EFFECTS OF DIFFERENT MEDICATIONS USED TO TREAT ALZHEIMER'S DISEASE ON CAENORHABDITIS ELEGANS

Abstract

The most prevalent kind of dementia is Alzheimer's disease, which develops when the brain no longer functions properly. The main causes of issues are with behaviour, thinking, and memory. A complex ailment called Alzheimer's is characterized by abnormal protein accumulation in the brain regions. Although the precise molecular causes of this disease are not yet known, it is well acknowledged that a protein homeostasis network exists. When they serve as an enzyme substrate or a signaling molecule to activate a biochemical pathway, metabolites have an impact on crucial steps in cellular pathways. It is relatively new to model Alzheimer's disease in a basic microscopic organism like *C. elegans*. Worms that have the human amyloid beta peptide genetically altered to be expressed in muscle cells build up immunoreactive deposits of amyloid beta 1-42 as well as insoluble beta amyloid, which is seen in senile plaques in AD brains. The impact of various medications can be studied in *C. elegans*

Keywords: Alzheimer’s Disease, *Caenorhabditis elegans*, Amyloid beta, Amyloid Precursor Protein

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I. INTRODUCTION

1. **Alzheimer's disease:** German psychiatric Alois Alzheimer noticed an amyloid plaque and loss of memory before the death of patient which was a serious medical condition known as Alzheimer's disease (Zeinab Breijyeh et al., 2020). In Emil kraeplien 8th edition of psychiatry handbook Alzheimer's disease as a medical condition mentioned (Gabriele et al., 2011). There are 50 million AD patients at present worldwide which can be double every 5 years (Yiannopoliz et al., 2020). Some treatments that are available only to improve the symptoms, no permanent cure is there (Livingstone G et al., 2020). In Alzheimer's disease two types of neuropathological changes occur:

   - Positive lesions which include senile plaque formation and neurofibrillary tangles.
   - Negative lesions which include synaptic loss (Zeinab Brijyeh et al., 2020) At present, two types of hypotheses for Alzheimer's disease.

2. **Cholinergic Hypothesis:** Ach (Acetyl Choline) is secreted in brain and responsible of physiological activities like attention, memory, learning etc. In AD degeneration of cholinergic neurons was found which directly affect to memory loss (Ferreira veria et al., 2016).

3. **Amyloid hypothesis:** According to the amyloid hypothesis, ageing or pathological conditions slow down the degradation of Aβ, which is produced by secretase, which causes amyloid beta peptide (Aβ40 and Aβ42) to accumulate. An increase in the Aβ42/Aβ40 ratio causes the development of amyloid fibrils, which cause neurotoxicity, tau disease, and ultimately the death and degeneration of neuronal cells. Amyloid beta catabolism and anabolism were discovered to be affected by AD risk factors and mutations of multiple genes, including APP, PSEN1 and PSEN2, which quickly lead to an accumulation of and quick progression of neurodegeneration (Paroni et al., 2019, Kametani et al., 2018, Ricciarelli et al., 2017).

   There are several risk factors for Alzheimer's disease.

4. **Aging:** Aging is a significant risk factor for AD. Hardly ever does this disease affect young people, and most cases of AD begin in persons over 65. (Guerrieo et al., 2015).

5. **Genetics:** A number of genetic factors are crucial in the development of AD. The majority of instances were linked to genetic variables such apolipoprotein E, presenilin 1 and 2, and amyloid precursor protein. (Van Cauwebreghe et al., 2016, Khanahmadi et al.; 2015). Environmental factors – AD is also impacted by environmental risk factors such as air pollution, nutrition, metals, oxidative stress, and inflammation. (Wainania et al., 2014, Grank et al., 2002). Medical factors – Elderly AD sufferers frequently have other health issues, such as diabetes, obesity, and cardiovascular disease (Stampfer 2006 , Santos et al., 2017).

II. ELEGANS USE AS MODEL ORGANISM

In 1963, Sydney Brenner developed the *C. elegans* which belongs to phylum Nematode of the animal kingdom. It is a transparent nematode and multicellular organism having a 1mm length (Brenner 1988; Brenner 2002). The organism is fully sequenced genome and the size of genome is 9.7×10^7 bp (White *et al.* 1986). *C. elegans* has 60-80% homology similar to humans. It has a simple nervous system having 302 nerve cells. *C. elegans* is widely used for genetic study and to research on human disease. *C. elegans* used as a model organism because they are small, easy and inexpensive to rear in the lab as well as it reproduce quickly (Sulston and Horvitz 1977; Kimble and Hirsh 1979; Sulston *et al.* 1983).

Usage of Drugs for Alzheimer's Disease

(Emeline Teo et al.,2019, Michele perni et al.,2021, M Obulesu et al.,2011)

Compounds	Mode of action
Mito Q	It is an oral antioxidant with the capacity to specifically target mitochondrial dysfunction.
N- acetylcysteine	It is antioxidant and used in treatment of cancer.
Butylated Hydroxy-anisole	It is lab made chemical which is used for food preservative and treatment in genetic disorder.
Met formin	It is a metabolic modulator that improve stress resistance.
Alpha -ketoglutarate	It extends life span via ATP synthesis and mTOR inhibition.
Lithium	It is type of medicine known as mood stabilizer. It is a salt which is used as psychiatric medication, primarily for bipolar disorder. It is metabolic modulator and anti- aggregation class.
Rapamycin	It is also called sirolimus, which suppress the immune system which led to prevention of transplant rejection. It is produced by soil streptomyces hygroscopicus.
Thioflavin -T	It is a anti aggregation and reduces paralysis. It extends life span and involve in the gene of protein homeostasis. It is stain which is used for monitor invitro amyloid fibril formation

Curcumin	It is anti -aggregation, antioxidant and anti-inflammation drug. It is stress resistance and induces stress response gene
Carnosine	It is used for preventing aging and complication of diabetes such as nerve damage kidney problem.
Kynurenic acid	It acts as an ant excitotoxic and anticonvulsant. This acid possesses neuroactive activity

III. EFFECT OF DRUGS ON C. ELEGANS

Three approaches exist for focusing on drugs: (S.S. Davis 1997, R. K. Keservani et al., 2017) First-order drugs target a particular organ or tissue; second-order drugs target individual cells; and third-order drugs target an intracellular compartment, such as the nucleus, endoplasmic reticulum, or mitochondria.

1. **Morphological study:** Mito Q

Circular mitochondrial DNA serves as the self-replicating genome of mitochondria (mtDNA). One animal cell typically has 100–10,000 copies of mtDNA. Ribosomal RNA, mitochondrial transport RNA, and a few hydrophobic ETC proteins are all encoded by human mtDNA. Inherited mitochondrial illnesses are caused by mutations in either mtDNA or nuclear DNA genes that code for mitochondrial proteins. (D. C Wallace et al., 2010).

The amyloid cascade theory has eclipsed others to date, although the pathophysiology of AD is still not fully understood. Nevertheless, none of the medication candidates blocking amyloidβ -peptide buildup have even been able to postpone this condition. Consequently, it was believed that the fundamental factor causing amyloid -peptide (Aβ) deposition, synaptic degeneration, and the development of neurofibrillary tangles in the sporadic type of AD is mitochondrial malfunction. (R. H Swerdlow et al.,2010) The findings of mitochondrial malfunction in AD patients lends credence to this theory (I. G. Onyango et al., 2016).also in animal models (M. Manczak et al., 2006; J.W. Lustbader et al., 2004). Additionally, according to P. I. Mereia et al. (2010), this method pinpoints novel therapeutic targets found in the mitochondria. A covalent bond between a ubiquinone molecule and the lipophilic decyltriphenylphosphonium (dTPP) cation is used to form the mitochondria-targeted ubiquinol known as mitoQ. (kelso et al., 2001). Due to the high mitochondrial membrane potential, MitoQ is intended to concentrate specifically several hundred times in the mitochondrial matrix. (J .M Macmanus et al.,2011, R.A smith et al., 1999). By using various assays, such as the paralysis assay and the stress tolerance assay, the Alzheimer's disease model CL2006 demonstrated that mitoQ may represent a potential therapy against Aβ- induced toxicity and oxidative stress. (Li fang Ng et al., 2014).

2. **N- Acetylcysteine:** In mucolytic therapy and to treat paracetamol overdose, N-acetylcysteine (NAC), a precursor to L-cysteine, is frequently utilised. (Giuseappe Tardiolo et al., 2018). NAC is regarded as a drug that exhibits pro-neurogenic and neuroprotective qualities. (R. S Bavarsad et al., 2014). Moreover, it is applied in treatments for mental and neurodegenerative illnesses. (L.s Ooi et al., 2018). NAC has undergone testing as a drug, serving as a precursor for the creation of GSH, and displaying potential actions that suggest an alternative as a potential future drug. (Y Hara et al.,2017). NAC's down-regulation suggests that it may contribute to the transcription of the APP gene. In actuality, neuroblastoma cells don't have any detectable APP mRNA levels. Such activity is associated with decreased NF-kB binding activity, which is boosted by oxidative stress and Aβ. (R. Studer et al., 2001). In *C. elegans* strain CF512, ASSNAC enhances glutathione concentration, enzyme activity, and the expression of the GST gene. This boosts the organisms' tolerance to oxidative stress and lengthens their life span. (Napthtali savior et al., 2018). examined the potential benefits of NAC supplementation on resistance to a range of environmental stressors, such as heat shock, UV irradiation, and oxidative stress in vivo on the N2 wild type strain of C. elegans. It also examined the effect of NAC on fertility, which is closely linked to ageing. Oh, Seung II, and colleagues (2015).

3. **Butylated Hydroxy-Anisole:** Three phenol model molecules are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and bisphenol-A (BPA). (Bashar Alhoch et al., 2019) Several prevalent human diseases, such as cancer, diabetes, inflammatory conditions, and various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, are characterised by a pathological stress response. **(**S. Fuida et al., 2010, R karam et al., 2015). BHA exhibits an antioxidant effect and is a food supplement that affects the gastrointestinal tract and extends life in N2 wildtype strain. (Sebastain schmeisser et al.,2013, M. Scheieber et al., 2014) and further mechanism is not studied yet.

4. **Met formin:** For managing hyperglycaemia in diabetics, metformin is a highly effective first- line medication. It is a desirable candidate for medication repurposing to treat age-related diseases that are reliant on and independent of glycaemic control. (Jie Zheg et al., 2022). Incident dementia is one of the main objectives in large trials like Targeting Aging with Metformin (TAME). (N Brazilai et al., 2018). Studies have shown a strong correlation between Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD), pointing to possible shared pathophysiological processes between the two conditions. Glucose metabolism balance, reduced amyloid plaque formation, normalised tau protein phosphorylation, and increased autophagy are possible effects of metformin. (Ping Ping Ning et al., 2022). The lifetime extension effect of metformin is mediated by both v-ATPase-mediated TORC1 inhibition and v-ATPase-AXIN/LKB1-mediated AMPK activation in *Caenorhabditis elegans*. (Jie Chen et al., 2017). In the *C. elegans* Parkinson's disease model, met formin exhibits neuroprotective effects via α synuclein aggregation. (Nada Saewanee et al., 2019).

5. **Alpha – Ketoglutarate:** They play a crucial role in the study of Alzheimer's and mitochondrial illness because they are a crucial Krebs cycle enzyme that is very susceptible to the damaging effects of ROS. (Dora Csaban et al., 2021). Astroctyes' ability to produce ATP is compromised by oxidative stress brought on by a variety of conditions,

including aluminium poisoning, as a result of malfunctioning mitochondria. As a result, globular, glycolytic, lipogenic, and ATP-deficient astrocytes are produced, which are cerebral traits that are typical in AD patients. (S C Thomas et al., 2015). The development of transgenic AD strains (GRU102) in *C. elegans* that express human Aβ-peptides in various cell types, including neurons, muscle, or specific subsets of neurons, and under various promoters, including temperature-inducible or constitutive, has confirmed AD-like pathology with varying disease severity. (Emelyne Teo et al., 2019). Some medications that affect metabolism have positive effects on AD, but the underlying mechanisms are still poorly understood. Hence, significant metabolic abnormalities seen in GRU102 may partially be explained by aKGDH activity inhibition, as seen in this mutation. Inactivation of aKGDH may explain the general decrease in spare respiratory capacity and energy deficits in the GRU102 animal in addition to the observed fall in levels of TCA cycle metabolites downstream of aKG (malate and fumarate). (Butterfield et al., 2006).

6. **Lithium:** In the treatment of Alzheimer's disease and its prodromal stages, lithium may be a therapeutic alternative. But more research on its medicinal effectiveness is necessary. Head-to-head comparisons with recognised dementia therapy alternatives should be a part of future research. Due to the therapeutic toxicity of lithium, careful patient selection and intensive therapeutic monitoring are required. (Robert Haussmann et al, 2021). For more than 60 years, bipolar disorder (BD) has been treated pharmacologically using lithium as a mood stabiliser. After oral treatment, lithium is quickly and completely absorbed from the digestive tract. Its concentration is initially highest in the serum before being clearly dispersed to other tissue compartments. (Jinhuawen et al., 2019). Lithium and metformin were studied in combination in transgenic animals using the GRU102 strain in an effort to prolong lifespan. (Emelyne Teo and Sheng Fong et al., 2020).

7. **Rapamycin:** Rapamycin, originally discovered in soil samples from Easter Island by Georges Nogrady in the late 1960s, is a macrocyclic lactone produced by the bacterium Streptomyces hygroscopicus. This compound is also commercially available under the trade names sirolimus and rapamune (Ramasamy Selvarani et al., 2021).

   Maf1, a kinase associated with the mechanistic target of rapamycin (mTOR), plays a role in the regulation of aging. However, its precise involvement in lifespan extension induced by mTOR inhibition, such as calorie or dietary restriction, remains unclear. In our study, we found that the deletion of maf1 hinders the extension of lifespan in the budding yeast S. cerevisiae through dietary or calorie restriction. Interestingly, this effect does not manifest in C. elegans due to the presence of mafr-1 (Ying Cai and Yue Hua Wei, 2016).

8. **Thioflavin –T:** The thioflavin T assay, along with behavioral, biochemical, and histological analysis, were used to assess the anti-aggregating effects of natural chemical compounds given to transgenic mouse models of Alzheimer's disease. (Stefanescu-Raluca and others, 2020). Thioflavin T molecules are bound by beta-amyloid fibrils and oligomers, but not by beta-amyloid monomers, which have no chemical interactions. (M .Biancalana et al., 2010 , I Maezawa et al., 2008). The created a peptide called A3 that, by increasing the aggregation kinetics, considerably improved the creation of amorphous aggregates of Aβ. Thioflavin T fluorescence tests showed faster A monomer

aggregation along with diminished Aβ cytotoxicity. The paralysis caused by the buildup of Aβ oligomers in transgenic *Caenorhabditis elegans* over-expressing amyloid precursor protein might be reduced by giving the worms A3 peptide. These data imply that the Aβ-aggregation-promotion effect may be helpful for creating Aβ-toxicity reduction techniques. (Aihua Yang et al., 2017).

9. **Curcumin:** Turmeric, derived from the Curcuma longa plant, also known as Haldi, is a common ingredient in the spicy dishes of India, Asia, and the Middle East (Shrikant Mishra and Kalpana Pulanivelu, 2008). Throughout history, it has held a significant place in Ayurveda, the Indian system of medicine, where it has been employed as an effective pain reliever and anti-inflammatory agent to alleviate muscle and skin discomfort. It has also exhibited anti-cancer properties (S Shishodia et al., 2005, HP Ammon et al., 1991).

   Maintaining the right diet and practicing calorie restriction is crucial for healthy aging. Curcumin, a polyphenolic compound derived from Curcuma longa, has recently shown its potential for promoting anti-aging effects. Research on curcumin and its impact on age-related conditions in model organisms has indicated that curcumin and its metabolites can extend the average lifespan of certain aging model organisms, including C. elegans (Aliabbas Zia et al., 2020).

10. **Carnosine:** Natural endogenous chemical carnosine has been the subject of intense research in recent years because of its potential to have positive impacts on human health. (Cristina Solana Manrique et al., 2022). Along with its analogues homocarnosine, anserine, and ophidine/balenine, carnosine (β-alanyl-L-histidine) is a histidine-containing dipeptide (HCD) that is extensively found in mammalian tissues (F. Bellia et al., 2014, G. Caruso et al., 2019). Though it's also present in the brain, the skeletal and cardiac muscles contain the most of this dipeptide. Identified carnosine (-alanyl histidine) as a potential treatment for vascular dementia after searching numerous agricultural products for compounds that shield neurons from Zn2+-induced neurotoxicity. (Masahiro Kawahara et al., 2020).
11. **Kynurenic acid:** Tryptophan's metabolite kynurenic acid (KYNA), which is produced by the kynurenine pathway, has neuroprotective properties both in vitro and in vivo. As new KYNA targets were found, the compound's unique biological function came to light. In Huntington's, Parkinson's, and Alzheimer's disorders, absolute or relative deficit of KYNA in comparison to neurotoxic kynurenines suggests that increasing brain KYNA levels may have therapeutic significance. (Aleksandra ostapiuk and Ewa M. Urbanska 2022). Analogs of the naturally occurring chemical kynurenic acid (KYNA) are being created, and their pharmacological effects are being studied, in hopes of developing multifaceted treatments for Alzheimer's disease (AD). The effects of synthesized KYNA analogues on NMDA receptor binding, mGluR5 binding and function, acetylcholinesterase (AChE) inhibition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and interference with the amyloid peptide (Aβ) fibrillation process were studied in transgenic Caenorhabditis elegans strain GMC101 expressing full-length Aβ42. (Girdhar Singh Deora et al., 2017). They investigated the roles of two metabolites, carnosine and kynurenic acid, in the breakdown of protein aggregates. In a C. elegans model of AD, these two metabolites reduce Aβ42 aggregation by inducing a cytosolic unfolded protein response mediated by HSF-1 and higher levels of the J-proteins DNJ-12 and DNJ-19 (Priyanka Joshi et al., 2021).

IV. MOLECULAR STUDY

The Alzheimer's disease hypothesis states that certain proteins and genes cause AD. Hence, some molecular methods for protein profiling and gene expression were investigated. These techniques were used to examine the effects of various medicines in *Caenorhabditis elegans*, including metformin, N-acetylcysteine, and kynurenic acid.

Gene expression by Real time polymerase chain reaction: Real-time quantitative PCR (RT-qPCR) is a technique employed to monitor PCR reactions as they unfold. RT-qPCR serves two primary purposes, categorized as absolute and relative quantification. Relative quantification is typically applied in genomics and functional transcriptomics to assess gene expression in biological experiments. In contrast, absolute quantification is widely utilized across various domains, such as microbiology, food technology, and biotechnology, to measure parameters like microbiological load, the presence of adulterants in products, and copy numbers, respectively (Ravikumar Harshita and Durai pandian Rex Arunraj, 2021).

V. PROTEIN PROFILING

1. **Sds Page:** By forming a complex with the potent cationic detergent sodium dodecyl sulfate (SDS) and undergoing separation through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the migration distance of a protein can be leveraged for the determination of its apparent molecular weight (Hiroyuki Matsumato et al., 2019).

2. **Western blotting:** Biologists frequently employ the well-known molecular biology technique known as Western blotting (WB), also referred to as immunoblotting, to explore a variety of protein properties, from basic protein analysis to disease detection. WB is a straightforward, distinctive, speedy, routine instrument with simple interpretation and clear outcomes. (Habeebunnisa Begum et al.,2022)

VI. CONCLUSION

Only a few drugs are now available due to the Alzheimer's disease's unclear pathological mechanism, which limits the creation of novel treatments. Several medications have a favorable morphological impact on *C. elegans*. However, certain aspects, such as genetic factors presenilin 1 & 2, and protein hypothesis, will determine if the molecular study will be a novel technique. The basic nervous system of *C. elegans* can be utilized as a model organism. A model of Alzheimer's disease that has been genetically altered can be utilized to profile protein and gene expression. The effect of a medicine on a certain gene or protein is understood, which will help with further clinical trials to determine which drug is more effective for AD, an inherited neurological degenerative illness. Using bioinformatics tool like docking can be used to assess the impact of medication on amyloid beta protein.

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