ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Assessment of Phosphodiesterase Inhibitors in Alzheimer's Disease Experimental Models

Received: 24 October 2022, **Revised:** 22 November 2022, **Accepted:** 26 December 2022

Dr. A. V. Chavda
Tutor Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Dr. V. M. Thorat
Professor& HOD. Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed ToBe University",Karad – 415110,Maharashtra

Dr. D.V. Sarvaiya
Tutor. Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Key Words:
Phosphodiesterase, Alzheimer, Medications, Disease.

Abstract:
Millions of people all over the globe suffer with AD. This complicated illness has a latency period of 20 years or more. Alzheimer's disease research has exploded in the past several decades, and finding a cure for this progressive neurological disease has become a global health priority. Despite this, modifying clinical symptoms of Alzheimer's disease and delaying the illness's start remains an important strategic aim, despite the persistent failure of drugs in clinical trials. In this publication, the authors evaluate phosphodiesterase inhibitors in animal models of Alzheimer's disease.

1. Introduction

Degeneration of neurons and other complicated neurological difficulties are only two of the many health problems associated with ageing, making it one of the most unexpected periods of life. "Alzheimer's disease (AD) is a neurodegenerative disorder that affects the brain and causes memory loss and mortality as a result of a variety of age-related factors. It's a leading suspect in the escalation of dementia; a serious public health issue throughout the world .Early-onset (EO) Alzheimer's disease and late-onset (LO) Alzheimer's disease are two types of the illness. Neuropathological hallmarks of Alzheimer's disease (AD) include amyloid beta (A) accumulation, the formation of neurofibrillary tangles inside cells caused by hyperphosphorylated tau protein, the loss of synapses, and the death of neurons." It begins with injury to the hippocampus' CA1 and CA3 areas, then spreads to the cerebral cortex. Numerous studies have implicated several pathogenic components and a few ideas, including the amyloidosis hypothesis, the tau hypothesis, or the GSK-3 hypothesis, in understanding the onset and progression of neurodegeneration in this disease. Even though a specific research route has been dedicated to clarifying the etiopathology of Alzheimer's disease, many unanswered questions remain. The intricacies of Alzheimer's disease can only be understood by first gaining a thorough understanding of the illness's neurobiology and pathology.[1]

STZ is a glucosamine-nitrosourea molecule that, upon ingestion, causes the production of a cytotoxic agent that particularly destroys cells in the pancreatic islet, hence inducing diabetes mellitus. Patients with Alzheimer's disease (AD) have biochemical changes in the brain that are similar to those seen in long-term (LT) rats given STZ intracerebroventricularly (ICV). These changes include impaired cerebral glucose or energy metabolism and impaired learning and memory. Amyloid deposition in the meningeal capillaries and increased production of phosphorylated tau protein in the cerebral cortex of ICV-STZ rats are both hallmarks of AD and provide evidence for the disease's progression in this paradigm.[2-3]

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

The Amyloid Hypothesis is a well-known and widely accepted pathogenic explanation for Alzheimer's disease, suggesting that an increase in intracellular and extracellular A is the driving event that causes the genesis of neuronal damage in this condition. In the amyloidogenic pathway, beta- and gamma-secretase cleave the APP several times, leading to the overproduction of A in neurons. Disruptions in cholinergic neurotransmission play a major role in the cognitive declines of today. [4]

AChE enzyme inhibitors are the standard therapy for Alzheimer's disease nowadays. AChE inhibitors, including donepezil and rivastigmin, are widely accepted as the standard of care, despite their ineffectiveness and the high expenses they incur. There is also evidence that neuroinflammatory reactions contribute to the development of Alzheimer's disease. The release of A profibrils in neurons is a key pathogenic event in the progression of Alzheimer's disease. This event stimulates microglia, which in turn causes pro-inflammatory responses that result in the generation of neurotoxic cytokines. This calls for an innovative multi-target strategy to mitigate the problems and slow the disease's progression.[5]

2. Material and Methods

Chemicals and Medications

Each medication solution was made from scratch just before the experiment began. Sigma Aldrich (USA) was contacted in order to purchase roflumilast, tadalafil, and A1-42. In terms of sourcing, we sourced streptozotocin and acetylthiocholine iodide from Sigma Aldrich in the United States and sourced donepezil from Cipla Pharmaceutical Company in India. We purchased TNF-, IL-1, and IL-6 ELISA kits from R&D Systems, Inc., and cAMP and cGMP ELISA kits from Krishgen Biosystems, all located in India. Indian Immunologicals Limited and Miracalus Pharma Pvt. Ltd. supplied the ketamine and xylazine, respectively.

Animals

Central Animal House Facility, Lucknow provided Wistar albino rats weighing between 250 and 300g in total. “The animals were acclimated in polypropylene cages at 25 2 °C and 55-65% relative humidity, with a 12 h light/12 h dark cycle, and a commercial pellet meal and water were provided free of charge. The CPCSEA rules were adhered to throughout all the studies.” Protocols 1531 and 1815 were registered with the Jamia Hamdard IAEC and given the green light to proceed with the experiments.

Dosage determination

The prescribed human dosage of roflumilast (500 g) was translated to a rat dose of 0.05 mg/kg p.o. using the formula for dose conversion described in a previous article. Daily batches of RFM were made by dissolving it in a solution of 98% methylcellulose and 2% Tween 80.

TDF (Tadalafil):

The oral TDF dosage of 0.51 mg/kg was calculated by dividing the recommended human dose of 5 mg by the body weight of a rat . Each day, 0.5% w/v sodium carboxymethyl Cellulose was used to make new TDF.

(DPZ) Donepezil

The prior literature suggested a dose of DPZ of 5 mg/kg p.o. In this experiment, DPZ served as a kind of control group.

Study: On day 1, rats in Group II (A1-42) were given 5 g of A1-42 ICV- bilaterally into each lateral ventricle to induce AD. On day 1, rats in groups III and IV received A1-42 intracerebroventricularly (ICV) in a volume of 5 l in each lateral ventricle. For 15 days, rats in Groups V and VI were given oral doses of 0.05 mg/kg/day and 0.51 mg/kg/day, respectively. On day 1, stereotactic surgery was done, and over the next 15 days, test medication therapy was administered. On days 14 and 15, rats in each treatment group were put through a NOR task, and from days 16 through 21, they were put through a MWM test. Brains were obtained for biochemical and histological investigation from rats that were slaughtered on day 22.

Administering A1-42 intracerebroventricularly

After administering a ketamine and xylazine cocktail to Wistar rats. The stereotactic coordinates are as follows: from below the surface of the brain, move dorsally by -3.5 mm, anteriorly by -0.8 mm, and posteriorly by 1.6 mm, all with reference to the sagittal suture. After incubating A1-42 oligomers in normal saline at 37 degrees Celsius for four days, a

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

solution of aggregated oligomers was obtained. Both cerebral ventricles were injected with A1-42 (5 g/5 l) dissolved in saline .

cAMP, cGMP, A1-42, & BACE-1 enzyme estimation

Using an ELISA kit for rats, we quantified cAMP, cGMP, A1-42, and BACE-1 enzyme as per the manufacturers' instructions. This measurement is performed using a sandwich enzyme immunoassay. The microplate has been pre-coated with a monoclonal antibody specific to rat cyclic AMP, cyclic GMP, amyloid beta (A1-42), and -secretase (BACE-1). After removing any unbound material with a wash solution, we added an enzyme-linked polyclonal antibody specific for rat cAMP, cGMP, A1-42, and BACE-1 and washed the wells again. There was a blue colour produced by the enzyme process, but as the stop solution was added, the colour became yellow. Rat cAMP, cGMP, A1-42, and BACE-1 binding is correlated with colour intensity as evaluated by spectrophotometry. The standard curve was used to make sense of the sample data. Statistical analysis resulted in values being presented as a mean S.E.M.

Biomarkers for oxidative stress

- **Thiobarbituric acid reactive compound estimation**

Previous studies' methods were used to make estimates of TBARS concentration. The homogenate was diluted to 0.25 ml and then placed in a 15 x 100 mm test tube before being incubated in a metabolic shaker at 37 °C for 1 hour. The same amount of homogenate was piped into a centrifuge tube, frozen at 0 degrees Celsius for an hour, and labelled as an incubation time of zero hours. After incubating for an hour, we added 0.25 millilitres of 5% (w/v) cooled TCA and 0.5 millilitres of 0.67% TBA and centrifuged at 4000xg for 10 minutes to remove the excess liquid. The supernatants were then transferred to new test tubes and heated in a water bath at boiling temperature for 10 minutes. The resulting pink colour had a red absorption of 535 nm. Using a molar extinction value of 1.56 105 M-1 cm-1, the TBARS concentration was determined and reported as nmol TBARS generated per hour per milligramme of protein.

- **Calculating Glutathione S-Transferase Activity**

According to previous studies, we measured the reduced GSH activity in brain tissue. Sulfosalicylic acid (4%), in 0.1 ml, was used to precipitate the PMS content (0.1 ml). “After storing the samples at 4 degrees Celsius for an hour, they were centrifuged at 1200 g for 15 minutes. Assay mixture volume was 2.0 ml and comprised 0.1 ml of filtered aliquot, 1.7 ml of PB (0.1 M, pH 7.4), and 0.2 ml of 5-5'- dithiobis- 2-nitrobenzoic acid.” At 412 nm, the yellow colour was quickly produced and read. Moles of GSH per milligramme of protein were determined using the molar extinction coefficient.

- **estimate Nitrite concentration**

Nitrite, a byproduct of NO, was quantified in the supernatant of brain tissue using a colorimetric test with Greiss reagent (0.1% N- (1- naphthyl) ethylenediaminedihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid). Green et al. (1982) and Ahmad et al. (2016) provide supporting evidence. After incubating the supernatant with Greiss reagent at room temperature and in the dark for 10 minutes, the absorbance of the supernatant was measured at 540 nm. Nitrite content (expressed as mol/ml) in the supernatant was evaluated using a sodium nitrite standard curve.

Histopathologicalanalysis

- **Staining with hematoxylin and eosin**

Histopathological examination with H&E staining was used to identify pyknotic neurons in the rat hippocampus. The brains were preserved in 10% formalin for 12 hours before being imbedded in paraffin wax. Using a microtome, 5-m thick coronal slices containing the CA1 area of the hippocampus were cut and mounted on glass slides for staining. After being deparaffinized with xylene and progressively rehydrated with graded alcohol, the slices were stained with H&E dye and mounted on saline-coated slides. The Moticam microscope was used to take pictures at a 20x magnification.

- **Dyeing using cresyl violet**

Neurons that stained positive for Nissl in the rat hippocampus were visualised using cresyl violet

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

staining. After 12 hours of preservation in 10% formalin, the brains were embedded in paraffin wax for permanent storage. Hippocampal coronal slices 5 m thick were cut from the CA1 region and placed on glass slides using a cryostat microtome. The slides were exposed to a Cresyl violet solution at 0.5% for 5-10 minutes.

- **A smear of Congo red**

"Rat hippocampal amyloidosis was analysed using Congo red staining. Cryostat microtome slices of the brain, were taken in a coronal plane and were 5 m thick." Following fixation on glass slides, the sections were dyed for 15-20 minutes with 1% Congo red solution. Images were taken using an Olympus microscope at 20x magnification.

Analysis

Graph Pad Prism software was used for statistical analysis of the neurobehavioral and metabolic parameters obtained after intravenous injection of streptozotocin (STZ) and anakinra (A1-42) in rats. The information was shown as a mean SEM. Two-way analysis of variance (ANOVA) with post hoc Bonferroni's multiple comparison test was used to compare the total amount of time spent exploring the familiar and new item in T1 and T2 for MWM and NOR, respectively. All additional factors were analysed using one-way ANOVA, and then Turkey's Kramer post hoc multiple comparison test was used to determine statistical significance between groups. Statistical significance was assumed when p 0.05.

3. Results

Non-spatial memory impairment caused by RFM and TDF in rats injected with ICV-A1-42

When non-spatial memory was evaluated using the new object recognition test, no significant differences were found between the groups throughout the familiarisation phase. Two-way repeated-measures testing was performed on day 14 for all novel-object-exposed rats. "Differences in pharmacological treatment between familiar and new things ($F_{1,60} = 26.21$; p 0.001) and the interaction between familiar and novel objects ($F_{5,60} = 8.971$; p 0.001) were statistically significant, as determined by an analysis of variance followed by a Bonferroni multiple comparison test." There was no discernible difference between the A1-42 group's responses to familiar and unexpected things. Both RFM and TDF therapy showed statistically significant increases in new object recognitions compared to recognitions of known items. One-way analysis of variance followed by Tukey's multiple comparison test revealed that the discrimination index was significantly lower in the A1-42 group than in the sham group . When compared to the A1-42 group, the discrimination index of those treated with RFM and TDF was considerably higher (p 0.01). Comparing the RFM and TDF groups to the sham group, however, revealed no discernible difference.

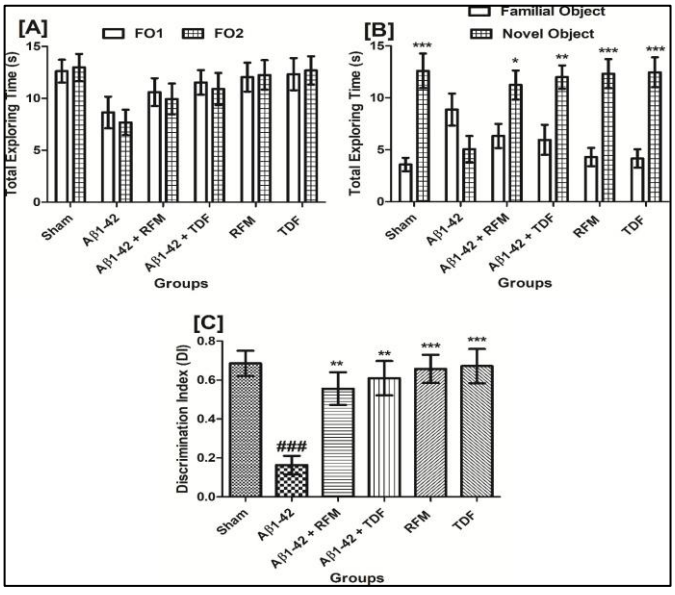

Fig.1: Non-spatial memory impairment caused by RFM and TDF in rats injected with ICV-A1-42

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Novel object recognition performance of ICV-A1-42 injected rats affected by RFM and TDF. Two-way repeated-measures ANOVA with post hoc Bonferroni multiple comparison test was used to compare how much time was spent exploring two similar objects (FO1 and FO2) during the familiarisation phase, and how much time was spent exploring two different objects (Familiar and Novel) during the retention phase. Standard error of the mean (n = 7) displays the central tendency of the data.

Using ICV-A1-42 to study the impact of RFM and TDF on spatial memory in rats

The rats were trained for 5 consecutive days, with each day consisting of 4 trials in the Morris water maze test, to assess their long-term spatial memory. There was a statistically significant difference in drug treatment, a statistically significant difference in days ($F_{4,144} = 103.8$; $p < 0.001$), but there was no statistically significant difference in the drug treatment and days interaction, as determined by a two-way repeated-measures ANOVA with a post hoc Bonferroni multiple comparison test. There was no statistically significant difference in the groups' mean times to reach the concealed platform on day 1, however on days 2-5, the A1-42 groups showed considerably longer mean times ($p < 0.001$) compared to the sham group. The percentage of time spent in the target quadrant was considerably lower in the A1-42 group compared to the sham group ($F_{5,36} = 9.469$; $p < 0.001$). The percentage of time spent in the target quadrant after RFM and TDF treatment was considerably higher than in the A1-42 group. This was indicative of a consolidated memory and secure sense of place over time. When comparing the RFM and TDF groups to the sham group, Table shows that neither group significantly increased nor decreased the amount of time they spent in the target quadrant.

Table1: ICV-A1-42 injected rats: RFM and TDF's impact on spatial memory

EscapeLatency(S)						
Groups(n=7)	**Sham**	**RFMperse**	**Aβ1-42 +RFM**	**TDFperse**	**Aβ1-42**	**Aβ1-42 +TDF**
Day 1	68.369±5.266	73.798±4.172	81.503±2.171	71.940±4.957	83.058±4.563	79.085±2.939
Day 2	44.973±4.665	49.401±4.812	60.073±3.421	55.632±4.915	78.459±3.870	55.828±3.614
Day 3	32.071±4.627	37.357±5.766	49.713±4.860	35.499±7.426	67.311±4.727	46.240±4.967
Day 4	22.143±3.186	25.143±3.904	37.427±3.458	29.000±5.409	56.776±5.767	34.231±5.130
Day 5	18.090+5.227	22.804+2.046	37.754+5.596	26.960+3.296	59.493+4.497	30.780+3.999
Percentage of time spent in "sweet						

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

spot"					
TDFper se	Aβ1-42+ TDF	Aβ1-42+ RFM	Aβ1-42	RFMperse	Sham
44.199±3.98 1	43.186±4.01 2	39.423±3.9 67	18.61±3.435	47.613±4.23 3	52.184+3.118

Morris Water Maze tests were performed on rats given ICV-A1-42 to see how RFM and TDF affected their ability to remember where they had been before. “For this analysis, we employed a two-way repeated-measures ANOVA followed by a Bonferroni multiple-comparison post-test to look at the acquisition trial's data. A1- 42 infused animals administered with RFM and TDF spent significantly more time in the target sector than A1-42 infused rats after the acquisition phase, during the probe trial, as determined by one-way ANOVA accompanied by the post hoc Tukey Kramer multiple comparison test. Average and standard deviation (n=7) displays the data. Statistically, the A1-42 group is quite different from the sham group. Roflumilast, talinocept, or both given intravenously.”

Hippocampal GSK-3, A1-42, and p-Tau in rats: RFM and TDF's effect

Increased levels of A1-42 in the brain are a pathological characteristic of Alzheimer's disease. “Increased GSK-3 activation has been associated to increased tau protein hyperphosphorylation in the brain. In our study, we injected rats with ICV A1-42 to cause degenerative alterations similar to Alzheimer's disease. The levels of GSK-3 and A1-42 in the cerebral cortex of the A1-42 group were statistically greater than those of the sham group, as determined by one-way ANOVA followed by the Tukey Kramer numerous comparison test. And p-Tau increased in step with this pattern.”

Table 2: ICV-A1-42 injected rats: effect of RFM and TDF on GSK-3, A1-42, and p-Tau

Groups (n = 6)	**Sham**	**RFM per se**	**Aβ1-42**	**Aβ1-42 + TDF**	**TDF per se**	**Aβ1-42 + RFM**
Aβ1-42 (pg/ml)	181.70 ± 39.349	212.86 ± 40.356	529.83 ± 32.618	295.56 ± 47.811	249.53 ± 38.740	318.69 ± 28.936
GSK-3β (ng/ml)	1.520 ±0.2929	1.819 ± 0.3662	5.158 ± 0.5511	2.450 ± 0.4281	1.584 ± 0.2886	3.011 ± 0.3857
p-Tau (ng/L)	36.076 ± 5.914	36.841 ± 4.290	71.106 ± 4.994	47.515 ± 6.490	39.008 ± 4.934	41.902 ± 5.670

Table shows that after 15 days of oral treatment of RFM and TDF, the levels of GSK-3, A1-42, and p-Tau in the A1-42-infused rats dropped dramatically. The Tukey-Kramer multiple comparison test is used after a one-way analysis of variance has been performed. The information is shown as a mean with a standard deviation of six. When comparing the markers to the sham group and the A1-42 group, there are statistically significant differences. Phosphorylated tau (p-Tau), amyloid beta 1-42

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

(A1-42), tadalafil (TDF), roflumilast (RFM), and glycogen synthase kinase-3 beta (GSK-3).

Analysis of RFM and TDF's influence on cAMP, cGMP, and BDNF levels in rat hippocampi

We used specialized ELISA kits to measure cAMP, cGMP, and BDNF concentrations. We found that in the hippocampus of rats administered ICV A1-42, compared to the sham group, the levels of cAMP, cGMP, and BDNF were significantly lower. In contrast, the cAMP and BDNF levels of rats treated with RFM for 15 days exhibited a striking rise compared to the A1-42 group. The levels of cGMP and BDNF in the hippocampus were also found to be considerably higher in the TDF group than in the A1-42 group (p 0.001).

Table 3: Analysing the role of RFM and TDF on cAMP, cGMP, & BDNF in rats given ICV-A1-42

Groups (n = 6)	**Sham**	**Aβ1-42 + TDF**	**Aβ1-42**	**RFM per se**	**TDF per se**	**Aβ1-42 + RFM**
cGMP (pmol/ml)	68.103 ± 4.356	65.649 ± 5.663	33.194 ± 3.570	61.550 ± 5.127	64.865 ± 4.584	48.144 ± 5.681ns
cAMP (pmol/ml)	51.532 ± 5.593	33.505 ± 2.621ns	21.068 ± 3.1627	49.153 ± 3.137	46.811 ± 4.398	48.860 ± 5.645
BDNF (pg/ml)	341.83 ± 22.430	315.17 ± 30.685	153.75 ± 19.691	339.75 ± 17.819	342.25 ± 23.417	298.33 ± 30.060

"The results show that RFM and TDF affect cAMP, cGMP, and BDNF levels in the hippocampus of ICV A1-42 rats. Exposure to A1-42 dramatically reduced cAMP, cGMP, and BDNF levels in the rat hippocampus, as determined by a one-way analysis of variance or a post hoc Tukey Kramer numerous comparison test." The data (n = 6) are shown as a mean SEM. Markers indicate significant differences. Roflumilast (RFM), Tadalafil (TDF), Statistical Insignificance (ns), cAMP, cGMP, and Brain-Derived Neurotrophic Factor (BDNF) are some examples of these.

Changes in oxidative stress indicators in the rat hippocampus after treatment with RFM and TDF:

Lipid peroxidation (MDA), nitrite, and glutathione peroxidase (GSH) levels were measured to evaluate the free radical production generated by intravenous injection of A1-42. We found that the levels of MDA (F5,30 = 9.101; p 0.001) and nitrite (F5,30 = 9.129; p 0.001) in the hippocampus of A1-42 group rats were significantly higher than in the sham group rats, whereas the levels of GSH (F5,30 = 5.770; p 0.001) were significantly lower.

Table 4: How ICV-A1-42 infusion affects oxidative stress in rats treated with RFM and TDF

Groups (n = 6)	**Sham**	**Aβ1-42 + RFM**	**RFM per se**	**Aβ1-42 + TDF**	**TDF per se**	**Aβ1-42**

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

TBARS (nMol/mg protein)	1.809 ± 0.2588	3.440 ± 0.5570	2.493 ± 0.6731	3.068 ± 0.7451	2.193 ± 0.4755	6.459 ± 0.5022
GSH (µMol/mg protein)	5.467 ± 0.7284	4.053 ± 0.8269	5.117 ± 0.6980	4.566 ± 0.6064	4.550 ± 0.5356	1.187 ± 0.3106
Nitrite (µMol/ml)	10.681 ± 1.549	16.865 ± 2.115	13.481 ± 1.950	14.814 ± 1.872	11.481 ± 1.908	27.172 ± 2.461

When given orally to rats for 15 days, RFM and TDF mitigated the oxidative damage caused by A1-42. The anti-oxidant activity of these PDE inhibitors was shown in the rat hippocampus by a decrease in MDA and nitrite and an increase in GSH. After analysing the data using one-way ANOVA, the researchers applied the Tukey-Kramer test for multiple comparisons. Standard error of the mean (n = 6) is shown as the data distribution. The symbols indicate significant differences at the 0.05, 0.01, and 0.001 level when comparing the A1-42 group to the sham group and the A1-42 group to itself. Reductions in thiobarbituric acid reactive substances (TBARS), glutathione (GSH), and tadalafil (RFM) have been seen.

Rats' hippocampi show the effects of RFM and TDF on histopathological change

The use of hematoxylin and eosin:

Tissue sections showing pyknotic nuclei, scattered vacuolization, shrunken pyramidal cells, and neuronal inflammation following A1-42 treatment demonstrated considerable loss of neurons. “One-way ANOVAshowed that the number of pyknotic neurons in the CA1 area of the hippocampus was substantially higher in the A1-42 group than in the sham group. However, compared to the A1-42 group, the pyknotic neuron count in the CA1 area of the hippocampus was dramatically reduced after administration of RFM and TDF.”

Using cresyl violet as a stain: Compared to sham group rats, animals treated with A1-42 showed significantly more neuronal damage in the CA1 area of the hippocampus as seen by cresyl violet staining. “Numbers showed a significant drop in Nissl positive staining in the CA1 area of the hippocampus between the A1-42 and sham groups. Nissl positive staining in the CA1 area of the hippocampus was shown to be considerably higher (p 0.05) after administration of RFM and TDF compared to the A1-42 group.”

Staining with Congo red: “Here, RFM and TDF therapy reduced the increased Congo red staining in CA1 areas of the hippocampus seen in A1-42 infused animals compared to sham group rats. Quantitative analysis confirmed an abundance of A deposition in the CA1 area of the hippocampus, as shown by positive staining with Congo red. In the CA1 area of the hippocampus, the expression of amyloid- beta deposition was shown to be considerably reduced after treatment with RFM and TDF compared to the A1-42 group.” The expression of amyloid-beta deposition was not significantly different between the RFM and TDF groups and the sham group.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

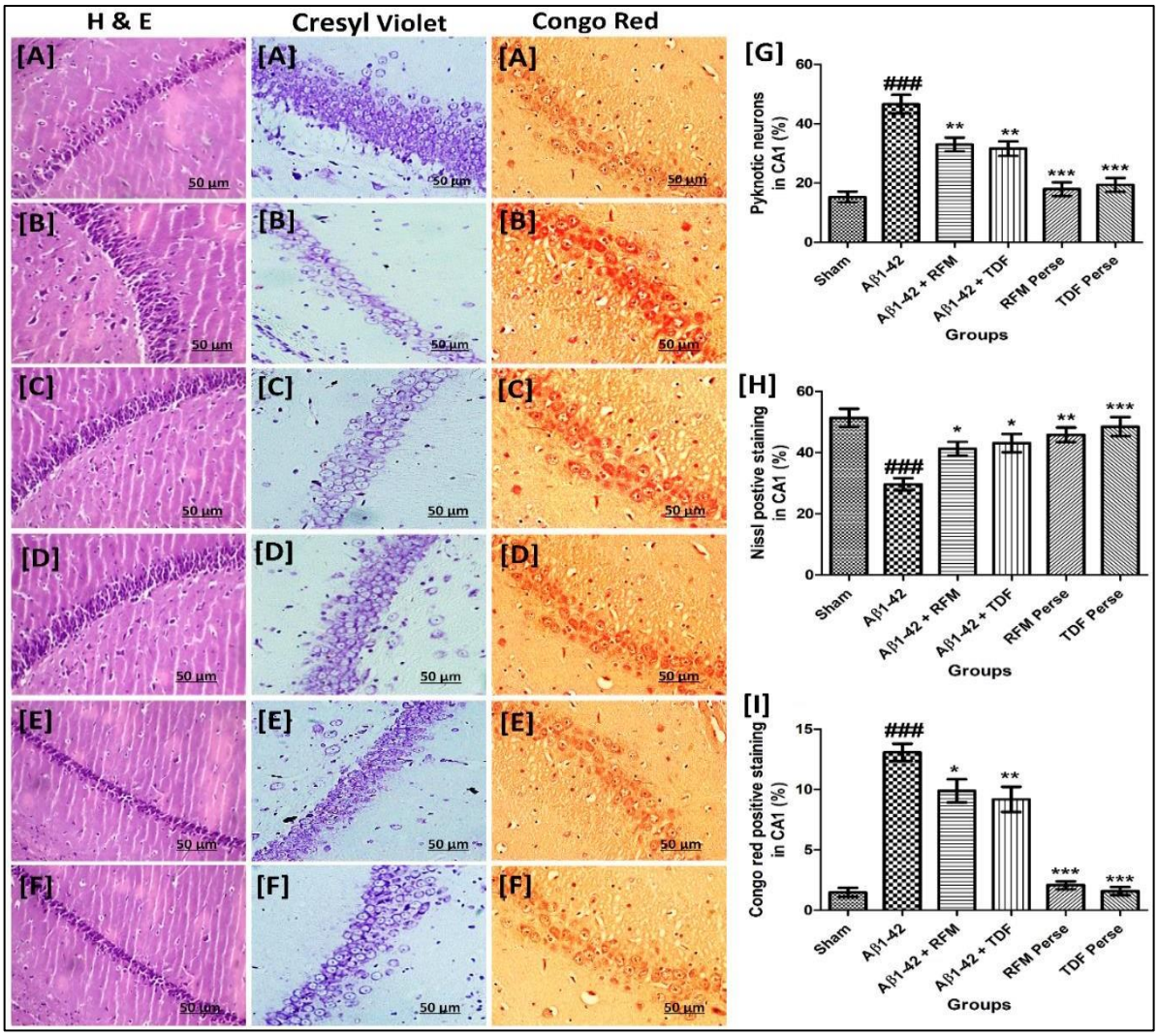

Fig. 2: Rats' hippocampi show the effects of RFM & TDF on histopathological change

Histopathology of the CA1 area of the hippocampus after 21 days of the experimental regimen, stained with H&E, Cresyl violet, & Congo red. Images show (A) a control group, (B) an A1-42 group, (C) an A1-42 group with RFM, (D) an A1-42 group with TDF, (E) an RFM per se, and (F) an RFM without TDF, all at 20x magnification with a scale bar of 50 m. H&E, Cresyl, & Congo red staining photos were captured using a Moticam and an Olympus BX51 microscope, respectively.

4. Discussion

Consistent with earlier results, A1-42 decreased rats' ability to distinguish between familiar and unfamiliar objects compared to rats in the sham group. However, rats given an oral combination of RFM and TDF for 15 days showed enhanced capacity to distinguish between known and unfamiliar objects.[6]

This result demonstrated that RFM and TDF may enhance rats' non-spatial memory. The rat was then put through MWM test, in which it was required to remember where it had been shown a concealed platform inside the MWM's four quadrants.[7]We found that rats in the ICV A1-42 group exhibited an increase in the escape latency during the acquisition phase and a decrease in the time spent in the target quadrant during the probe trial, both of which portrayed an impairment in the spatial memory.[8]

Injecting A1-42 into brain ventricles has been shown in many studies to enhance oxidative stress by boosting lipid peroxidation and nitrite production while suppressing GSH production . Our findings

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

corroborated those of earlier studies showing that A1-42 produced oxidative stress may be mitigated by RFM and TDF therapy. This indicated the PDE inhibitors' ability to quench free radicals. Findings from the current research support the use of PDE inhibitors like RFM and TDF to treat AD-related neurobehavioral and neuropathological abnormalities.[9-10]

5. Conclusion

Amyloid beta (A) deposition is a key factor in the pathogenesis of AD, contributing to the onset of cognitive and cholinergic impairments, neuroinflammation, including neuronal death. Here, we provide evidence that the intracerebroventricular (ICV) injection of A1-42 to rats causes neuropathological alterations in the hippocampus that are comparable to those seen in Alzheimer's disease. “Some of the negative effects of A1-42 injection might be reduced with the use of RFM and TDF treatment. The infusion of A1-42 ICV into either cerebral ventricle in rats is a well-established paradigm for inducing AD. The hippocampus, which controls cognitive processes including spatial recall and learning, receives information from the cerebral ventricle, a network of cavities containing cerebral spinal fluid.”

References

[1] Yao L, Chen Y, Li J, et al. Phosphodiesterase Inhibitors for Alzheimer's Disease: A Systematic Review and Meta-Analysis. Am J Alzheimers Dis Other Demen. 2018;33(5):283-296.

[2] Singh AK, Kaur N, Chaudhary A, et al. Phosphodiesterase Inhibitors: A Novel Targeted Therapeutic Approach for Alzheimer's Disease. CurrNeuropharmacol. 2019;17(9):823-836.

[3] Kroker KS, Brunner B, Bathke J, et al. Effect of phosphodiesterase inhibitors on cognitive function and cholinergic markers in a rat model of Alzheimer's disease. Behav Brain Res. 2018;347:441-447.

[4] Chen F, Chen X, Yang D, et al. Inhibition of Phosphodiesterase-5 Improves Alzheimer's Disease-Related Phenotypes and Modulates Brain Indoleamine 2,3-Dioxygenases 1 /Tryptophan 2,3-Dioxygenases-Kynurenine Pathway Abnormalities in AβPP/PS1 Mice. Neurotherapeutics. 2019;16(4):1230-1243.

[5] Oliveira LF, Goulart VA, Santos JR, et al. Phosphodiesterase-5 inhibition attenuates neuroinflammation and improves cognitive function in rats with induced Alzheimer's disease. J Neuroimmunol. 2017;305:90-98.

[6] Shimizu-Albergine M, Rybalkin SD, Rybalkina IG, Feil R, Wolfsgruber W, Hofmann F. Individual roles of cyclic nucleotide phosphodiesterase isoforms in signal processing in mouse cerebellar granule cells. J Biol Chem. 2020;278(12):10508-10513.

[7] Wang R, Li B, Li X, et al. PDE5 inhibitor sildenafil protects against ischemic stroke-induced neuroinflammation and cognitive impairment by suppressing peripheral and central immune responses. J Neuroinflammation. 2018;15(1):11.

[8] Yu Y, Wang H, Peng G, et al. Phosphodiesterase 5 inhibitors attenuate neuroinflammation induced by middle cerebral artery occlusion in mice. Oncotarget. 2017;8(46):80817-80830.

[9] Hendrix MJ, Sano M. Pharmacological treatments for Alzheimer's disease: beyond cholinesterase inhibitors. Aging Health. 2021;7(4):565-574.

[10] Niewöhner J, Bohrmann B, Collin L, et al. AAV9-mediated central nervous system–targeted gene therapy for a mouse model of Niemann-Pick disease type A. Sci Transl Med. 2018;6.