PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Study protocol for a phase II, double-blind, randomized controlled trial of cannabidiol (CBD) compared to placebo for reduction of brain neuroinflammation in adults with chronic low back pain
AUTHORS	Pike, Chelsea; Kim, Minhae; Schnitzer, Kristina; Mercaldo, Nathaniel; Edwards, Robert; Napadow, Vitaly; Zhang, Yi; Morrissey, Erin; Alshelh, Zeynab; Evins, Anne; Loggia, Marco; Gilman, Jodi

VERSION 1 – REVIEW

REVIEWER	Alfonso Romero-Sandoval, Edgar Wake Forest University, Department of Anesthesiology
REVIEW RETURNED	04-Jul-2022

GENERAL COMMENTS	• The protocol contains the methods, material, and procedures of a phase II, double-blind, randomized, placebo-controlled, 4-week clinical trial (6 weeks follow-up) to test whether cannabidiol (p.o., CBD) reduces radiological markers of glial activation in the thalamus (neuroinflammation - primary outcome) and pain and depressing symptoms (secondary outcomes) in patients with low back pain. Other secondary and exploratory outcomes are proposed. • CBD dosing and placebo administration are well planned and based on existing clinical data. • Inclusion and exclusion criteria are adequate. Diagnosed chronic low back pain, more than six months is the target adult patient population: 80 patients, 40 per group. • Neuroinflammation (primary outcome) will be determined using PET/MRI and [11C]PBR28 (peripheral benzodiazepine receptor), which is believed to be a biomarker of glial reactivity/activation which is indicative of “neuroinflammation.” • Since some individuals show low binding to [11C]PBR28, which is associated with Ala147Thr TSPO polymorphism, participants will be tested for this. • Informed consent and IRB protocol are in place. • Blinding, randomization, and treatment allocation procedures are well-designed. • Data management, statistical plan, and data and safety monitoring are sound. Strengths: - The investigation of CBD as potential drug to treat chronic back pain is an unmet need. Currently, there is no high-quality clinical trial that studies the potential effects of CBD in chronic pain. This study is well-designed, even though the primary outcome does not seem to be well supported (glial modulation – see below). The

	effects of CBD on pain and pain-related comorbidities are well supported and worth exploring. Limitations: - The idea that CBD will reduce glial reactivity/activation and that this effect will be detected by PET [11C]PBR28 is not compelling. So far, [11C]PBR28 increases have been shown in patient populations where glial cells are believed to display an activation state. However, there is no evidence that glial modulators (currently used in the clinic for other indications) could reduce the PET [11C]PBR28 signal. Strong evidence exists that multiple glial modulators have failed to mitigate drug-induced headaches and neuropathic pain conditions. However, CBD is not a glial modulator per se. Thus, as CBD has mild anti-inflammatory effects will likely fail to inhibit glial activation. True potent glial modulators (minocycline, propentofylline, ibudilast, etc.) could have been included as potential "positive" controls or would have been more suitable to be tested in this patient population. Similarly, if these glial modulators have failed in chronic pain states, how a less potent drug (CBD) is expected to be effective in chronic back pain? In other words, there is a disconnect between the potential effects of CBD in a chronic pain condition and its potential mechanism of action (glial modulation). Furthermore, minocycline has shown to reduce PET [11C]PBR28 signal in patients (with brain trauma, PMID: 29272357). However, minocycline has not shown meaningful clinical effects in patients with lumbar radicular neuropathic pain (PMID: 25373391). - The major mechanism of action of CBD is not neuroimmune modulation. In fact, recent studies have demonstrated that CBD acts on voltage-gated sodium channels in neurons. It is logical to think that this is the mechanism by which CBD acts in neurological conditions (i.e. epilepsy). For example, it is known that anticonvulsants and antidepressants that somehow reduce chronic pain act on neurons. Thus, the proposed mechanism of action (glial modulation), which is the primary outcome, is not well supported. - Another limitation is the heterogenicity of chronic back pain, which is not taken into consideration. How are the investigators correct for clinical issues such as disc herniation, root compression, spinal cord compression, vertebral instability, etc.?

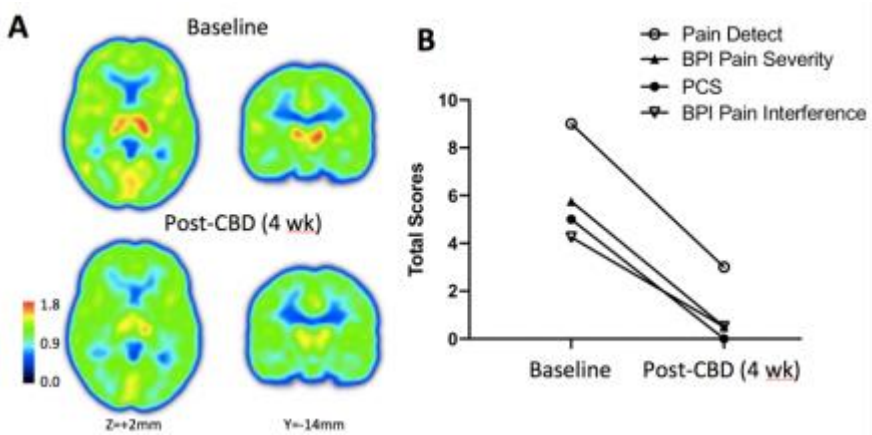
VERSION 1 – AUTHOR RESPONSE

Response to Reviewer:

We would like to thank Dr. Romero-Sandoval for his expert comments. In addition to pointing out multiple strengths (of which we are grateful), he has very eloquently expressed concerns, primarily about the rationale underlying the study of the effect of CBD with TSPO imaging. We address his comments below.

The idea that CBD will reduce glial reactivity/activation and that this effect will be detected by PET [11C]PBR28 is not compelling. So far, [11C]PBR28 increases have been shown in patient populations where glial cells are believed to display an activation state. However, there is no evidence that glial

modulators (currently used in the clinic for other indications) could reduce the PET [11C]PBR28 signal.



Our Reply: We agree that the effect of glial modulators on the TSPO signal has never been evaluated in pain populations (which underscores the novelty of the study). However, as the reviewer points out below, a recent study does show that minocycline reduces [^{11}C]PBR28 signal in traumatic brain injury (Scott et al., Brain 2018). This, we feel, contributes to a rationale for the use of [^{11}C]PBR28 PET signal as an outcome for trials on glial modulators. Ultimately, while we understand, and to large extent share, the Reviewer's viewpoints, our preliminary data in a single, pilot participant revealed a clear reduction in the brain [^{11}C]PBR28 signal, in addition to a reduction in pain ratings and pain catastrophizing (see figure below). While we are very cautious with interpreting data from a single patient, we feel that these preliminary results would be promising enough to justify this exploratory mechanistic clinical trial.

Figure. Imaging and behavioral effects of CBD in a single patient treated with a four-week course of CBD (Epidiolex).

Strong evidence exists that multiple glial modulators have failed to mitigate drug-induced headaches and neuropathic pain conditions.

Our Reply: We agree that the evidence for efficacy of glial modulators in pain has so far been underwhelming, with at least one high-profile pharmacological agent failing to demonstrate efficacy (propentofylline, in post-herpetic neuralgia patients; Landry et al., Experimental Neurology 2012). Other studies, though, have suggested at least some efficacy (e.g., minocycline, Vanelderen et al., Anesthesology 2015; Syngle et al., Neurol Sci 2014). As the reviewer himself points out below, the pain reduction in the Vanelderen study was statistically, but not clinically, significant (vs. placebo). However, it is important to point out that a number of factors might explain these small (e.g., Vanelderen trial) or null effects (e.g., Landry trial). For instance, in the absence of a method of detecting neuroinflammation (absent in all of those studies), it remains unknown whether the specific populations tested in those trials do exhibit neuroinflammation. In our study, we include chronic back pain patients which we have shown in multiple papers (Loggia et al., Brain 2015; Torrado-Carvajal et al., Pain 2021) to exhibit [^{11}C]PBR28 signal elevation, thus mitigating this concern.

However, CBD is not a glial modulator per se. Thus, as CBD has mild anti-inflammatory effects will likely fail to inhibit glial activation. True potent glial modulators (minocycline, propentofylline, ibudilast, etc.) could have been included as potential "positive" controls or would have been more suitable to be tested in this patient population. Similarly, if these glial modulators have failed in chronic pain states, how a less potent drug (CBD) is expected to be effective in chronic back pain? In other words, there is a disconnect between the potential effects of CBD in a chronic pain condition and its potential mechanism of action (glial modulation). Furthermore, minocycline has shown to reduce PET [11C]PBR28 signal in patients (with brain trauma, PMID: 29272357). However, minocycline has not shown meaningful clinical effects in patients with lumbar radicular neuropathic pain (PMID: 25373391).

Reply: While we agree that CBD's mechanism of action is complex, it does also act on CB receptors, including those expressed in glial cells (Ehrhart et al., J Neuroinflammation 2005; Gomes et al., Schizophr Res 2015). Again, our preliminary data also showed an effect of CBD on pain ratings, supporting the rationale for this study.

The major mechanism of action of CBD is not neuroimmune modulation. In fact, recent studies have demonstrated that CBD acts on voltage-gated sodium channels in neurons. It is logical to think that this is the mechanism by which CBD acts in neurological conditions (i.e. epilepsy). For example, it is known that anticonvulsants and antidepressants that somehow reduce chronic pain act on neurons. Thus, the proposed mechanism of action (glial modulation), which is the primary outcome, is not well supported.

Reply: The reviewer makes a good point, and we agree that there's growing evidence that CBD may also act on voltage-gate sodium channels in neurons. However, we would like to stress that a positive outcome in our trial will not depend on whether CBD's action is mediated by a direct effect on glia. In fact, it is entirely possible (and, indeed, we have been proposing over the years) that the neuroinflammatory signal we are detecting in cLBP patients may be driven by aberrant neural activity ("neurogenic" neuroinflammation). As such, CBD may reduce neuroinflammation by modulating activity in voltage-gated sodium channels in neurons. We have added a sentence about this to our introduction.

Another limitation is the heterogenicity of chronic back pain, which is not taken into consideration. How are the investigators correct for clinical issues such as disc herniation, root compression, spinal cord compression, vertebral instability, etc.?

Reply: This is another important observation, and we agree that the umbrella definition of chronic low back pain is quite broad, encompassing mechanistically different etiologies. We have added a sentence to the limitations section of the paper mentioning that chronic low back pain is a broad category and could limit the ability to identify a specific mechanism of action of CBD. In our prior work, we show that patients with different symptom presentations might have slightly different cortical TSPO signal (e.g., radicular cLBP> axial cLBP) in some cortical regions (the primary somatosensory cortex; Alshelh et al., Brain 2022). However, in the thalamus we observed very consistent signal elevations that could be detected in each patient examined in our first study (Loggia et al., Brain 2015) and were not different across etiologies (Alshelh et al., Brain 2022). This is why we focused on the thalamus as our primary region-of-interest. Because the thalamus appears to be indifferent to the clinical presentation, we think that including a heterogeneous group of cLBP patients will be adequate to achieve the aims of our trial, while at the same time presenting us with the opportunity to explore potential differences across presentations and etiologies, in exploratory subanalyses.

VERSION 2 – REVIEW

REVIEWER	Alfonso Romero-Sandoval, Edgar Wake Forest University, Department of Anesthesiology
REVIEW RETURNED	23-Aug-2022

GENERAL COMMENTS	I appreciate the candid responses from the reviewers. I think they (and I) recognize that it is virtually impossible to have a perfect clinical trial, but this study is important and well-designed overall. My last recommendation would be to incorporate the multiple confounders that this type of research and approach entails. Of course, the strengths are evident and they will definitively be highlighted, but a balanced data interpretation would strengthen the article. Thanks for the professional discussion.

VERSION 2 – AUTHOR RESPONSE

Response to Reviewer:

I appreciate the candid responses from the reviewers. I think they (and I) recognize that it is virtually impossible to have a perfect clinical trial, but this study is important and well-designed overall. My last recommendation would be to incorporate the multiple confounders that this type of research and approach entails. Of course, the strengths are evident and they will definitively be highlighted, but a balanced data interpretation would strengthen the article. Thanks for the professional discussion.

We thank Dr. Romero-Sandoval for this comment. Since we are randomizing the treatment groups, confounding variables should be balanced between the groups – and thus we do not plan to adjust for confounding variables. However, if we do find that despite randomization, there are imbalances between groups, we will adjust for potential confounding variables using directed acyclic graphs (DAGs) to determine which confounders may be an issue, and will control for these variables.