PENTOXIFYLLINE (TNFα-INHIBITOR) ATTENUATES COLCHICINE INDUCED APOPTOTIC AMNESIA IN MICE

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Summary

Tumor necrosis factor alpha (TNFα) is a protein that induced cell death by apoptosis. Cell death of brain neurons is associated with the memory loss or amnesia. The present study was designed to investigate the effect of pentoxifylline (TNFα-inhibitor) in normal and apoptotically amnesic mice, using water maze test. Mice of control, pentoxifylline and colchicine treated groups were administered with normal saline (10ml kg^{-1} i.p.), pentoxifylline (3mg kg^{-1} i.p.) and an apoptotic agent, colchicine (4mg kg^{-1} i.p.), respectively, 30 min before the first acquisition trial for 4 consecutive days, and with distilled water (10ml kg^{-1} i.p.), 30 min before the first retrieval trial on 5^{th} day and vice-versa. Apoptotically amnesic mice developed by colchicine treatment further treated with pentoxifylline (3mg kg^{-1} i.p.) 30 min before the first acquisition trial for 4 consecutive days and were administered normal saline (10ml kg^{-1} i.p.) 30 min before the first retrieval trial on 5^{th} day. Escape latency time (ELT) from water during the acquisition trials and time spent (TS) in target quadrant (TQ) in search of missing platform, of each mouse were noted. Normal saline treated mice exhibit decrease in ELT and increase in TS during the acquisition and retrieval trials, respectively, but colchicine produced apposite effects to normal saline. Pentoxifylline (3mg kg^{-1} i.p.), did not produce any significant difference in ELT and TS in TQ of normal mice as compare to normal saline treated control group. Pentoxifylline (3mg kg^{-1} i.p.), significantly decrease the ELT and increase the TS in TQ of apoptotically amnesic mice. On the basis of these observations, it can be concluded that pentoxifylline did not improves learning and memory of normal animals, but it attenuates memory impairment of apoptotically amnesic mice. This may be attributed due to its potent TNFα-inhibiting property and protection of neuronal cells from apoptotic death.

Key words- Pentoxifylline, Colchicine, TNFα, Amnesia, Apoptosis, Water maze, Mice

Introduction

Tumor necrosis factor alpha (TNFα) or cachetin is a pleiotropic-pro-inflammatory cytokine; predominately produced by monocytes and activated macrophages. TNFα binds to two distinct cell surface receptor i.e. TNFR1 (55kd) and TNFR2 (75kd), which have been identified in human myocardium [1, 2] and other human tissues. Members of the TNFα family of receptors (TNFR) initiate apoptosis, some initiate cell proliferation, and other initiates both. Activation of one of the TNFα receptors by the cytokine, TNFα can leads to apoptosis by inducing the association of the receptor with the adaptor protein (TNFR-adaptor protein with a death domin).

This association ultimately activates caspase, responsible for cell death by apoptosis. Apoptosis related neurodegenerative disorders, associated with free radicals generation [3] may causes impairment of learning and memory in animals and human beings [4]. Ageing and age related memory impairment is also associated with apoptosis or cell suicide [5]. Memory impairment in various neurodegenerative disorders such as Alzheimer's disease [6], Parkinson's disease, Stroke, Huntington's disease, is closely related to ageing and apoptosis [7]. Above reports reveal that TNFα is one of those whose induced apoptosis or cell death is responsible for memory impairment in aged, and neurodegenerative subjects [8].

Pentoxifylline a derivative of theobromine and reported to improve learning and memory in glutamate lesioned rats possibly by interacting with central muscarinic receptor of acetylcholine [9]. Pentoxifylline is also reported to suppress gene transcription of cytokines like TNFα [10] and to prevent cell death or improve cell survival and memory. The pentoxifylline analogus like, propentophylline (PPF) also prevents neurodegenration by blocking excessive Ca^{2+} influx into ischaemic neurons. PPF and denbuphylline are selective cAMP phosphodiesterase inhibitor, noted to improve cerebral glucose metabolism in patients with senile dementia [11]. It is clear from the above reports that apoptosis is related to memory loss and pentoxufylline improves learning and memory, but none of the studies explain how pentoxifylline attenuates apoptosis induced amnesia. Therefore, the present study was designed to evaluate the effect of pentoxifylline on apoptotically induced amnesia in mice, using water maze test [12].

Materials and Methods

Animals

Swiss albino mice (28-36g) of either sex procured from Indian Veterinary Research Institute (IVRI) Izatnagar, Bareilly-243022 India, were housed in animal house provided with 12 hours light and dark cycle, free access to water and standard laboratory diet (Kisan feed Ltd. Mumbai). All the animals were naive to water maze. The experiments were conducted between 10.00 to 17.30 hrs in a semi-sound proof laboratory. The research was conducted as per the guidelines of "committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Drugs and Solutions

All drug solutions were freshly prepared prior to use. The solutions of pentoxifylline (Sun pharmaceuticals Ltd, Silvassa) and Colchicine (SISCO Research Laboratories Pvt. Ltd. Mumbai) were prepared in distilled water.

Apparatus: Escape latency time (ELT) and time spent (TS) for all animals were measured by employing the water maze test. The test allows the evaluation of spatial memory. Water provides a uniform intramaze environment, thus eliminating any olfactory interference. Food and water deprivation is not required in this test as required in other models. Water maze consists of a circular pool, made of a galvanized iron sheet having a diameter of 150 cm and a height of 45 cm. The pool was filled with water upto a height of 30 cm and water was made opaque with commercially available white color and maintained at $25^{O}C$. The pool was hypothetically 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 platform ($11cm^2$) of 29 cm height, was placed in the centre of one of these four quadrants i.e. target quadrant (TQ). The platform was submerged 1 cm below the water surface. Utmost care was taken

not to change the relative location of water maze with respect to any object serving as a visual clue in the laboratory.

Acquisition trials: Each mouse was placed in water maze apparatus for four consecutive days and four trials were conducted on each day. Each trial was conducted with an interval of five minutes and each trial was started from the midpoint of peripheral wall of each quadrant (Q) with animal facing towards the wall of water maze. The mice were allowed to swim for 120 s. After locating the hidden platform the mice were permitted to remain on it for 10 s before returning to the home cage. Mice that fail to locate the hidden platform within 120 s were placed on it by hand and scored it as 120 s. The time taken by the mice to locate the hidden platform was noted down and was termed as escape latency time. Mean of the four-escape latency times was calculated for each day. This mean was used as an index of acquisition or learning. Starting position on each day to conduct four acquisition trials was changed as follows:

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

Retrieval trials: On 5^{th} day, platform was removed. Mouse was placed in water maze and allowed to explore the maze for 120 s. Each mouse was subjected to four such trials and each trial was started from different quadrant. Mean time spent in target quadrant in search of missing platform was noted down. It was taken as an index of retrieval of memory.

Experimental Protocol

Eight groups of mice (n=10) were employed. All pharmacological agents [Pentoxifylline (3mg kg^{-1}), Colchicine (4mg kg^{-1})] and their vehicle [distilled water (10ml kg^{-1})] were administered intraperitonialy (i.p.), 30 min before the first acquisition trial for 4 consecutive days and 30 min before the first retrieval trial on 5^{th} day only. Pentoxifylline was administered 5 min after the administration of colchicine.

Statistical Analysis

All the results were statistically interpreted using one-way analysis of variance (ANOVA) followed by Dunnett test. A value of $P<0.05$ was considered statistically significant.

Results

Effect of normal saline and distilled water (vehicle) on learning and memory

In control experiments mice were administered normal saline 30 min before acquisition trials and they demonstrated significant decrease in escape latency time (ELT) as a result of consecutive acquisition trials conducted on day 2,3 and 4 as compared to acquisition trial conducted on day 1 (Fig.1). Moreover, control mice administered normal saline (NS) 30 min before retrieval trial spent significant more time in search of missing platform as compared to time spent in other quadrants i.e. Q1, Q3 and Q4 during retrieval trial conducted on day 5 (Fig.2). The administration of distilled water (DW) used as vehicles for making drug solutions and administered to mice, 30 min before acquisition trials conducted on day 1 to day 4 and retrieval trial conducted on day 5, produced no

marked effect on decrease in ELT (Fig.1) and more time spent in target quadrant as compared to normal saline treated control experiments (Fig.2). It suggests that distilled water (vehicle) treatment produced no marked effect on normal acquisition and retrieval of memory.

Effect of colchicine on learning and memory

The colchicine (4mg kg^{-1} i.p.) administered 30 min before acquisition trials conducted on day 1 to day 4 and retrieval trial conducted on day 5, significantly attenuated the decrease in ELT (Fig.1) and increase in time spent in target quadrant in search of missing platform (Fig.2). It demonstrates that colchicine treatment has produced impairment of learning and memory.

Effect of pentoxifylline (TNF-α Inhibitor) on learning and memory.

The administration of pentoxifylline (3mg kg^{-1}i.p.) 30 min before acquisition trials conducted on day 1 to day 4 and retrieval trial conducted on day 5, demonstrated no marked effect on decrease in the ELT (Fig.1) and increase time spent in target quadrant in search of missing platform (Fig.2). It suggested that pentoxifylline per se treatment did not affect acquisition and retrieval of memory.

Effect of pentoxifylline (TNF-α Inhibitor) on colchicine induced amnesia.

The administration of pentoxifylline (3mg kg^{-1}i.p.), 5 min after the colchicine treatment, 30 min before acquisition trials conducted on day 1 to day 4 and retrieval trial conducted on day 5, attenuated decrease in the ELT as compare to colchicine treated group (Fig.1) and increase time spent in target quadrant in search of missing platform (Fig.2). It suggested that pentoxifylline (3mg kg^{-1} i.p.) significantly modulate colchicine (4mg kg^{-1} i.p.) induced amnesia.

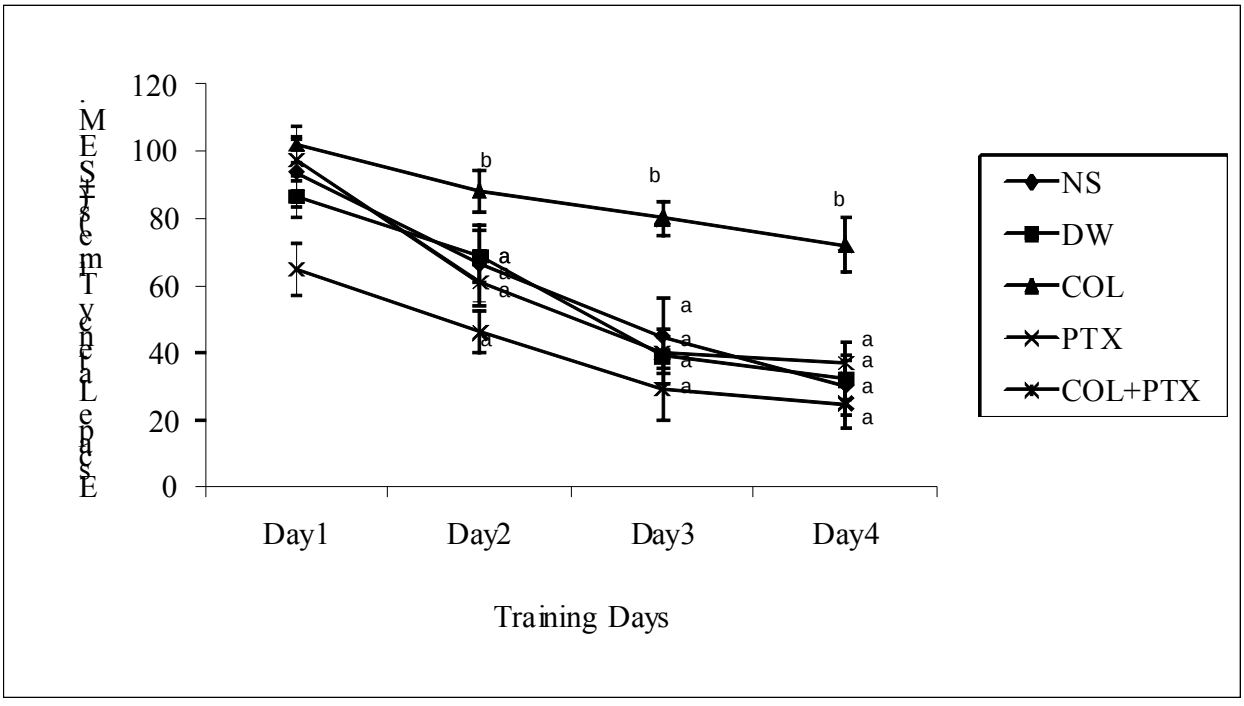

Fig.1: NS (control), DW, COL, PTX and COL+PTX, represents administration of normal saline (10ml kg^{-1} i.p.), distilled water (10ml kg^{-1} i.p.), colchicine (4mg kg^{-1} i.p.), pentoxifylline (3mg kg^{-1} i.p.) and colchicine (4mg kg^{-1} i.p.)+pentoxifylline (3mg kg^{-1} i.p.) respectively in mice, 30 min before the first acquisition trial for 4 consecutive days. Each value represents mean±S.E.M. (n=10). a= $p<0.05$ Vs ELT on day1.b=$p<0.05$ Vs ELT of control group for the same day.

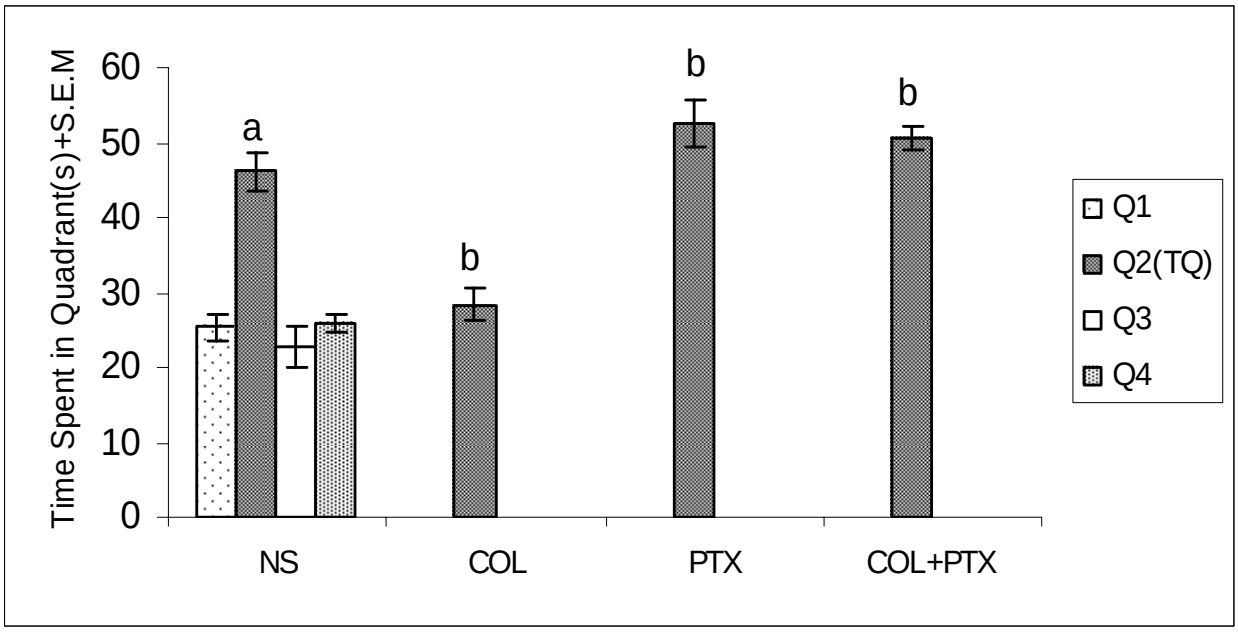

Fig.2: NS (control), COL, PTX and COL+PTX, represents administration of normal saline (10ml kg^{-1} i.p.), colchicine (4mg kg^{-1} i.p.), pentoxifylline (3mg kg^{-1} i.p.) and colchicine (4mg kg^{-1} i.p.)+pentoxifylline (3mg kg^{-1} i.p.), respectively in mice, 30 min before the first retrieval trial on 5th day only. Each value represents mean±S.E.M. (n=10). a= $p<0.05$ Vs TS in other quadrants i.e. Q1 Q3 and Q4. b=$p<0.05$ Vs TS in target quadrant (TQ) i.e. Q2 in control group.

Discussion

The survival of a multicellular organism is dependent on a sophisticated balance between the life and death of its components cells. Cell death can occur either by necrosis or apoptosis. Necrosis is the more common type of cellular death after exogenous stimuli and is manifested by severe cell swelling, denaturation and coagulation of proteins, breakdown of cellular organelles and cell rupture. Apoptosis occurs within an organism and cells die through activation of an internal suicide program. The function of this process is to eliminate unwanted cells selectively with minimal disturbance to surrounding cells and the host. It is thought to be responsible for numerous physiologic and pathologic events, such as programmed destruction of cells during embryogenesis. Cellular death produced by a variety of injurious stimuli that are capable of producing necrosis, when given in low doses induce apoptosis i.e., radiation, mild thermal injury, cytotoxic anticancer drugs. The term apoptosis coined by [13] an active gene directed process of cell death [14], and accelerated by free radicals. Apoptosis plays a significant role in the process of embryonic development [15]. Mutation in the death process may be responsible for various disorders such as AIDS [16], cancer [17], autoimmune diseases [18], neurodegenerative disorders [19] memory impairment. This process is identical in all cells, regardless of cell type or the killing agent. Cells undergoing apoptotic cellular suicide rapidly shrink due to efflux of water, either through simple osmosis or through aquaporins water channels [20] and lose their normal intercellular contacts and subsequently exhibit dense chromatin condensation, nuclear fragmentation, cytoplasmic blabbing and cellular fragmentation [21-22] into small apoptotic bodies. These apoptotic bodies are quickly phagocytosed and digested by neighboring healthy cells or macrophages [23-24]. The apoptotic process can be modulated by

various stimuli including hormones, cytokines, growth factors, bacterial or viral infections, immune responses and oxidative stress [25]. Apoptosis or programmed cell death (PCD) played a significant role in neurodegenerative disorders like, Alzheimer's disease, Parkinson's disease, Huntington's disease and motor neuron disease, and the sufferers exhibited a unique symptom, memory impairment. Alzheimer's disease (AD) is a major cause of dementia, associated with degeneration of cholinergic neurons in the brain. The mode of neuronal death in AD is a matter of debate either by apoptosis or necrosis [26]. Implications of apoptotic death of neuronal cells in several disorders that are commonly showed impairment of memory as a major sign. In senile dementia, over expression of gilial derived tumor necrosis factor alpha (TNFα) is also seen. An increased caspase activity in neurons is reported during the PCD of neurons and pathological conditions such as stroke, which establish the crucial importance of this enzyme in normal development of the nervous system [27]. Abnormalities, mainly results from decreased neuronal apoptosis, which leads to marked alteration in brain structures. The overall brain mass found to be is substantially larger than that of normal mice. A variety of hyperplasias in the cerebellum, cortex, hippocampus and striatum were reported to be present. In addition absence of apoptotic cells at sites of major morphologenetic change during normal brain development was reported, which indicates the absolute requirement of caspase-3 activation during neuronal PCD [27]. In contrast mice lacking the gene for caspase-1 and caspase-2 [28], did not show any evidence of disrupted brain development [29]. Caspase-3 levels can be also up-regulated following permanent occlusion of the middle cerebral artery [30]. Growth factor deprivation is a common method used to induce apoptosis in various neuronal cells, which mimic PCD during development due to limiting trophic factor. Caspase-3 has been implicated in the induction of apoptosis in human cerebellar granule cells, due to k^+/serum deprivation [31]. Caspase-3 concentration and activity play a critical role in neurodegenerative and apoptotic brains of human beings [27, 31]. Caspase-3 is also present in hippocampus [32], cortex and striatum, seats of learning and memory [33] and interferes with the long-term spatial memory storage [32]. Jeltsch *et al.* [34] and Nakayama & Sawada [35] also observed memory impairment by colchicine. Colchicine [36-37] induced apoptotic stimuli in human cerebellar cells, possibly due to increase concentration of caspase-3. Colchicine damaged dentate granule cells and caused dose-dependent memory impairment in rats [34]. Colchicine impaired memory at doses exhibiting no evidence of histological changes in the dentate granule cells [35]. Colchicine mediated impairment of memory is possibly due to induction of apoptosis via increased caspase-3 activity [38].

In the present study normal mice shown a marked decrease in escape latency time (ELT) during the learning trials and an increase in the time spent (TS) in target quadrant (TQ) in searching of the missing platform during the retrieval trial. The observations suggest normal learning and retrieval of learning in mice. The results were in agreement with previous observations of Saraf et al., [39]. The administration of pentoxifylline (3mg kg^{-1}i.p.), 5 min after the colchicine treatment, 30 min before acquisition trials conducted on day 1 to day 4 and retrieval trial conducted on day 5, attenuated decrease in the ELT as compare to colchicine treated group and increase time spent in target quadrant in search of missing platform. It suggested that pentoxifylline (3mg kg^{-1} i.p.) significantly modulate colchicine (4mg kg^{-1} i.p.) induced amnesia. The present observation supported by the previous reports that shown pentoxifylline suppressed gene transcription of cytokines like TNFα [10] and to prevent cell death or improve cell survival and memory. The pentoxifylline analogus like, Propentophylline (PPF) also prevents neurodegenration by blocking excessive Ca^{2+} influx into ischaemic neurons. PPF and denbuphylline are selective cAMP phosphodiesterase inhibitor, noted to improve cerebral glucose metabolism in patients with senile dementia [11].

Conclusion

On the basis of present observations, it can be concluded that pentoxifylline attenuates apoptotic memory impairment. This may be attributed due to its potent TNFα-inhibiting property and protection of neuronal cells from apoptotic death, atleast in mice species.

Acknowledgement

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