Project title

IMMUNOLOGICAL CHANGES AND NEURODEGENERATION IN COLCHICINE INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

(Final Report)

BY

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Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. TITLE OF THE PROJECT

Immunological changes and neurodegeneration in colchicine induced rat model of Alzheimer's disease

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR

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3. NAME AND ADDRESS OF THE INSTITUTION

University Colleges of Science and Technology, University of Calcutta, 92, APC Road, Kolkata 700 009

4. UGC APPROVAL LETTER NO. AND DATE

UGC MRP No. : F. No. 42 – 532/ 2013 (SR) dated 22nd March, 2013.

5. DATE OF IMPLEMENTATION

01.04.2013

6. TENURE OF THE PROJECT

01.04. 2013 to 31.03.2016 (3 years)

7. TOTAL GRANT ALLOCATED

Rs. 13, 44, 253/-

8. TOTAL GRANT RECEIVED

Rs. 12, 57, 798/-

9. FINAL EXPENDITURE

Rs. 13, 47, 007/- (Vide Audit Report sent to UGC from CU on 24.11.2016 and a copy of the audit report is attached with this final report)

10. TITLE OF THE PROJECT

Immunological changes and neurodegeneration in colchicine induced rat model of Alzheimer's disease

11. OBJECTIVES OF THE PROJECT

Vide Enclosure - 1

12. WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS)

Yes all of the objectives were achieved. (Vide Enclosure - 2)

13. ACHIEVEMENTS FROM THE PROJECT

Vide Enclosure - 3

14. **SUMMARY OF THE FINDINGS (IN 500 WORDS)**

Vide Enclosure - 4

15. CONTRIBUTION TO THE SOCIETY (GIVE DETAILS)

Vide Enclosure - 5

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

Yes the project fellow has been awarded Ph.D. degree from the University of Calcutta on 10.05.2016.

Name of the candidate: Ms. Susmita Sil

PhD registration number: No. 1999 PhD. (Sc.) Proceed/13 dt. 05/04/2013

Titile of the thesis: Effects of some non-steroidal anti-inflammatory drugs on memory and immune functions in colchicine induced neurodegeneration model of rats.

17. **NO. OF PUBLICATIONS OUT OF THE PROJECT (PLEASE ATTACH)**

Ten research papers have been published in reputed journals and six abstracts were published in conference proceedings out of the project. Vide Enclosure - 6

24.3.2021

(PRINCIPAL INVESTIGATOR)

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(REGISTRAR/PRINCIPAL)

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UNIVERSITY OF CALCUTTA

(Seal)

Vide Enclosure – 1

OBJECTIVES OF THE PROJECT

The aim of this study was to investigate the immunological changes in colchicine induced AD rats and to explore its association with the neuropathological changes of brain and alteration of cognitive functions.

The objectives were

1. To study the alteration of memory (incorrect choice and latency) in radial arm maze, histopathology of the brain (Nissl granules staining, staining of plaques and tangles), haematological changes (TC of RBC and WBC and DC of WBC) and immunological changes (phagocytotic activity of blood WBC, Phagocytosis of splenic mononuclear cells, Leucocyte adhesive inhibition index and cytotoxicity of splenic MNC, Delayed type of hypersensitive reaction, serum level of (IL1,2,6,TNFα and IFN-γ), in colchicine induced AD rats at 2 weeks and 4 weeks after i.c.v. injection of colchicine. The serum corticosterone will also be measured.These parameters will also be measured in control and sham operated rats.

2. To study the above mentioned parameters after administration of Naproxen (5,10,20,40 mg/kg body wtp.o) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

3. To study the above mentioned parameters after administration of Celecoxib (5, 10, 20, 40 mg/kg body wt, p.o) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

4. To study the above mentioned parameters after administration of vitamin C (5,10,20,40 mg/kg body wt, p.o) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

5. To study the above mentioned parameters after administration of Aminoguanidine (5, 10, 20, 40 mg/kg body wt, p.o) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

6. To study the above mentioned parameters after administration of Naproxen + Vitamin C (optimum dose) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

7. To study the above mentioned parameters after administration of Naproxen + Aminoguanidine (optimum dose) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

8. To study the above mentioned parameters after administration of Naproxen + Vitamin C + Aminoguanidine (optimum dose) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

9. To study the above mentioned parameters after administration of Mannitol (that can temporarily disrupt blood-brain barrier) (1.4-1.6 M/kg body wti.v.) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v. administration of colchicine.

10. To study the above mentioned parameters after administration of Mannitol + Naproxen (optimum dose) in control, sham operated and colchicine induced AD rats after 2 weeks and 4 weeks of i.c.v.administration of colchicine.

Vide Enclosure – 2

WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS)

The major objectives of the project was to investigate the immunological changes in the intracerebroventricular (icv) colchicine injected AD rats (cAD rats) and to explore its association with neurodegeneration and memory impairments. The hippocampal and serum inflammatory markers [TNF α, IL 1β and Reactive Oxygen Species (ROS), nitrite] were progressively increased in cAD rats and probably resulted in neurodegeneration (increased chromatolysis and plaques in hippocampus) and memory impairments in the two time duration studies. COX2 and PGE2 in hippocampus were increased and serum corticosterone level was decreased in cAD rats. The peripheral immune parameters (Leukocyte adhesion inhibition index, Phagocytic index of blood WBC, Phagocyic activity of splenic polymorphonuclear cells and cytotoxicity of splenic mononuclear cells) were found to be related with neuroinflammation, serum inflammatory markers and corticosterone in this animal model. The blood brain barrier (BBB) as assessed in cAD rats appeared to be leaky, probably due to neuroinflammation. The resultant effect was the passage of brain inflammatory markers to the periphery that caused changes of peripheral immune parameters. The mechanism of induction of neuroinflammation in cAD rats was investigated by the use of blocker of different inflammatory inducer. The inhibitor of COX (naproxen) and COX 2 (etoricoxib), oxidative stress (vitamin C) and nitric oxide synthase (NOS inhibitor aminoguanidine) were able to reduce neuroinflammation, neurodegeneration, peripheral immune changes and memory deficits in cAD rats in a dose dependant manner. It appears form these studies that the neuroinflammation in cAD rats is induced by COX, NOS and oxidative stressor. The interactions of inducers of neuroinflammation in cAD rats were assessed by the concurrent blocking of COX/oxidative stress, COX/NOS and oxidative stress/NOS by administering two compounds simultaneously in the same rats. A common mechanism is probably operating for neuroinflammation in cAD rats by different inducers of inflammation. Hippocampal TNFα, IL1β and ROS were found to be associated with the number of amyloid plaques in hippocampus and with memory parameters WME, RME.

Objectives achieved out of the project:

(1) The hippocampal inflammatory markers [TNFα, IL1β and Reactive Oxygen Species (ROS), nitrite] and serum inflammatory markers (TNFα, ROS, nitrite) were increased in cAD rats

compared to that of control and sham operated rats. The changes were more in rats of 21-day study duration than that of 15-day study duration. IL1β level in serum was not significantly changed in cAD rats. The serum corticosterone level was decreased in cAD rats. There was no significant difference in TC and DC of WBC in cAD rats. Increased phagocytic activity of blood WBC and splenic polymorphonuclear cells (PMN), and increased cytotoxicity and decreased leucocyte adhesive inhibition index (LAI) of splenic mononuclear cells (MNC) were noted in cAD rats. The changes are more in rats of 21-day study duration than that of 15-day study duration. Increased chromatolysis and presence of amyloid plaques in hippocampus were found in cAD rats and again these changes are more in 21-day study than that of 15-day study duration. In cAD rats the working memory error (WME) and reference memory error (RME) were increased, and the latency to enter first baited arm (LE1A, prospective memory) and latency to enter four baited arms (LE4A, spatial memory) were also increased than that of control and sham operated rats. The memory parameters in 21-day study showed higher changes than that of 15-day study duration. The colchicine induced neuroinflammation is probably resulted in neurodegeneration, memory impairments and peripheral immune responses. Hippocampal TNFα, IL1β and ROS were found to be associated with the number of amyloid plaques in hippocampus but not with the intensity of Nissl stain. Hippocampal TNFα, IL1β, ROS and nitrite showed significant association with WME and RME.

(2) The administration of three doses of naproxen, a non- specific COX inhibitor (5, 10, 20 mg/kg body wt, p.o.) in cAD rats in two study durations (15 and 21- day) showed a dose dependant inhibition of the parameters of neuroinflammation (TNFα, IL1β, ROS, nitrite), neurodegeneration (chromatolysis and amyloid plaques in hippocampus) and memory impairments (increased WME, RME, latency to enter first baited arms and latency to enter four baited arms) compared to that of the naproxen treated control and sham operated rats. The higher inflammatory markers in serum (TNF α, ROS, nitrite) of cAD rats were also inhibited by naproxen and here again a dose dependant response was observed in both the study durations. The decreased serum corticosterone level in cAD rats showed gradual recovery to the control value with the graded dose of naproxen and regained the control value at the dose of 20 mg of naproxen/kg body wt. The increased phagocytic activity of blood WBC and splenic PMN in cAD rats was inhibited by naproxen. The increased cytotoxicity of splenic MNC was similarly inhibited by naproxen. After administration of naproxen the lower LAI in cAD rats gradually recovered to the value on control rats treated

with naproxen. The dose dependant response of naproxen was observed in these immune parameters in both the study duration (15-day and 21-day study). The importance of cox enzyme in the colchicine induced neuroinflanmmation is evident from these results. Hippocampal TNFα and nitrite level were found to be associated with the number of amyloid plaques in hippocampus but not with the intensity of Nissl stain. Hippocampal nitrite was found to be associated with RME, LE1A (prospective memory) and LE4A (spatial memory).

(3) The role of COX2 in the neuroinflammation and neurodegeneration, memory impairments, peripheral immune responses was assessed by administration of three doses of etoricoxib, a specific cox 2 blocker (10, 20 and 30 mg/kg body wt, p.o.) in two study durations (15 and 21- day). Control and sham operated rats were similarly treated with etoricoxib. All the deficits in memory parameters (increased WME, RME, latency to enter first baited arms and latency to enter four baited arms) of cAD rats showed gradual recovery with the increasing doses of etoricoxib in two study durations (15 and 21- day). The increased level of the markers of neuroinflammation (TNFα, IL1β, ROS, nitrite, COX2 and PGE2), neurodegeneration (chromatolysis and amyloid plaques in hippocampus) in hippocampus also showed a gradual inhibition with increasing dose after administration of etoricoxib in cAD rats in two study durations. In serum inflammatory markers (TNF α, ROS, nitrite) in cAD rats were similarly inhibited after administration of etoricoxib. The lower serum corticosterone level in cAD rats also regained control value gradually with increasing doses of etoricoxib. The changes of peripheral immune responses in cAD rats were also inhibited /blocked gradually with increasing doses of etoricoxib. The results shows COX2 played a role in the colchicine induced neuroinflammation which resulted in reurodegeneration, memory impairments and peripheral immune changes in this rat model of AD. Hippocampal nitrite was found to be associated with WME, and RME and LE1A (prospective memory).

(4) The role of oxidative stress on the neuroinflammation, neurodegeneration and memory impairments and peripheral immune responses was assessed by administration of three doses (200, 400 and 600 mg/kg body wt. p.o.) of vitamin C (oxidative stress blocker) in a 21- day study. Control and sham operated rats were similarly treated with vitamin C. Administration of vitamin C in cAD rats at the dose of 200 and 400 mg/kg body wt resulted in the recovery of the impairments of memory parameters (working memory error, reference memory error, latency to enter first baited arms and latency to enter four baited arms).

However, the memory impairments were not recovered at the dose of 600 mg/kg body wt of vitamin C. The increased neuroinflammation in cAD rats was also inhibited by vitamin C at dose of 200 and 400 mg/kg body wt. Vitamin C at the dose of 600 mg/kg body wt not only failed to inhibit these parameters but increased it further. The higher chromatolysis and increased number of amyloid plaques in cAD rats were inhibited by vitamin C at the dose of 200 and 400 mg/kg body wt but chromatolysis and number of amyloid plaques were further increased in cAD rats treated with vitamin at the dose of 600 mg/kg body wt. The increased serum inflammatory markers (TNF α, ROS, nitrite) in cAD rats was inhibited after administration of vitamin C at the dose of 200 and 400 mg/kg body wt but these markers were further increased at the dose of 600 mg/kg body wt. Serum IL1β which was not significantly increased in cAD rats showed a pronounced higher level after administration of vitamin C at the dose of 600 mg/kg body wt but other lower doses of vitamin C (200 and 400 mg/kg body wt) did not show any effect on the serum IL1β level. The lower serum corticosterone level in cAD rats however regained the control level after administration of all the three doses of vitamin C (200, 400 and 600 mg/kg body wt). The changes of peripheral immune responses in cAD rats were inhibited/blocked at the lower doses of vitamin (200 and 400 mg/kg body wt). Vitamin C at the dose of 600mg/kg body wt not only failed to inhibit the changes of immune responses in cAD rats but also increased them further. Thus the results showed a dual role of vitamin C on the icv colchicine induced neuroinflammation, neurodegeneration, memory impairments and peripheral immune responses.

(5) Aminoguanidine [nitric oxide synthase (NOS) blocker] was administered in cAD rats at two doses (30 and 50 mg/kg body wt, p.o.) in a 21- day study to assess the role of nitrosative stress on the neuroinflammation, neurodegeneration and memory impairments and peripheral immune responses induced by colchicine in rats. Control and sham operated rats were similarly treated with aminoguanidine. Administration of aminoguanidine in cAD rats resulted in recovery of neuroinflammation (at 50 mg/kg body wt) and partial prevention of neurodegeneration which could be corroborated with partial recovery of memory impairments in this model. The higher serum inflammatory markers (TNF α, ROS and nitrite) in cAD rats was inhibited by aminoguanidine and the control level was attained at the dose of 50 mg/kg body wt. The lower serum corticosterone also regained the control value with the administration of 50 mg/kg body wt of aminoguanidine. The changes in peripheral immune responses in cAD rats were inhibited/blocked by aminoguanidine and

complete recovery occurred at the dose of 50 mg/kg body wt. This study shows the nitric oxide synthase played a role on the colchicine induced neuroinflammation in this rat model.

(6) Combined dose of naproxen (20 mg) and vitamin C (400mg) was given in cAD rats in a 21-day study to assess the individual participation of COX enzyme and oxidative stress in the colchicine induced neuroinflammation in the present rat model. These doses were selected from previous study which produced maximum inhibition on neuroinflammation. The observed parameters of neuroinflammation, neurodegeneration, memory impairments and peripheral immune responses did not show additive effects by these two compounds. The individual compound showed recovery of the parameters to the control value in study of 2 and 4. Thus it appears oxidative stress and COX are inducing neuroinflammation by a common mechanism.

(7) Combined dose of naproxen (20 mg) aminoguanidine (50 mg/kg body wt) was given in cAD rats in a 21-day study to assess the individual participation of COX and NOS in the colchicine induced neuroinflammation in the present rat model. The markers of neuroinflammation, neurodegeneration, memory deficits and peripheral immune responses showed the effects that were elicited by the use of naproxen in study of 2. The effects of aminoguanidine on the parameters of neurodegeneration and memory impairments as observed in 5 were increased in this study of combined dose. From these results it may be surmised that the oxidative and NOS are inducing neuroinflammation in cAD probably through a common pathway.

(8) Combined dose of aminoguanidine (50 mg/kg body wt from the study in 5) and vitamin C (400 mg/kg body wt. from the study in 4) was given in cAD rats in a 21-day study to assess the individual participation of oxidative stress and NOS in the colchicine induced neuroinflammation in the present rat model. These doses were selected from previous study which produced maximum inhibition on neuroinflammation. The combined dose of aminoguanidine and vitamin C showed that the hippocampal neuroinflammatory markers (TNF α, IL 1β, ROS and nitrite) did not show pronounced additive effects as the individual drug produced maximum inhibition. However, the effect of combined dose on the parameters of neurodegeneration and memory impairments were more than individual effect of aminoguanidine, but similar to that of vitamin C. These parameters were partially recovered with 50 mg/kg body wt of aminoguanidine in study 5. The serum inflammatory

markers did not show prominent additive effect by these two compounds. The additive effects were also not found on peripheral immune responses. Thus, it appears the oxidative and nitrosative stress on neuroinflammation in cAD rats are probably operating through a common pathway.

(9) The status of blood brain barrier in cAD rats was assessed by measuring hippocampal and serum level of inflammatory markers and peripheral immune response in two time points after iv injection of 1M mannitol (30 and 60 min) in cAD rats in a 21- day study. Control and sham operated rats were similarly treated with mannitol and the parameters were measured as in cAD rats. Although the hippocampal inflammatory markers did not further change after mannitol injection in cAD rats, the serum inflammatory markers and peripheral immune responses were altered and these changes were greater after 60 min than that of 30 min of mannitol injection. It can be concluded that the blood brain barrier (BBB) is impaired in cAD rats and this impairment is related with the serum inflammatory markers and peripheral immune responses observed in that condition.

(10) The effect of naproxen (COX inhibitor) on the BBB in cAD rats was investigated by measuring hippocampal and serum level of inflammatory markers and peripheral immune response in two time points after iv injection of 1M mannitol (30 and 60 min)) in cAD rats treated with naproxen (20mg/kg body wt) a 21- day study. The changes of serum inflammatory markers and peripheral immune responses in cAD rats treated with naproxen were lower than that of the cAD rats. The inhibition of neuroinflammation by naproxen probably helped to maintain the status of BBB in cAD rats treated with naproxen and mannitol could not make it more leaky like that of cAD rats without naproxen treatment.

Vide Enclosure - 3

ACHIEVEMENTS FROM THE PROJECT

(a) The peripheral immunological changes in icv colchicine injected AD rats (cAD rats) were first reported from the study of this project. In the human AD patients some peripheral immunological changes were reported previously.

(b) It has also been first identified and reported that the leaky blood brain barrier in cAD rats is probably the cause of peripheral immunological changes in cAD rats.

(c) The association between neuroinflammation and neurodegeneration/memory deficits was also revealed from two study durations in cAD rats. The increasing neuroinflammation in cAD rats with time is related with greater memory deficits and neurodegeneration. The colchicine induced neuroinflammation is mediated through COX, COX2, NOS and oxidative stress as the specific blockers were able to inhibit neuroinflammation which resulted in the reduction of neurodegeneration and memory deficits. The level of COX 2 and PGE2 in hippocampus was measured directly and found to be increased in cAD rats. COX 2 and PGE2 in hippocampus were inhibited by etoricoxib in a dose dependant manner.

(d) The interactions of inducers of neuroinflammation in cAD rats were assessed by the concurrent blocking of COX/oxidative stress, COX/NOS and oxidative stress/NOS by administering two compounds simultaneously in the same rats. A common mechanism is probably operating for neuroinflammation in cAD rats by different inducers of inflammation.

(e) It was found that the hippocampal TNFα, IL1β and ROS were associated with the number of amyloid plaques in hippocampus and with memory parameters WME, RME.

(f) Ten Journal articles have been published in different journals on the basis of this study (Vide Enclosure 6).

(g) Recognitions and awards received by the project fellow for paper presentation based on the results of this project work:

1. Paper entitled "Effect of Naproxen on the cognitive-immune association in colchicine induced rat model of Alzheimer's disease" got selected for **Young Scientist Award Programme** in Indian Science Congress Conference 2013, Kolkata, West Bengal.

2. Received **R. Nath Memorial Travel Award** in IAN [Indian Academy of Neurosciences, Allahabad, India (2013)].

3. Received **Best Poster Presentation Award** on "Neuroinflammation linked neurotoxicity in experimental rat model of Alzheimer's disease" in Indian Science Congress Conference 2014, Jammu, India.

4. Paper entitled **"Regional pattern of neuroinflammation linked with neurodegeneration and cognitive impairment in intracerebroventricular colchicine injected rats"**got selected for Young Scientist Award Programme in Indian Science Congress Conference 2015, Mumbai, India.

7. Paper got selected for **Young Scientist's Award programme (Tulsabai Somani Educational Trust Award)** for the annual conference of Indian Academy of Neurosciences at Panjab University, 31st Oct – 2nd Nov, 2015.

Vide Enclosure - 4

SUMMARY OF THE FINDINGS (IN 500 WORDS)

Alzheimer's disease (AD) is characterised by progressive cognitive impairments which is a consequence of extensive neuronal loss. The mechanism of neurodegeneration in AD is not clearly understood. Intracerebroventricular (icv) injection of colchicine in rats induces memory impairments and histopathological changes that are found in AD. The aim of this study was to investigate the immunological changes in icv colchicine injected AD (cAD) rats and to explore its association with the neuropathological changes of brain and alteration of cognitive functions.

Neuroinflammation in hippocampus and serum, neurodegeneration in hippocampus, memory impairments and some peripheral immune responses were measured in cAD rats, control and sham operated rats in this his study. The memory parameters such as working memory error (WME), reference memory error (RME), latency to enter first baited arm (LE1A for assessment of prospective memory) and latency to enter four baited arm (LE4A for assessment of spatial memory) were measured in radial arm maze. The hippocampal and serum inflammatory markers [TNF α, IL 1β and Reactive Oxygen Species (ROS), nitrite] and hippocampal COX2 and PGE2 were progressively increased in cAD rats and probably resulted in neurodegeneration (increased chromatolysis and amyloid plaques in hippocampus) and memory impairments (increased WME, RME, LE1A and LE4A) in the two time duration studies. The serum corticosterone level was decreased in cAD rats. The peripheral immunological changes such as increased phagocytic activity of blood WBC and splenic polymorphonuclear cells (PMN), and increased cytotoxicity and decreased leucocyte adhesive inhibition index (LAI) of splenic mononuclear cells (MNC) in cAD rats were found to be related with neuroinflammation, serum inflammatory markers and corticosterone in this animal model. The mechanism of induction of neuroinflammation in cAD rats was investigated by the use of blocker of different inflammatory inducer. The inhibitor of non-specific COX (naproxen) and COX 2 (etoricoxib), oxidative stress (vitamin C) and nitric oxide synthase (NOS inhibitor aminoguanidine) were able to reduce neuroinflammation, neurodegeneration, peripheral immune changes and memory deficits in cAD rats in a dose dependant manner. It appears form these studies that the neuroinflammation in cAD rats is induced by COX, NOS and oxidative stressor. The interactions of inducers of neuroinflammation in cAD rats were assessed by the concurrent blocking of COX/oxidative stress, COX/NOS and oxidative stress/NOS by administering two compounds simultaneously in the same rats. A common mechanism is probably operating for neuroinflammation in cAD rats by different

inducers of inflammation. The blood brain barrier (BBB) as assessed in cAD rats appeared to be leaky, probably due to neuroinflammation. The resultant effect was the passage of brain inflammatory markers to the periphery that caused changes of peripheral immune parameters. The association of neuroinflammation with the neuropathological changes of hippocampus and alteration of memory parameters has been examined in this study. It was found that the hippocampal TNFα, IL1β and ROS were found to be associated with the number of amyloid plaques in hippocampus and with memory parameters WME, RME. Thus the present study characterized the immunological changes in cAD rats and the association of these changes with neurodegeneration and memory deficits.

Vide Enclosure - 5

CONTRIBUTION TO THE SOCIETY (GIVE DETAILS)

Alzheimer's disease (AD) is considered to be a degenerative brain disease and is characterized by progressive cognitive impairments including memory deficit and personality changes. More than 25 million people in the world are currently affected by dementia, most suffering from AD, with around 5 million new cases occurring every year. The World Health Organization (WHO) predicts that by 2025, about 75% of the estimated 1.2 billion people aged 60 years and older would reside in developing countries. It is estimated that the number of people living with dementia will almost double every 20 years to 42.3 million in 2020 and 81.1 million in 2040. There are 2 million AD patients in India in 2020 and it is expected to be 2.7 million in 2030 and 4.6 million in 2050. The prevalence of familial AD (FAD) is much lower (5-10 % of the patients) compared to sporadic AD (SAD) (90-95 % of the patients). At present there is no cure of this disease. As the aged population is increasing worldwide and even in India, it is expected that the health care system will be over burdened in future for increasing AD patients. Though animal study cannot be extrapolated to human patients, the present study attempted to pave the path for future management of AD patients.

The neurodegeneration in FAD has been explained on the basis of amyloid cascade hypothesis. Although the amyloid cascade hypothesis has been considered important for explaining the mechanism of neurodegeneration in AD patients for the last 20 years, several reports indicate the inadequacies of this hypothesis specially in SAD. Thus, it appears that the amyloid cascade hypothesis explaining the neurodegeneration in AD needs to be reconsidered as it is inadequate to explain the neurodegeneration in most of SAD. An alternative hypothesis is probably required to explain the mechanism of neurodegeneration in AD which may provide alternative therapeutic strategies for AD. SAD is a heterogeneous disease in which the process of neurodegeneration is probably more complex and different in individual AD patients, and is not clearly known.

The icv colchicine injected rat model is considered as an animal model of sporadic AD. The immunological changes of this animal model has been characterised from this study. On the basis of the present finding this model may better be used for the development of drugs for sporadic AD.

The results of the present study showed that non-specific COX and COX 2 blocker is effective in inhibiting the colchicine induced neuroinflammation. Involvement of the inflammation and immune system in neurodegeneration of AD patients was indicated by epidemiological studies which had shown that the use of non-steroidal anti-inflammatory drugs (NSAID)in people suffering from arthritis might reduce the risk of this disease. Though NSAID are not found to be suitable from the preliminary studies in AD patients, once the disease process has been initiated, the possibility of the use of NSAID for inhibiting the progression of AD cannot be ruled out. This study indicated the possibility of NSAID in AD patients may be re-examined.

The results of this study shows that the mechanism of neuroinflammation may be inhibited by inhibitor of oxidative and nitrosative stress. The natural food rich in antioxidants (specially vitamin C) or antioxidant enriched foods may be recommended to healthy population for prevention of AD in old age. The life style is related with stresses and different well recognised methods to reduce stress such as yoga and exercise may be recommended for population as preventive measure for AD.

Vide Enclosure – 6

PUBLICATIONS FROM THE PROJECT

Research Publications

1. Sil S., Goswami AR., Dutta G., Ghosh TK. Effects of naproxen on immune responses in a colchicine induced rat model of Alzheimer's disease. *Neuroimmunomodulation,* 2014: 21, 304-321. DOI: 10.1159/000357735

2. Sil S., Ghosh TK. Amelioration of anxiolytic behavior in intracerebroventricular colchicine injected rats by naproxen. *Asian journal of Pharmaceutical and Clinical Research,* 2015: 8, 189 - 196.

3. Sil S, Ghosh R, Sanyal M, Guha D and Ghosh T K. A Comparison of neurodegeneration linked with neuroinflammation in different brain areas of rats after intra-cerebroventricular colchicine Injection. *J Immunotoxicology* , 2016: 13(2), 181-190. http://dx.doi.org/10.3109/1547691X.2015.1030804

4. Sil S, Ghosh T K, and Ghosh R: NMDA receptor is involved in neuroinflammation in intracerebroventricular colchicine-injected rats. *J Immunotoxicology* 2016: 13(4), 474-489. DOI/10.3109/1547691X.2015.1130760.

5. Sil S., Ghosh TK. Role of cox-2 mediated neuroinflammation on the neurodegeneration and cognitive impairments in colchicine induced rat model of Alzheimer's Disease. *Journal of Neuroimmunology*. 2016: 291,115-124. DOI: 10.1016/j.jneuroim.2015.12.003

6. Sil S., Ghosh TK. Cox 2 plays a vital role on the impaired anxiety like behavior in colchicine induced Alzheimer rats. Behavioral Neurology. 2016: 5, 1-8. DOI: 10.1155/2016/1501527

7. Sil S, Ghosh Arijit and Ghosh TK: Impairment of blood brain barrier is relatedwith the neuroinflammation induced peripheral immune status in intracerebroventricular colchicine injected rats: An experimental study with mannitol. *Brain Res* 2016: 1646, 278–286. doi:10.1016/j.brainres.2016.05.052,

8. Sil S, Ghosh TK, Gupta P, Ghosh R, Kabir S N , Roy A: Dual role of vitamin C on the neuroinflammation mediated neurodegeneration and memory impairments in colchicine induced rat model of Alzheimer Disease. *Journal of Molecular Neuroscience*, 2016: 60, 421-435. DOI 10.1007/s12031-016-0817-5.

9. Sil S, Ghosh TK, Ghosh R, Gupta P: Nitricoxide synthase inhibitor, aminoguanidine reduces intracerebroventricular colchicine induced neurodegeneration, memory impairments and

changes of systemic immune responses in rats. *J Neuroimmunology,* 2017: 303, 51–61. http://dx.doi.org/10.1016/j.jneuroim.2016.12.007.

10. Sil S and Ghosh TK: Etoricoxib inhibits peripheral inflammation and alters immune response in intracerebroventricular colchicine injected rats. *J Neuroimmunology,* 2018*:* 317, 15–23. DOI: 10.1016/j.jneuroim.2018.01.018.

Published proceedings

1. Susmita Sil, Tusharkanti Ghosh. Effect of Naproxen on the cognitive-immune association in Colchicine induced rat model of Alzheimer's disease. Proceedings of 100th Indian Science Congress, Kolkata, 2013, (Y.S. Abstracts) Section: Medical Sciences (including Physiology), page no. 42.

2. Susmita Sil, Tusharkanti Ghosh. The role of cyclooxygenase in the colchicine induced neurodegeneration in rats. Proceedings of Annals of Neurosciences, Vol. 20, Annual conference of Indian Academy of Neurosciences, Allahabad, India, 2013, page no. 50.

3. Susmita Sil, Tusharkanti Ghosh. Neuroinflammation linked neurotoxicity in experimental rat model of Alzheimer's disease. Proceedings of 101st Indian Science Congress, Jammu 2014, (Abstracts of Best Poster Award Programme) Section: Medical Sciences (including Physiology), page no. 21-22.

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ANNEXURE-X

UNIVERSITY GRANTS COMMISSION

BHADUR SHAH ZAFAR MARG, NEW DELHI-110 002

EVALUATION REPORT

It is certified that the final report of the UGC Major Research Project entitled "Immunological changes and neurodegeneration in colchicine induced rat model of Alzheimer's disease" (vide UGC approval Number UGC MRP No. : F. No. 42-532/2013 (SR) dt. 22nd March, 2013) by Prof Tusharkanti Ghosh, Dept of Physiology, University of Calcutta, 92 APC Road, Kolkata 700 009, West Bengal has been assessed by the "Evaluation Committee" consisting of the following members for final submission to the University Grants Commission, New Delhi.

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The final report is found to be satisfactory.

MChakrabarti 17/3/2020

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