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Commentary

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Endotoxin for Alcohol Research: A Call for Experimental Medicine Using Lipopolysaccharide Challenge

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Abstract

Studies of inflammation in alcohol use disorder (AUD) are overwhelmingly preclinical, and translation to clinical samples is necessary. Endotoxin administration has been used successfully in humans to study mood disorders, offering a translational, reliable and safe model that may be validated in AUD research. We argue for the use of endotoxin challenge to elucidate the interplay between AUD and inflammation.

COMMENTARY

There is a great deal of interest in the role of inflammation in psychiatric disorders, including alcohol use disorder (AUD). While neuroinflammation appears to be a key component of AUD, the existing literature is overwhelmingly preclinical and findings in humans are largely correlational. Experimental approaches that establish a causal link between inflammation and AUD phenotypes are currently lacking and are necessary for the next step in the translation of this hypothesis. One reason for this limited translation of preclinical findings to human and clinical samples is the lack of reliable methods to study inflammation. Therefore, to improve the translation of preclinical findings to clinical samples, experimental manipulations that can give rise to a phasic state of high inflammation are required and may allow for experimental medicine models to be tested in clinical populations.

One promising experimental method by which transient inflammation can be provoked is through the administration of purified bacterial endotoxin (lipopolysaccharide, LPS). Endotoxin administration in humans yields a reliable, transient and safe response, with clear dose–response relationships. LPS is administered as an intravenous infusion, usually at low doses between 0.4 and 1.0 ng/kg body weight. This infusion induces a phasic inflammatory response, with peripheral cytokine levels—biomarkers of systemic immune response—peaking around 2 h post-infusion and returning to baseline within four to 6 h. At these low doses, endotoxin administration has been shown to safely and briefly mimic low-grade inflammatory response, raising cytokine levels and subtly affecting neuropsychiatric symptoms, including depressed mood and anxiety, while causing limited changes in vital signs including heart rate, blood pressure and temperature. This method has been used in many past human challenge studies and remains the World Health Organization standard for endotoxin assays used in the pharmaceutical industry (Suffredini and Noveck, 2014). While this method has been used successfully in human subjects to better understand the contribution of inflammation to mood disorders, it has not been widely implemented in the context of AUD. In this commentary, we make an argument for the use of endotoxin challenge in AUD research to elucidate the complex interplay between AUD and inflammation.

First, inflammatory signaling is significantly implicated in AUD in both preclinical and clinical models. Individuals with AUD have

increased plasma levels of proinflammatory cytokines, and animal models with chronic alcohol exposure show long-lasting increases in systemic inflammation. In healthy humans, alcohol administration has been reported to modulate—either raising (Afshar *et al*., 2015) or lowering (Monnig *et al*., 2020)—endogenous LPS levels. Furthermore, LPS endotoxin-induced inflammation in mice produces prolonged increases in alcohol consumption. On the whole, preclinical and clinical studies show that alcohol exposure affects inflammation, and preclinical studies indicate that the reverse may be true as well. Therefore, we contend that an LPS endotoxin challenge in humans provides a valid forward translation of the role of inflammation in AUD.

In support, endotoxin challenges have been used in clinical studies of affective disorders (Lasselin *et al*., 2020). These studies not only offer proof of safety and reliability in human subjects but also strengthen the reasoning that endotoxin challenge will be an effective method for studying AUD, as there is a well-established relationship between AUD and emotion regulation. A negative mood can induce alcohol-seeking due to its effects on craving, and alcohol use inhibits negative emotion regulation. This suggests that in humans, endotoxin challenge may also affect the secondary symptomology of AUD through its effects on mood and behavior. To date, however, this method has not been applied broadly to AUD research, and in fact, there is some skepticism about the application of this paradigm.

Perhaps the most common criticism of the endotoxin challenge approach is driven by uncertainty regarding the degree to which central nervous system inflammatory response reflects the systemic response measured by peripheral cytokines, as well as differences between the sustained inflammatory state seen in chronic alcohol consumption and the transient state induced by endotoxin administration. However, these questions can be answered empirically. LPS administration offers the potential to explore these outstanding questions by conducting longitudinal and neuroimaging studies. Indeed, neuroimaging findings show that endotoxin-induced inflammation is linked to neural response (Lasselin *et al*., 2020), a promising indicator that the systemic inflammatory state induced by LPS administration is reflected in the brain. The endotoxin challenge proposed in this commentary is aimed at inducing a transient/phasic immune response distinct from and beyond the chronic/tonic levels likely present at baseline for subjects with AUD. LPS models of chronic diseases have been used successfully in animals (i.e. circulating LPS via osmotic minipump, Lindros and Järveläinen, 2005); however, chronic administration of low-dose endotoxin in humans is hampered by endotoxin tolerance (Kiers *et al*., 2017). Additional preclinical and longitudinal studies will likely be necessary to investigate the chronic nature of inflammation in AUD. It is also worth noting that the only published study using endotoxin administration in the context of AUD found that LPS did not induce anxiety or alcohol craving. However, this study was conducted within a clinical trial of the neuroimmune drug pioglitazone and was prematurely terminated after 16 subjects, with only eight subjects undergoing the LPS challenge in the placebo group (Schwandt *et al*., 2020). Therefore, these findings are considered highly preliminary and indicate that endotoxin challenge requires further assessment in studies of AUD. Despite its limitations, this study provides valuable evidence that LPS administration in human subjects with AUD is indeed safe.

Importantly, both physical and behavioral responses to LPS are largely conserved across vertebrate species from animals to humans, allowing for forward- and reverse-translation of findings. Thus, the endotoxin challenge presents exciting opportunities for treatment development. Given the wealth of preclinical and clinical research into neuroimmune therapies for AUD, one can envision an experimental medicine study in which an endotoxin challenge is used to elicit a transient inflammatory response as well as behavioral alcohol-related outcomes, and one such neuroimmune drug is used to either block or 'rescue' both the immune and behavioral responses. This type of approach can provide valuable proof-of-mechanism regarding these therapies for AUD.

LPS administration presents a multitude of possibilities for clinical research. One can imagine exploring the impact of inflammation in alcohol consumption, through self-administration paradigms, cue-reactivity (neuroimaging or behavioral) and secondary factors such as mood-related outcomes. This method could even shed light on the relationship between alcohol, the gut microbiome and alcohol-associated liver disease (ALD). Studies suggest that the gut microbiome contributes to the pathogenesis of AUD (Temko *et al*., 2017), which in turns play an important role in ALD via inflammatory mechanisms (Hosseini *et al*., 2019). Furthermore, the gut microbiome might play a role in alcohol craving and seeking behaviors (Leclercq *et al*., 2014). This area is of high interest but is lacking in clinical mechanism-oriented studies—thus, one can envision combining the LPS endotoxin challenge with a gut microbiome study to directly interrogate these inflammatory mechanisms.

To date, the vast majority of studies implicating inflammation in AUD are preclinical in nature. Therefore, it is crucial for the field to progress to experimental models that can effectively probe the role of neuroinflammation in AUD phenotypes in humans. Endotoxin challenge offers a translational, reliable and safe model of inflammation that may be validated through use in studies of AUD. Endotoxin administration presents a method through which the complex relationship between AUD and inflammatory signaling may be elucidated, and may aid in the development of neuroimmune treatments for AUD. We argue for the utilization of this paradigm in AUD research, with a focus on translating a large preclinical body of evidence for the relationship between inflammation and AUD into a more clinically useful and applied knowledge base.

CONFLICT OF INTEREST STATEMENT

None declared.

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