Colchicines-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type

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Abstract:
Alzheimer's disease (AD) is the most common type of dementia disorder of elderly affecting millions of people. The pathophysiology of the disease is complex and involves multiple pathways of neuronal damage. Sporadic dementia of Alzheimer's type (SDAT) has been shown to be associated with microtubular dysfunction and is characterized by the appearance of specific cytoskeletal cellular abnormalities, including neurofibrillary tangles and senile plaques. Intracerebroventricular (*icv*) administration of colchicine, a microtubule-disrupting agent, causes cognitive dysfunction as evidenced by poor retention of memory in both Morris water maze and elevated plus-maze task paradigms that is associated with excessive free radical generation. Biochemical analysis revealed that *icv* colchicine injection significantly induced lipid peroxidation, increased nitrite and depleted reduced glutathione (GSH) and acetylcholinesterase (AChE) level in rat brains. Chronic treatment with rivastigmine (0.625 and 2.5 mg/kg, *po*) twice daily for a period of 25 days beginning 4 days prior to colchicine injection significantly improved the colchicine-induced cognitive impairment and reduced AChE level. The results of the present study clearly indicated that colchicines-induced cognitive impairment and oxidative stress in animals and can be used as an animal model for drug screening for Alzheimer's disease.

Key words:
colchicine, neurotoxicity, rivastigmine, Alzheimer's disease

Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder of the elderly, is characterized by cognitive dysfunctions, behavioral and social deterioration. The disease pathophysiology is complex and involves multiple distinct and overlapping redundant pathways of neuronal damage. Hippocampus, limbic system and cortex are the primary neuronal injury regions involved in disease pathophysiology [31]. Its heterogeneous etiology makes it difficult to define clinically the most important factor in determining the onset and progression. Sporadic dementia of Alzheimer's type (SDAT) has been shown to be associated with microtubular dysfunction and is characterized by the appearance of specific cytoskeletal cellular abnormalities, including neurofibrillary tangles and senile plaques [1, 24, 30]. Of particular interest are the SDAT-associated changes in the cholinergic markers because of possible association between these alterations and deficits in cognitive abilities [4, 33, 50].

Animal models are essential tools in the elucidation of the pathophysiology of the disorders and for the development of newer therapeutics. Until now, attempts to devise treatments for ADF have been hindered by the lack of such a model. Based on the present knowledge on validity, the available models can

be categorized into homologous, analogous and correlation models. The homologous model requires all the critical factors of AD to be similar to the clinical syndrome. This includes etiology, biological basis of the symptoms, response to treatment course and outcome, as well as unique features such as individual vulnerability. None of the animal models showed the neuropathological characteristics, such as, senile plaques and neurofibrillary tangles, the two major hallmarks of AD. The analogous models require some of the critical features between the preclinical and clinical syndrome to be similar and other features may not be similar. Development of therapeutics for AD requires appropriate *in vitro* or *in vivo* models that reflect the errant biochemical pathways and reflect the pathological hallmarks of the disease as well as the clinical manifestations. The correlation models in AD research are obscured by the fact that it is an age-related disorder with complex neurobiology, and it is difficult to correlate particular symptom(s) to clinical features of AD. However, these models are also associated with some drawbacks particularly differential development of precursor protein in discrete areas of brains with age.

The characteristics of AD approximated by an animal model of the syndrome include [1] behavioral changes, particularly alterations in working and reference memory due to the dysfunction of cholinergic system, [2] decreased acetylcholinesterase (AChE) level, a presynaptic cholinergic marker, that is characteristic of extensive cholinergic cell loss [3, 37, 39], alteration in oxidative stress markers if any. Further, the model should show delayed development or abolishment of cognitive deficits or improved cognitive dysfunction with cholinergic agents with or without protection from or prevention of neuronal loss.

Thus, the present study was carried out to establish colchicine-induced cognitive dysfunction as an animal model of sporadic dementia of Alzheimer type in rats [42]. In addition, the model was validated by using rivastigmine, a dual inhibitor of both AChE and butyrylcholinesterase [21, 51].

Materials and Methods

Animals

Male Wistar rats bred in Central Animal House of the Panjab University, Chandigarh, weighing 180–200 g at the start of the surgery were used. Animals were acclimatized to laboratory conditions prior to experimentation. Following surgery, the animals were kept under standard conditions of light and dark cycle with food and water *ad libitum* in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 09:00–15:00 h. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

Surgery and intracerebroventricular administration of colchicines

Surgery was performed according to previously described protocol [27]. All animals were anesthetized with sodium pentobarbital (45 mg/kg, *ip*) and positioned in a stereotaxic apparatus. The head was positioned in a frame and a midline sagittal incision was made in the scalp. Two holes were drilled through the skull for placement of injection cannula into the lateral cerebral ventricle. The scalp was then closed with suture. In sham-operated rats, the surgery was identical except for drilling of holes and placement of the cannula. Coordinates for the injection were 0.8 mm posterior to bregma, 1.8 mm lateral to sagittal suture and 3.6 mm beneath the cortical surface. After surgery, all animals received gentamicin (5 mg/kg, *ip*) to prevent sepsis. Rats were intracerebroventricularly (*icv*) infused with either artificial cerebrospinal fluid (ACSF; in mM: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 dextrose) or 15 μg colchicine dissolved in ACSF. Solutions (5 μl) were injected using a Hamilton microsyringe positioned in the injection cannula. To promote diffusion, the microsyringe was left in place for a period of 2 min following injection. Special care was taken during the postoperative period to provide food and water inside the cage of the rat.

Behavioral assessment

Assessment of cognitive performance

Elevated plus maze paradigm

The elevated plus maze consisted of two opposite open arms (50 × 10 cm), crossed with two closed arms of the same dimensions with 40 cm high walls. The arms are connected with central square (10 × 10 cm). Acquisition of memory was assessed on day 13 after colchicine injection. Rats were placed individu-

ally at one end of an open arm facing away from the central square. The time taken to move from open arm and enter one of the closed arms was recorded as initial transfer latency (ITL). Animals were allowed to explore the maze for 30 s after recording ITL and returned to home cage. After 24 h (day 14) and 8 days (day 21) of ITL, the rat was placed similarly on the open arm and retention latency was noted again and termed as first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively [44, 47].

Spatial navigation task

The acquisition and retention of a spatial navigation task was examined using a Morris Water Maze [18]. Animals were trained to swim to a platform in a circular pool (180 cm diameter × 60 cm) located in a test room. The pool was filled with water (28 ± 2°C) to a depth of 40 cm. A movable circular platform 9 cm in diameter, mounted on a column, was placed in the pool 2 cm above the water level for maze acquisition test and another movable platform 9 cm in diameter, mounted on a column, was placed in the pool 2 cm below the water level for maze retention test. Four equally spaced locations around the edge of the pool (N, S, E, W) were used as start points and divided the pool into 4 quadrants.

Maze acquisition test (Training)

Animals received a training session consisting of 4 trials with an inter-trial interval of 6–10 min on day 13. In all 4 trials the starting positions were different. A trial began by releasing the rat into the maze facing the wall of the pool at one of the starting points. The latency to find the escape platform was recorded up to a maximum of 3 min. If a rat did not escape onto the platform within that time, it was placed on the platform where it remained for 15 s. The platform was fixed in the centre of one of the 4 quadrants and remained in that location for the duration of experiment. The time taken by a rat to reach the platform was recorded as initial acquisition latency (IAL). At the end of a trial, rat was returned to its home cage and approximately 5 min elapsed before beginning the next trial.

Maze retention test (Testing for retention of the learned task)

Following 24 h (day 14) and 8 days (day 21) after IAL, a rat was randomly released at any one of the edges (N, S, E, W) facing the wall of the pool and tested for the retention of the response. The time taken to reach the hidden platform on day 14 and on day 21 following colchicine injections was recorded and termed as first retention latency (1st RL) and second retention latency (2nd RL), respectively.

Assessment of gross behavioral activity (Closed field activity)

Gross behavioral activity was assessed on days 13, 14 and 21 following *icv* colchicine injection. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light sensitive photocells using digital photoactometer and values were expressed as counts per 5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room.

Biochemical tests

Biochemical tests were carried out 24 h after the last behavioral test on day 21 following colchicine injections i.e. on day 22. Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with 10 times (w/v) ice cold 0.1 M phosphate buffer (pH = 7.4). The homogenate was centrifuged at 10,000 × g for 15 min and aliquots of supernatant was separated and used for biochemical estimation.

Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills [52]. The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The values were calculated using molar extinction coefficient of chromophore (1.56×10^5 M/cm) and expressed as percentage of control.

Estimation of reduced glutathione

Reduced glutathione (GSH) in brain was estimated according to the method described by Ellman [15]. Supernatant (1 ml) was precipitated with 1 ml of 4% sulfosalicylic acid and cold-digested at 4°C for 1 h.

The samples were centrifuged at 1200 × g for 15 min at 4°C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 M, pH = 8) and 0.2 ml of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) were added. The yellow color developed was read immediately at 412 nm using Perkin Elmer lambda 20 spectrophotometer. Results were calculated using molar extinction coefficient of chromophore (1.36×10^4 M/cm) and expressed as percentage of control.

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) according to Green at al. [20]. Equal volumes of supernatant and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature in the dark and the absorbance at 540 nm was determined with Perkin Elmer lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as percentage of control.

Acetylcholinesterase (AChE) levels

AChE is a marker of extensive loss of cholinergic system in the forebrain. The quantitative measurement of acetylcholinesterase levels in brain was performed according to the method of Ellman [14]. The level of acetylcholinestrase is a marker of loss of cholinergic neurons. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M 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 using Perkin Elmer lambda 20 spectrophotometer. Results were calculated using molar extinction coefficient of chromophore (1.36×10^4 M/cm) and expressed as percentage of control.

Protein estimation

The protein content was measured by biuret method using bovine serum albumin as standard [19].

Drugs and treatment schedule

Colchicine (Sigma Chemicals Co., St. Louis, MO, USA) and rivastigmine (Ranbaxy Ltd., India) solutions were made freshly at the beginning of each experiment. Colchicine was prepared in ACSF such that 15 μg dose was delivered in a volume of 5 μl injection for intracerebroventricular administration. For oral administration, rivastigmine was suspended in 0.5% sodium carboxymethylcellulose and administered in 1 ml/100 g body weight. Animals were randomly divided into seven groups of 6–8 animals each. The first group, sham-operated group, received vehicle for rivastigmine. The second group, vehicle-treated group, received ACSF (5 μl, *icv*). The third group received colchicine (15 μg/5 μl, *icv*). The second and third groups also received equivalent volume of vehicle for rivastigmine throughout the study period. The fourth and fifth groups received rivastigmine only at doses of 0.625 and 2.5 mg/kg orally, daily for a period of 25 days. The sixth and seventh groups, received rivastigmine orally at doses of 0.625 and 2.5 mg/kg orally, daily for a period of 25 days beginning 4 days before colchicine injection. The doses of rivastigmine were selected on the basis of those reported in literature.

Statistical analysis

Values are expressed as the mean ± SEM. The behavioral data were analyzed by a repeated measure two-way analysis of variance (ANOVA) with drug-treated groups as between- sessions and as within-subject factors. The interaction of drug treatment × session was considered to test for drug effect on retention. The biochemical estimations were separately analyzed by one-way ANOVA. *Post-hoc* comparisons between groups were made using Tukey's test; $p < 0.05$ was considered significant.

Results

Effect of rivastigmine on memory performance in elevated plus maze paradigm in colchicine-injected rats

In the present experiment, the mean ITL on day 13 for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm

within 60 s. Following training, sham-operated, ACSF-injected, and rivastigmine *per se* treated (0.625 and 2.5 mg/kg/day, *po*) rats entered closed arm quickly and the mean retention transfer latencies (1st RTL and 2nd RTL) to enter closed arm on days 14 and 21 were shorter as compared to ITL on day 13 of each group, respectively (Tab. 1). In contrast, the colchicine-injected rats performed poorly throughout the experiment and did not show any change in the mean retention transfer latencies on days 14 and 21 as compared to pre-training latency on day 13 which demonstrates that colchicine-induced marked memory impairment. Chronic administration of rivastigmine (0.625 and 2.5 mg/kg/day, *po*) beginning prior to colchicine injection significantly decreased the mean retention latencies on days 14 and 21 following colchicine injection ($p < 0.05$ compared to *icv* colchicine group) (Fig. 1) indicating an improvement of memory impairment. Rivastigmine *per se* (0.625 and 2.5 mg/kg/day, *po*) treatment had no effect on the mean transfer latencies as compared to *icv* ACSF-injected group on all the days of experimentation (Tab. 1). Repeated measures ANOVA indicated a significant drug treatment × session interaction.

Effect of rivastigmine on spatial navigation task in colchicine-injected rats

Sham-operated, ACSF-injected, and rivastigmine *per se* (0.625 and 2.5 mg/kg/day, *po*) groups of animals quickly learned to swim directly to the platform in the Morris water maze on day 13 (Tab. 2). Colchicine-injected rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 13. Rivastigmine *per se* (0.625 and 2.5 mg/kg/day, *po*) groups of rats also performed similarly during the acquisition of a spatial navigation task on day 13 (compared to ACSF-injected group) (Tab. 2). There was a significant difference in the mean IAL of colchicine--injected group compared to ACSF-injected group on day 13 indicating colchicine-induced impairment of acquisition of spatial navigation task ($p < 0.05$). In contrast, rivastigmine (0.625 and 2.5 mg/kg/day, *po*) treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to colchicine-injected rats on day 13 following colchicine injection ($p < 0.05$) (Fig. 2).

Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform

Tab. 1. Effect of ACSF and rivastigmine (Riv) *per se* treatment on memory performance in elevated plus maze paradigm in rats

Treatment	Mean transfer latency (s)		
	ITL	1st RTL	2nd RTL
Sham operated group	53.45 ± 12.3	12.34 ± 2.3[a]	8.23 ± 1.2[a]
ACSF injected group	52.36 ± 5.0	10.41 ± 2.5[a]	9.93 ± 3.0[a]
Riv 0.625 mg/kg *per se*	60.22 ± 5.6	11.12 ± 2.0[a]	8.23 ± 1.5[a]
Riv 2.5 mg/kg *per se*	59.86 ± 5.34	9.67 ± 1.9[a]	7.12 ± 1.2[a]

Values are expressed as the mean ± SEM. [a] $p < 0.05$ as compared to ITL of the respective group on day 13 (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons). ITL: initial transfer latencies on day 13; RTL: retention transfer latencies on days 14 (1st) and 21 (2nd)
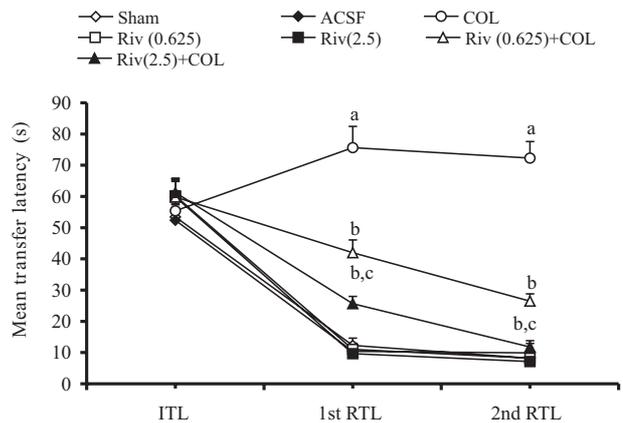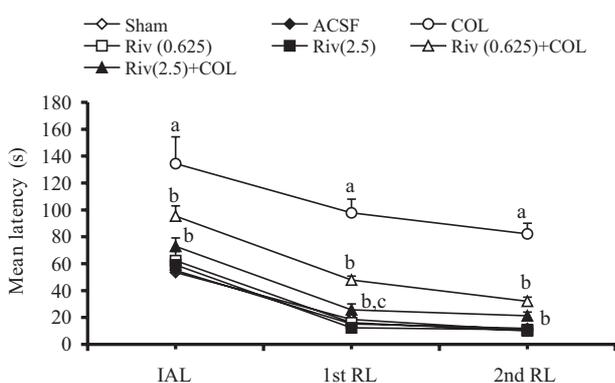

Fig. 1. Effect of rivastigmine (Riv; 0.625 and 2.5 mg/kg/day, *po*) on memory performance in elevated plus maze paradigm in intracerebroventricular colchicine (COL)-injected rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to ACSF-injected rats on day 14 or 21, [b] $p < 0.05$ as compared to *icv* colchicine injected rats on day 14 or 21, [c] $p < 0.05$ as compared to Riv (0.625) + COL-treated group on day 14 or 21. (Repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons). ITL: initial transfer latencies on day 13; RTL: retention transfer latencies on days 14 (1st) and 21

were significantly decreased in sham-operated and ACSF-injected rats on days 14 and 21, respectively, as compared to IAL on day 13 following colchicine injection. Rivastigmine alone treatment did not induce any alteration in the 1st and 2nd RL on days 14 and 21, respectively (compared to ACSF-injected group). On the contrary, the performance in the colchicine-injected rats was changed after initial training in the water maze on days 14 and 21, with significantly higher mean retention latencies compared to ACSF-

Tab. 2. Effect of rivastigmine (Riv) on spatial navigation task in colchicine-injected rats

Treatment	Mean latency (s)		
	IAL	1st RL	2nd RL
Sham operated group	53.46 ± 3.0	19.58 ± 2.7^a	12.45 ± 1.2^a
ACSF injected group	55.23 ± 5.6	15.15 ± 1.4^a	12.91 ± 1.4^a
Riv 0.625 mg/kg *per se*	69.34 ± 9.9	16.67 ± 1.9^a	10.24 ± 1.9^a
Riv 2.5 mg/kg *per se*	59.13 ± 5.3	12.34 ± 2.6^a	10.90 ± 1.6^a

Values are expressed as the mean $\pm$ SEM. [a] $p < 0.05$ as compared to IAL of the respective group on day 13 (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons). IAL: initial acquisition latencies on day 13; RL: retention transfer latencies on days 14 (1st) and 21 (2nd)

Fig. 2. Effect of rivastigmine (Riv; 0.625 and 2.5 mg/kg/day, *po*) on spatial navigation task in intracerebroventricular colchicine (COL)-injected rats. Values are the mean $\pm$ SEM. [a] $p < 0.05$ as compared to ACSF-injected rats on day 13, 14 or 21, [b] $p < 0.05$ as compared to *icv* colchicine-injected rats on day 13, 14 or 21, [c] $p < 0.05$ as compared to Riv (0.625) + COL-treated group on day 14. (Repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons). IAL: initial acquisition latencies on day 13; RL: retention transfer latencies on days 14 (1st) and 21 (2nd)

injected group ($p < 0.05$). The results suggest that colchicine caused significant cognitive impairment. However, chronic rivastigmine (0.625 and 2.5 mg/ kg/day, *po*) pre- and post-treatment resulted in a significant decline in the 1st and 2nd RL on days 14 and 21, respectively, following colchicine injection ($p < 0.05$ compared to *icv* colchicine-injected rats) (Fig. 2) and improved the retention performance of the spatial navigation task. Repeated measures ANOVA indicated a significant effect of drug treatment, session, and a significant drug treatment × session interaction.

Effect of rivastigmine on gross behavioral activity in colchicine-injected rats

In the present series of experiments, the mean score of gross behavioral activity on day 13 for each rat was relatively stable and showed no significant variation. The mean scores in sham-operated, ACSF- and colchicine-injected rats remained unchanged from the mean scores of gross behavioral activity observed on day 13 throughout the entire observation period. Chronic administration of rivastigmine *per se* (0.625 and 2.5 mg/kg/day, *po*) had no effect on the gross behavioral activity as compared to ACSF-injected rats on day 14 and 21. Further, both the doses of rivastigmine (0.625 and 2.5 mg/kg/day, *po*) in colchicine-injected rats did not cause any alteration in the gross behavioral activity as compared to colchicine-injected rats on day 14 and 21. Repeated measures ANOVA revealed that there was no significant effect of drug treatment, session, and a significant drug treatment × session interaction.

Effect of rivastigmine on brain lipid peroxidation and reduced glutathione levels in colchicine-injected rats

Intracerebroventricular administration of ACSF had no effect on brain MDA levels and GSH as compared to sham-operated rats. Central colchicine administration caused marked increase in free radical generation, lipid peroxidation, and decline in antioxidant defense as indicated by a significant rise in brain MDA levels and depletion of GSH as compared to ACSF injected rats. Further, there were no alteration in the brain MDA levels and GSH levels due to rivastigmine *per se* (0.625 and 2.5 mg/kg/day, *po*) treatment as compared to ACSF-injected rats. Chronic rivastigmine (0.625 and 2.5 mg/ kg/ day, *po*) administration produced no alteration in the brain MDA levels and GSH levels as compared to colchicines-treated rats.

Effect of rivastigmine on brain nitrite levels in colchicine-injected rats

Central ACSF injection did not cause any change in brain nitrite levels as compared to sham-operated rats. Similarly, rivastigmine (0.625 and 2.5 mg/kg/day, *po*) *per se* treatment had no effect on brain nitrite levels as compared to ACSF-injected rats. Intracerebroventricular colchicine administration caused significant increase in brain nitrite levels. Further, rivastigmine

(0.625 and 2.5 mg/kg/day, *po*) did not alter nitrite levels as compared to colchicine-injected rats.

Effect of rivastigmine on brain acetylcholinesterase levels in colchicine-injected rats

Intracerebroventricular administration of ACSF had no effect on brain acetylcholinesterase levels as compared to sham-operated rats. In contrast, central colchicine injection showed significant decline in the brain AChE levels as compared to ACSF-injected rats. Rivastigmine (0.625 and 2.5 mg/kg/day, *po*) *per se* treatment did not cause any change in the brain AChE levels as compared to ACSF-injected rats. However, chronic oral administration of rivastigmine (0.625 and 2.5 mg/kg/day, *po*) caused significant reduction in AChE levels compared to colchicine-injected group (Fig. 3).
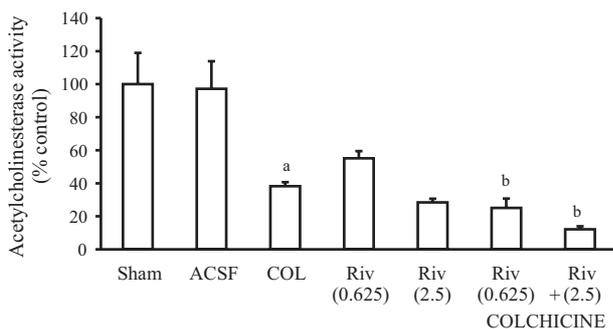

Fig. 3. Effect of rivastigmine (Riv; 0.625 and 2.5 mg/kg/day, *po*) on brain acetylcholinesterase levels in *icv* colchicine (COL)-injected rats.Values are the mean ± SEM. ^{a} p < 0.05 as compared to artificial cerebrospinal fluid (ACSF)-injected group; ^{b} p < 0.05 as compared to colchicine-injected group. (Repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons)

Discussion

Experimentally, it has been demonstrated that central administration of microtubule-disrupting agents can result in cell death associated with cognitive impairment, which resembles the microtubule dysfunction in AD [17, 45, 46]. Microtubules are conspicuous components of the neuronal cytoskeleton and play a crucial role in a wide range of cellular cascade including

growth and differentiation, axonal and dendritic transport [43].

Colchicine, a cytotoxicant, binds irreversibly to tubulin dimers and prevents addition of tubulin molecules to the fast growing end, thereby inhibiting microtubule assembly and disrupting microtubule polymerization [34]. It blocks mitosis and axoplasmic transport [23]. Colchicine produces marked destruction of hippocampal granule cells, mossy fibers and septohippocampal pathways. It induces neurofibrillary degeneration [29, 48, 53], which is associated with loss of cholinergic neurons and decrease in presynaptic cholinergic parameters, with a reduction in acetylcholinesterase and in choline acetyltransferase activity, thereby resulting in decreased ability to learn and in loss of memory [13].

In the present study, central administration of colchicine caused significant loss of memory as evident from the increased transfer latencies in elevated plus maze and Morris water maze paradigms. Further, the learning and memory performances of animals can be well delineated during the time course of colchicine administration. In addition, these behavioral changes associated with marked reduction in AChE levels, which further supports the involvement of cholinergic system. It has been reported that central administration of colchicine produces a time- and dose-dependent anatomical, behavioral and neurochemical changes maximum at 2–3 weeks following colchicine administration [16, 33]. Within 9 weeks of colchicine administration, a behavioral and neurochemical recovery occurs indicating cholinergic plasticity [11]. Cognitive impairment has a slow and insidious onset, which takes about 14–21 days. Thus, the *icv* model can be considered as a relevant model to explain sporadic dementia of Alzheimer's type (SDAT). It is characterized by a progressive deterioration of cognitive functions, microtubular destruction and decrease in acetylcholinesterase and choline acetyltransferase activity [7, 33].

It has been well established that generation of free radicals and subsequent oxidative stress occurs prior to cytopathology and plays a key role in pathogenesis of AD [28]. Recent study has demonstrated that central administration of colchicine causes an increase in free radical generation and the subsequent oxidative stress [27]. Colchicine induces a direct inflammatory response in the CNS [12, 26]. The pathological cascade of inflammation is associated with cholinotoxicity resulting in morphological, neurochemical and

behavioral changes [13]. It has been reported that alteration in glucose/energy metabolism in brain impairs endogenous antioxidant defenses and causes neuronal damage [5]. This resultant oxidative stress in turn provokes changes in macromolecules, lipid membranes, enhances the release of glutamate, thus completing a vicious cycle that leads to free radical-induced neurotoxicity [9]. Thus, central administration of colchicine causes an increase in free radical generation and the subsequent oxidative stress that leads to cognitive impairment.

The cholinergic hypothesis [3, 4] has led to the development of procholinergic compounds to enhance cognitive functioning. Cholinesterase inhibitors are the best-established class of therapeutics for treatment of dementia of Alzheimer's type (DAT) [22]. Cholinesterase inhibitors increase the synaptic availability of acetylcholine (ACh) by reducing its degradation. Acute and chronic treatment with cholinesterase inhibitors has been shown to improve cognitive function in animal models and in patients with DAT [35]. The drugs improve performance in behavioral tasks such as in radial arm maze [10, 36], Morris water maze [8, 40], the five choice serial reaction time [25], the object recognition task [41] and the spatial cone field task [38].

In the present study, rivastigmine was effective in ameliorating memory deficits in both the paradigms employed [6, 49]. However, it failed to prevent oxidative stress suggesting that generation of free radicals might influence cholinergic neuronal function by some unknown mechanism. Further, the data suggest that free radicals are generated secondary to excitotoxicity or inflammation due to colchicine. Even though this model correlates with SDAT in humans, it has certain setbacks. The colchicines-treated rats exhibit an elevated acoustic startle response that may alter behavioral reactivity [44]. Colchicine produces depletion of dynorphin with decreased threshold to pain. Penetration of colchicine to subarachnoid space results in jumpy and irritable behavior. Colchicine-treated rats may develop myoclonic twitches, exhibit a loss in body weight and become aggressive. Central administration of colchicine sometimes results in poor appetite and transient diarrhea. Central administration of colchicine results in adipsia and aphasia for about 7–10 days after the lesion. The other limitation of this model is that it is time-consuming and requires large number of animals due to extensive mortality.

In summary, *icv* colchicine model can be further used to understand better SDAT pathogenesis in humans. It has several features that are consonant with SDAT in humans, including an insidious onset, time-dependent changes in behavioral and biochemical patterns. The impairment of working and reference memory, the cardinal symptom of AD and increased oxidative stress, are the hallmarks in the pathophysiology of AD which suggests that *icv* colchicine administration could be a suitable animal model to study pathophysiology of AD [32]. Further, colchicine-induced cognitive dysfunction is attenuated by rivastigmine as in patients with AD further demonstrating the relevance of this model to study the pathophysiology of AD.

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