Canurta

SIRT2/MAGL/FAAH Whitepaper

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Introduction

The world of medicine is constantly evolving and demands innovation in order to treat certain illnesses and diseases. Pharmaceutical companies need to develop safe and effective drug therapies that can combat disease and prolong life. As the world's population continues to grow and the average person starts to live longer, the prevalence of many diseases will rise. The world recently experienced a pandemic that devastated the economy and healthcare. Even today, the healthcare system is struggling to keep up with the number of people that are sick and most people are unable to receive the treatments they need. Canurta has the potential to enter the market with products that can help alleviate the pain commonly associated with diseases such as cancer, sickle cell anemia, A lzheimer disease and other aging related disorders. The issue being addressed in this report is based on the understanding of how there are unmet needs in healthcare and how Canurta should move forward in providing an effective treatment option for certain diseases that are less prevalent or have relatively few remedies available. The three enzymes outlined in this report were researched to identify and understand their role in regulating inflammation in the body. They include fatty acid amide hydrolase (FAAH), Sirtuin 2 (SIRT2) and Monoacylglycerol lipase (MAGL). This review outlines the biological activity of each inhibitor, disease states impacted by each target, and the potential therapeutic benefits of flavonoids as anti-inflammatory treatments. It also explains the market potential for Canurta and assesses current drug trials and avenues to explore when designing new drug treatments.

Background

SIRT2 is present primarily in the cytoplasm and deacetylates the major component of microtubules [10]. SIRT2 also transiently migrates to the nucleus during G2/M transition of mitosis and deacetylates histone H4, thereby modulating chromatin condensation during metaphase [10]. There is a link between SIRT2 and neurodegeneration through the transcription factor, nuclear factor kappa B (NF-κB) [10]. NF-κB plays a pivotal role in regulating gene expression related to aging and inflammation. It does this by inducing the expression of pro-inflammatory cytokines which are classified as signaling molecules [10]. Once the cytokines are active they essentially elicit an immune response in the body and promote the production of white blood cells [10]. *In vivo* and *in vitro* evidence suggests that SIRT2 is a crucial regulator of neurological function in the differentiation and survival of various nerve cells [11]. SIRT2 gene knockout procedures in mice have led to impairments in the normal function of the nervous system associated with aging [11]. Additional research examining the knockout of SIRT2 in different nerve cells of mice will further our understanding of its function in the nervous system [11].

There still needs to be more research to determine the direct effect of SIRT2 on Alzheimer's disease (AD) [10]. The expression of SIRT1 and SIRT2 during aging and the progression of Alzheimer's disease differ considerably. SIRT1 mRNA expression levels were significantly decreased in both older people and AD patient groups compared to young people; however, the reverse is seen for SIRT2 [12]. The potential increase in SIRT2 expression can be indicative of a possible link between treating these diseases and inhibiting SIRT2. The inhibition

of SIRT2 may play an essential role in the improvement of cognitive abilities and the modulation of relevant pathways in the progression of AD [12]. It is important to think about the overall impact of inhibiting SIRT2 and how an effective treatment may require the coordination of multiple inhibitors and drugs. For example, histone deacetylase (HDAC) inhibitors increase histone acetylation and enhance memory and synaptic plasticity [13]. This paired alongside a SIRT2 inhibitor or SIRT1 activator may provide a therapeutic advantage compared to single drug treatments.

The second inhibitor under investigation was fatty acid amide hydrolasse (FAAH). There is a great deal of evidence suggesting that FAAH can be targeted to treat inflammatory disorders [1]. Blockade of FAAH has been shown to reduce pain sensation, inflammation, and anxiety/depression without substantial effects on motility or cognition [2]. The beneficial effects caused by FAAH blockade are predominantly mediated through the activation of CB1 or CB2 receptors in the brain [1]. Endocannabinoids are produced on-demand by post-synaptic neurons, rather than being stored in synaptic vesicles like classical neurotransmitters [2]. The endocannabinoid system (ECS) is composed of the two cannabinoid receptors CB1 and CB2, the endogenous cannabinoid ligands: N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes involved in endocannabinoid synthesis and degradation [4]. The most well-studied FAAH inhibitor is URB597, a carbamate that irreversibly inhibits FAAH with high selectivity in the nervous system [2]. Development of a FAAH inhibitor that displays optimal combination of efficacy and selectivity has remained an intensely pursued objective for companies and researchers [2]. Another available FAAH inhibitor is PF-3845. It covalently inactivates FAAH by carbamylation of the enzymes serine nucleophile [2]. Studies have shown that PF-3845 completely inhibited FAAH activity and did not react with

other serine hydrolases *in vivo* [2]. This means that it did not have any immediate adverse effects on the model organism and could be an optimal approach to treating relevant disease states [2]. In addition, PF-3845 is a highly selective and efficacious inhibitor of FAAH that should prove to be a valuable pharmacological tool for investigating the function of the endocannabinoid system [2].

The last inhibitor studied was MAGL. Monoglyceride lipase (MGL) , also referred to as monoacylglycerol lipase (MAGL), is the major enzyme catalyzing the hydrolysis of MGs into glycerol and fatty acids [3]. The most prominent sugar species acting as signaling lipid is 2-arachidonoyl glycerol (2-AG) which is the most abundant endogenous agonist of cannabinoid receptors in the body [3]. Recent observations demonstrate that 2-AG is needed for arachidonic acid (AA), the precursor of prostaglandins and other inflammatory mediators [3]. MAGL inhibitors have also been shown to exert anti-inflammatory action in the brain and protect against neurodegeneration [5]. MAGL appears to be the main enzyme involved in AA synthesis in the brain, liver, and lungs [6]. The inhibition of MAGL promotes neuron survival in chronic neurodegenerative disorders and reduces brain edema and neuronal loss [6]. The majority of anti-inflammatory drugs used are COX inhibitors because they decrease neuroinflammation and the chance of getting a neurodegenerative disorder [3]. Since MAGL inhibitors do not exert control over AA and prostaglandin pathways in the gastrointestinal system, they do not exhibit the toxicity commonly associated with the COX1/COX2 inhibitors [5]. *In vitro* experiments have shown that 2-AG may exhibit its anti-inflammatory effects through CB1 or CB2 receptors [14]. MAGL metabolizes 2-AG in presynaptic membranes of neurons and releases arachidonic acid and glycerol, leading to downstream prostaglandin synthesis and inflammation [14]. Inactivation of MAGL can reduce Aβ plaque production in a mouse model of Alzheimer's disease (AD). The

inhibition also exerted anti-inflammatory effects that improved synaptic functions and cognitive skills in AD animal models [17]. The pharmacological inhibition of MAGL prolongs the lifetime of the endogenous 2-AG [25]. By prolonging 2-AG activity, an indirect activation of CB1 and CB2 results, which reduces inflammation and pain; with less likelihood of producing the side effects associated with natural or synthetic cannabinoids, including dependency, cognitive impairment, sedation and psychological problems [25].

Disease Targets

Aging

Aging has been outlined in numerous research articles as a potential target for flavonoids and anti-inflammatory treatments. It has been reported that proteins involved in nucleosome assembly, ER nuclear signaling, and the response to unfolded proteins increase, whereas the abundance of proteins involved in metabolism (fatty acid, carbohydrate, and amino acid) decreases during aging [42]. Epigenetic regulation in cooperation with transcriptional regulators could act as markers of aging [42]. Compounds under investigation that increase lifespan include: metformin, rapamycin (immunosuppressive agent), resveratrol (polyphenol and sirtuin activator), spermidine, aspirin and masoprocol (antioxidant and anti-inflammatory properties) [42]

FAAH inhibitors have had the greatest promise in reducing the inflammation associated with arthritis and other aspects of aging. Experimental studies have demonstrated that one of the most important functions of the endocannabinoid signaling system is tissue protection against injury [9]. Fatty acid amide hydrolase (FAAH) activity was analyzed in human cortical membrane samples and in membranes from adult and aged rat brains [9]. FAAH participation in AEA hydrolysis was demonstrated using URB597, a FAAH specific and irreversible inhibitor.

URB597 partially inhibited AEA degradation. This indicates that other enzymes, besides FAAH, are responsible for AEA hydrolysis and the control of aging in the body [9]. Mental health deterioration due to chronic neurodegenerative diseases represents the largest cause of disability in the world [22]. There is significant potential for Canurta to treat aging with their flavonoid molecules particularly in the elderly population. A dietary supplement can ensure that individuals receive a naturally sourced treatment that reduces inflammation caused by aging.

Alzheimer's Disease

It is estimated that 6.5 million Americans age 65 and older are living with Alzheimer's in 2022 [49]. About 1 in 9 people aged 65 and older have Alzheimer's and it is projected by 2050 that the number of Alzheimer's patients may grow to 12.7 million [49]. Alzheimer's disease (AD) is mostly diagnosed by the detection of amyloid plaques in the brain [8]. Inflammation has been proposed as the main contributing factor in the development of AD [8]. The occurrence of amyloid β peptide culminates in pathways of synaptic and cognitive dysfunction and neuronal death [28]. AD also results in neurofibrillary tangles (NFTs) in the brain [14]. This leads to memory loss, cognitive and motor impairment, and changes in personality and behaviour [14]. This irreversible and debilitating disease is the most common cause of dementia, and there are no clinically effective treatment options available [14]. Most studies support that increased risks of AD and cognitive decline are correlated with higher saturated fatty acids (SFAs) in dietary intake [17]. Inheritance of the apolipoprotein E (APOE) gene is the only well-established genetic risk factor for sporadic AD [20]. Some studies have identified age as another major risk factor for AD because the number of plaques and tangles increases as people grow older [20]. N eurodegeneration in AD is estimated to start twenty to thirty years before the appearance of clinical symptoms [20].

Limited bioavailability and rapid metabolization of flavonoids restricts their beneficial effects [8]. Flavonoids are able to cross the blood-brain barrier with chronic or acute administration suggesting that these compounds can feasibly have a direct effect on the brain, so these chemical compounds could be used to slow down the progression of AD [28]. Cannabinoid agonists may represent a potential therapy in normal aging that would favor high AEA neurotransmitter levels, leading to neuroprotection and anti-inflammation [9]. Some inhibitors that require further study include cholinesterase inhibitors which are designed to combat impairment of cholinergic neurons by slowing degradation of acetylcholine, the neurotransmitter “memory molecule”, after its release at synapses [21]. G- Secretase inhibitors have reached clinical trials, but published results are limited. LY450139, was well tolerated and reduced the amount of (Aβ) plaques in the brain [21]. Since g-secretase also cleaves other substrates, including Notch, the inhibitors can have deleterious effects on embryogenesis in organisms and on lymphoid and gastrointestinal tissues in mammals [21]. This means that treating Alzheimer's disease is a very complicated process because of the myriad mechanisms by which the disease affects the brain, and the potential adverse effects and high toxicity associated with these current pharmacotherapy .

Sickle Cell Anemia

According to reports, 20 to 25 million people worldwide are living with inherited sickle cell traits and about 300,000 infants are born with the disease every year [47]. Sickle cell disease is common among people of African, Middle Eastern, and South Asian descent [47]. The pain associated with s ickle c ell a nemia seemed to be something that Canurta could possibly explore as a holistic treatment option. Acute and vaso-occlusive pain are hallmarks of the disease

and the primary cause of hospitalization [7]. The classical view of sickle cell anemia has always focused on the genetic defect that creates abnormal hemoglobin that polymerizes when deoxygenated. Polymerization within the red blood cell causes it to deform, obstruct blood flow and produce acute and chronic tissue damage [18]. A holistic view examines the hypothesis that the oxidizing sickle red cell is an irritant that provokes an inflammatory response as it obstructs flow. The tissues not only receive poor blood circulation, but are also exposed to inflammatory cytokines and other growth factors that can cause pain [18].

Opioids are the primary treatment option for severe pain but high doses are needed due to the altered pharmacokinetics of morphine which increases undesirable side effects such as sedation, nausea, constipation, dependence and fatal overdose risks [27]. The activation of cannabinoid receptors, both type 1 (CB1) and type 2 (CB2), decreased hyperalgesia. The CB2 receptor agonist JWH-133 reduced deep tissue hyperalgesia and attenuated mast cell degranulation (cellular process that releases antimicrobial cytotoxic or other molecules from secretory vesicles called granules found inside some cells) [27]. Blocking the enzyme that breaks down the endocannabinoid anandamide, decreased sensitization of nociceptors and hyperalgesia in mice. Thus, increasing AEA in the periphery by blocking its hydrolysis may have beneficial effects in managing pain associated with SCD [27]. More than one-third of SCD patients have self-reported the use of cannabis for relief of pain but experience sedation and effects on mood [27]. This means that there is a lot of potential for Canurta to offer cannabis free products that do not produce the same sedation and mood changes.

There are certain molecules currently under evaluation that try to target the pain associated with sickle cell anemia. The primary mechanism for the acute anti-

hyperalgesic effect of URB597 appears to be the activation of CB1 receptors by increased levels of AEA associated with FAAH inhibition [27]. URB597 elevated levels of AEA in the skin without altering levels of other endocannabinoids [27]. A potential concern is that inhibition of FAAH not only increases the level of AEA, but also impacts the level of other fatty acid amides. [27]. These compounds may not be endogenous ligands for cannabinoid receptors, but alterations in their tone could cause non-specific effects that are not captured by studies focusing on the endocannabinoid system [27]. Therefore, when proceeding to treat SCA with FAAH inhibitors, mindfulness of the cascading effects and potential toxicity in other systems of the body will be necessary.

Gut Microbiota

Another aspect of wellness that has become increasingly popular these last few years is gut microbiome health. There is bidirectional communication between the brain and gut microbiota, which plays an important role in brain activity and the gastrointestinal tract [23]. The gut microbiome regulates the permeability of the blood brain barrier, which acts as a mesh in the brain and prevents certain molecules from leaving the blood [23]. Investigation of the gut microbiota and their metabolites may lead to the development of new drugs for the treatment of many diseases [23].

One of the most important things that affects the gut is the composition of a healthy diet [23]. Consuming fiber-rich diets or indigestible carbohydrates promotes the production of short chain fatty acids (SCFAs) via fermentation by microbes and maintains a healthy gut environment [23]. Recent studies have suggested that both the phenolic substrates supplied to the gut bacteria

through dietary intake and the aromatic metabolites produced may in turn modulate the composition of the microflora populations through selective prebiotic effects and antimicrobial activities against gut pathogenic bacteria [24]. The health properties attributed to beneficial bacteria for human hosts include protection against gastrointestinal disorders and pathogens, nutrient processing, reduction of serum cholesterol, reinforcement of intestinal epithelial cell-tight junctions and modulation of the intestinal immune response through cytokine stimulus [24]. In return, the gut microbiota can contribute to the metabolism and bioavailability of polyphenols and their metabolites [26]. Polyphenols are potent anti-inflammatory compounds that could provide an interesting alternative candidate in pain management. Studies performed on strawberry anthocyanins have highlighted the antioxidant, nervous and immune system benefits of polyphenols in the gut and identify natural health products, specifically prebiotics, as a lucrative industry to explore [26].

Model Organisms

Zebrafish are commonly used to study the early stages of development. Zebrafish, unlike *C.elegans*, possess most of the major organ systems in humans such as a heart, eyes and kidneys so organ development can be studied in this species [41]. Zebrafish models can be easily injected with DNA or RNA to permanently modify their genetic makeup in order to generate transgenic or knock-out zebrafish lines. Working with mice in this way is much more complicated. Mouse embryos develop inside the mother and to access and manipulate them, the mother would have to be sacrificed [44]. The ability of zebrafish to generate numerous embryos during breeding makes them useful for high throughput drug screening [44]. To determine if loss of function of a gene could cause the symptoms of disease, the same gene is mutated or “knocked-out” in zebrafish,

and then the fish are examined for similar symptoms [44]. The exact mutation that a specific patient has could be introduced into zebrafish as well [44]. If one or more of the patient's symptoms are observed in the zebrafish model, the zebrafish can be used for further studies to help determine why the mutation in that gene causes the disease. For a patient with a neurological disease, the neurons of embryos can be fluorescently labeled to see if they form incorrectly [44]. It is important to note that z ebrafish are not useful models for human diseases that mainly take place in a tissue type or body part that zebrafish do not have (prostate, mammary glands, lungs) [44]. While the model may have a lot of upside there are still some restrictions in how their use to test certain diseases. For example, a disease such as sickle cell anemia which originates from mutations that arise in human genes cannot be tested in z ebrafish but could potentially be tested in *C.elegans*.

The *Caenorhabditis elegans* genome possesses homologs of about two-thirds of all human disease genes. Based on its physiological aging characteristics and superiority, the use of *C. elegans* as a model system for studies on aging, age-related diseases, mechanisms of longevity, and drug screening has been widely acknowledged in recent decades [42]. *C.elegans* have a high genetic homology with humans, availability of complete genome sequence, conserved biological molecular responses and high fertility rates that make them a useful model organism to study with respect to aging in humans [42]. The short lifespan of this organism and small size are favorable for the screening of anti-aging drugs due to the reduced experimental costs and their usability for high throughput screening experiments [42]. However, *C. elegans* lack certain anatomical features including a blood transport system, a blood-brain barrier and blood filtration in the kidney which is required to transport a drug that treats Alzheimer's and the pain associated with sickle cell anemia. Thus, not making them an ideal model for brain related

diseases [42]. They also have poor absorption due to their tough cuticles [43]. Advantages for both organisms include the presence of a nervous system and alimentary canal which allows them to be a good indicator of oral toxicity [43].

Drugs On The Market

MAGL inhibitor JZL184 decreased the pro-inflammatory response of microglia and reduced the total Aβ load in transgenic mouse models [17]. URB602 is a selective MAGL inhibitor that exhibited an improvement in neuroprotection by reducing cyclooxygenase-2 (COX-2) elevation and NF-κB phosphorylation [17]. A Phase 2 trial with the Pfizer FAAH inhibitor PF-04457845 was recently completed and the compound was well tolerated in osteoarthritis patients but there was a lack of analgesic effect in the knee [19].

MAGL inhibitors can be divided into two main categories: compounds that irreversibly bind the enzyme and compounds that inhibit the protein reversibly. Irreversible inhibitors represent the first developed MAGL inhibitors [25]. Presence of the carbamate moiety is typical of irreversible inhibitors because the carbamate group is able to acylate the nucleophilic Ser122 of MAGL active site [25]. This forms a more stable enzyme complex, which causes the irreversible inactivation of the enzyme [25]. These inhibitors were tested *in vitro* and *in vivo* to determine their potency. All compounds showed IC50 values for human MAGL lower than or equal to 100 nM. Reversible inhibitors do not have the problems of irreversible inhibitors in preclinical studies but they often suffer from poor inhibition activity [25].

Quercetin is a flavonoid found naturally in plants including cannabis and among other foods such as onions, apples, broccoli, and red wine. It presents pharmacological, antioxidant,

cardioprotective, and antiapoptotic properties [28]. Naringin belongs to a family of polyphenol compounds that have an altered carbon atom chain and exist in grapefruits and other citrus fruits. This flavonoid has been shown to elicit antioxidant and anti-inflammatory effects and improve recognition [28]. Treatments with n aringin increased the amount of Nrf2 proteins (regulatory factor of the expression of antioxidant protein genes) and subsequent activation of antioxidant response element (ARE) pathway genes [28]. Naringin's mechanism of action is through the inhibition of oxidative cellular stress, which will then reduce neuronal loss typically associated with aging related diseases [28]. Oxidative stress is a strong mechanism of neuroinflammation and has pertinent effects in neurodegenerative diseases [28]. Ironwood P harmaceuticals disclosed a series of FAAH inhibitors for the treatment of pain, inflammation, and anxiety [38]. Compound IW-6118 was evaluated in a Phase 1 clinical study and demonstrated favorable pharmacokinetics and dose related elevation of biomarkers confirming that IW-6118 inhibits FAAH in humans [38].

Failed Drugs

Experimental single-target drugs that are effective in animal models of neurodegenerative diseases have not been effective in clinical trials of AD [14]. Reasons for the failure include: the drug not binding in the human target, the drug not reaching the human target in vivo, the drug-target interaction not having enough of an impact to reduce the signs and symptoms of the disease, the drug is administered to patients at an advanced stage of the disease when it is already too late to modify the disease process, and inconsistent data [14]. There are three primary avenues of multi-target pharmacology: multiple medication therapy (MMT), multiple compound medication (MCM), and multi-target directed ligands (MTDLs) [14].

Phase 1 clinical trials with BIA 10-2474 (from the Bial pharmaceutical company) were terminated owing to the death and sickening of some volunteers from off-target effects [19]. Dual FAAH and MAGL inhibition may induce catalepsy and THC-like effects [25]. This was demonstrated by the in vivo administration of the dual inhibitor JZL 195 [25]. In addition, differences in plant genetics and growing conditions such as soil, light, temperature, humidity, and time of harvesting may lead to significant differences in the concentration of potential active or toxic constituents [29].

Effective Assays

Bioassay for quality control reflects the drug's known or intended mechanism of action and pharmacodynamic or surrogate biomarkers [29]. Multiple-batch analysis looks at the effects on clinical endpoints. This allows quantification of potential heterogeneity in clinical outcomes for subjects who receive different batches and shows that a clinical response is not sensitive to a certain dosage, demonstrating that the studied doses are more effective than active treatment options [29].

The GOT-IT recommendations provide a structured approach to assemble an optimal path between the starting point or identification of a potential new target and the achievement of specific goals. The framework should help to increase the value of a drug discovery program [15]. In spectrophotometric enzymatic assays, MAGL inhibitors were tested at different concentrations (ranging from 25 μM to 1.7 nM) and the absorbance was recorded [25]. The most promising MAGL inhibitor showed IC50 values in the range of 3.0–5.0 nM [25]. Although the mechanism of inhibition is not always specified, it is presumed that most MAGL inhibitors are reversible [25].

Application To Canurta

Gut Health is Wealth

Fermented foods with high levels of beneficial microorganisms can contribute to maintaining gut microbiota diversity and facilitating the production of bioactive metabolites from polyphenols and their absorption and bioavailability [33]. Prebiotic effect can be defined as the selective stimulation and growth of host microbial species that confer health benefits to the host [32]. Since polyphenols are an excellent source of prebiotics, Canurta has the potential to enter the market as an innovative product and solution to promoting gut health [32]. Overall, polyphenols enhance the growth and establishment of the probiotic bacterial family *Bifidobacteriaceae* and *Lactobacillaceae* and inhibit pathogenic bacteria [32]. Knowing all of this, there is still a need for research on polyphenol uptake, bioavailability and interaction with receptors and enzymes [32].

Current research has demonstrated how polyphenols act locally to protect the gastrointestinal tract (GIT) against oxidative damage [32]. Metabolites of polyphenols can get absorbed into the systemic circulation and modulate various parameters involved in the vascular system. This can prevent platelet aggregation, reduce blood pressure and improve the plasma lipid profile [32]. As polyphenols support host metabolism by absorbing the nitrites and certain secondary bile salts that act as substrates for complex enzymatic reactions, they promote longevity and increase lifespan [32]. Flavonoids designed to promote gut microbiota result in decreased availability of nutrients to pathogenic bacteria, thus acting as antimicrobial agents [32].

Comparative Sources of Dietary Flavonoids

Phenolic acids such as ferulic and *p*-coumaric acids of cereal grains (rice, wheat, corn) and pulses (beans, chickpeas, lentils) are also involved in modulation of the immune response and are found to contribute to gut health and metabolism [32]. Polyphenols in cranberry extract decreased the accumulation of triglycerides and strengthened insulin sensitivity in humans [32]. Flavonoids in foods and beverages made from cocoa beans have improved the function of endothelial tissues, altered glucose metabolism, and reduced oxidative stress [32]. Understanding how certain polyphenols are being used currently will help with the research and development of new health products. Herbal supplements enriched with flavonoids are frequently reported for their ameliorative effects in the management of pain [40]. Flavonoids found in green tea, can produce microglial activation and protect against inflammation in Alzheimer's disease [40]. By collecting information on how certain flavonoids are used we are able to isolate potential areas of expansion for Canurta and determine which markets have become saturated with competitors.

Digestive Pathway

The biotransformation of polyphenols takes place in the enterocytes of the small and large intestines [32]. Gut microbiota and the liver were found to be crucial sites for flavonoid metabolism. The gut contains microorganisms that use the unabsorbed polyphenols as substrates for their enzymatic activities [32]. After their uptake through the intestinal epithelium, dietary polyphenols and metabolites undergo phase I and II metabolism and are excreted in urine.

Formulations/Administration

Multi-target-directed-ligands (MTDLs) are single molecules capable of interacting with multiple targets. Designing and testing new MTDLs could be a research strategy for developing a cure for neurodegenerative diseases such as AD [14]. MTDLs have an increased molecular

weight making it challenging to synthesize an MTDL that is effective as a single-target drug [14]. The preferred route of administration would be oral for diseases such as Alzheimer's, Sickle Cell Anemia and promoting the gut microbiota because the drug will then be able to elicit a response in the stomach and enter blood circulation. Flavonoid co-administration with carbohydrate-dense foods exhibited enhanced absorption and bioavailability. A fatty matrix can also increase the uptake of flavonoids and slow down their clearance. On the other hand, protein co-administration and flavonoid protein interactions significantly reduce the oral bioavailability of many flavonoids [40].

MAGL/FAAH Inhibitors and Disease

Preventing 2-AG degradation by MAGL inhibition may be an effective therapeutic strategy for reducing neuroinflammation [14]. MTDLs containing pharmacophores that inhibit MAGL may effectively slow down the inflammation that contributes to neurodegenerative diseases [14]. MAGL inhibitors have been hypothesized to ameliorate pain-like behavior and neuronal hyperexcitability [7]. Pharmacological modulation of FAAH could be exploited to treat neurodegenerative disorders, depression and pain and magnify the neuroprotective action of AEA [9]. However, when designing a therapeutic for neurodegenerative disorders you need to make sure that the drug is able to cross the blood brain barrier in order to elicit a response [17].

FDA Regulations

Drugs are safe to proceed with their proposed phase 1 or 2 clinical studies after addressing quality and safety concerns [29]. Reasons for holds or withdrawals include inadequate human use experience, lack of product information to assess the safety of the proposed doses, or serious adverse effects associated with the botanical product [29]. Once a

product has been shown to be safe and effective, the methods used in manufacturing the drug and the controls used to maintain the drug's quality are adequate to preserve the drug's consistent identity, strength, quality, and purity, it can be approved and sold to the public as a prescription or over the counter (OTC) drug [29]. Since Canurta can ensure consistent product quality and safe manufacturing techniques, drugs that are designed to reduce inflammation will seamlessly pass FDA regulations.

Natural Health Products

Applicants must give detailed information about the product to Health Canada that includes medicinal ingredients, source, dose, potency, non-medicinal ingredients and recommended use [39]. Evidence may include clinical trial data or references to published studies, journals, pharmacopeias and traditional resources. The type and amount of supporting evidence required depends on the proposed health claim of the product and its overall risks [39]. Apparently, classifying a drug as a homeopathic medicine under the title of a NHP will allow it to be approved faster compared to traditional drug treatments which require extensive clinical studies to become approved [39].

Clinical Exploration

A balanced diet includes plant-based foods, bioactive compounds, fatty acids, and polyphenols [30]. Phenolic compounds found in a healthy diet can balance the gut microbiome and promote wellness, indirectly treating the pain associated with certain disease states targeted in this paper. The best potential drug for Canurta to market and develop quickly can be achieved

through a dietary supplement which promotes health and wellness. A more traditional drug design will require approximately ten years of clinical studies and can still prove to be ineffective in the end.

Market Research

The most extensively studied MAGL inhibitor is JZL184 [36]. JZL184 acts as a potent irreversible inhibitor of MAGL with an approximate 300-fold selectivity versus FAAH [36]. However, repeated administration to mice of a high dose of JZL184 results in a down-regulation of CB1 receptors and a tolerance to both the effects of the compound itself and of exogenous cannabinoids *in vivo* [36]. So MAGL inhibitors might be more useful in conjunction with non-steroidal anti-inflammatory drugs (NSAIDs), since JZL184 provides protection against gastric hemorrhages produced by diclofenac in mice [36].

The process of discovering and developing a pharmaceutical agent can take over a decade and costs $2.8 billion on average. Even then, nine out of ten therapeutic molecules fail Phase II clinical trials and regulatory approval [37]. Chemical properties such as solubility, partition coefficient (logP), degree of ionization and intrinsic permeability along with pharmacokinetic properties must be considered when designing a new drug [37]. Cell-based in vitro assays are often used as preliminary studies, followed by animal studies to identify the toxicity of a compound, increasing the expense of drug discovery [37].

Revenue of existing therapeutics

The global Alzheimer's disease therapeutics and diagnostics market is anticipated to generate revenue of $9.97 billion by 2028. Active companies in the markets today include: Hoth Therapeutics, Regeneron Pharmaceuticals, Sanofi, Cassava Sciences and AbbVie company [46].

The global sickle cell disease treatment market size was valued at USD $2.1 billion in 2017 [47]. Emerging markets such as Nigeria, Ghana, South Africa, Brazil, Mexico, Saudi Arabia, and India are expected to exhibit lucrative growth due to favorable government policies, growing awareness about hematological malignancies, increased investment, and improved healthcare infrastructure [47]. The treatment market is expected to witness the introduction of gene therapies such as bluebird bio's Lentiglobin, Bellicum Pharmaceuticals BPX 501, and Gamida Cell's Cordin in the near future [47]. The major players in the aging drugs market include LG Life Science, Merz Pharma, Bloomage and SciVision Biotech [48].

Current standard of care

The current treatment for chronic sickle cell disease is hydroxyurea and blood transfusions [47]. Hydroxyurea was the only FDA-approved drug for the treatment of the disease. In 2017, Emmaus Life Sciences drug Endari, received FDA approval for the treatment of severe complications associated with SCD in adult and pediatric patients. Endari was the second drug to be approved by the FDA [47]. Galantamine, rivastigmine, and donepezil are cholinesterase inhibitors that are prescribed for mild to moderate Alzheimer's symptoms [50]. Aducanumab is the only disease modifying medication currently approved to treat Alzheimer's [50]. The FDA has also approved donepezil, rivastigmine and memantine for the treatment of moderate to severe Alzheimer's [50], but none of these drugs have had more than minimal impact on the condition or longevity of its afflicted patients in the longterm.

Conclusion

Canurta will be focused on entering the market as a high-activity anti-inflammatory product for nutraceutical product manufacturers, and all the necessary steps that are required thereof. The healing properties of hemp can be attributed to high levels of essential fatty acids, amino acids and dietary fiber [34]. The polyphenols extracted by Canurta is an excellent source of dietary fiber which is necessary for healthy digestion [35]. In addition to alpha-linolenic acid and linoleic acid, hemp also contains gamma-linolenic acid (GLA). The fatty acid may help with the hormone imbalances that occur during menopause, as well as inflammation but this something that Canurta can continue to explore and requires more research [35]. Some of the most popular applications of hemp include smoothies, topping salads or yogurt, baking hemp seeds into muffins and using them as tea extracts [35]. As thse products hit the market, some of the easiest applications for our products include everyday life, not just treating chronic pain associated with terminal diseases. There are various avenues for Canurta to explore regarding their flavonoid molecules and potential drug treatments. This review outlined three enzymes and their role in regulating inflammation in the body. The two inhibitors discussed were FAAH and MAGL. The biological activity of each inhibitor, disease states impacted by each target and the potential therapeutic benefits of flavonoids as anti-inflammatory treatments was explained in this paper. Overall, the whitepaper highlights the beneficial effects of polyphenols in healthcare, how to get a drug approved by Health Canada and the steps Canurta can take to enter the market.

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