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ANTICHOLINERGIC BURDEN AND RISK OF COGNITIVE IMPAIRMENT IN OLDER ADULTS

A thesis (or dissertation) submitted in partial fulfillment of the requirements for the degree of
Master of Pharmaceutical Sciences with a concentration in Pharmacotherapy and Outcome
Sciences at Virginia Commonwealth University

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Aging Populations Core

Virginia Commonwealth University Richmond, Virginia

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DEDICATION

I dedicate this project to *my husband*, who taught me it's never too late to start a career to pursue your true passion, and that context is everything.

I would also like to dedicate my thesis to my parents, Ammi and Abbu and siblings, Saad, Zunera, Nusra, and Fahad.

“Even though miles apart, you guys believed in me, and showed your continuous support emotionally, spiritually, and morally. Thank you, and love you always. Can’t wait to see you guys again.”

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ABBREVIATIONS

ACM- Anticholinergic medications

ACB - Anticholinergic cognitive burden

ADR - Adverse Drug Reactions

AD – Alzheimer’s disease

AGS - American Geriatrics Society

Ach – Acetylcholine

ALS – Amyotrophic lateral sclerosis

CI - Cognitive impairment

CNS – Central Nervous System

ChAT- Choline acetyltransferase

C_{\max} – maximum concentration

cDNA - Complementary Deoxyribonucleic Acid

CYP2D6 – Cytochrome P450 2D6

CREB1 – CAMP responsive element binding protein 1

CCL2- Chemokine ligand 2

CXCL5 – C-X-C motif chemokine 5

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

GRM1 – Glutamate metabotropic receptor 1

GRM2 – Glutamate metabotropic receptor 2

GCLM – Glutamate- cysteine ligase modifier

IL-6 - Interleukin - 6

IL-1 β – Interleukin 1 beta

mRNA – messenger ribonucleic acid

MCP-1 - Monocyte chemoattractant protein

NHA - Normal Human Astrocytes

NHBMECs - Normal Human Brain Microvascular Endothelial Cells

PCR – Polymerase chain reaction

PPARG – peroxisome proliferator-activated receptor gamma

RNA – Ribonucleic acid

RT-PCR – Real-time polymerase chain reaction

SSRIs – Selective serotonin reuptake inhibitors

SOD1 – Superoxide dismutase

STOPP - Screening Tool of Older Persons Prescription

START - Screening Tools to Alert Doctors to Right Treatment

TCAs – Tricyclic antidepressants

US – United States

WHO- World Health Organization

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ABSTRACT

ANTICHOLINERGIC BURDEN AND RISK OF COGNITIVE IMPAIRMENT IN OLDER ADULTS

By, SYEDA REHMA HASHIMI, PharmD

A thesis (or dissertation) submitted in partial fulfillment of the requirements for the degree of Master of Pharmaceutical Sciences with a concentration in Pharmacotherapy and Outcome Sciences at Virginia Commonwealth University.

Virginia Commonwealth University, 2020

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Aging Populations Core

Studies reveal that 10-27% of older adults chronically use anticholinergic medications. Increased cumulative anticholinergic burden scores are associated with increased risks of dementia. The mechanisms by which anticholinergic drugs negatively impact cognition remain elusive. However, researchers speculate that the drug's impairment of cholinergic neurons promotes neuroinflammation.

Research question or Hypothesis

We hypothesize that drugs with anticholinergic properties will induce inflammation in the brain. MCP1 and IL 6 are chemokines that contribute to neuroinflammation. We investigated the influence of diphenhydramine (Benadryl) on the production of MCP1 and IL-6 in Normal Human Astrocytes and Paroxetine on the production of MCP1 in Normal Human Astrocytes and Normal Human Brain Microvascular Endothelial Cells.

Methods

Normal Human Astrocytes were cultured (seeded at 5,000 cells/cm²) and treated with a clinically relevant concentration (equivalent to clinically observed C_{max} for the respective drugs) of diphenhydramine (66ng/ml) and Paroxetine (1.67×10^{-4} mM). RNA was isolated and converted into cDNA, and then gene expression was measured via RT-PCR. MCP1 and IL-6 protein production were measured from cell culture supernatants by ELISA. One-way ANOVA compared MCP-1 and IL-6 protein concentrations with Tukey correction for multiple corrections. The gene expression was reported with relative significance ($p = 0.05$) using Student's t-test.

Conclusions

Drugs with anticholinergic properties are considered to be pro-inflammatory. However, our data from normal human astrocytes treated suggest that diphenhydramine possesses anti-inflammatory properties. This preliminary finding suggests the possibility of pathological mechanisms of anticholinergic medications with dementia that may not be associated with inflammation.

Our data from Paroxetine in normal human brain microvascular endothelial cells suggest that Paroxetine possesses pro-inflammatory properties. Our preliminary findings indicate the possibility of a pathological pro-inflammatory mechanism associated with Paroxetine. Further evaluation of anticholinergic drugs in the human brain is warranted.

1. INTRODUCTION

In the 20th century, astonishing population growth was aging in the United States. In 1900, 3.1 million Americans were 65 years and older, representing 4% of the total population. According to the Census Bureau, in 1986, the population had grown to 29.2 million persons or 12.1% of the total population. The population projections of the 21st century still indicate the continuation of growth. Those 85 years and older are the most rapidly growing population in terms of actual numbers and percentages of the total population. Concurring to the Social Security Administration, the number of individuals at or above age 65 is expected to rise from thirty-four million in 1995 to eighty-seven million in 2080. Whereas, the population at or above age eighty-five is expected to grow from 3.8 million in 1995 to 18.3 million in 2080. (Biology of Aging- Handbook)

1.1 AGING

Aging is the process of growing older. It is a gradual, continuous, and complex process associated with dynamic biological, physiological, psychological, and behavioral changes. Even though aging is associated with mild changes such as graying of hair, some significant age-related changes can decline daily-life activities and increase vulnerability to various diseases, frailty, and disability. Aging is a substantial chance for many acute and chronic conditions, including neurodegenerative disease. There is no single principle to explain aging. However, studies have shown that aging can be slowed, suggesting that we can delay the appearance or reduce the number of diseases and increase older adults' health span by aiming for aging. Researchers are presently centering on hereditary, natural life-style, behavioral and

social components, and their effect on the initiation and progression of age-related diseases and neurodegenerative conditions. (www.nia.nih.gov)

1.2 COMPLEXITY OF AGING

More than 248 million of roughly 418 million people aged 65 years and over (59%) now reside in the developing countries. The world's elderly distribution will be shifting considerably in the upcoming decades, with a growing proportion of 67% by 2020. Asia has the first noteworthy number of older adults, 217 million. China alone has 87 million people (6.8 percent of its populace) aged sixty-five year and older, and this will increment to 167 million (11.5 percent) by 2020.¹

With advancing age, there is an increased vulnerability to chronic health problems. New diagnoses will further add complexity to the health status of older adults with pre-existing health problems. Many health disorders experienced by older individuals are commonly managed with medications. Antibiotics, cardiovascular, psychotropics, anticholinergics are used routinely to treat health disorders observed in older adults. However, drug development clinical trials often exclude older adults.² A survey conducted in Australia, New Zealand, Canada, United Kingdom, and the United States, showed that approximately 75% of those aged 65 took at least one prescription drug regularly for chronic medical conditions.³

Multimorbidity that increases with social deprivation and age is also associated with functional decline, more reduced quality of life, increased mortality, and significantly more healthcare usage. Multimorbidity is characterized by two or more chronic conditions that cannot be cured and are controlled through medications and other treatments.⁷

The prevalence of polypharmacy is also common in this segment of the population. Polypharmacy defined as prescribing a threshold of five to seven medications or more. Inappropriate medication use, improper drug use, inappropriate prescribing, potentially inappropriate prescription are the terms used to recognize under-prescribing, over-prescribing, and mis-prescribing practices ⁴. Multiple cohort studies have recently reported increased, potentially inappropriate medicines in acute and long-term settings in community-dwelling older people. Furthermore, these studies revealed associations between inappropriate medications and increased risks of adverse drug reactions, morbidity, and mortality. ⁵ The use of multiple medicines, especially with high anticholinergic properties in older adults, leads to increased cognitive function risks, impaired judgment, and a decline in intellectual understanding. During the hospital stay, older adults with cognitive impairment are also at an increased risk of delirium, resulting in extended hospital stays and increased thirty-day mortality rates. ⁶

Although drug therapy is considered central in most chronic medical conditions experienced by older adults, a substantial number of these individuals also experience adverse drug reactions (ADR). ADRs are a severe health problem, and it is reported that older adults are seven times more likely than younger adults to have ADRs that require hospitalization. The number of prescription drugs is the primary indicator of risk for an adverse drug reaction. As the number of prescriptions increases in older adults, the odds of an adverse drug reaction, especially cognitive impairment, are increased. ⁸ Cognitive impairment (CI), in older people, is broadly definable ADR. Some drugs are associated with increased risks of depression, suicide, seizures, and confusion. These symptoms are more challenging to assess and can impact the cognitive abilities of older adults.

Drugs with anticholinergic properties are prescribed widely to older adults, but adverse effects often restrict their use. Many commonly used medications, such as digoxin, psychotropics, and anticholinergic (muscarinic-blocking) properties, have been consistently associated with increased cognitive impairment risks. Several alterations in intrinsic physiological factors, such as pharmacokinetics and pharmacodynamics changes, also put older adults at increased chances of developing cognitive impairment from medications. ²

2. TOOLS TO ASSESS QUALITY OF GERIATRIC CARE

In recent years, several criteria have been developed to classify medication usage's appropriateness while protecting older adults from adverse events. The Beer's criteria, Screening Tool of Older Persons Prescription (STOPP), and Screening Tools to Alert Doctors To Right Treatment (START) are the best known and widely studied in the US and Europe. ⁹

2.1 BEER'S CRITERIA

The American Geriatrics Society (AGS) Beer's criteria for Potentially Inappropriate Medication (PIM) Use in Older Adults is utilized broadly by clinicians, educators, researchers, healthcare administrators, and regulators. The AGS Beers Criteria is a list of potentially inappropriate medications that should be avoided by older adults in most cases or under specific situations, such as in certain diseases or conditions. The five types of criteria in the 2019 updated Beers criteria include:

1. Medications that are potentially inappropriate in most older adults.
2. Those that should typically be avoided in older adults with certain conditions.

3. Drugs to use with caution.
4. Drug-drug interactions.
5. Drug dose adjustment based on kidney function.

The Beers criteria are considered for use in adults 65 years and older in all ambulatory, acute, and traditional care settings, except for the hospice and palliative care settings. To improve medication selection, educate clinicians and patients, reduce adverse drug events, and serve as a tool for evaluating the quality of care, cost, and drug use patterns of older adults. The primary objective of the 2019 update proceeds to be improving the care of older adults by diminishing their exposure to Potentially Inappropriate Medications (www.americangeriatrics.org).

3. LITERATURE REVIEW

Several investigators have reported the use of anticholinergics associated with sustained cognitive defects in older adults. A prospective population-based cohort study was conducted to examine if cumulative anticholinergic use is associated with an increased risk for incident dementia. The researchers used data from the Adult Changes in Thought study in Group Health. The study included 3434 participants aged 65 or older with no dementia. The most anticholinergic classes used were tricyclic antidepressants, first-generation antihistamines, and bladder antimuscarinics. They observed that during the mean follow-up of 4.8 years, 23.2% developed dementia, whereas, 79.9% considered to have possible Alzheimer's disease (AD).¹⁰

A longitudinal study was conducted between 2 cohorts (participants taking medium or high anticholinergic medications compared to those not taking these medications) to determine the association between anticholinergic medications use and cognition, glucose metabolism and

brain atrophy in cognitively healthy older adults from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Indiana Memory and Aging Study (IMAS). They found that medications with medium or high anticholinergic activity were associated with imperfect memory, brain hypometabolism, brain atrophy, and increased cognitive impairment risk. This study was the first to examine the in vivo brain structural and functional differences.¹¹

Selective Serotonin Reuptake Inhibitors, including Fluoxetine, Paroxetine, Citalopram, Sertraline, Escitalopram is the most prescribed type of antidepressants. But little is known if there is a connection between depression and dementia. A prospective cohort study was conducted in community-dwelling individuals aged 65 years and older adults without dementia, to determine if antidepressants (serotonin reuptake inhibitor- Paroxetine, tricyclic antidepressants, serotonin antagonist and reuptake inhibitors) use was associated with increased risks of dementia. The findings concluded that 25% of the participants developed dementia, whereas 22% developed possible Alzheimer's disease. Paroxetine was found to be most associated with increased dementia risk.¹²

4. ANTICHOLINERGICS

A vast number of medications on the updated (2019) Beer's list are considered potentially inappropriate due to their anticholinergic properties. Older adults subjected to increased anticholinergic load due to the extensive use of anticholinergic/antimuscarinic medications and the medications with anticholinergic side effects (antihistamine, antidepressants, skeletal muscle relaxant, antipsychotics, antispasmodic). Older adults widely use anticholinergics medications as an OTC or as a prescription for diverse conditions such as seasonal allergies, overactive bladder, and depression. The predominance of anticholinergic use in older adults ranges from 8-37%.¹³ The risks may outweigh the benefits of using anticholinergics in older adults. Well-known chances of anticholinergic burden are falls, worsened functioning, and worsened cognition. Older adults are more sensitive to anticholinergic effects due to pharmacokinetic and pharmacodynamic changes, diminished acetylcholine transmission in the brain, and increased permeability of the blood-brain barrier. It is suspected that the cognitive impairment associated with these agents can be reversible on the medications' discontinuation. Several investigators have still reported that the anticholinergics can be related to sustained cognitive defects such as mild cognitive impairment or dementia.¹⁰

4.1 ANTICHOLINERGIC BURDEN LIST

Anticholinergic medications are classified on an anticholinergic burden list. The ACB list has been previously used to identify cognitive impairment risks in many populations using anticholinergic drugs. The Anticholinergic Cognitive Burden List identifies anticholinergics with

varying degrees of anticholinergic activities. Medications on the Anticholinergic Cognitive Burden list are classified into two groups:

1. Medications with possible anticholinergic effects: those with *in-vitro* anticholinergic activity with no reported evidence of clinical anticholinergic properties.
2. Medications with definite anticholinergic activity: those with functional and clinically relevant effects on cholinergic signaling. ⁶

It is recommended that clinicians, pharmacists, and healthcare professionals consider the anticholinergic properties of medications when adding or changing the patient's drug regimen to minimize the anticholinergic side effects (Rochon, Paula., 2020)

4.2 MECHANISM OF ACTION OF ANTICHOLINERGICS

Generally, anticholinergics produce their effects by blocking acetylcholine (ACh), which acts as a neurotransmitter at the presynaptic terminal at the central and peripheral nervous system. They inhibit the parasympathetic nerve impulses by selectively blocking the binding of acetylcholine to nerve cells. Apart from producing therapeutic effects, some anticholinergics produce some therapeutic results that are not primary therapeutic effects. The mechanism by which they produce these effects is still unknown, but researchers have speculated that the cholinergic neuron's direct impairment may underlie these effects. ¹⁰

5. THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

The inflammatory reflex is stimulated when the afferent vagus nerve senses the inflammatory products such as cytokines, damage-associated pathogen patterns through the cytokine receptor, and pattern recognition receptors. The nerve activity is transfer through the central nervous system (CNS) to the efferent vagus nerve. The first pathway involves the splenic nerve, a coordinate association between the afferent vagus nerve and splenic nerve is still debatable. Triggered splenic nerves release norepinephrine from their terminals, which interacts with β 2-adrenergic receptors, expressed on the choline acetyltransferase (ChAT)-positive T cells within the spleen, causing the release of acetylcholine (ACh) for specific T cell. Acetylcholine binds to α 7 nicotinic acetylcholine receptors (α 7nAChRs) expressed on macrophages, resulting in suppressing proinflammatory cytokine production by macrophages.¹⁴

5.1 HYPOTHESIS

Cholinergic signaling is anti-inflammatory; we are hypothesizing that anticholinergic medications will induce inflammation in the brain.

6. SPECIFIC AIMS

- 1. To characterize the effect of Diphenhydramine on the physiological and pathophysiological properties of normal human astrocytes.*
- 2. To characterize the effects of Paroxetine on the physiological and pathophysiological properties on normal human astrocytes.*
- 3. To characterize the effects of Paroxetine on the physiological and pathophysiological properties on normal human brain microvascular endothelial cells.*

7. DIPHENHYDRAMINE

Diphenhydramine hydrochloride is an antihistamine drug with a chemical name of 2-(diphenylmethane)-N, N -dimethyl ethylamine hydrochloride with a molecular formula $C_{17}H_{21}NO \cdot HCl$ (molecular weight 291.82) (www.accessdata.fda.gov)

7.1 PHYSICOCHEMICAL PROPERTIES

It occurs as a white odorless, crystalline powder and soluble in water and alcohol.

7.2 DOSAGE

Adults: 25 to 50 mg, three or four times daily.

The nighttime sleep-aid dosage is 50 mg at bedtime.

7.3 STORAGE

Keep tightly closed—store at 20° to 25°C (68° to 77°F). Protect from freezing and light.

7.4 PHARMACOLOGY OF DIPHENHYDRAMINE

Diphenhydramine hydrochloride is an antihistamine with anticholinergic and sedative effects.

Diphenhydramine (Benadryl) is a first-generation H₁ antagonist that acts as a reversible competitive inhibitor of histamine, which binds to the H₁ receptor.

7.5 USAGE

Diphenhydramine hydrochloride is used to relieve runny nose, itching of the nose or throat, and itchy, watery eyes from hay fever or upper respiratory allergies, for active and prophylactic treatment of motion sickness and nighttime sleep aid.

7.6 PHARMACOKINETICS OF DIPHENHYDRAMINE

Diphenhydramine pharmacokinetics in adults for single doses of 25 and 50 mg diphenhydramine HCl have been reported in several studies. Following the intravenous administration of 50 mg, diphenhydramine is observed at a maximum concentration of 66ng/ml (concentration used in the experiments to treat the normal human astrocytes and normal human brain microvascular endothelial cells). Diphenhydramine's total systemic clearance is 6.16 mL/min/kg, the volume of distribution is 4.54 L/kg, and its exponential half-life is 8.5 hours. Following oral administration of 50 mg in studies conducted, diphenhydramine attained maximum concentrations at 2.3 and 2.2 hours and had exponential half-lives of 9.2 and 9.8 hours. Diphenhydramine undergoes the first-pass metabolism after oral administration with an absolute bioavailability of $72\% \pm 8\%$, with about 2% of the dose remaining unchanged in the urine. Diphenhydramine is metabolized via demethylation to N-demethyl diphenhydramine, which is demethylated to N, N-didemethyl diphenhydramine further metabolized by oxidative deamination to diphenylmethoxyacetic acid. The initial N-demethylation has not been reported to be related to cytochrome p450 2D6 (CYP2D6) activity. Still, the subsequent N-demethylation has reduced in subjects who were phenotype as CYP2D6 poor metabolizers. In drug-interaction pharmacokinetic studies with venlafaxine and metoprolol, diphenhydramine was shown to be an inhibitor of CYP2D6 ¹⁵

8. NORMAL HUMAN ASTROCYTES

Michael von Lenhossék introduced the term astrocyte (Astron, star, and kytos, a hollow vessel, later cell, i.e., star-like cell) in 1895. Astroglia belongs to a class of neural cells (also known as astrocytes) of ectodermal, neuroepithelial origin that maintain homeostasis and provide a defense of the central nervous system (CNS). Astrocytes are highly diverse in form and function and exhibit high adaptive plasticity, which helps in functional maintenance of the CNS in development and aging. Astrocytes are involved in controlling homeostasis of the CNS at all levels of the organization. Through transporting the significant ions and protons, removing and catabolizing neurotransmitters, releasing neurotransmitter precursors, and scavengers of reactive oxygen species, astrocytes maintain the molecular homeostasis. Astrocytes are also involved in supporting the neurotransmission and controlling cellular homeostasis through embryonic neurogenesis (that occurs from radial glia) and adult neurogenesis (which includes stem astrocytes of neurogenic niches). By synthesizing glycogen and supplying neurons with energy substrates, astrocytes regulate the metabolic homeostasis.¹⁶

9. CHEMOKINES (MCP-1) AND CYTOKINE (IL- 6)

Chemokines are small heparin-binding proteins that constitute a large family of peptides (60–100 amino acids) related to cytokines structurally. The primary function is to regulate cell trafficking. Chemokines can be classified into four subfamilies based on the number and location of the cysteine residues at the N-terminus of the molecule and are named CXC, CC, CX3C, and C. The structure of chemokines is composed of three distinct domains: (1) a highly

flexible N-terminal domain, which is constrained by disulfide bonding between the N-terminal cysteine, (2) a long loop that leads into three antiparallel β -pleated sheets; and (3) an α -helix that overlies the layers. Chemokines are divided into two main functional subfamilies: inflammatory and homeostatic chemokines. Inflammatory chemokines control the recruitment of leukocytes in inflammation and tissue injury, whereas, homeostatic chemokines attain housekeeping functions such as navigating leukocytes to and within secondary lymphoid organs and the bone marrow and the thymus during hematopoiesis.

Chemokines induce chemotaxis by activating G-protein-coupled receptors (GPCRs), including adhesion molecules and glycosaminoglycans (GAGs). Chemokines bind to specific cell surface transmembrane receptors coupled with heterotrimeric G proteins, which leads to the activation of intracellular signaling cascades, which further causes the migration towards chemokines.

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family and a potent chemotactic factor for monocytes. Located on chromosome 17 (chr.17, *q11.2*), human MCP-1 is composed of 76 amino acids. MCP comprises four members (MCP-1, -2, -3, and -4). CCL2 is produced by various cell types, either by oxidative stress, cytokines, or growth factors. CCL2 is produced by many cell types, such as endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells. CCL2 regulates monocytes migration and infiltration, memory T lymphocytes, and natural killer (NK) cells.¹⁸

Cytokines are small proteins, Interleukin-6 (IL-6) is a cytokine which induces the maturation of B cells into antibody-producing cells. The essential sources of IL-6 in the CNS are neurons, astrocytes, microglia, and endothelial cells. IL-6 participates in neurogenesis, influencing both

neurons and glial cells, oligodendroglialogenesis, and astroglialogenesis. IL-6 is a prototypical four-helix bundle cytokine.¹⁹

10. NEURODEGENERATIVE GENES

Glutamate is the major excitatory neurotransmitter of the nervous system and is involved in memory and neuronal activity. In neuronal death, the overstimulation of glutamate has been reported. There are two forms of glutamate receptors, ionotropic and metabotropic. Metabotropic glutamate receptors (mGluRs) have eight subtypes so far. They are divided into subgroups (Group I: mGluR1 and mGluR5, Group II: mGluR2 and mGluR3 and Group III: mGluR4, mGluR6, mGluR7, and mGluR8) based on cell signaling activation and sequence homology. Metabotropic glutamate receptors are G protein-coupled receptors and are widely spread in the central nervous system and implicated in various neurodegenerative diseases, including Alzheimer's disease and Parkinson and Huntington's disease. Elevated levels of mGluR1 have been found in the neurons of the olfactory bulb, cerebellar cortex, ventral pallidum, globus pallidus, entopeduncular nucleus, lateral septum, magnocellular preoptic nucleus, and thalamic nuclei.⁵⁷ The widespread presence was also reported in cerebellar Purkinje cells. Group, I mGluR expression has been observed in the substantia nigra, globus pallidus, lateral septum, and thalamic relay nuclei.⁵⁸

CREB signaling has also been reported for long-lasting changes in synaptic plasticity and further mediates short-term memory conversion to long-term memory. CREB signaling has been implicated in several brain pathological conditions, including cognitive and neurodegenerative

disorders. The β -amyloid (A β) peptide, which plays a crucial part in Alzheimer's disease's pathogenesis, also alters the hippocampal-dependent synaptic plasticity and memory and mediates the synapse loss through CREB signaling pathway. Alteration in CREB signaling has been associated with other cognitive disorders, including Huntington's disease and Rubinstein-Taybi and Coffin-Lowry syndromes suggesting an essential role of CREB signaling in cognitive dysfunction.⁵⁹

Mitochondria are essential organelles for energy metabolism and are critical for adaptation to oxidative stress, calcium homeostasis, and cellular viability and function regulation. Mitochondrial dysfunction in many psychiatric disorders has been reported in many neuroimaging, GWAS, and postpartum brain analysis. Mitochondrial impairment can increase the production of reactive oxygen species, altering the cellular functions. The accumulation of oxidative damage in the brain results in the loss of neuronal plasticity and function. Several GWAS studies have identified CACNA1C as one of the relevant genetic risk factors for developing bipolar disorder and major depressive disorder.⁶⁰

An explorative gene expression analysis will be performed to find the drug's unexplained effect on the brain. We are interested in seeing if drugs with high anticholinergic properties modulate any genes that are already associated with neurodegeneration. We are focusing on adverse drug reactions or how the mechanism of the ADR happens so if there is any genetic variance in neurodegenerative genes that can cause a disease process or overexpression of any of the pathways is linked to disease process, it is possible that drug with high anticholinergic properties can interact with it. For this reason, we are studying Drug-Gene Interaction.

11. EXPERIMENTAL APPROACH

11.1 CELL CULTURE EXPERIMENTS

Normal human astrocytes (Lonza, Walkersville Inc, Walkersville, MD, USA) at pass two were seeded at a density of 5000 cells/cm² and cultured at 80% confluence in growth media (AGM-2, Lonza) at physiological temperature. Serum-free media (ABM-2, Lonza) was used 24 h prior to treatment. Cell viability was assessed by trypan blue (Sigma) staining for normal human astrocytes (NHA) treated with Diphenhydramine (Benadryl) 66ng/ml. All working solutions of Diphenhydramine were dissolved in RNA free water (Sigma). The effects of Diphenhydramine on MCP-1 and IL-6 proteins were observed as described below. Treatment groups included control (untreated), and Diphenhydramine (66ng/ml) alone. Diphenhydramine was obtained from Sigma-Aldrich. Each experiment was performed four times.

11.2 PROTEIN QUANTIFICATION AND GENE EXPRESSION

MCP-1 and IL-6 protein concentrations were measured in duplicate by immunofluorescence detection (R&D Systems, Minneapolis, USA) and normalized to total protein content. If Diphenhydramine induced changes in protein concentrations, gene expression was performed. RNA (Ribonucleic acid) is isolated from the normal human astrocytes using the RNeasy mini kit (Qiagen Inc., Valencia, USA) and converted to Complementary deoxyribonucleic acid (cDNA) using a high capacity cDNA reverse transcription protocol (Applied Biosystems, Foster City, USA). RNA and cDNA quality was assessed by absorbance (Thermo Scientific™ NanoDrop™ One). Real-time PCR (RT-PCR) was performed using primer and probe sets for the *CACNA1C*,

CYP2D6, *GCLM*, *GRM1*, *GRM2* and *CREB1* and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Taqman Gene Expression Assays, Applied Biosystems Inc.)

11.3 STATISTICAL ANALYSIS

One-way ANOVA compared MCP-1 and IL-6 protein concentrations with Tukey correction for multiple corrections. The gene expression was reported with relative significance ($p= 0.05$) using Student's t-test.

12. RESULTS

12.1 EFFECT OF DIPHENHYDRAMINE ON IL-6 AND MCP-1 PROTEIN PRODUCTION

Diphenhydramine produces a 10% non-significant decrease in MCP-1 protein production (mean \pm SEM: 3866 \pm 49.73 pg/mg vs 3200 \pm 327.1pg/mg, $p=NS$). (Fig 1) In contrast, Diphenhydramine at doses 66ng/ml produces a statistically significant 20% decrease in IL-6 protein (mean \pm SEM: 85.45 \pm 3.78 pg/mg vs 68.65 \pm 1.94, pg/mg, $p=0.029$). (Fig 2)

MCP-1 Production by Normal Human Astrocytes

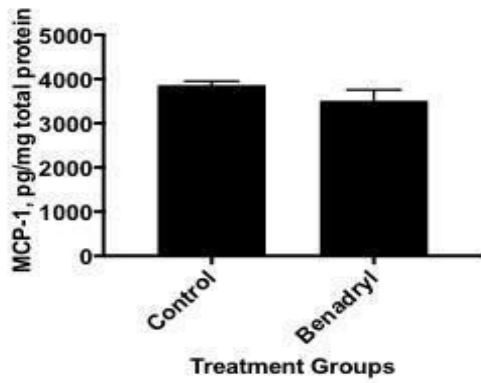


Fig 1: Effect of Diphenhydramine on MCP-1 protein production ($p < 0.05$ denoted by *)

IL6 Protein Production by Normal Human Astrocytes

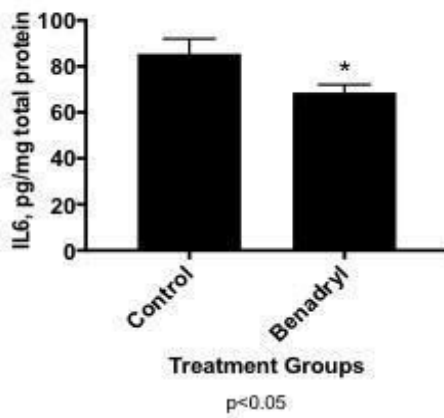


Fig 2: Effect of Diphenhydramine on IL-6 protein production ($p < 0.05$ denoted by *)

12.2 EFFECT OF DIPHENHYDRAMINE ON GRM-1 mRNA PRODUCTION

After observing the IL-6 protein lowering effects, we tested if diphenhydramine modulated mRNA expression of neurocognitive genes. Diphenhydramine at doses 66 ng/ml significantly decreased *GRM1* mRNA expression (Fig 3). In contrast, diphenhydramine at doses 66 ng/ml did not produce a significant effect on *CACNA1C*, *CYP2D6*, *GCLM*, *GRM2*, and *CREB1* mRNA expression (Fig 3).

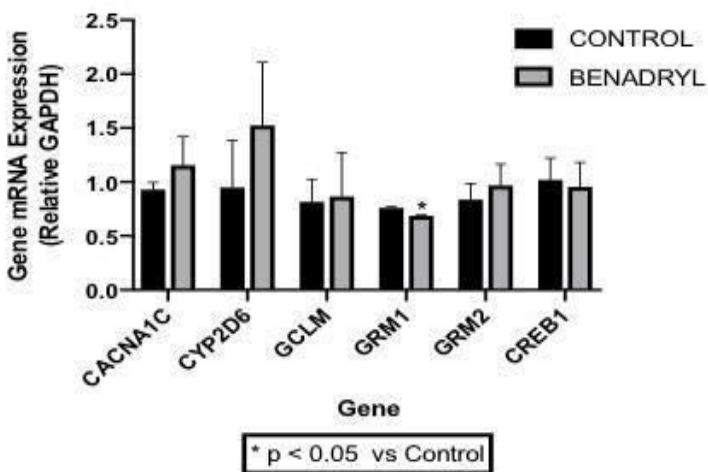


Fig 3: Effect of Diphenhydramine on Gene mRNA production ($p < 0.05$ denoted by *)

13. DISCUSSION

Diphenhydramine, a first-generation antihistamine, is commonly available to treat the rash, dry and itchy eyes, common cold, hay fever, and allergies. It is also evident from different reports that long-term usage of drugs with high anticholinergic activity such as antihistamines can produce cognitive impairment or decline in older adults. Anticholinergic drugs are hypothesized to be pro-inflammatory, but in our current experimental model of normal human astrocytes, diphenhydramine demonstrated an anti-inflammatory effect. In the present study, we observed

diphenhydramine's effects on MCP-1 and IL-6 protein production at physiologic drug concentrations (66ng/ml). In this study, we found no significant reduction of diphenhydramine on MCP-1. Animal studies suggest that MCP-1 levels were elevated in older and young heterochronic parabionts, a young mouse whose vascular system is joined with an older mouse.^{20,21} In a longitudinal analysis of an asymptomatic older adult cohort, increased MCP-1 levels were associated with longitudinal decline in verbal episodic memory.²² To add support to their findings, Lee indicated that higher baseline MCP-1 levels are related to decreased cognitive function over time²³

We observed a statistically significant reduction in IL-6 protein production. In the Rotterdam Study, IL-6 was found to be associated with an increased risk of dementia. Neuroimaging studies also demonstrated more robust associations with IL-6. In a multiethnic cohort study, elevated serum IL-6 levels were also associated with a global measure of cognitive function. In addition to this, higher levels were also found in Alzheimer's disease patients' spinal fluid.

In addition to the effects on IL-6 protein levels, diphenhydramine lowered basal *GRM1* mRNA expression. It is a G-protein coupled receptor for glutamate. Ligand binding causes a conformational change that triggers signaling via guanine nucleotide-binding proteins (G proteins) and modulates downstream effectors' activity. Signaling activates a phosphatidylinositol-calcium second messenger system. It also participates in glutamate's central action in the CNS, such as long-term potentiation in the hippocampus and long-term depression in the cerebellum. After CNS injuries such as trauma or ischemia or in CNS neurodegenerative disorders, the properties of astrocytes change, the cells become more reactive. Stable expression of metabotropic glutamate receptors (*GRM1* and *GRM5*) was also

reported. Animal studies suggest that inherited ataxia can be associated with defective expression of mGlu receptors in Purkinje cells. Ataxic transgenic mice have shown mutations of *GRM1* gene and reduced expression of mGlu receptors²⁴⁻²⁶

In conclusion, we have observed a novel anti-inflammatory effect of diphenhydramine. Further studies of cotreatment with IL-1b to simulate aging and characterize diphenhydramine's effects on different brain cell populations are warranted. Diphenhydramine prominently reduced *GRM1* expression, thus potentially impacting the glutamatergic pathway. The impact of diphenhydramine on *GRM1* mRNA expression should also be tested in additional models of neurocognitive diseases.

The limitations of the studies include generating the hypothesis from *in vitro* cell culture models. For long-term clinical outcomes, we did not consider the impact of other therapy that could interact with diphenhydramine. We also did not consider additional pathogenic factors besides neuroinflammation.

14. PAROXETINE

PAXIL (paroxetine hydrochloride) is an orally administered psychotropic drug. It is the hydrochloride salt of a phenylpiperidine compound identified chemically as (-)-trans-4R-(4' fluorophenyl)-3S-[(3',4'-methylenedioxyphenoxy) methyl] piperidine hydrochloride hemihydrate and has the empirical formula of $C_{19}H_{20}FNO_3 \cdot HCl \cdot 1/2H_2O$. The molecular weight is 374.8 (329.4 as a free base) (www.accessdata.fda.gov)

14.1 PHYSICOCHEMICAL PROPERTIES OF PAROXETINE HYDROCHLORIDE

Paroxetine hydrochloride is an odorless, off-white powder with a melting point range of 120° to 138°C and a 5.4 mg/mL solubility in water.

14.2 PHARMACOLOGY OF PAROXETINE

Paroxetine is a selective 5-HT reuptake inhibitor. Studies at clinically relevant doses in humans have shown that paroxetine blocks serotonin's uptake into human platelets. In vitro studies in animals demonstrated that paroxetine is a potent and highly selective inhibitor of neuronal serotonin reuptake and has feeble effects on norepinephrine and dopamine neuronal reuptake. In vitro radioligand binding studies also indicated that paroxetine has some affinity for muscarinic, alpha1-, alpha2-, beta-adrenergic-, dopamine (D2)-, 5-HT1-, 5-HT2-, and histamine (H1)-receptors. (www.accessdata.fda.gov)

14.3 PHARMACOKINETICS OF PAROXETINE

Paroxetine is well absorbed from the gastrointestinal tract and undergoes first-pass metabolism. Due to its lipophilic amine character, paroxetine is extensively distributed into tissues. Its plasma

protein binding is about 95%. Paroxetine metabolism occurs within the liver and is mediated by cytochrome CYP2D6, CYP3A4, and possibly other cytochrome enzymes. Genetic polymorphism of CYP2D6 may alter the pharmacokinetics of paroxetine. The renal clearance of Paroxetine is negligible. Single-dose studies reveal that the pharmacokinetics of paroxetine is non-linear in most subjects. Also, steady-state pharmacokinetic parameters are not predictable from single-dose data. In many subjects, daily administration of 20-50mg of paroxetine leads to little or no disproportionality in plasma levels with dose, although this phenomenon is evident in a few subjects. Steady-state plasma concentrations are generally achieved within 7 to 14 days. The maximum plasma concentration of Paroxetine is 61.7ng/ml ($1.67 \cdot 10^{-4} \text{mM}$) after 30 daily doses. The terminal half-life is about one day. In elderly subjects, there is wide interindividual variation in steady-state pharmacokinetic parameters, with statistically significantly higher plasma concentrations and slower elimination than in younger adults.²⁷

15. METHOD

15.1 CELL CULTURE EXPERIMENTS

Normal human astrocytes (Lonza, Walkersville Inc, Walkersville, MD, USA) at pass two were seeded at a density of 5000 cells/cm² and cultured at 80% confluence in growth media (AGM-2, Lonza) at physiological temperature. Serum-free media (ABM-2, Lonza) was used 24 h prior to treatment. Cell viability was assessed by trypan blue (Sigma) staining for normal human astrocytes (NHA) treated with Paroxetine ($1.67 \cdot 10^{-4} \text{mM}$). All working solutions of Paroxetine were dissolved in RNA free water (Sigma). The effects of Paroxetine on MCP-1 protein.

Treatment groups included control (untreated), Paroxetine ($1.67 \times 10^{-4} \text{mM}$) alone, IL-1B (2ng/ml) and Paroxetine with IL-1B. Paroxetine was obtained from Sigma-Aldrich.

15.2 PROTEIN QUANTIFICATION AND GENE EXPRESSION

MCP-1 was measured in duplicate by immunofluorescence detection (R&D Systems, Minneapolis, USA) and normalized to total protein content. If Paroxetine induced changes in protein concentrations, gene expression was performed. RNA (Ribonucleic acid) was isolated from the normal human astrocytes using the RNeasy mini kit (Qiagen Inc., Valencia, USA) and converted to Complementary deoxyribonucleic acid (cDNA) using a high capacity cDNA reverse transcription protocol (Applied Biosystems, Foster City, USA). RNA and cDNA quality was assessed by absorbance (Thermo Scientific™ NanoDrop™ One). Real-time PCR (RT-PCR) was performed using primer and probe sets for the *CACNA1C*, *CYP2D6*, *CREB1*, *PPARG* and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Taqman Gene Expression Assays, Applied Biosystems Inc.)

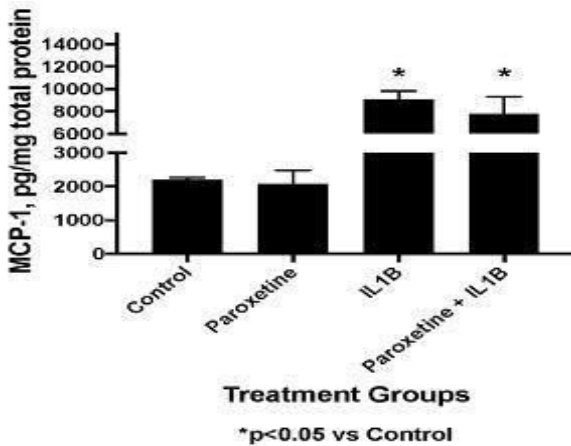
15.3 STATISTICAL ANALYSIS

One-way ANOVA compared MCP-1 concentration with Tukey correction for multiple corrections. The gene expression was reported with relative significance ($p = 0.05$) using Student's t-test.

16. RESULTS

16.1 EFFECT OF PAROXETINE ON IL-1B STIMULATED MCP-1 PRODUCTION

In basal conditions, Paroxetine at doses $1.67 \cdot 10^{-4} \text{mM}$ did not produce any significant effects on MCP-1 production (Fig 4). Furthermore, Paroxetine did not inhibit the induction of MCP-1 by pro-inflammatory cytokine IL-1B (Fig 4).



(Fig 4: Effect of Paroxetine on IL-1B stimulated MCP-1 protein production ($p < 0.05$ denoted by *))

16.2 EFFECT OF PAROXETINE ON IL-1B STIMULATED CACNA1C AND CYP2D6 mRNA PRODUCTION

After investigating the impact of Paroxetine on MCP-1 protein production, we tested whether Paroxetine modulated basal or stimulated CACNA1C and CYP2D6 mRNA expression. In basal conditions, with Paroxetine at doses $1.67 \cdot 10^{-4} \text{mM}$, we did not see any statistically significant

effect. However, Paroxetine inhibits the IL-1B induced expression of CACNA1C (Fig 5) and CYP2D6 (Fig 6).

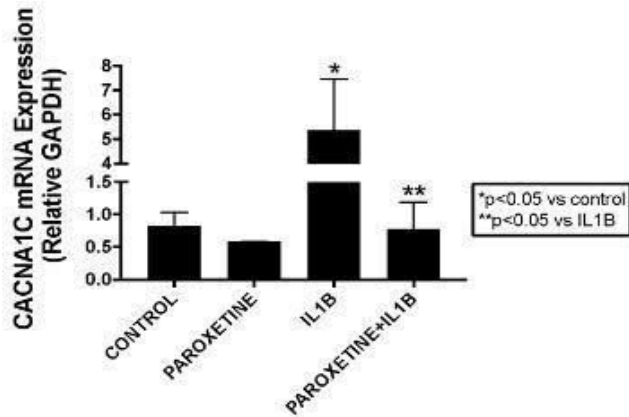


Fig 5: Effect of Paroxetine on IL1B stimulated CACNA1C mRNA expression ($p < 0.05$ denoted by *)

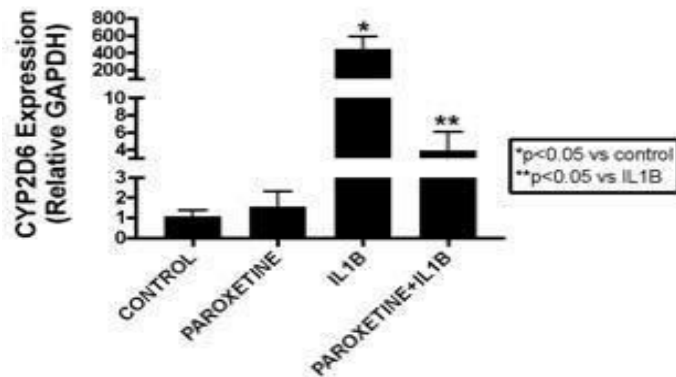


Fig 6: Effect of Paroxetine on IL-1B stimulated CYP2D6 mRNA expression ($p < 0.05$ denoted by *)

16.3 EFFECT OF PAROXETINE ON IL-1B STIMULATED CREB 1 AND PPARG mRNA EXPRESSION

We tested whether Paroxetine at physiologic drug concentration $1.67 \times 10^{-4} \text{mM}$ modulated basal or stimulated *CREB1* and *PPARG* mRNA expression. Under basal conditions, Paroxetine did not produce any significant effects. However, Paroxetine blunted IL-1B induction of *CREB1* and *PPARG* (Fig 7 & Fig 8).

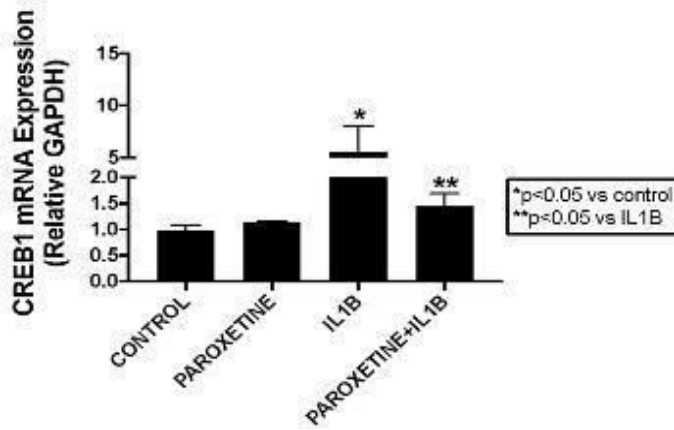


Fig 7: Effect of Paroxetine on IL-1B stimulated *CREB 1* mRNA expression ($p < 0.05$ denoted by*)

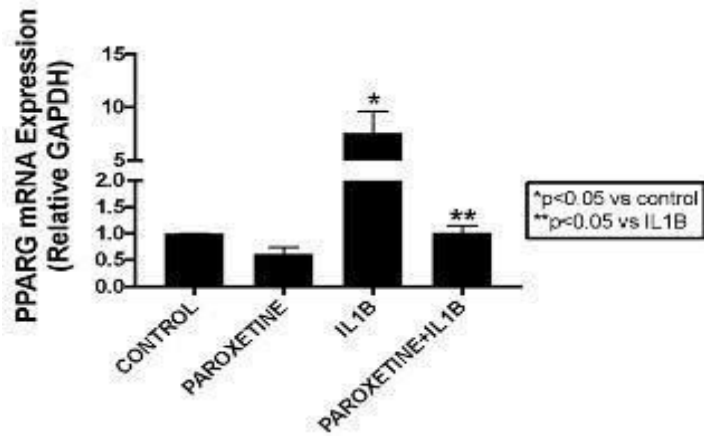


Fig 8: Effect of Paroxetine on IL-1B stimulated PPARG mRNA expression ($p < 0.05$ denoted by*)

17. DISCUSSION.

The prevalence rate of depression in elderly rates around 10-15% for those living in the community. Selective Serotonin Reuptake Inhibitors, including Fluoxetine, Paroxetine, Citalopram, Sertraline, Escitalopram is the most prescribed type of antidepressants. In 1990, selective serotonin inhibitors (SSRIs) became the first treatment for depression, replacing the tricyclic antidepressant (TCAs). Many recent clinical ^{28,29} and preclinical studies ³⁰⁻³² have shown that the brain's inflammatory processes are involved in depression.

In the current experimental model of normal human astrocytes, we examined the effects of Paroxetine on IL-1B stimulated production of MCP-1 at physiologic cMax concentration ($1.67 \times 10^{-4} \text{mM}$). In the current study, we observed that Paroxetine does not impact inflammation in normal human astrocytes. Lehto et al. and Grassi-Oliveria et al. reported that depression was associated with lower MCP-1 concentration than healthy controls.^{33,34} Myung et al., 2016

reported lower MCP-1 levels in a study conducted in sixty-six Korean patients with major depressive disorder receiving antidepressant monotherapy for six weeks (Escitalopram, sertraline, paroxetine, fluoxetine, and mirtazapine).³⁵ MCP-1 is known to attract peripheral monocytes to the brain, which results in an inflammatory reaction. One case-control study investigated the role of G-2518A polymorphism of the MCP-1 gene and found that A allele is associated with increased psychopathology. In a Korean population, subjects with A allele were found to have an increased risk of developing the major depressive disorder and psychotic features compared to those with the G allele.³⁶ Recently it is shown that lower serum levels of MCP-1 predict the lack of response of antidepressants³⁷

We also examined the effect of Paroxetine on basal and IL-1B stimulated *CACNA1C*, *CYP2D6*, *CREB-1*, and *PPARG* mRNA expression. We observed that Paroxetine prevents inflammation-induced mRNA expression of *CACNA1C*, *CYP2D6*, *CREB-1*, and *PPARG*.

CACNA1C encodes for the pore-forming 1C subunit of the Ca_v1.2 L-type calcium channel (LTCC). *CACNA1C* mediates the influx of calcium ions into the cell upon membrane polarization, plays an essential role in dendritic development, neuronal survival, synaptic plasticity, and memory/learning.

CACNA1C has been considered a promising candidate gene associated with psychiatric disorder.³⁸ Polymorphisms in *CACNA1C* gene have been recognized by Genome-wide association studies (GWAS) with the risk of developing schizophrenia, bipolar disorder, and major depressive disorder.³⁹⁻⁴¹ In healthy volunteers, risk-associated single nucleotide polymorphisms in *CACNA1C* have predicted higher scores on depression, anxiety, negative mood, interpersonal sensitivity.^{42,43} In rodents, the pharmacological blockade of LTCCs resulted

in antidepressants effect in learned helplessness paradigm and forced swim and tail suspension tests.⁴⁴⁻⁴⁶ In a large sample study conducted by Beitelshes et al, the investigators screened 46 polymorphisms within CACNA1C to investigate associations between cardiovascular disease outcomes and treatment responses. They discovered that rs1051375 variant status had a significant interaction with antihypertensive treatment strategy.⁴⁷

CYP2D6 is a polypeptide of 497 amino acids. Even though it accounts for only a small percentage of total hepatic CYP450 content, its role in drug metabolism is significant.⁴⁸ CYP2D6 is a major drug-metabolizing enzyme responsible for eliminating 20% clinically used drugs. Substrates of CYP2D6 include tricyclic antidepressants (desipramine), selective serotonin reuptake inhibitors (paroxetine), B blockers (metoprolol), opioid analgesics (codeine) and anti-cancer drugs (tamoxifen).⁴⁹ CYP2D6 mediated drug metabolism exhibits large inter-individual variability. Based on the urinary metabolic ratio, an individual can be classified as ultra-rapid metabolizer (UM), normal metabolizer (NM), intermediate metabolizer (IM) and poor metabolizer (PM) of CYP2D6.⁵⁰ Paroxetine plasma levels are higher in older adults. A double-blind, randomized trial in the elderly depressed study with Paroxetine and Nortriptyline, conducted by Solai et al., revealed that 66 elderly depressed patients with both antidepressants were found to have a significant correlation between pretreatment CYP2D6 activity and steady-state plasma concentration.⁵¹

In the current experimental model in normal human astrocytes, we examined the effects of Paroxetine on IL-1B stimulated CREB-1 and PPARG mRNA expression. We observed that Paroxetine at a physiologic drug concentration ($1.67 \times 10^{-4} \text{mM}$) blunted inflammation-induced CREB1 and PPARG mRNA expression in normal human astrocytes.

CREB is a 43kDa transcriptional factor that belongs to the leucine zipper family and it is a final molecular target of several signaling pathways such as c-AMP, calcium, and Ras-dependent kinases. CREB exists in activated phosphorylated or inactive phosphorylated forms. The phosphorylated CREB further binds to the CREB sequence on the DNA promoter region, and the transcription process then starts.⁵² Nibuya reported that after chronic administration of fluoxetine, desipramine, and mRNA CREB levels were increased in the rat hippocampus region.⁵³

Peroxisome proliferator-activated receptor-gamma, also known as the glitazone receptor or NR1C3, is a type II nuclear receptor that in humans is encoded by PPARG. PPARG is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. It forms a heterodimer with retinoid x receptor (RXR) to bind to specific response elements in its target genes' promoter region. (genecards.org). We observed that Paroxetine at a physiologic drug concentration ($1.67 \cdot 10^{-4} \text{mM}$) blunted inflammation-induced PPARG mRNA expression in normal human astrocytes.

18. NORMAL HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Brain microvascular endothelial cells (BMECs) are the major component of the blood-brain barrier, limiting the passage of soluble and cellular substrates from the blood into the brain. They have some unique features such as tight intercellular junctions that slow paracellular flux and display high electrical resistance, the absence of fenestrae and a reduced level of pinocytic activity, and asymmetrically localized enzymes and carrier-mediated transport systems. These

features distinguish them from peripheral endothelial cells (Diane et al., 1995). Alterations in blood-brain barrier properties can contribute to neurodegenerative and neuroinfectious pathogenesis and exacerbation.

19. METHOD

19.1 CELL CULTURE EXPERIMENT

Human brain microvascular endothelial cells were grown in EBM-2 medium (Lonza, Walkersville, MD). The cells were grown at 5% CO₂, 37 C, and 95% relative humidity and when reached 90% confluency were treated with Paroxetine ($1.67 \times 10^{-4} \text{mM}$). Cell viability was assessed by trypan blue (Sigma) staining for normal human brain microvascular endothelial cells (NHBMECs) treated with Paroxetine ($1.67 \times 10^{-4} \text{mM}$). All working solutions of Paroxetine were dissolved in RNA free water (Sigma). The effects of Paroxetine on MCP-1 protein was observed. Treatment groups included control (untreated), Paroxetine ($1.67 \times 10^{-4} \text{mM}$) alone, IL-1B (2ng/ml) and Paroxetine with IL-1B. Paroxetine was obtained from Sigma-Aldrich.

19.2 PROTEIN QUANTIFICATION AND GENE EXPRESSION

MCP-1 was measured in duplicate by immunofluorescence detection (R&D Systems, Minneapolis, USA) and normalized to total protein content. If Paroxetine induced changes in protein concentrations, gene expression was assessed via RT-PCR. RNA (Ribonucleic acid) was isolated from the normal human brain microvascular endothelial cells using the RNeasy mini kit (Qiagen Inc., Valencia, USA) and converted to Complementary deoxyribonucleic acid (cDNA) using a high capacity cDNA reverse transcription protocol (Applied Biosystems, Foster

City, USA). RNA and cDNA quality was assessed by absorbance (Thermo Scientific™ NanoDrop™ One). Real-time PCR (RT-PCR) was performed using primer and probe sets for the *CCL2*, *CXCL5*, *GRM1*, *SOD1* and *CYP2D6* and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Taqman Gene Expression Assays, Applied Biosystems Inc.)

19.3 STATISTICAL ANALYSIS

One-way ANOVA compared MCP-1 concentrations with Tukey correction for multiple corrections. The gene expression was reported with relative significance ($p= 0.05$) using Student's t-test.

20. RESULTS

20.1 EFFECT OF PAROXETINE ON IL-1B STIMULATED MCP-1 PRODUCTION

In basal conditions, Paroxetine at $1.67 \times 10^{-4} \text{mM}$ in normal human brain microvascular endothelial cells did not produce any effects on inflammation (Fig 9). Furthermore, Paroxetine did not inhibit MCP-1 induction by IL-1B (Fig 9).

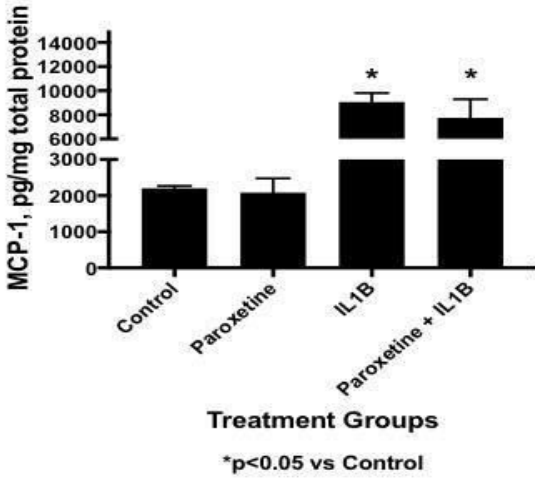


Fig 9: Effect of Paroxetine on IL-1B stimulated MCP-1 protein production ($p < 0.05$ denoted by *)

20.2 EFFECT OF PAROXETINE ON IL-1B STIMULATED CCL2 AND CXCL5 mRNA EXPRESSION

We tested whether Paroxetine lowered basal or stimulated *CCL2* and *CXCL5* mRNA expression. Paroxetine at $1.67 \times 10^{-4} \text{mM}$ did not produce any significant effects on basal or IL-1B treated normal human brain microvascular endothelial cells (Fig 10 & Fig 11).

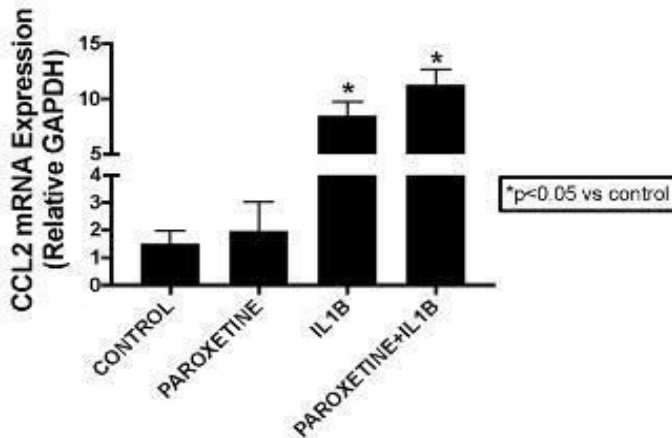


Fig 10: Effect of Paroxetine on IL-1B stimulated CCL2 mRNA expression ($p < 0.05$ denoted by *)

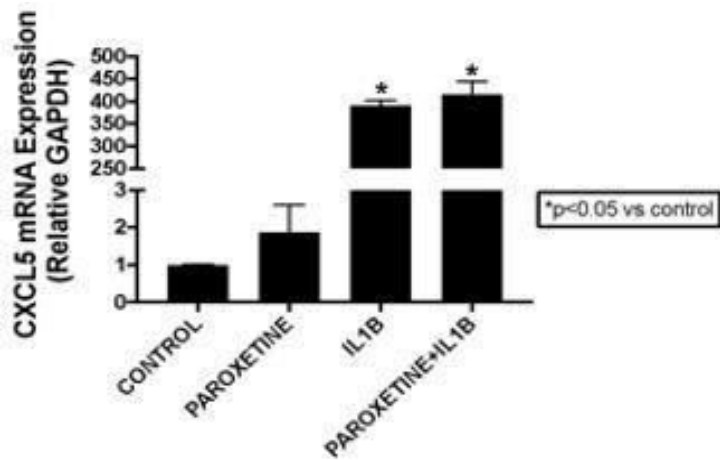


Fig 11: Effect of Paroxetine on IL-1B stimulated CXCL5 mRNA expression ($p < 0.05$ denoted by *)

20.3 EFFECT OF PAROXETINE ON IL-1B STIMULATED GRM-1 AND SOD1 mRNA EXPRESSION

In basal or IL-1B stimulated conditions, Paroxetine at doses $1.67 \times 10^{-4} \text{mM}$ did not produce any significant effects on *GRM-1* in normal human brain microvascular endothelial cells (Fig 12). However, basal Paroxetine treatment induced the expression of *SOD-1*(Fig 13).

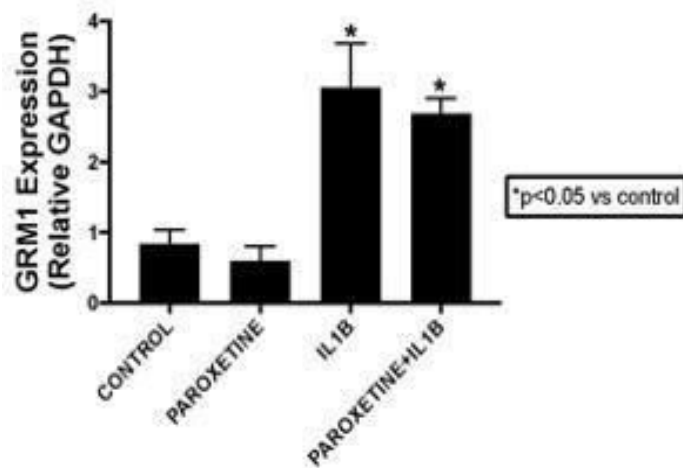


Fig 12: Effect of Paroxetine on IL-1B stimulated GRM1 mRNA expression ($p < 0.05$ denoted by *)

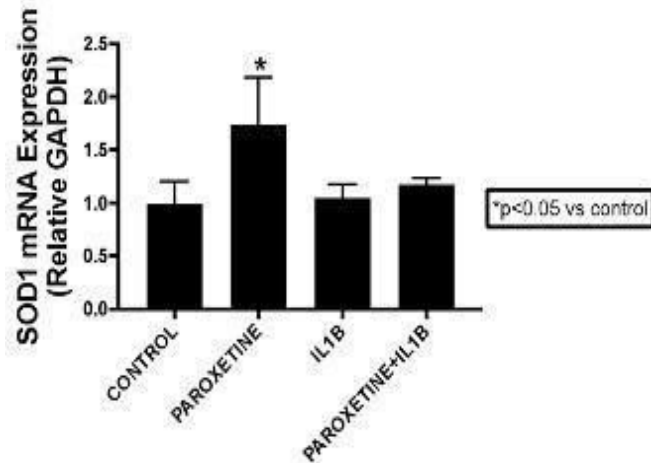


Fig 13: Effect of Paroxetine on IL-1B stimulated SOD1 mRNA expression ($p < 0.05$ denoted by *)

20.4 EFFECT OF PAROXETINE ON IL-1B STIMULATED CYP2D6 mRNA EXPRESSION

In basal conditions, Paroxetine at $1.67 \times 10^{-4} \text{mM}$ did not produce any significant effects on CYP2D6 expression in normal human brain microvascular endothelial cells. However, Paroxetine prevents IL-1B induced CYP2D6 mRNA expression in normal human brain microvascular endothelial cells (Fig 14).

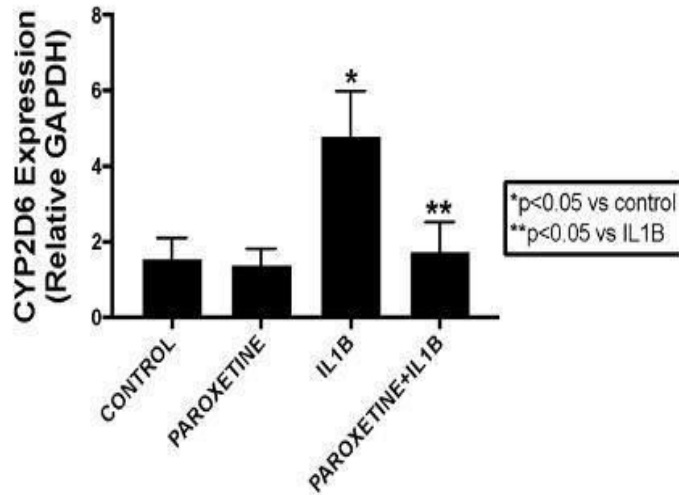


Fig 14: Effect of Paroxetine on IL-1B stimulated CYP2D6 mRNA expression ($p < 0.05$ denoted by *)

21. DISCUSSION

In the current experimental model of normal human brain microvascular endothelial cells, we examined the effects of Paroxetine on IL-1B stimulated production of MCP-1 at a physiologic drug concentration ($1.67 \times 10^{-4} \text{mM}$). In the current study, we observed that Paroxetine does not impact inflammation in normal human brain microvascular endothelial cells, similar to what we saw with normal human astrocytes. We noted that Paroxetine prevents inflammation-induced CYP2D6 mRNA expression similar to what we found in normal human astrocytes. Furthermore, Paroxetine induced the expression of *SOD1* in the current experimental model of normal human brain microvascular endothelial cells.

SOD1 or Cu/Zn superoxide dismutase is a soluble protein that converts harmful superoxide radicals to molecular oxygen and hydrogen peroxide. The human SOD1 gene located on chromosome 21q22.11 and codes for monomeric SOD1 polypeptide. SOD1 expression studies still produce conflicting data about whether increased or inhibited regulation is harmful.^{54,55}

In a study conducted, *SOD1* gene expression was elevated in specific nervous systems (brainstem and spinal cord), commonly affected by Amyotrophic Lateral Sclerosis but not in other brain regions (cerebellum and cerebral cortex) affected during neurodegenerative processes.⁵⁶

Further studies of cotreatment with IL-1b to simulate aging, and also to characterize the paroxetine effects on different populations of brain cells are warranted. The impact of paroxetine to induce the expression of SOD1 mRNA expression should also be examined in additional models of neurocognitive diseases.

The limitations of the studies include generating the hypothesis from in-vitro cell culture models. For long-term clinical outcomes, we did not consider the impact of other therapy that could interact with paroxetine. We also did not consider additional pathogenic factors besides neuroinflammation.

OVERALL CONCLUSIONS

We did not observe a shared biologically plausible pathway shared between the anticholinergic drugs that we examined (i.e., pro-inflammatory cascade). Therefore, Comprehensive approaches that examine the pharmacologic properties of anticholinergic drugs within the aging brain are warranted.

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