The Endocannabinoidome: Expanding the Approach to Treating Traumatic Brain Injury Using the Endocannabinoid System

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Abstract

This paper explores the potential use of endocannabinoidome molecules as a therapeutic approach to treating traumatic brain injury (TBI). Google Scholar was used to obtain the primary research literature analyzed for this review. Studies which manipulate the endocannabinoid system through methods such as administration of 2-AG or AEA ligands, inhibiting breakdown enzymes, and using CB1 and CB2 agonists or antagonists have shown promising results in treating TBI; however, no pragmatic clinical therapy has been found so far. The discovery of similar molecules and receptors has resulted in the expansion of the endogenous system and bred the term endocannabinoidome, which incorporates the newly discovered molecules and receptors. Ligands of the endocannabinoidome produce similar therapeutic benefits for TBI but act by different receptor pathways, which may allow one to overcome current existing problems of manipulating the endocannabinoid system for TBI treatment. Currently, therapies used to treat TBI have many unwanted side effects, establishing the need for alternative research options. This paper examines three of these endocannabinoidome molecules that have been previously researched for treating TBI and illuminates their specific receptor pathways and how these receptor pathways operate differently from the ordinary pathways of the endocannabinoid system. Gaining an understanding of the receptor pathways used by endocannabinoidome molecules will open a new field of research for therapeutics to treat TBI.

Keywords: endocannabinoidome, traumatic brain injury, endocannabinoid system
Brain injuries are among the most prevalent injuries in the general population. Specifically, traumatic Brain Injury (TBI) is a debilitating injury that many people suffer from worldwide and it can result from workplace incidents, sports, accidents, etc., with physical, sensory, and cognitive symptoms (Ghajar, 2000). In environments wherein participants suffer repetitive TBI, such as contact sports and the military, a progressive neurodegenerative disease known as chronic traumatic encephalopathy (CTE) can result (Omalu, 2014). TBI can be classified as either primary or secondary injury. The primary injury results from the initial impact, whereas the secondary injury is the damage that occurs following the initial impact. This secondary injury happens because of complex cellular processes and biochemical cascades that occur in the neurons that surround the primary injury site (Ghajar, 2000).

There have been several clinical trials and treatments to explore how to treat the disease; however, none have been successful so far. One potential method involves manipulating a newly discovered system called the endocannabinoid system. Manipulations by administrating endocannabinoids, receptor antagonists and agonists, and inhibiting synthesizing enzymes have shown promising results in rat/mouse studies but unfortunately have led to unsuccessful trials in humans (Mechoulam & Shohami, 2007). However, newly discovered molecules that are structurally related to the endocannabinoidome, the ensemble of endocannabinoid receptors, ligands, and other similar molecules may provide some possible therapeutics in treating TBI. There are few studies published about the endocannabinoidome system. It has been suggested that these compounds work via a mechanism that does not involve the traditional endocannabinoid receptors, which may allow them to bypass the current problems associated with manipulating the endocannabinoid system to treat TBI (Arturo & Fabiana, 2018). Experimentation to this end has resulted in conflicting answers with no clear path forward. Furthermore, there are almost no comprehensive review papers that consider the endocannabinoid system for treating TBI. The purpose of this research review paper is to explore the differences between endocannabinoidome neurotransmitters and endocannabinoids and how these differences influence the role of endocannabinoidome molecules as therapeutic agents.

Endocannabinoid System and the Endocannabinoidome

The endocannabinoid system is a biological system that involves three main parts: the endocannabinoids, the cannabinoid (CB) receptor proteins, and the enzymes involved in synthesizing and breaking down the endocannabinoids. The endocannabinoids are endogenous, lipid-based, mediating ligands/neurotransmitters found in the brain and peripheral tissues. The main endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The CB receptors that endocannabinoids act on include CB1 (found in the central nervous system), CB2 (found in peripheral nervous system/immune cells), and other receptors. AEA has a high affinity for CB1, whereas 2-AG favours CB2 (Pacher et al., 2013). N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) is an enzyme mainly involved in AEA synthesis. sn-1-diacylglycerol lipase (DAGL) is responsible for 2-AG production. Fatty acid amide hydrolase-1 (FAAH-1) degrades the biological activity for AEA. Finally, a specific monoacylglycerol lipase (MAGL) degrades 2-AG (Battista et al. 2012). Most endocannabinoid system functions are still relatively unknown, but recent studies have shown that activation of this system may induce changes in synaptic plasticity, brain development, conditioning, appetite, and pain (Ligresti et al., 2016). The endocannabinoid system’s ability to activate multiple processes makes it an excellent candidate for the treatment of TBI of the secondary injury type, which results in many injury processes. Previous experimental manipulations have included the administration of endocannabinoids, using enzyme inhibitors to increase endocannabinoid levels, and CB receptor agonists and antagonists.

Recently, new endocannabinoid-like molecules have been discovered in the bovine brain (Tan et al. 2010). Dozens of these endogenous molecules have similar structures to endocannabinoids, but their functions remain a mystery. As a result of their discovery, questions regarding the relationship between these molecules and the endocannabinoid system have emerged (Mechoulam et al. 2014). These endocannabinoid-like molecules act on GPR55, GPR18, GPR119, TRPA1, and several non-receptor targets in addition to the CB1, CB2, and TRPV1 receptors which endocannabinoids act on. This discovery led some researchers to term the newly discovered endocannabinoid-like molecules and all of its metabolic enzymes and receptor targets as the “endocannabinoidome” to represent an expanded view of the endocannabinoid system. Many of these endocannabinoid-like molecules are derived from fatty acids and any shift in their quantity can have dire consequences on the homeostasis of the body (Di Marzo et al. 2014). This makes the endocannabinoidome an interesting target in changing the homeostasis of the body.

Issues with Manipulating the Endocannabinoid System

The administration of traditional endocannabinoids AEA and 2-AG showed auspicious laboratory results, but clinical trials involving synthetic endocannabinoids ultimately led to
disappointing results (Di Marzo et al., 2009; Saul, 2007). After TBI, upregulation of the endocannabinoids was observed which has been shown to mitigate some of the processes of secondary injury such as edema, inflammation, reactive oxygen species, and apoptosis (Mechoulam, Panikashvili, & Shohami, 2002). However, conflicting results pose a difficult challenge in developing a proper therapeutic agent based on these studies. In one study, mice were subjected to TBI in the form of a closed head injury (CHI). An increase in 2-AG levels was observed, reaching a peak at 4 hours and then declining shortly after. This was unlike the controls without CHI. The mice were then subjected to synthetic 2-AG at times of either 15 min before or 1 hour after the injury. A neurological severity scoring system (NSS) was also used to measure the rats’ neurological function. The NSS operates by assigning one point for every task failed and zero points for every successful task. Mice that received 2-AG had a significant recovery of function, observed by an increased change in NSS (ΔNSS) scores, measured 1 hour and 24 hours after the initial injury (Panikashvili et al., 2002). In addition, there was a significant reduction of edema in tissues of treated mice compared to untreated mice. This suggests that 2-AG upregulation plays a role in reducing edema and neurological damage following TBI (Mechoulam & Shohami, 2007). Other studies found that 2-AG also inhibits tumor necrosis factor-α (TNF-α) production in macrophages (an important inflammatory mediator) and injection also suppresses the formation of radical oxygen intermediates (Gallily et al., 2000). However, contradictory results appeared in a study by Hansen et al. (2002) showing that rather than 2-AG, AEA was upregulated in the ipsilateral cortex following concussive head trauma in rats with no changes in 2-AG levels. Activation of glutamate excitotoxicity via injection of NMDA resulted in an observed 13-fold increase in AEA. Thus, it remains uncertain as to which ligand is upregulated following TBI. Additionally, an underlying issue for administering these ligands is that they are broken down quickly. The endocannabinoid system produces these lipid mediators on demand rather than storing them in vesicles and breaks them down rather quickly as well; therefore, the effects are not long-lasting and would not be useful in treatment of a chronic condition.

Research into the inhibition of endocannabinoid-breakdown enzymes has also shown promising results, but application into clinical trials has led to disappointing outcomes. Administering PF-3845 (FAAH inhibitor) to a TBI mouse model caused an increase in AEA levels, along with a reduction in TBI-induced anxiety-like behaviour and impairments to fine motor movement control (Tchantchou et al., 2014). However, other studies, including one involving PF-04457845 (irreversible FAAH inhibitor), saw an increase in endocannabinoids but no pain relief in patients with osteoarthritis suggesting lack of efficacy (Huggins et al., 2012). Even more concerning was a clinical trial using an experimental FAAH-inhibitor that ended with the death of a participant and four others suffering permanent brain damage (Kaur et al., 2016). Explanations of these results have suggested that the extended time of inhibition using this particular FAAH inhibitor and possible inhibition of enzymes other than FAAH may have triggered an autoimmune response in the brain. Overall, there is a lack of data on FAAH inhibitor usage in humans which further complicates determining safe dosages (Kaur et al., 2016).

Likewise, MAGL inhibitors have shown promising results but were similarly difficult in application. CTE was reproduced in a mouse model of repetitive mild CHI and administration of MAGL inhibitor promoted neurological recovery and reduced astrogial reactivity, expression of amyloid precursor protein, and formation of Aβ. This suggests that preventing the degradation of 2-AG into prostaglandin precursors can reverse tau protein aggregation and neurodegeneration, which are hallmarks of CTE (Zhang et al., 2015). However, a lack of reversible human MAGL inhibitory compounds has impeded the development of any therapeutics, the development of new classes of MAGL inhibitors are currently being investigated (Tuccinardi et al., 2014). Another concerning issue is that human MAGL is involved in many different biochemical pathways such as storage of triglycerides (Karlsson et al., 2003). This makes selective targeting of MAGL inhibition another aspect to consider when developing a therapeutic agent to prevent unwanted side effects.

The discovery of CB1 and CB2 receptors has led to the development of agonists and antagonists for treating TBI. CB1 activation results in psychoactive effects whereas CB2 activation results in anti-inflammatory effects (Sim-Selley, 2003; Turcotte et al., 2016). Since psychoactive side effects are unwanted in clinical treatment, drugs that act as CB1 antagonists were sought after for therapeutic usage. Research into CB1 receptor blockers found that the use of the antagonist with 2-AG after CHI led to a decreasing ΔNSS and brain water content compared to mice that were not treated with the antagonist (Mechoulam et al., 2007). CB1 antagonists have also been found to act as anti-obesity agents, a conclusion made based on THC’s ability to stimulate appetite. The drug rimonabant was marketed as such but never received Food and Drug Administration (FDA) approval and was eventually removed from the market due to its suicidal and anxiety-inducing side effects (Di Marzo et al., 2009; Saul, 2007). These side effects may be attributed to the ubiquity of CB1 receptors in the brain where antagonism produces unwanted side effects. Agonists of CB2 receptors have also been explored. CB2 receptor activation has anti-inflammatory properties, as evidenced by their location on the immune cells. However, a lack of understanding of the exact location of CB2 receptors in the body makes the development of a therapeutic agent difficult. It has been observed that the use of CB2 agonists is most effective prior to the insult but can still promote inflammation if given after the trauma (Pacher et al., 2013). Again, inconsistent results
demonstrate the difficulty in creating a viable clinical trial using either agonists or antagonists.

One area receiving less research attention is the effect of non-CB1/CB2 receptors for therapeutic targets such as the TRPV1 receptors for TBI. Another area that has not been extensively studied is the allosteric modulation of CB1 and CB2 receptors (Schurman, & Lichtman, 2017). However, new studies into the endocannabinoidome's molecules present themselves as allosteric modulators or as new alternative molecules that can act on non-CB receptors or. This may provide a different route for developing a therapeutic treatment using the endocannabinoid system. Many of these endocannabinoidome compounds exist, but they are beyond the scope of this paper. The endocannabinoidome ligands and receptors highlighted in this paper are N-arachidonoyl-L-serine (AraS), N-arachidonoyl glycine (NAGly), and palmitoyl serine (PalmS).

AraS (N-arachidonoyl-L-serine)

AraS was discovered in bovine brain in a study conducted by Cohen et al. (2011). Based on its structural similarities to AEA, AraS was also thought to have conveyed neuroprotective properties such as vasodilation by binding onto TRPV1 receptors. AraS can also reduce inflammation via suppression of reactive oxygen intermediates, nitric oxide production, and TNF-α formation observed in the murine macrophage cell line (Godlewski et al., 2009). In addition, AraS has been shown to reduce apoptosis and neuronal loss, highlights of both primary and secondary injury. When CHI mouse models were subjected to an injection of synthetic AraS, a significant decrease in NSS score was seen in the AraS-treated mice compared to the controls. This suggests that AraS has neurobehavioral protection abilities. In order to specify that anti-apoptosis is the method of protection, the researchers stained the tissue with TTC (2,3,5-triphenyltetrazolium chloride), which binds only to live tissue. Overall, a 45% reduction in lesion volume was observed via TTC staining for the AraS treated mice, indicating an overall decrease in apoptosis and neuron cell death (Cohen-Yeshurun et al., 2011). A follow-up study by the same researchers investigated the proneurogenic properties that AraS may possess. Cerebral cortical cultures of neuroprogenitor cells (NPCs) were prepared using 14-day murine embryos. An in vitro culture was grown and single NPCs proliferated to form clonally derived floating sphere colonies known as neurospheres, which continually renew and can differentiate into either neurons or glial cells. The neurospheres were treated with AraS, which showed an increase in size after 4 days compared to vehicle-treated cells. Also, it was discovered that AraS reduces differentiation of the NPCs, evident by a reduction in the expression of astrocytic marker glial fibrillary acidic protein and neuronal marker TUJ1 (Cohen-Yeshurun et al., 2013).

From these results, it can be concluded that AraS promotes neurogenesis and may also inhibit astrogliosis processes such as glial scar formation. AraS is made of neuroprotective properties similar to the traditional endocannabinoid molecules such as AEA and 2-AG.

One important aspect is the variety of receptors that AraS binds to. The researchers determined AraS did not bind to CB1, CB2, or TRPV1 receptors. Rimonabant was administered with AraS which resulted in no changes in the recovery or lesion volume reduction. This result demonstrates that the therapeutic effects of AraS do not operate in a manner requiring the CB1 receptors. Subsequent testing with CB2 antagonist (SR144528) and TRPV1 receptor antagonist (capsazepine) showed significantly higher NSS values compared to AraS treated receptors, along with a two-fold increase in lesion volume for TRPV1 antagonist treated mice. This suggests that TRPV1 channels and CB2 receptors have a role in the AraS neuroprotective effects. Paxilline (big potassium channel blocker) was also introduced with AraS-treated mice and resulted in changes in therapeutic effects providing evidence of BK channel's involvement (Milman et al., 2006). However, the proneurogenic properties of AraS have shown some dependency on the traditional CB receptors. Using CB1, CB2, and TRPV1 antagonists, along with AraS, resulted in a loss of the proliferative effect in the AraS-treated cells, demonstrating that each of the endocannabinoid receptors contribute to the proliferative effects of AraS on NPC (Cohen-Yeshurun et al., 2011). Therefore, it remains inconclusive which neuroprotective properties are mitigated by traditional CB receptors. It can be concluded that AraS acts through a different mechanism than AEA to mitigate TBI. This possibility is important because it may provide an opportunity to circumvent the problems existing with CB1/CB2 receptor agonism or antagonism. Previously, it was mentioned that the antagonism of CB1 results in unwanted psychosomatic side effects, such as suicidal thoughts and anxiety. Lack of involvement of CB1 receptor means that the drug would not need CB1 antagonism, which would make the development of a drug using AraS as a blueprint a much simpler design. Since only mouse/rat models have been used, ascertaining the psychological effects on humans from these studies is difficult because of the complexity of human behaviour in comparison to rodent behaviour. Additionally, more is known about what receptors AraS does not bind to than those it does bind to. One theory is that the endocannabinoidome molecule interacts with the GRP55 receptor. To test this, the researchers treated both GRP55 siRNA-transfected cells and siRNA-transfected cells (control) with AraS. Overall, a significant reduction in AraS-induced endothelial migration was seen in the cells with GRP55 expression knock outs. To clarify the importance of the interaction, atypical cannabinoid O-1918, an antagonist of GRP55 receptors, was used. Cells treated with O-1918 and AraS produced very little endothelial proliferation and...
migration when compared to those treated with only AraS. This led to the conclusion that GRP55 receptor may interact with AraS to activate its neuroprotective functions. As a result of this discovery, some scientists have termed GPR55 as the “third” CB receptor known as CB3 (Zhang et al., 2010). Further research on the effects of this receptor is needed. Overall, AraS presents itself as a new molecule for TBI treatment because of its novel receptor pathway.

PalmS (Palmitoyl-Serine)

The structure of PalmS resembles AraS which made some researchers think that PalmS conveys neuroprotective properties. Only one extensive study has been done on PalmS so far. Similar NSS values were observed between PalmS treated mice and control mice within the first hour after the injury, but after 35 days, the ΔNSS was 2.6 units higher in PalmS treated mice than in vehicle-treated mice. From these results, it was concluded that PalmS improved the neurological outcomes for mice recovering from TBI. The researchers then wanted to determine which specific injury process PalmS mitigates. It was postulated that PalmS provides improved neurobehavior via apoptosis inhibition. Western blot analysis of pro-survival mediators pERK and pAkt, along with anti-apoptotic molecules Bcl-xL shows that there was a significant increase in Akt phosphorylation for the PalmS treated group. A lack of ERK or Bcl-xL phosphorylation was observed as well (Mann et al., 2015). In addition, testing on neuroinflammation was done because of its novel receptor pathway. PalmS assistance in the palmitoylation process can provide improved neurobehavior via apoptosis inhibition.

One theory developed by the researchers to explain this allosteric modulation property is that PalmS is involved in palmitoylation of proteins (a post-translational modification involving the covalent attachment of fatty acids, such as palmitic acid, to cysteine) (Linder, 2001). Palmitoylation of a receptor increases the number of functions and regulatory control of a receptor beyond its genetic code. The palmitoylation of G protein-coupled receptors (GPCRs) allows for processes such as protein trafficking, activating functions of membrane proteins, and shutting of intracellular compartments upon receptor binding. PalmS assistance in the palmitoylation process can therefore modify the activity of the endocannabinoid receptors instead of acting as a full agonist (Mann et al., 2015).

NAGly (N-arachidonoyl Glycine)

Similar to the discovery of AraS, NAGly was found in brains of cattle and rodents (Huang et al., 2001). In addition, NAGly was thought to have vasodilation properties based on its similar structure to AEA. Vasorelaxant properties were observed via activation of BKCa (big potassium calcium channels) through NAGly binding onto an unknown Gi/o-coupled receptor (Parmar et al., 2010). NAGly activation was also found to cause BV-2 microglia migration, similar to the effects of Abn-CBD (abnormal cannabinoid) receptor binding activation. This evidence suggests that the receptor for NAGly is GPR18. The researchers concluded that NAGly acts on GPR18 to cause migration, proliferation, and other processes via a lipid-based signaling mechanism (McHugh et al., 2010). Another study investigated the basis of NAGly’s anti-inflammatory effect and discovered that NAGly causes mouse macrophages to undergo apoptosis. Knocking out GPR18 results in attenuation of this apoptosis induction by NAGly, providing further evidence of their ligand-receptor relationship (Takenouchi et al., 2012). For its ability to inhibit pro-inflammatory responses, vasodilation effect, and microglia migration, NAGly demonstrates itself as a potential avenue as a therapeutic molecule for TBI.
The way in which NAGly promotes therapeutic effects is unclear, but there are many proposed theories to explain this mechanism. One study purported that NAGly’s therapeutic effects were due to the inhibition of FAAH. The administration of NAGly to rats led to a significant elevation of AEA. There was also evidence that NAGly does not bind onto CB1 and CB2 receptors. From these observations, the researchers concluded that NAGly promotes anti-inflammation via a mechanism involving FAAH inhibition (Cascio et al., 2004). However, further studies involving rat, mouse, and human trials showed that NAGly was most potent as an FAAH inhibitor in mice and rats but not humans. However, out of the 12 other NAAs (N-arachidonoyl-amino acids) tested, two were found to be potent for FAAH binding. Perhaps the most promising result was that N-arachidonoyl-isoleucine (NAIle), one of the two compounds mentioned, is much more potent on human FAAH than rat or mouse FAAH (Cascio et al., 2004). As previously mentioned, clinical trials of FAAH inhibitor had disastrous results, but the discovery of NAGly as a potential FAAH inhibitor may provide a more endogenous route to manipulate AEA upregulation (Kaur et al., 2016). This may prove to be a safer and more specific method to target FAAH inhibition overall.

Another hypothesis postulates that AEA activates the non-CB receptors via degradation into NAGly which can then bind onto said receptors. This suggests that NAGly is ultimately the therapeutic molecule, rather than AEA, as previously believed. This study found that AEA undergoes oxidative metabolism along with conjugation of glycine to arachidonic acid (from FAAH hydrolysis of AEA) to produce NAGly. This NAGly then goes on to activate GPR18 and GPR92 (Bradshaw et al., 2009). Vasodilation using NAGly contrasts with AEA as the latter requires CB receptors to produce its vasodilation properties while the former does not (Parmar et al., 2010). Similar to AraS and PalmS, the lack of use of traditional CB receptors suggests that drug development using NAGly can help avoid the problems previously discussed. However, contradictory results have emerged in recent years. A recent study attempted to dispel the different findings associated with the function of GPR18 receptor and NAGly. Previous studies that have concluded NAGly binds onto GPR18 in order to activate Gi/o pathways could not be replicated experimentally, thus casting doubt on the actual mechanism involved (Finlay et al., 2016). Regardless of the ambiguity, NAGly has been shown to not involve CB1 nor CB2 receptors in any of the aforementioned studies. Further research of the compound will be needed before NAGly can be used to design a clinical drug.

**Limitations and Future Research**

The discovery of the endocannabinoidome seems to raise more questions about the endocannabinoid system than answer them. Currently, the most substantial limitation lies...
with experimental mouse/rat models that do not translate well directly to human behaviour. For example, it is difficult to observe any psychological side effects such as suicidal thoughts due to differences between human and rat/mouse social and psychological behaviours. In addition, it remains difficult to study the biochemical basis of these molecules as it seems that each molecule operates via a different pathway mechanism. Therefore, the most beneficial studies would involve a more proficient technique to study the receptor pathways involved in these molecules. However, before this can be accomplished, the complete characterization of the endocannabinoidome must occur using a lipidomic approach termed “endocannabinoidomics” (Piscitelli, & Bradshaw, 2017; Fig. 1). Future experiments will involve testing the neuroprotective properties of other endocannabinoidome ligand families in TBI mouse models such as $N$-arachidonoyl-dopamines, $N$-acyl-serotonins, $N$-acyltaurines, and other $N$-acyl amino acids members (Arturo & Fabiana, 2018). The dependency of the traditional CB receptors of new endocannabinoidome ligands can be tested by using the same method as the PalmS study: using CB1, CB2, and TRPV1 antagonists, along with receptor knockout models to see if there are any effects on the ability of the molecule to mitigate TBI damage (Mann et al., 2015; Fig. 2). These findings will be a stepping stone towards the development of a full theory encompassing all lipids of the endocannabinoidome/endocannabinoid system which may one day lead to a TBI therapeutic agent.

**Conclusion**

While the endocannabinoid system is an interesting novel system that shows therapeutic potential, the lack of consistent studies in addition to the inability to create a pragmatic and efficient synthetic drug has left the field stagnant. However, recent discoveries of endocannabinoid-like lipid moieties, known as endocannabinoidome molecules, have acted as catalysts for future research. The endocannabinoidome molecules offer neuroprotective effects against TBI by working in a manner that does not involve the traditional CB1, CB2, and TRPV1 receptors, which may circumvent the current problems existing with endocannabinoid system manipulations. Understanding the relationship between the endocannabinoid system and these endocannabinoidome molecules may one day aid in the development of a clinical drug that assists in the treatment of TBI.
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