K5 An Evaluation of Cannabinoid 2 Receptor and Endogenous Cannabinoid 2-Arachidonylglycerol in the Central Nervous System

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After attending this presentation, attendees will better understand conclusions drawn during drug and toxicology-based testing. Cannabinoids (CBs) are constituents of marijuana (phytocannabinoids) and marijuana-like compounds, which are endogenously or synthetically produced.

This presentation will impact the forensic science community by the elucidation of cannabinoid receptor 2 (CB2R) brain expression as revealed by the interdisciplinary approaches of immunocytochemistry, Western blot, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation evaluates results generated in an *in vitro* study regarding analysis of endogenously produced cannabinoids and CB2R expression in the astrocyte using Western and liquid chromatography tandem mass spectrometry LC/MS/MS analysis. This research could impact the forensic science community in relation to evidence of CB2R's upregulation and potential targeting in the central nervous system. Cannabinoids (CBs) are constituents of marijuana (phytocannabinoids) and marijuana-like compounds which may be endogenous, or synthetically produced.[1,2] When CBs bind to cannabinoid receptors, they are able to produce their effects. Cannabinoid receptor 1 (CB1R) as opposed CB2R, generates negative psychoactive manifestations such as short-term memory loss, hallucinations, and impaired motor function.

Botanicals, including marijuana, contain over 460 known compounds. Approximately 60 of these compounds are unique substances to cannabis named cannabinoids.[3] Evidence shows that these compounds are helpful during neurodegenerative disease states which impair the normal functions of neuronal activity. In the brain, astrocytes provide neurotrophic and metabolic support for neurons facilitating their ability to function properly. Therefore, the study of astrocytes is important to ensure proper protection of neurons in disease states. Astrocytes take on the morphological change represented in fibrous astrocytes when immersed in an inflammatory state named reactive astrogliosis.[4] During this period of reactivity, the astrocyte produces a number of pro-inflammatory cytokines and anti-inflammatory cytokines. These cytokines both help and at times cause damage to the cell in an attempt to return to its homeostatic state. It is unclear whether astroglial cells undergoing gliosis produce anti-inflammatory responses that are expressed via a CB2R-mediated pathway. Astroglial-mediated neuroprotection would be potentially invaluable since astroglial cells outnumber neurons and microglia in brain. Up-regulation of existing CB2R in this type of cell population would enhance the neuroprotection that is desired. This study aimed to solidify the expression of CB2R in astrocytes to establish its potential use with naturally occurring cannabinoids while avoiding psychoactivity, caused by activation of CB1R.

Sprague-Dawley rats were harvested at one to three days old and their brains utilized for cell culture. Astrocytes were then isolated from microglia and neurons then plated in pure astrocyte cultures in 35mm and 100mm dishes. Subculture of astrocytes up to passage three (p3) was performed before experimentation. Lipopolysaccharide (LPS)

was added to samples at 0, 0.010µg/mL, 0.10µg/mL, 1.00µg/mL, and 10.0µg/mL. Quantitative analysis of CB2R was implemented using Western blot. LC/MS/MS quantitative experimentation was executed following liquid-liquid extraction of lipids from our homogenized in vitro samples to detect endocannabinoid 2-arachidonoylglycerol (2-AG) concentrations. Concentrations of 2-AG increased dose-dependently by 12% at 1.0µg/mL of LPS. CB1R was identified but there was no change in concentration compared to the control. CB2R was detected at a dose-dependent 45% increase in rat cortical astrocytes (RCAs).

These findings imply that astrocytes express CB2R protein and endogenous cannabinoid 2-AG during inflammation in astrocytes. 2-AG was readily profiled using LC/MS/MS analysis. Quantitative analysis of both CB2R and 2-AG lead to implications that ligand-receptor binding of the cannabinoid system, specifically via CB2R signaling, can be explored in the astrocyte. Therefore, it is pertinent that in prospective studies specification of CB2R is targeted. Despite the controversial recent findings of CB2R in astrocytes, we have shown that they are, in fact, present in RCAs. This is very beneficial to brain tissue that has undergone any type of traumatic insult regarding its functionality. It is potentially an implication for isolating modulation of CB2R so that its anti-inflammatory properties could be taken advantage of, providing a novel route to anti-inflammation. Moreover, cannabinoid ligands are an ideal route of administration in that they bypass the psychoactive side effects that are characteristic of CB1R binding and activation.

Reference(s):

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Astrocyte, Cannabinoid Receptor, Endogenous Cannabinoid