolved in aCSF in a volume of 10 µl in each ventricle on day 1 and 3 and the animals were treated with normal saline containing as a vehicle of minocycline) for 21 days.

Group 4: MIN10 (STZ + MIN, 10mg/kg, i. p.) Rats infused with i. c. v. streptozotocin on day 1 and 3 and immediately after first streptozotocin infusion, treated with 10 mg/kg i. p. minocycline for 21 days.

Group 5: MIN20 (STZ + MIN, 20mg/kg, i. p.), Rats infused with i. c. v. streptozotocin) were treated with minocycline at doses of 20 mg/kg, i. p. respectively for 21 days following 1st streptozotocin infusion.

Group 6: MIN40 (STZ + MIN, 20mg/kg, i. p.) Rats infused with i. c. v. streptozotocin) were treated with minocycline at doses of 40 mg/kg, i. p. respectively for 21 days following 1st streptozotocin infusion

Group 7: per se group of normal animals were treated with 40mg/kg, i. p. of minocycline.

The vehicle and doses of Minocycline were selected based on previous reports [17, 19, 20] in literature.

Materials: Streptozotocin was purchased from Sigma–Aldrich, USA. Minocycline was used in the form of marketed preparation (Minoz, Ranbaxy, India). All other chemicals used in the study were of analytical grade. Solutions of the drug and chemicals were freshly prepared before use.

Intracerebroventricular (i. c. v.) Infusion of Streptozotocin: Male Wistar rats weighing 230-250 g were anaesthetized with ketamine (100mg/kg, ip) and xylazine (5mg/kg, ip). The head was placed in position in the stereotaxic apparatus and a midline saggital incision was made in the scalp. Two holes were drilled through the skull for placement of infusion cannula into the lateral cerebral ventricles using following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to saggital suture; 3.6 mm ventraly8 from the surface of the brain [21]. Streptozotocin was dissolved in 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 (pH 7.4)] and slowly infused (1 µl/min) using Hamilton microsyringe in a volume of 10 µl into each cerebral ventricle (bilateral i. c. v.) on day 1 and 3 [22]. After ICV injection, povidone-iodine solution was applied and the cut skin was sutured after second injection followed by daily application of Neosporin^R. The body weights were continuously monitored.

Biochemical Parameters:

Brain Homogenate Preparation: Animals were sacrificed by decapitation and brains were remove and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice-cold 0.1 M phosphate buffer (pH7.4) in a volume 10 times the weight of the tissue. The

homogenate was centrifuged at 10,000×g for 15min and aliquots of supernatant separated and used for biochemical estimation.

Protein Estimation: Protein was measured in all brain samples by the method of Lowry et al. (1951) [23] using bovine serum albumin (BSA) (1 mg/ml) as a standard.

Estimations of level of Acetylcholinesterase: The quantitative measurement of acetylcholinesterase activity in brain was performed according to the method described by Ellman *et al.* (1961) [24]. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412 nm spectrophotometrically. The acetylcholinestrase activity in the supernatant was expressed as nmol per mg protein.

Statistical Analysis: The results are expressed as means ± S. E. M. The behavioral and biochemical values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P < 0.05 was considered statistically significant.

RESULTS:

Effect of Minocycline on Brain Acetylcholinesterase Activity in i. c. v. Streptozotocin Infused Rats: The activity of acetylcholinestrase was increased significantly in brain homogenate of i. c. v. streptozotocin infused rats compared with those of sham group (P<0.001). Minocycline treatment in streptozotocin infused rats dose dependently decreased the enhanced acetylcholinesterase activity compared with streptozotocin infused rats (**Table 1**).

TABLE 1: EFFECT OF MINOCYCLINE ON BRAIN ACETYLCHOLINESTERASE ACTIVITY IN I. C. V. STREPTOZOTOCIN INFUSED RATS

GROUPS	AChE (nM/mg protein)
Sham	172.16 ±8.9
aCSF	169.5 ±7.91
MIN (per se)	170.66 ± 4.71
ICV STZ	400.87 ± 8.55 [a]
MIN 10	318.33 ± 7.49 [b]
MIN 20	266.66 ± 9.14 [c]
MIN 40	259.21 ± 6.27 [d]

Values are expressed as mean ± S.D. (n=10). The acetylcholinesterase activity was significantly increased in i. c. v. streptozotocin group compared with sham group ([a] P<0.05 vs sham group). Minocycline significantly decreased streptozotocin induced increase in acetylcholinesterase activity compared with streptozotocin group [[b] P<0.05 vs i. c. v. streptozotocin group, [c;] P<0.05 vs i. c. v. streptozotocin and Minocycline at 10 mg/kg groups, [d] P<0.05 vs i. c. v. streptozotocin and Minocycline at 20 mg/kg].

aCSF = artificial cerebrospinal fluid; MIN5, MIN10 and MIN 20 = Minocycline at 5, 10 and 20 mg/kg dose respectively, Per se=administered Minocycline at a dose of 40 mg/kg in normal animals

Minocycline at a dose of 40 mg/kg was found to be comparatively most effective in ameliorating streptozotocin induced increase in acetylcholinesterase activity (P<0.001). However, the same treatment (40 mg/kg) in normal animals did not modify the basal acetylcholinesterase activity compared with those of sham and aCSF group of animals (P>0.05, Table 1).

DISCUSSION: Present study demonstrates that ICV STZ injection in rats lead to significant increase in acetylcholinesterase activity and these results are consistent with the earlier findings [25,26] and treatment of rats with Minocycline (10,20 and 40 mg/kg/day) for 3

weeks could significantly decreased the raised level. Acetylcholine (ACh) is the first neurotransmitter whose diffusion from the central nervous system was investigated and whose extracellular levels variations were correlated to changes in neuronal activity [27].

Acetylcholine is a prominent neurotransmitter of the peripheral and the central nervous system. In the central nervous system, acetylcholine is involved in attention, learning, memory, consciousness, sleep, and control of voluntary movements [28-32]. Dysfunction of the cholinergic system is implicated in major neurological disorders such as schizophrenia, Alzheimer's disease, Parkinson's disease and Huntington's disease [33]. Acetylcholine is formed from its precursor's choline and acetyl coenzyme A by choline acetyltransferase and released from cholinergic nerve terminals into the synaptic cleft between presynaptic and postsynaptic neurons [34]. The resulting chemical signal conveyed by acetylcholine is terminated by its enzymatic degradation. Acetylcholine is rapidly metabolized to acetate and choline by acetylcholinesterase, but a small fraction leaks out of the synaptic cleft into the extracellular fluid [34].

Cholinergic system plays an important role in memory formation and retrieval [35, 36]. The hippocampus, amagdala and cortical regions of the brain are mainly involved in cholinergic transmission to monitor learning and memory processing, and seem to be more prone to oxidative damage and pathogenesis of Alzheimer's disease [37, 38]. Acetyl cholinesterase activity is a marker of extended loss of the cholinergic system in the brain [39]. In summary, the present study has shown that Minocycline is effective in decreasing raised levels of acetyl cholinesterase thereby cholinergic dysfunction. The same action of Minocycline may be the probable reason for the amelioration of cognitive deficits in experimental models of Alzheimer's disease Sharma et al. 2010 [40]. Although Minocycline has proved to be good target in dementia resembling Alzheimer's type in several experimental models even then more extensive research efforts are still needed to verify the claims.

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