Metformin as a Curing Agent in Chemotherapy-Induced Cognitive Impairments

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A Thesis submitted to the Department of Neuroscience in partial fulfillment of the requirements for the degree of Bachelor of Science

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I pledge my word of honor that I have abided by the Washington College Honor Code while completing this assignment.

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Abstract

Chemotherapy is a type of cancer treatment that uses drugs to kill cancer cells, working by slowing or stopping the growth of cancer cells. It can be used to treat or ease cancer symptoms. Chemotherapy kills other cells other than just cancer. Incidence of memory deficits tends to increase in cancer patients after receiving chemotherapy. Chemotherapy-induced cognitive impairment (CICI) is generally not discussed when talking about the damages of chemotherapy. Novel treatments are being used to address CICI, including type II diabetes drug metformin. Metformin is a curing drug used by physicians that exhibits an ability to alter cellular energy metabolism, allowing for the regulation of the AMPK pathway and increasing hippocampal neurogenesis. Overall, metformin is a unique treatment as it is cheap and harmless and has minimal side effects, successfully decreasing the cognitive impairments caused by chemotherapy.

Chemotherapy-Induced Cognitive Impairment (Introduction)

Cancer is a disease characterized by the development of abnormal cells that divide uncontrollably and have the ability to destroy normal body tissue (Mayo Clinic, 2022). Trillions of cells compose the human body and go through a process known as cell division to grow and multiply. This process occurs to replace aging or damaged cell to keep body systems intact. For cells to become cancerous, genetic mutations occur from a varying array of factors. These include inherited mutations, ones that develop over time as our genes wear out, or environmental factors that cause epistatic changes (Canadian Cancer Society, 2023). When a gene mutation occurs, normal instruction within cells is bypassed and cells grow out of control or do not die when needed. The genetic mutations that most commonly contribute to cancerous growths effect proto-oncogenes, tumor suppressor genes, and DNA repair genes (NIH, 2021). Gene changes in proto-oncogenes disrupt the regulation of normal cell growth and division, allowing cells to grow and survive when they should not. Tumor suppressor genes also control cell growth and division and modifications to these genes allow cells to grow in an uncontrollable manner.

Cancer serves as a major problem worldwide in public health, with an approximate projection in 2023 of 1,958,310 new cases, and 609,820 deaths in the United States alone (World Health Organization, 2021). The most common forms include cancers in the respiratory, digestive, and genital systems, with almost 20% of deaths stemming from respiratory cancers (Siegel et al., 2023). Future cases of cancer will likely increase over time due to increasing prevalence factors that increase risk, such as smoking, unhealthy diet, and physical inactivity (ACS, 2023).

Any cancerous growths, known as tumors, can spread and invade nearby tissues or travel to distant places in the body to form new tumors (NCI, 2021). A cancer that has spread from its

place of origin to another part of the body is called metastatic cancer. Most of cancer related deaths occur through metastatic disease due to the aggressive nature this form possesses (ACS, 2022). These tumors block the function of a body system, disrupting normal processes that help us maintain base level health (Siegel et al., 2023). As a tumor grows, the cancer cells push onto normal tissue beside the tumor, causing an issue in how those normal cells function. Treating cancer is a necessity as it prevents incidents of cancer from occurring later in life and allows the organs it effects to properly function.

To eliminate cancerous tissue from the body, a wide range of established treatment alternatives are being used. In cases where cancerous cells are in areas of low risk, surgery is used to remove the cancerous tissue (Mayo Clinic, 2022). This is done in multiple capacities such as removal of the tumor with scalpels, cryosurgery with liquid nitrogen to freeze cancerous tissue in its early stages, or lasers used to cut through tissue as a form of precise therapy (Mayo Clinic., 2022). All these surgical treatments work to prevent metastatic potential of cancer cells before cancer develops, debulk the tumor because not all cancerous cells can be eliminated, and to ease cancer symptoms to improve quality of life. Radiation therapy is a cancer treatment in which x-rays and other forms of intense energy are used to kill cancerous cells. This is commonly used in cancer cases to kill cancer cells and shrink tumors over multiple weeks. The radiation therapy does not kill cancer cells right away, instead it damages the DNA that is already damaged by cancer, thus causing it to die eventually (NIH, 2019). Overall, the genetic material that controls how cells grow and divide are targeted, preventing the growth of new cancer cells and causing them to die (Mayo Clinic., 2020). Both surgery and radiation therapy are paired commonly with pharmaceutical treatments to increase efficacy (NIH., 2012). One of these treatments is target therapy, a treatment that targets proteins that control how cancer cells

grow, spread and divide. This precise treatment is broken down into categories including small-molecule drugs and monoclonal antibodies (NIH, 2022). Specifically, these two sub therapies of target treatment help the immune system destroy cancer cells or stop signals that form new blood vessels. This process, known as angiogenesis, prevents blood flow to the cancer cells, stopping chemical signals from being released, and the thus formation of new cancer cells will be halted (NCI., 2018).

No cancer treatment comes as close as chemotherapy does to benefits and detriments on the patient. Chemotherapy is a type of cancer treatment that uses drugs to kill cancer cells. It works by slowing or stopping the growth of cancer cells and can be used to treat or ease cancer symptoms. In use, chemotherapy is used to treat many different forms and stages of cancer. When in use with other treatments, chemotherapy shrinks the tumor before surgery or radiation therapy or kills any remaining cells after treatment. However, chemotherapy doesn't come without side effects on treated patients. Not only does it kill the cancerous cells within the body, but it also slows the growth of the healthy cells that are needed for normal function within the body. The most common normal cells to be damaged by chemotherapy include hair follicles, blood-forming cells in the bone marrow, and cells in the mouth, digestive tract, and reproductive systems (ACS, 2020). Hair loss is a well know side effect as most patients that receive chemotherapy have cells in their hair follicles die throughout treatment. Another common side effect of chemotherapy includes fatigue and anemia. This occurs as the cells that prevent infection and regenerate blood in the bone marrow are attacked by treatment, preventing the ridding of toxins and other phages throughout the body (ACS, 2020). Additionally, if cells in the mouth, digestive tract, and reproductive systems are damaged, normal processes in these systems will be disrupted. This could include sores in the mouth, loss of teeth, nausea, vomiting, and

fertility problems (ACS, 2020). These effects are generally short term but may take months or years to go away completely.

Although these side effects are dangerous and make a physical impact, cognitive impairment is generally not discussed when talking about the damages of chemotherapy. Chemotherapy-induced cognitive impairment (CICI) is often caused by direct neurotoxic injury to the brain, a reduction in new neurons being formed, or white matter abnormalities (Whittaker et al., 2022). Commonly, impairments to visual processing, executive function, and attention are all affected in various severities. The severity generally differs from person to person, with some people being unable to return to work and others regaining full ability to think and function (Mayo Clinic, 2023). These impairments have patients experiencing a lack of ability in recalling words, having confusion, or requiring a greater effort to perform everyday activities (Whittaker et al., 2022). As many as 3 out of 4 people that received chemotherapy admit to changes in their mental sharpness (Swiner, 2022).

It has been found that incidence of memory deficits tends to increase in cancer patients after receiving chemotherapy (Sritawan et al., 2020). The chemicals used in chemotherapy induce negative effects on neurons and neurotransmitters involved in cognitive processing (Acevedo, 2012). The disruption of cellular proliferation in key memory centers of the brain is ultimately linked to CICI effects in cancer patients.

Although some traditional therapies are used to lessen or alleviate these symptoms, novel drug treatments are being used. Metformin is a curing drug used by physicians for type II diabetes that exhibits an ability to alter cellular energy metabolism (Saraei et al., 2019). The use of metformin in conjunction with cancer treatment is still in early stages. However, biological effects are highly studied. Metformin reduces the production of endogenous reactive oxygen

species (ROS) and oxidative stress associated with DNA damage by inhibiting mitochondrial respiration (Saraei et al., 2019). In addition to the reduction of ROS, metformin exhibits an antioxidant activity which aids in restoring cognitive function (Saraei et al., 2019). In response to metformin reducing gluconeogenesis through kinase signaling, an increase glucose uptake in cells occur, and ultimately leads to a decrease in glucose levels. This process allows for the regulation of the AMPK pathway, preventing memory dysfunction (Alhowail & Chigurupati, 2021).

Overall, metformin is a unique treatment as it is inexpensive and harmless and has minimal side effects. It has the highest total mean availability around the world with (>80%) among of surveyed medicines (Babar et al., 2019). Pricing remains generally low as it is an essential drug that is used for type II diabetes. The side effects of metformin include nausea and vomiting, but only in 2% of people worldwide (NHS, 2022). This number is much lower than the number of people who receive chemotherapy and experience side effects. It is my suggestion that metformin is used to lower cancerous cell proliferation, and decrease the cognitive impairments caused by chemotherapy.

Chemotherapy as a Cancer Treatment

Chemotherapy has been used as a cancer treatment for over a century and aims to eliminate cancerous tumors by reducing rates of cancer cell proliferation (DeVita & Chu, 2008). There are many kinds of chemotherapy, all of which work through the cell cycle to eliminate cancerous cells (ACS, 2019). Cells must go through a process known as the cell cycle in which they replicate and make two new cells, all while stopping at multiple regulatory checkpoints to ensure proper division. As cancer cells tend to form new cells quicker than normal cells, these checkpoints are not activated, allowing under replicated or damaged DNA to go undetected, which causes increased cellular proliferation (Collin et al., 1997). This increase in damaged cells leads to the buildup of cancerous cells, causing other body systems to be affected.

Since cancer cells are mutated, and thus have defects that make them more susceptible to chemotherapy's chemical structure, chemotherapy treatment is more successful at eliminating these damaged cells than healthy cells (ACS, 2019). However, chemotherapy drugs are unable to differentiate healthy cells from those that are cancerous, meaning normal cells will be damaged along with the cancer cells that are being targeted (ACS, 2023). This process of normal cell damage results in very heavy side effects including hair loss, fatigue, anemia, and infection (ACS, 2020). Chemotherapy is effective as cancerous cells are usually unable to recover from the potent nature of the drugs, and healthy cells can recover from the effects over time (ACS, 2023). Although chemotherapy is an effective therapy, many negative side effects arise in treated patients that can alter everyday function and quality of life.

The hippocampus is one of the main structures in the brain effected by chemotherapy treatment (ACS, 2020). As chemotherapy drugs pass through the blood brain barrier and into the temporal portion of the cerebral cortex, known as the hippocampus, specific cells and functions

are attacked in addition to cancerous cells (Anand & Dhikav, 2012). Stellate cells and pyramidal cells are highly affected through reductions in spine density, and these are the primary cell types which the hippocampus is composed of (Grujicic, 2022). The hippocampus is important in many brain processes, namely learning and memory (Anand & Dhikav, 2012). When healthy pyramidal and stellate cells are attacked, the process of neurogenesis, which is crucial to the regulation of memory, mood, and spatial learning, is reduced (Alexander & Hasselmo, 2018; QBI, 2020).

Another common result of chemotherapy treatment includes chemotherapy-induced cognitive impairments (CICI), or a decrease in the ability to control cognition. This can cause a range of impairments that have a negative impact on quality of life for patients (Whittaker et al., 2022). Up to 75% of all chemotherapy patients display CICI (Whittaker et al., 2022). For example, a study of 196 long term breast cancer patients, those who had received chemotherapy (Koppelmans et al., 2012) performed significantly worse on many cognitive tasks including functional execution and verbal memory compared to those who did not receive treatment. The mechanisms by which CICI work remain unclear, however it has been proposed that a decrease in neurogenesis, a disruption of hippocampal cell proliferation, increased oxidative stress, and the production of reactive oxygen species all contribute to memory dysfunction (Figure 1). Pro-inflammatory cytokines levels also increase in cancer patients, even before chemotherapy treatment (Alhowail & Chigurupati, 2021). Increasing levels of these cytokines can lead to cognitive impairment and cause a central inflammatory response (Alhowail & Chigurupati, 2021). With chemotherapy, cytokine levels significantly increase though release from the vagus nerve, potentially leading to CICI related effects (Alhowail & Chigurupati, 2021).

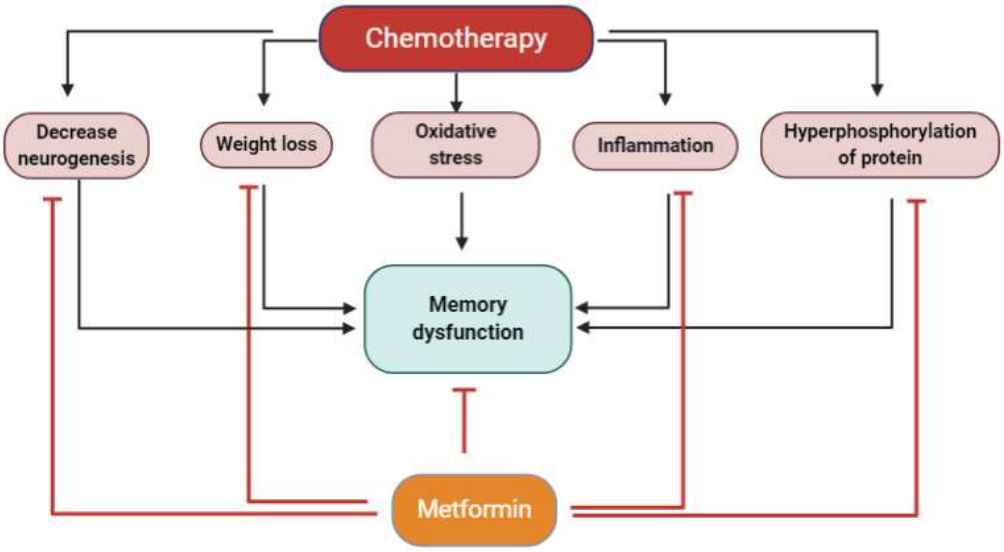

Figure 1: Mechanisms of CICI and potential protective effects of metformin. Mechanisms of CICI (black lines) and potential protective effects of metformin (red lines; Alhowail & Chigurupati, 2021)

Methotrexate (MTX) is a common antimetabolite chemotherapeutic agent that works by interfering with RNA and DNA by acting as a substitute to the normal building block of RNA and DNA, thus preventing a cell from reproducing more cancer cells (ACS, 2019). MTX is commonly used as an anti-cancer treatment as it treats many forms of cancer and has a high efficacy (ACS, 2019). However, MTX can reduce hippocampal neurogenesis associated with cognitive impairment leading to CICI (Sritawan et al., 2020).

Sritawan et al., (2020) did a study on how MTX effects rats in a novel object recognition task, and how its effect cell viability in the hippocampus. Sprague-Dawley rats were given 75 mg/kg dosage of MTX on days 7 and 14, exhibiting standard MTX treatment in humans. The rats were then placed in a 50 x 50 x 50 cm^3 black box where the animal was allowed to habituate for 30 minutes, followed by 24 hours of rest, and then 3 more minutes of habituation. Following this, the rats were allowed to explore the box with two objects (A & B; Figure 2). 15 minutes later, the rats were placed back in the box and allowed to explore again, but this time with a familiar

object (FO) and novel object (NO). Inherently, the rats should explore the novel object more than the familiar object (Sritawan et al., 2020). It was found that rats in the control group had high preference for the novel object, however the MTX group demonstrated equal preference for both objects ($p > 0.05$). It was found that through MTX treatment, the rats were unable to make the distinction of the new object ($p < 0.05$) compared to the control, thus exhibiting a reduced spatial memory.

Additionally, bromodeoxyuridine (BrdU) and DAPI immunostaining was used to determine cell survival and cell proliferation in the SGZ portion of the dentate gyrus in MTX treated rats (Figure 3). Each hemisphere was stored in a 30% sucrose solution before serial sections were cut along the coronal plane at 20 μm thickness. The 20 μm thick sections were treated with 2 N hydrochloric acid before being washed with 0.1 M borate buffer. The sections were incubated with primary anti-BrdU, and then with goat anti-rabbit secondary antibody. BrdU is an analog that incorporates DNA of cells during the synthesis phase of the cell cycle (Taupin, 2007). As such, BrdU can be used to determine cell viability in developing neuronal cells. Following this, all sections were counterstained with DAPI, a stain that helps determine number of nuclei living in cell samples (Taupin, 2007). These samples were run to determine cell survival in the SGZ of the dentate gyrus. Living cells can go under cell proliferation, an important process as it allows for an increase in cells being formed, increasing neural connections and thus cognitive function (NCI, 2023).

It was found that there was a significant decrease ($p < 0.0001$) in cell survival and proliferation in MTX treated rats compared to the control. This demonstrates that chemotherapeutic MTX treatment leads to a decrease in cell health and viability, causing cellular

processes to be altered in the hippocampus. When this happens, CICI related symptoms arise and can cause significant impacts in treated individuals.

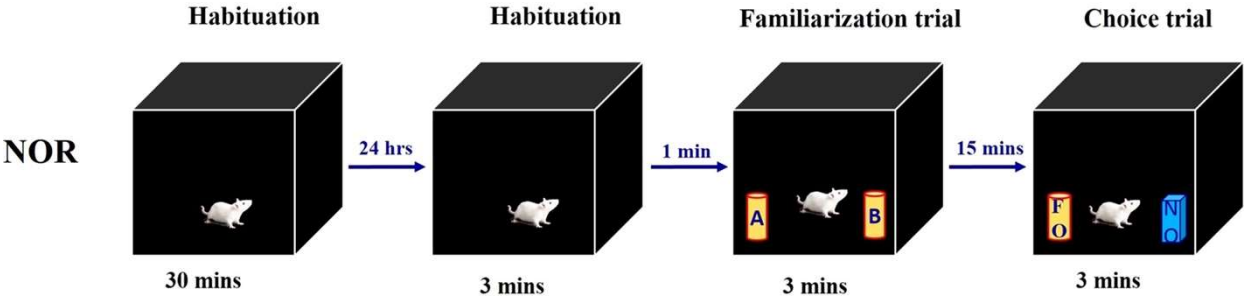

Figure 2: The novel object recognition protocol. (FO: familiar object, NO: novel object; Sritawan et al., 2020)

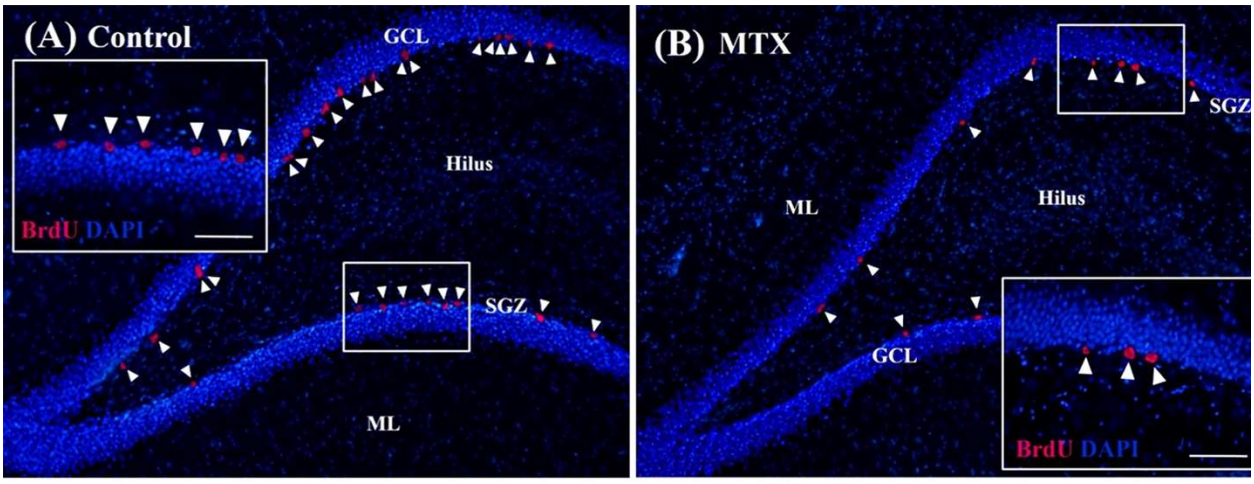

Figure 3: Representative images of BrdU (red) and DAPI (blue) immunostaining in the SGZ region of the hippocampus of rats, displaying cell survival. There is significant reduction in BrdU cells in the methotrexate group (B) compared to that of the control (A; Sritawan et al., 2020).

Another common chemotherapeutic, doxorubicin (DOX), is used as an anthracycline (anti-tumor antibiotic) to interfere with enzymes involved in copying DNA during the cell cycle (ACS, 2019). This prevents the ability of a cell to reproduce by binding to DNA, thus stopping cancerous cell division (ACS, 2019). DOX has also been associated with CICI related complications through actions of neurotoxicity (Alharbi et al., 2020).

Alharbi et al., (2020) did a study to determine the effect of doxorubicin on memory dysfunction in rats through behavioral testing. Forty male rats were divided into DOX treatment and control groups. Rats in the DOX treatment received intraperitoneal injections of 4 mg/kg DOX weekly for 5 weeks.

The first test was a novel object recognition test, in which the rats were allowed to explore two white teacups in a familiarization trial. The rats were then returned to their respective cages for 3 hours before being returned to a 40x 40 x 40 cm^3 wooden box in which the test was performed. In this trial, one of the two teacups was replaced with a novel object, and the time spent exploring the novel object was recorded over a 5-minute period. The time spent exploring the novel object was recorded alone versus as a differential ratio (time spent exploring novel object / total time spent exploring).

The second test is an elevated plus maze (EPM) test, which is used to measure anxiety as well as learning and memory processes. Rats were placed in a wooden apparatus consisting of two opposing arms: the open and closed arms (50 x 10 cm). The maze was placed 50 cm above the floor and during the training session, the rat was placed at the end of an open arm facing the central apparatus. They were then allowed to explore the apparatus for 10 minutes before returning to the maze 3 hours later for another trial to determine transfer latency time, which is the time it took the rat to move from the open arm to either of the closed arms.

In the novel object recognition test, DOX was significantly different ($p < 0.05$) than the control group. (Figure 11). In the EPM test, the transfer latency in the DOX-treated group significantly higher ($p < 0.05$) than those in the control group (Figure 12). These tests suggest memory was impaired by DOX to the control, showing DOX has neurotoxic effects that impact cell proliferation leading to CICI related effects.

In hopes of preventing CICI related effects, a novel treatment of metformin has been found to be one of the only known treatments (Alharbi et al., 2020). Growing evidence has shown the metformin may have the ability to be used in on diseases other than diabetes, including remedy in CICI effects.

Metformin: A Curing Agent

Although chemotherapy has been shown to be successful in removing cancer, the toxicity associated with chemotherapy leads to acute and chronic side effects including hair loss, anemia, fatigue, and chemotherapy-induced cognitive impairment (ACS, 2020). Cognitive dysfunction in patients can vary anywhere from moderate to severe, altering everyday function of affected individuals, and can affect emotional and mental status (Alharbi et al., 2020). CICI remains a major clinical challenge, effecting upwards of 75% of treated patients, with very few therapeutic strategies to address neurotoxic effects (Alharbi et al., 2020). Metformin, a type II diabetes drug, has shown beneficial effects for diseases other than diabetes alone, including remedy of CICI related symptoms (Whittaker et al., 2022; Li et al., 2022). As there have not been any other clinical treatments, including prescriptions or procedures, it is worthwhile to explore metformin as a potential treatment for CICI.

Metformin is a commonly prescribed drug for patients with type II diabetes mellitus (T2DM) and works by decreasing intestinal glucose absorption, improving peripheral glucose uptake, and increasing insulin sensitivity (Wang et al., 2017). Recognized as a 5'-AMP-activated protein kinase (AMPK) agonist that induces energy stress, metformin inhibits the mitochondrial respiratory chain complex I and decreases ATP production (Li et al., 2022). AMPK can sense low ATP levels, causing cells to switch from an anabolic to catabolic (Li et al., 2022). Metformin-mediated AMPK can increase insulin sensitivity (Figure 4) by inhibiting acetyl CoA carboxylase (ACC) activity and inhibiting the expression of gluconeogenic genes (Li et al., 2022).

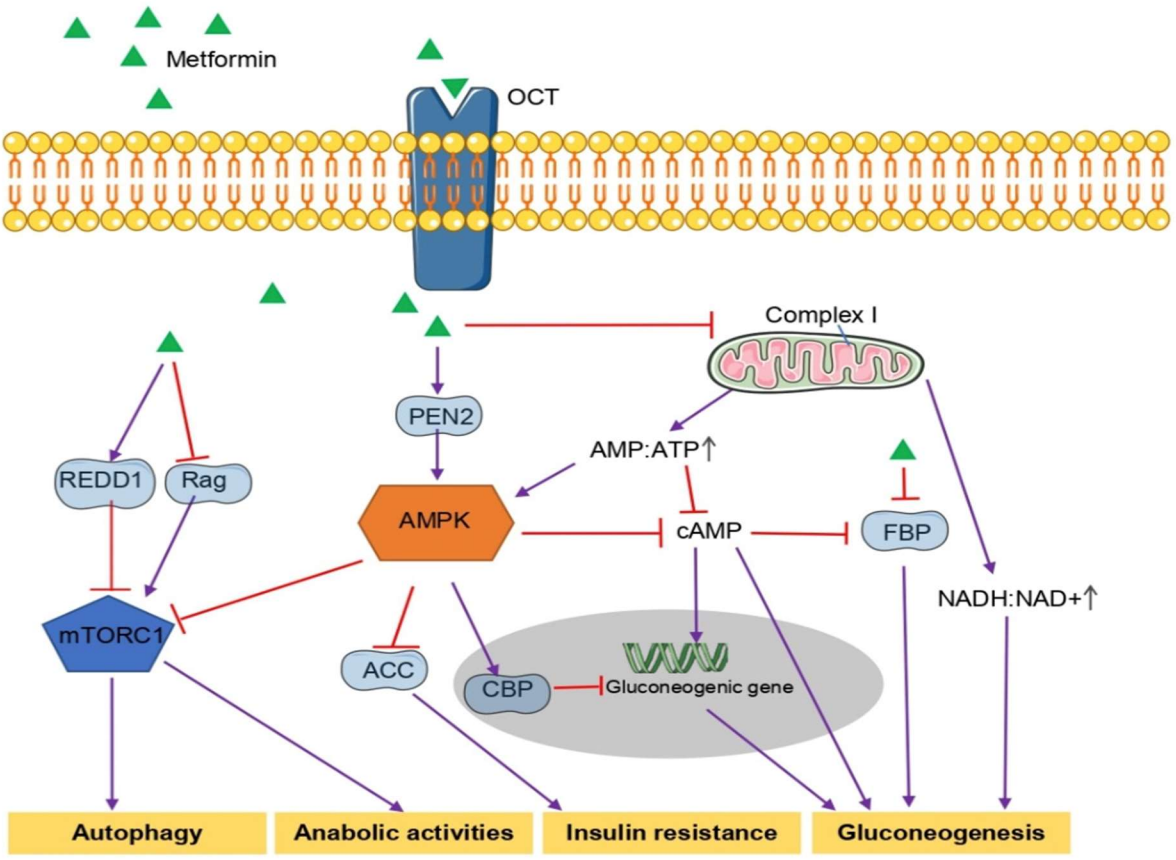

Figure 4: The general mechanism of underlying hypoglycemic effects of metformin. Metformin activates AMPK through mitochondrial mechanisms (Li et al., 2022).

In the brain, metformin can regulate autophagy by AMPK signaling pathways to alleviate abnormal protein aggregations thus causing a decrease in oxidative stress and a suppression of neuroinflammation (Figure 4; Li et al., 2020). Also, metformin can balance the excitatory: inhibitory neurotransmitter ratio by directly regulating the amount of presynaptic neurotransmitter release or binding availability of the postsynaptic membrane (Li et al., 2022). This is important as it allows metformin to significantly enhance synaptic plasticity in a variety of pathological conditions, thus regulating cognitive performance (Li et al., 2022).

In addition to being the first line of defense for T2DM for more than six decades, metformin has shown high pleiotropic activity by having effects on other body systems other than the intended (Drzewoski & Hanefeld, 2021).

Zhou, et al., (2016) investigated the effect of metformin on cisplatin-induced cognitive impairment using behavioral assays. Cisplatin is a platinum-based chemotherapy compound that led to an increase in coherency of myelin basic protein (MBP) fibers, causing a depletion in white matter integrity (Zhou et al., 2016). Drugs such as cisplatin are commonly used as standard treatment for numerous forms of cancer (Whitney et al., 2008). White matter depletion can cause behavioral abnormalities as well as morphological changes to neuron organization including a reduction in dendritic branching and astrocyte activation.

Fifty-six female mice, aged 8-10 weeks old, were intraperitoneally treated with 2.3mg/kg cisplatin per day or saline for 3 cycles consisting of 5 daily injections followed by 5 days without injections, resulting in a cumulative dose of 34.5 mg/kg over a 30-day period. In treatment, 100mg/kg of metformin or saline was given intraperitoneally for seven days starting 1 day prior to the first injection of cisplatin in each cycle and 1 day after the last dose. Following treatment, all mice were placed into a novel object recognition test to test memory (Figure 2). They are then habituated to the test arena for 5 min/day for 5 days. On day 6, the mice were placed back into the box with 2 identical objects in which they were allowed to explore for 5 minutes. Following this, the mouse returned to the box 7 minutes later and were allowed to explore the arena, but this time, there was a novel object in addition to the familiar object. The time spent sniffing, climbing, or touching the objects was scored and analyzed though a discrimination index (time with novel object / total exploration time of both objects).

After the novel object recognition task, a social discrimination test was performed two to three days later. During the training period, a 6-week-old juvenile mouse was placed in a mesh wire enclosure and into the home cage of the test mouse for 5 minutes. The test mouse was allowed to freely explore the new enclosure, and then removed 8 minutes later. They then place 2 juvenile mice, one familiar and one novel in the home cage of the test mouse for 5 minutes. A social discrimination index (time with novel mouse / total exploration time) was calculated and interpreted (Figure 6). A preference for the novel mouse is assessed as an indication of cognitive function.

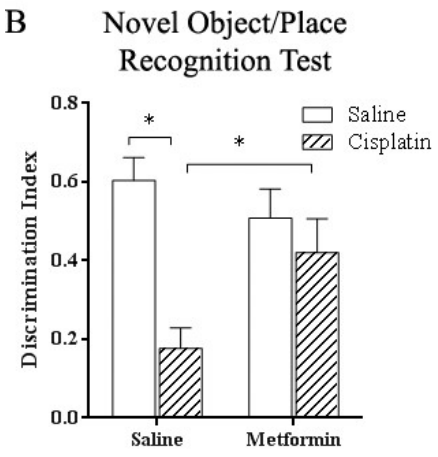

Figure 5: Effect of Metformin and Cisplatin on Cognitive Function in Mice. The discrimination index (time with novel object / total exploration time of both objects) is plotted as a value from 0-1 (Zhou et al., 2016).

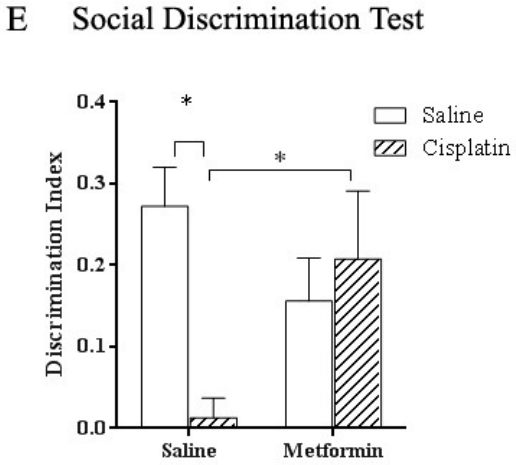

Figure 6: Effect of Metformin and Cisplatin on Cognitive Function in Mice. The social discrimination index (time with novel mouse/ total exploration time) is plotted as a value from 0-1 (Zhou et al., 2016).

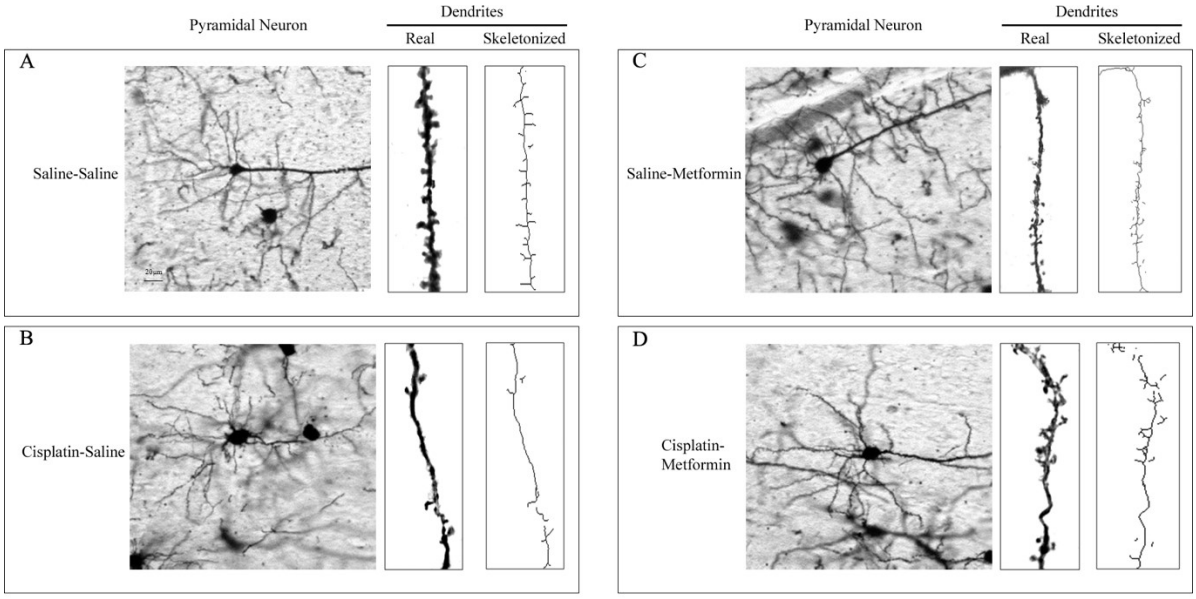

Figure 7: Effect of Cisplatin and Metformin on Dendritic Spine Density in the Cingulate Cortex of Mice. Brains were stained using a Golgi-staining kit and analyzed through Sholl analysis (Zhou et al., 2016).

Following the behavioral assessments, the treated mice were euthanized, and brain samples from the cingulate cortex were collected. Two pyramidal neurons from the cingulate cortex of each mouse were selected to determine dendric spine density and number. Dendritic spines are small protrusions off neuronal dendrites that aid in signaling between neurons (Runge et al., 2020). Although dendric spines vary in density over a lifespan, they play an important role in brain activity and function (Runge et al., 2020). Brains are then stained with Golgi immersion to screen for morphological abnormalities including a decrease in spine density and dendritic branches. Spine density of pyramidal cells can be quantified by the number of protrusions and branches per dendrite length (mm).

Zhou et al., (2016) find that the mice exposed to cisplatin + metformin treatment showed a significantly different (0.42 ± 0.09, $p < 0.05$) discrimination of the novel object compared to those that just received cisplatin treatment (0.18 ± 0.05; Figure 5). Similar results are found in the social discrimination test. Mice exposed to cisplatin + metformin treatment showed a

significantly different (0.21 ± 0.08, $p < 0.05$) discrimination index than those that just received cisplatin treatment (0.60 ± 0.06; Figure 6). Mice treated with cisplatin alone display a discrimination index score approximately 10 times less (0.02 ± 0.02) than mice treated with cisplatin and metformin (all data expressed as mean ± SEM). Cisplatin treatment caused deficits in spatial orientation and memory, leading to behavioral abnormalities and cognitive deficits. However, metformin treatment completely prevented cisplatin-induced cognitive deficits if taken one day before and one day after cisplatin treatment.

Through Golgi staining, it has been found that the number of dendritic spines in the cingulate cortex was significantly lower (153.07 ± 30.90, $p < 0.05$) in only cisplatin treated mice than in cisplatin + metformin treated mice (597.6 ± 88.41, $p < 0.05$; the number of dendritic spines/mm is expressed as mean ± SEM). The control treatment displayed (658 ± 66.96, $p > 0.05$) dendric spines/mm, suggesting that cisplatin + metformin treated rats showed little to no abnormalities. If dendric spines in the cingulate cortex are reduced, the synaptic contact between neurons will thus be reduced causing a slowing of signals passed within the brain, leading to cognitive impairments including CICI (Runge et al., 2020). However, metformin protects against the chemotherapy induced reduction in neural arborization.

Ultimately, the result of this study demonstrated the preventative effects of metformin on memory, cell proliferation, cell survival and neuronal deterioration caused by cisplatin treatment in a mouse model (Zhou et al., 2016). This may occur through the normalization of oxidative stress in the hippocampus and induction of cell proliferation, leading to increased cognitive performance. As cisplatin is a commonly used chemotherapy drug, it is important to recognize the adverse effects on cognition that may be induced through the heavy toxicity of this treatment.

Although the ability of metformin as a curing agent following CICI symptoms was not explored, metformin can be a preventative agent if taken in conjunction with cisplatin (Zhou et al., 2016).

In another study, Sritawan et al., (2020) explored the effects of metformin on hippocampal neurogenesis induced by methotrexate (MTX) chemotherapy. MTX is a common chemotherapeutic treatment that interferes with RNA and DNA function, preventing cellular replication (ACS, 2023). It has shown adverse effects to patients' cognition through decreasing hippocampal neurogenesis associated with cognitive impairment (Sritawan et al., 2020).

Thirty male Sprague-dawley rats, aged 4-5 weeks, were separated into 5 different groups and randomly assigned to either the control, MTX, metformin, preventive (co-administration of metformin before and during receiving MTX), or throughout (co-administration of metformin before, during, and after receiving MTX) groups (Table 1; Figure 8).

Rats were then run through a novel object recognition test to determine recognition memory (Figure 2). The rats were allowed to habituate to an empty open-field arena (50 x 50 x 50 cm^3) for 30 minutes before being placed back in the arena for a familiarization trial of 3 minutes. Here, the rats explore a pair of identical objects placed in set regions of the enclosure. After the familiarization trial, the rats were returned to an arena to explore one of the familiar objects, and a novel object for 3 minutes. The preference index value was determined as a percentage (time spent exploring novel object / combined time exploring both objects).

Following the novel object recognition task, rats were killed, and the brain from each rat was removed to determine cell proliferation in the SGZ of the dentate gyrus. The dentate gyrus is a part of the hippocampus critical for encoding memory and function (Piatti et al., 2013). Subsequentially, the SGZ (subgranular zone) region of the brain is in the dentate gyrus and is where adult neurogenesis occurs (Piatti et al., 2013). Each hemisphere was stored in a 30%

sucrose solution before serial sections were cut along the coronal plane at 20 μm thickness. The 20 μm thick sections were incubated with primary anti-Ki67 and then with a rabbit anti-mouse secondary antibody. Ki67 is a protein found in all vertebrates and can be marked through immunostaining (Miller et al., 2018). As Ki67 is indicated in every phase of the cell cycle, it allows researchers to tag it and locate proliferating cells by microscopy (Miller et al., 2018). Following this, all sections were counterstained with propidium iodide and cover slipped. These samples were run to determine cell proliferation in the SGZ of the dentate gyrus. Cell proliferation is an important process as it allows for an increase in cells being formed, increasing neural connections and thus cognitive function (NCI, 2023).

As a result, metformin and co-treatment groups exhibited a significant difference ($p < 0.05$) in preference index (PI) compared to MTX treatment alone (Figure 9). The PI of all groups were significantly over 50% ($p < 0.05$), suggesting no memory deficits in the rats. However, the MTX group was below a PI of 50% ($p > 0.05$), indicating possible memory impairment. This suggests that exposure to MTX chemotherapy impaired recognition memory and metformin reduced this impairment.

It was also found that MTX treatment significantly reduced proliferating cells compared to the control group ($p < 0.0001$). Those in the treatment groups had nearly double the number of proliferating cells compared to the MTX treatment, showing metformin significantly increases ($p < 0.01$) the number of proliferating cells in the SGZ of the dentate gyrus. The quantity of proliferating cells in the metformin, preventive, and throughout groups was similar to the control group, suggesting a strong efficacy of metformin in recovery from MTX (Figure 10). As proliferating cells are important in cognitive function, metformin having preventive and curing effects on MTX induced cognitive impairment is highly important in discussing CICI remedies.

Table 1: Treatment Groups and Respective Drug Dosages. All groups followed strict administration procedures. (i.p: intraperitoneal, i.v: intravenous; Sritawan et al., 2020).

Treatment Group	Drug Dosage
Control (n=6)	0.9% saline via (i.p.) and (i.v.) injection
MTX (n=6)	75 mg/kg methotrexate
Metformin (n=6)	200 mg/kg metformin via (i.p.) injection
Preventative (n=6)	75 mg/kg methotrexate 200 mg/kg metformin via (i.p.) injection
Throughout (n=6)	75 mg/kg methotrexate 200 mg/kg metformin via (i.p.) injection

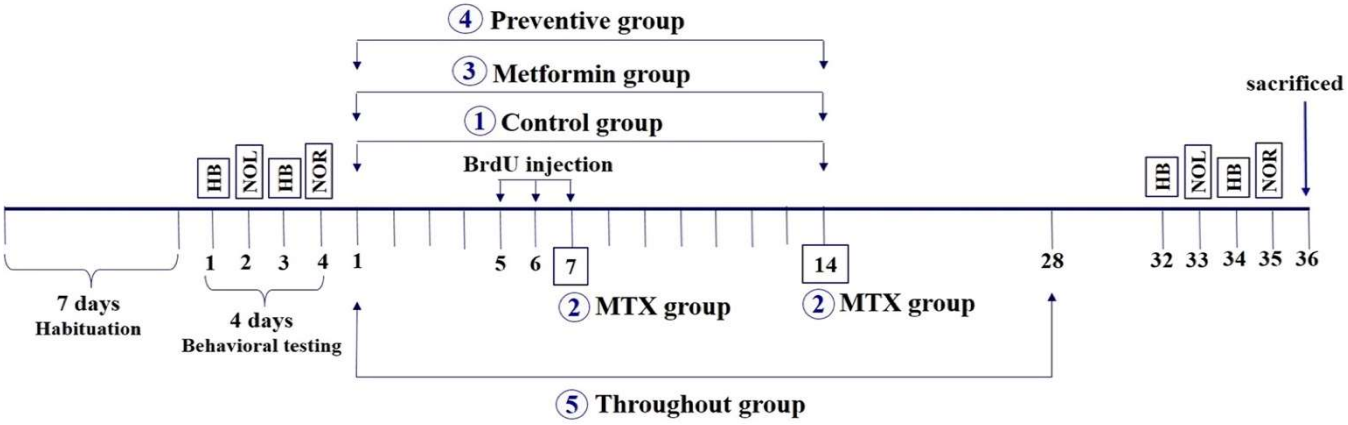

Figure 8: Timeline of Drug Administration, Behavioral Testing, and Euthanization. (HB: habituation, NOR: novel object recognition test; Sritawan et al., 2020).

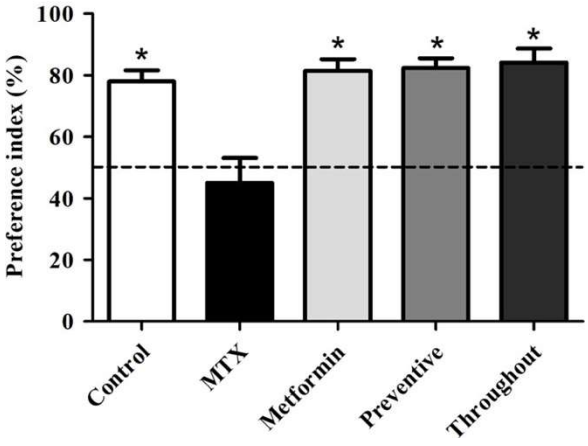

Figure 8: Effect of Metformin and Methotrexate on Cognitive Function in Rats. The preference index (time with novel object / total exploration time of both objects) is plotted as a value from 0-100% (Sritawan et al., 2020).

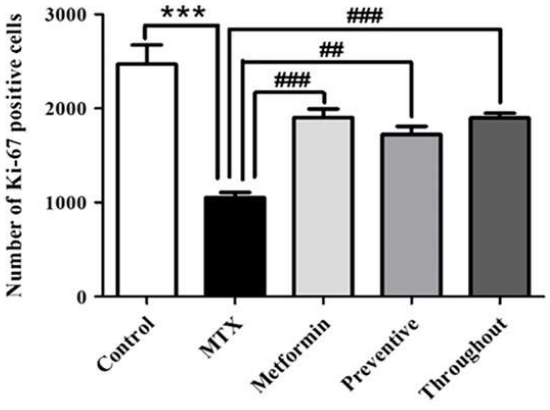

Figure 9: Effect of Metformin and Methotrexate on Proliferating Cells in Rats. The number of Ki-67 positive cells is plotted as a value from 0-3000 (Sritawan et al., 2020).

Sritawan et al., (2020) demonstrated the preventative effects of metformin on memory and cell proliferation caused by MTX chemotherapy in a rat model. Through the reduction of reactive oxygen species associated with DNA damage, mitochondrial respiration was inhibited by metformin and subsequentially reduced cognitive impairments associated with hippocampal structure. In addition, hippocampal neurogenesis was suppressed by MTX suggesting the production of newborn neurons are essential to the formation of hippocampus-dependent memories. When co-administered with MTX, metformin relieved any impairments of hippocampal neurogenesis, indicating a curing nature.

Alharbi et al., (2020) explored the effects of metformin on doxorubicin-induced memory dysfunction. Doxorubicin (DOX), a chemotherapy treatment that interferes with enzymes involved in copying DNA during the cell cycle, severely impairs hippocampus-dependent

cognitive function (ACS, 2023). These cognitive dysfunctions are associated with neurogenesis, alterations in protein function, and inflammation (Alharbi et al., 2020).

Forty male rats were divided into four separate groups: DOX treated, metformin treated, DOX + metformin treated, and control groups. Rats in the DOX treatment received intraperitoneal injections of 4 mg/kg DOX weekly for 5 weeks. The metformin treated rats had 3 mg/mL dissolved in drinking water daily. In the DOX + metformin treatment group, metformin was administered in the same way before DOX treatment. Following 5 treatments which represented a typical chemotherapy cycle, rats were placed in 2 unique behavioral tasks to determine cognitive effects of each treatment group.

The first test was a novel object recognition test, in which the rats were allowed to explore two white teacups in a familiarization trial. The rats were then returned to their respective cages for 3 hours before being returned to a 40 x 40 x 40 cm^3 wooden box in which the test was performed. In this trial, one of the two teacups was replaced with a novel object, and the time spent exploring the novel object was recorded over a 5-minute period. The time spent exploring the novel object was recorded alone versus as a differential ratio (time spent exploring novel object / total time spent exploring).

The second test is an elevated plus maze (EPM) test, which is used to measure anxiety as well as learning and memory processes. Rats were placed in a wooden apparatus consisting of two opposing arms: the open and closed arms (50 x 10 cm). The maze was placed 50 cm above the floor and during the training session, the rat was placed at the end of an open arm facing the central apparatus. They were then allowed to explore the apparatus for 10 minutes before returning to the maze 3 hours later for another trial to determine transfer latency time (time it took the rat to move from the open arm to either of the closed arms).

In the novel object recognition test, DOX, DOX + metformin and metformin alone groups all were significantly different ($p < 0.05$) than the control group. (Figure 11). In the EPM test, the transfer latencies in the DOX-treated and metformin treated groups is significantly higher ($p < 0.05$) than those in the control group (Figure 12). These tests suggest memory was impaired in all treatment groups compared to the control, showing metformin treatment has little to no effect on remedying CICI related symptoms.

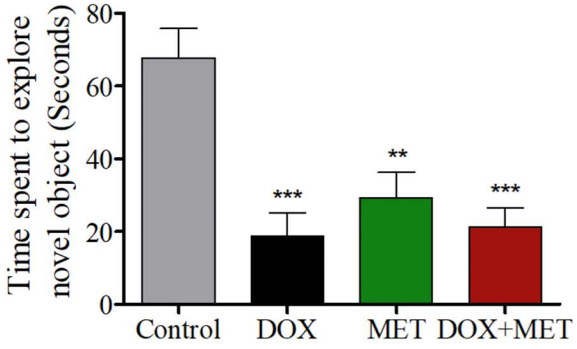

Figure 11: Effect of Metformin and Doxorubicin on Cognitive Function in Rats. Time spent exploring the novel object is plotted as a value from 0-80 seconds (Alharbi et al., 2020).

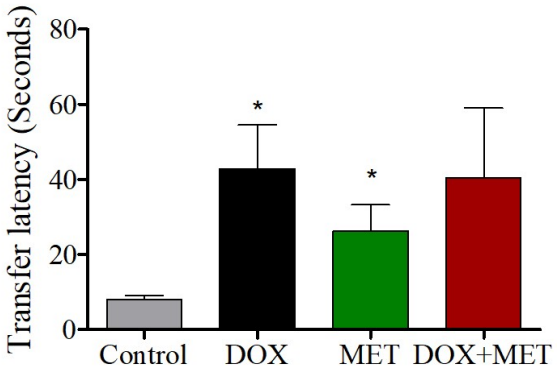

Figure 12: Effect of Metformin and Doxorubicin on Transfer Latency in Rats. Time spent moving from open arms to closed arms is plotted as a value from 0-80 seconds (Alharbi et al., 2020).

This study shows that DOX treatment can cause memory loss in behavioral tests. This occurs through DOX interference of AMPK signaling pathways, producing a negative feedback loop that negatively impacting cognition (Gratia et al., 2012). Even though metformin can reduce cytotoxic effects of several chemotherapy agents including cisplatin and methotrexate, it failed to reduce neurotoxicity induced by DOX. The mechanism by which this process occurs is unknown, and future studies are needed to investigate the protective effects of metformin on neurotoxicity in humans.

In addition to not working with all chemotherapeutic drugs, metformin has also exhibited the ability to cause dementia through treatment (Chin-Hsiao, 2019). In a case study with 7086

Alzheimer's patients showing dementia symptoms, there was a 41% increased risk of dementia associated with metformin use compared to those that did not use metformin (Imfeld et al., 2012). Conflicting effects of metformin on cognition were also observed in a small clinical study conducted in Australia (Moore et al., 2013), in which 35 metformin users and 91 non-users were evaluated on multiple cognitive tests. Metformin users showed an 8% worse cognitive performance on a mini-mental state examination (MMSE) test than those that did not use metformin (Moore et al., 2013). The MMSE test measures five areas of cognition responsible for everyday function. This may have occurred through patients prescribed metformin having lowered serum vitamin B_{12} levels, as the MMSE score of this group was 7% lower than the control (Moore et al., 2013). Although the risk of dementia has been proven in T2DM patients, it has not been found to effect or worsen symptoms of CICI. Moreover, a high number of chemotherapy treated individuals experience CICI effects, and this number drops to approximately 2% following metformin treatment (Whittaker et al., 2022). Additionally, since 2005 metformin has reduced the risk of cancer and effects of CICI by 23% worldwide (Saraei et al., 2019). This suggests a high efficacy of the drug and little to no reason not to use it. Surprisingly, metformin has the highest total availability among surveyed drugs around the world (>80%) (Babar et al., 2019). Pricing also remains generally low as metformin is an essential drug used around the world for type II diabetes.

As shown above, metformin has curing nature for some, but not all chemotherapy treatments. Although the mechanisms by which metformin works in the brain in conjunction with differing chemotherapeutic treatments is understudied, some investigators revealed that dose of metformin may influence efficacy (Kasznicki et al., 2014). In conjunction with

chemotherapy treatment, the dose of metformin needs to be carefully monitored and evaluated to prevent harmful drug-drug interactions (Kasznicki et al., 2014).

In conclusion, many different chemotherapy treatments have shown a negative effect on memory function and brain morphology in patients. Metformin has shown a high ability in curing chemotherapy patients of CICI related symptoms and increasing memory function by mediating the AMPK pathway and suppressing inflammation (Li et al., 2020). Any ability to reduce risk of side effects or occurrence of disease is seen as a benefit, and metformin clearly works as a treatment option. As shown through research by Alharbi et al., (2020) there is little to no harm in taking metformin in conjunction with chemotherapeutics, so it should be universally used.

Conclusions

Cancer remains as a public health issue, representing the second leading cause of death worldwide (World Health Organization, 2021). There are many treatment options to combat cancer, however none are as effective as chemotherapy. Chemotherapy can kill cancer cells, but it can also damage healthy cells. This can cause major side effects including hair loss, anemia, fatigue, and fertility issues (ACS, 2020). Chemotherapy-induced cognitive impairment (CICI) is a lesser-known side effect, but it effects as many as 3 out of 4 people that received chemotherapy. CICI is stimulated by damages to cellular proliferation in the brain, leading to impairments to visual processing, executive function, and attention, all affected in various severities. The severity from person to person generally differs, with some people being unable to return to work, and others regaining full ability to think and function (Mayo Clinic, 2023) (Swiner, 2022). Chemotherapy treatments, doxorubicin, methotrexate, and cisplatin have all shown neurotoxic effects by decreasing memory and social recognition in rat models (Alharbi et al., 2020; Sritawan et al., 2020; Zhou et al., 2016).

There has been growing evidence documented that supports the ability of metformin, a type II diabetes mellitus drug, to have beneficial effects for diseases other than diabetes alone, including remedy of CICI related symptoms (Whittaker et al., 2022; Li et al., 2022). Metformin is a readily accessible drug that exhibits an ability to alter cellular energy metabolism (Saraei et al., 2019). The side effects of metformin are generally minimal and include nausea and vomiting, occurring in only 2% of people worldwide (NHS, 2022). This number is much lower than the number of people who receive chemotherapy and experience side effects (Whittaker et al., 2022). In addition to being readily accessible worldwide, pricing remains generally low as it is an

essential drug that is used for type II diabetes. Metformin has the highest total mean availability around the world with (>80%) among of surveyed medicines (Babar et al., 2019).

However, metformin shows the ability to improve cognitive performance in variable behavioral tests and cure possible CICI effects, but not when paired with select chemotherapies (Alharbi et al., 2020; Sritawan et al., 2020; Zhou et al., 2016). Alharbi et al., (2022) showed that when doxorubicin (DOX) was paired with metformin treatment, CICI side effects were unable to mediated. Metformin did not cause any negative effects when paired with DOX treatment, signifying a low risk of harm.

` Metformin has also exhibited the ability to cause dementia through treatment in high dosage (Chin-Hsiao, 2019). Although the risk of dementia has been proven in T2DM patients, it has not been found to effect or worsen symptoms of CICI. Patients who are experiencing CICI effects have shown high recovery of cognitive function after metformin treatment, suggesting a strong efficacy (Drzewoski & Hanefeld, 2021). Moreover, a high number of chemotherapy treated individuals experience CICI effects (54%), and this number drops to approximately 2% following metformin treatment (Whittaker et al., 2022). Overall, metformin is a unique treatment as it is cheap mostly harmless and has minimal side effects.

However, metformin shows the ability to improve cognitive performance in variable behavioral tests and cure possible CICI effects, but not when paired with select chemotherapies (Alharbi et al., 2020; Sritawan et al., 2020; Zhou et al., 2016). Alharbi et al., (2022) showed that when doxorubicin (DOX) was paired with metformin treatment, CICI side effects were unable to mediated. Metformin did not cause any negative effects when paired with DOX treatment, signifying a low risk of harm.

Metformin can also be used to lower cancerous cell proliferation and increase healthy cell survival in treated patients (Saraei et al., 2019). The anti-proliferative effect of metformin is associated with AMPK activation and additional, less involved mechanisms (Saraei et al., 2019). Additionally, as metformin regulates AMPK and increases insulin sensitivity, cells can function at a greater level, leading to healthy cell survival.

In addition to decreasing CICI related symptoms, metformin has also shown the ability to be used in conjunction with cancer treatments to starve cancerous cells during treatment, acting as a second form of treatment (Saraei et al., 2019). In response to metformin reducing gluconeogenesis through kinase signaling, an increase glucose uptake in cells occur, and ultimately leads to a decrease in glucose levels. This process allows for the starvation of cancerous cells, causing cellular death. Since 2005, metformin has reduced the risk of cancer by 23% worldwide and reduced the chance of any cancers up to 88% (Saraei et al., 2019). Additionally, metformin may have anti-tumor effects by lowering insulin levels, thus activating AMPK which prevents the transcription of genes responsible for glycogenesis (Saraei et al., 2019).

It is my suggestion that metformin should be used to lower the decrease of cognitive impairments caused by chemotherapy. As there have not been any other clinical treatments, including prescriptions or procedures, it is worthwhile to further explore metformin as a potential treatment for CICI. Cognition is ultimately the premise of personality in humans, and any effect to this can alter how someone views the world and themselves. A simple treatment of metformin at low dosages is highly affordable, effective, and will not cause any additional harm.

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