Published in final edited form as:

Alcohol Clin Exp Res. 2017 March ; 41(3): 497–500. doi:10.1111/acer.13312.

Repurposing Tigecycline for the Treatment of Alcohol Use Disorder

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Abstract

Following acamprosate's FDA approval for treatment of alcohol use disorder (AUD) more than a decade ago, there have been no new medications added to the physician's therapeutic tool bag. All three FDA approved medications, disulfiram, naltrexone and acamprosate have limited treatment efficacy. Considering the complex nature of AUD, additional medications and investigation into potential biomarkers associated with each treatment is warranted with the hopes of benefiting its diverse array of patients. Although recent clinical trials continue to sketch future therapeutic strategies, many current clinical trials are stagnant as investigations for the above compounds have yet to yield definitive mechanisms of action. Thus, the scientific and medical community must push forward clinical trials by evaluating promising preclinical compounds. In this commentary, we discuss four highly coherent papers from Dr. Susan Bergeson and colleagues, where they investigate the tetracycline derivative tigecycline in reducing alcohol consumption, as well as alcohol induced pain and withdrawal in mice (Fig. 1). Although promising, these comprehensive studies must be validated in preclinical settings for effectiveness in alternative animal models to determine the molecular mechanisms by which these compounds reduce AUD symptomatology, thus facilitating progression of tetracycline derivatives into the clinic.

ANTIBIOTICS AND ALCOHOL USE DISORDER

Several studies report that tetracycline antibiotics minocycline and doxycycline (Agrawal et al., 2011; McIver et al., 2012) reduce alcohol consumption. Notably, other reports indicate that alcohol consumption can affect several components of the neuroimmune system (Blednov et al., 2012; Montesinos et al., 2016). The first report by Syapin et al. (Syapin PJ, 2016) explores how tigecycline outperforms doxycycline and minocycline in reducing binge alcohol drinking in the dark (DID). In agreement with their findings, Bergeson et al. (Bergeson SE, 2016b) further explores how tigecycline can reliably reduce alcohol

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

dependence in a combined chronic intermittent ethanol (CIE) vapor and 2-bottle choice alcohol consumption model.

The tetracycline class of broad spectrum antibiotics is effective against gram positive and gram negative bacterial microorganisms. This class of compounds allosterically inhibit the association between aminoacyl tRNA and the 30S and 50S ribosomal subunits, resulting in derailment of transcriptional activity (Griffin et al., 2011). While this class of antibiotics has seen decreased use as a result of microorganism resistance, the use of these compounds for their non-antimicrobial properties (reactive oxygen species [ROS] scavenging, inhibition of extracellular matrix proteolysis by matrix-metalloproteinases [MMPs], anti-apoptotic neuroprotection, and inhibition of pro-inflammatory cytokines) are the current focus of investigation (Griffin et al., 2011). In this regard, the dose-response study of tetracycline derivatives by Syapin et al., (2016) provides timely information and identified tigecycline as the best candidate. However, tigecycline appears to be the least efficacious of the compounds in reducing alcohol consumption. Although unlike the other tetracyclines, tigecycline does not alter water intake or induce toxicity. Thus, although both doxycycline and minocycline are more efficacious in decreasing alcohol consumption, they also significantly reduce water intake (Agrawal et al., 2011; Agrawal et al., 2014). Another important finding from Syapin et al., (2016) revealed that not all tetracycline derivatives reliably decrease alcohol consumption. Only minocycline and tigecycline were effective in doing so, as shown for both male and female C57BL/6J mice. The evidence for reduction of DID alcohol consumption by semi-synthetic tetracyclines provided by Syapin et al., (2016) is further strengthened in a second investigation by Bergeson et al. (2016b), which specifically focuses on the ability of tigecycline to significantly dampen alcohol dependence in a combined CIE vapor and 2-bottle choice alcohol consumption model. In this study, Bergeson and colleagues show that tigecycline was efficacious in reducing alcohol consumption in both male and female C57BL/6J mice, without affecting water consumption. However, it is unclear from this study whether tigecycline is targeting systems directly altered by chronic alcohol intake or whether these results are off-target effects common to this class of compounds. Tigecycline lowered alcohol intake in dependent (alcohol vapor-exposed) and non-dependent (air-exposed) mice, albeit without alcohol drinking differences between these two cohorts. Therefore, these results should be strengthened by future studies that test tigecycline on rodent models (i.e. high-alcohol-preferring P-rat or HAD1 rat model) known for their inherent high baseline of alcohol consumption (Bell et al., 2015). Taken together, Syapin et al. (2016) and Bergeson et al. (2016b) indicate that tigecycline shows significant efficacy in reducing binge alcohol intake as well as alcohol consumption in alcohol dependent male and female mice.

The results of these studies raise several questions in regards to how these compounds reduce alcohol intake. Although the authors do not show mechanistic evidence, they highlight the importance of a chemical modification at R6', where substitution of -OH for -H may be an important determinant of the non-antimicrobial properties of minocycline and tigecycline, including reduction of alcohol consumption. Therefore, we may speculate that this modification on tigecycline (or similar modifications on other tetracycline derivatives) may be an essential factor in the ability for this class of drug to disrupt MMP-mediated priming of microglia into reactivity or perhaps facilitate peripheral monocyte infiltration of

the blood brain barrier (BBB). As a result, tigecycline may share minocycline's ability to inhibit antigen presentation and extravasation into the BBB by circulating peripheral T-cells (Brundula et al., 2002; Kalish and Koujak, 2004). Tigecycline may have the ability to regulate alcohol-induced cytokine "mini-storms" (i.e. release of TNF α , IL-1 β and others cytokines/chemokines) that may form during presence of high levels of alcohol in brain (Crews and Vetreno, 2016; Lee and Kim, 2014). Indeed, this has been a mechanism reported for tetracycline derivatives in relation to neuroinflammation caused by brain injury, epilepsy and neurodegeneration (da Fonseca et al., 2014). While we propose potential mechanisms of action for tigecycline, we should emphasize that the four companion studies by Bergeson and colleagues do not address whether alcohol is inducing a neuroinflammatory response in their subjects. Moreover, neuroimmune and behavioral response systems are bidirectional, as recent evidence indicates that activation of the reward system can boost immune responses (Ben-Shaanan et al., 2016). Consequently, we stress careful consideration of the articles highlighted in this commentary when designing future studies aimed at elucidation of how tigecycline reduces alcohol consumption.

IS NEUROINFLAMMATION INVOLVED IN AUD?

Excessive long-term alcohol use exerts a physiologically detrimental effect on peripheral organs, including the pancreas, intestinal tract and liver (Natarajan et al., 2015). The mechanisms by which these damages occur vary and include increases in hepatic acetaldehyde production, increased ROS, fatty acid ethyl esters as well as the gut derived endotoxin lipopolysaccharide (LPS) (Natarajan et al., 2015). In particular, excessive alcohol consumption has been reported to damage intestinal epithelium resulting in leakage and systemic circulation of LPS, a molecule composed of the outer membrane of gram negative bacteria which has particular affinity for toll-like receptors (TLR) and in particular TLR-4 (Montesinos et al., 2016). Importantly, the innate immune system engages an inflammatory response in many cell types including monocytes, macrophages and central nervous system (CNS) microglia as a result of this innate immune interaction (Fernandez-Lizarbe et al., 2009). Consequently, NF κ B and AP-1 mediated transcriptional activity in microglia induce production and release of TNF- α and IL-1 β (Crews and Vetreno, 2016). Given alcohol's well known activation of LPS mediated innate immune responses, it is not surprising that recent investigations have revealed a role for microglia mediated neuroinflammation and neurodegeneration as a result of excessive alcohol exposure (Crews and Vetreno, 2016). Although it is obvious that these discoveries advance our mechanistic understanding of how peripheral and CNS immunity contributes to AUD, of equal importance are the potential new therapeutic approaches that are underlined by these investigations.

TIGECYCLINE AND SEX DIFFERENCES IN PAIN RESPONSE

Alcohol has long been used to buffer against acute pain, which is simply a concentration of pro-inflammatory cytokines that are released in response to injury or infection. Paradoxically, uncontrolled chronic alcohol abuse potentiates nociception in both males and females (Egli et al., 2012). Given the beneficial anti-inflammatory effects of tetracyclines (Garrido-Mesa et al., 2013), it is not surprising that Bergeson et al. (2016) found tigecycline-promoted anti-nociception in male C57BL/6J mice. What was surprising were the sex

specific differences revealed by Bergeson et al. (Bergeson SE, 2016a) in responses to pain, as tigecycline seemed to potentiate nociception in female C57BL/6J mice. Sex specific differences are well documented in terms of alcohol consumption. Interestingly, in rodent models of AUD females display increased alcohol consumption relative to males, while the converse is observed in humans (Becker and Koob, 2016). Therefore, the results of Bergeson et al. (2016a) are particularly interesting, given that in rodent models, females are reported to be less affected by pain from withdrawal severity (Becker and Koob, 2016). One possible explanation for the paradoxical pro-nociception reported for females in Bergeson et al. (2016) may include the differential effects of tigecycline on pain responses in male versus female mice, which could involve cell specific immune-related responses. For example, sex-dependent developmental expression of TLR in microglia (Lenz et al., 2013) in addition to differences in T-cell activation in rodents have been reported (Sorge et al., 2015). Notably, tetracyclines are known to affect the biochemical behavior of both microglia and T-cells (Brundula et al., 2002; Garrido-Mesa et al., 2013; Kalish and Koujak, 2004).

POTENTIAL MECHANISMS OF TIGECYCLINE ACTION ON AUD

As Syapin et al., (2016) and Bergeson et al. (2016b) point out in their discussion, follow up mechanistic studies are indeed warranted to understand how this class of antibiotics function to reduce alcohol consumption. One mechanism worthy of consideration is the potential effect of tigecycline on the expression of genes that regulate synaptic levels of glutamate. We suggest this avenue of study based on evidence showing that the antibiotic ceftriaxone, a cephalosporin class of antibiotic, has been specifically implicated in alcohol seeking and consumption (Lee et al., 2013), and in reducing alcohol withdrawal (AWD) symptomatology (Abulseoud et al., 2014) by altering expression of the excitatory amino acid glutamate transporter-2 (EAAT2, also known as GLT-1). Martinez et al 2016 provides convincing evidence that tigecycline significantly reduces AWD severity (Martinez JM, 2016), concluding that the convulsion reductions by tigecycline are yet another phenotype shared by the semi-synthetic tetracyclines. Interestingly, there is evidence suggesting that by preventing microglial activation and subsequent release of pro-inflammatory cytokines, this class of compounds may protect against AWD-mediated convulsions (Wang et al., 2012). More importantly, the authors stress that tigecycline may be used as an alternative to benzodiazepine treatment, as the latter is known to have high abuse potential. An additional benefit toward AUD therapy is tigecyclines long $t_{1/2}$ (between 40–60 h), making it an ideal treatment. In terms of treatment compliance, minimizing administration of doses reported to induce nausea, vomiting, and gastrointestinal intolerance (200–300mg/kg) should positively influence compliance in AUD patients (Muralidharan et al., 2005). Optimizing route of administration in favor of maximizing oral bioavailability (as opposed to intravenous administration), in the doses reported to reliably reduce alcohol intake (50 – 100 mg/kg in the four companion studies), should also enhance compliance. Finally, careful monitoring for potential hepatotoxicity or renal complications is also warranted, given tigecyclines extensive volume of distribution and reportedly longer $t_{1/2}$ in non-central compartments (bone, spleen, etc.) (Muralidharan et al., 2005).

CONCLUSION

In conclusion, this group of companion papers provides new evidence for the ability of tigecycline, a semi-synthetic tetracycline antibiotic, to reduce alcohol consumption, AWD symptomatology, and pain responses. Recent evidence implicates neuroimmune-neurocircuit feedback interactions in the regulation of behavior (Ben-Shaanan et al., 2016). Thus it is important to mechanistically determine how these seemingly disparate systems contribute to AUD via deciphering the role of tigecycline in immune responses. Importantly, the pre-clinical studies discussed in this commentary highlight the novel repurposing of tigecycline to aid in reduction of several AUD-related phenotypes. However, before widespread clinical use of antibiotics to treat AUD can be entertained, careful consideration must be given to the antibiotic resistance crisis currently facing our healthcare system (Seputiene et al., 2010).

Acknowledgments

We thank Dr. J.H. Song for his helpful comments on antibiotics. This work was supported by the Mayo Graduate School (AO), the Samuel C. Johnson for Genomics of Addiction Program at Mayo Clinic, the Ulm Foundation, the Godby Foundation, the David Lehr Research Award from American Society for Pharmacology and Experimental Therapeutics, and National Institute on Alcohol Abuse and Alcoholism (AA018779) to DSC.

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Fig. 1.

Summary of physiological effects of tigecycline on alcohol consumption and pain responses.

(A). Tigecycline reduces alcohol intake in male and female mice subjected to binge drinking in the dark (DID). (B) Tigecycline decreases alcohol intake in male and female alcohol dependent mice examined via the combined two-bottle choice/alcohol vapor exposure model. (C). Tigecycline suppresses alcohol withdrawal induced convulsion severity in male and female mice. (D) Tigecycline differentially affects nociception following DID alcohol consumption, as male mice showed reductions in pain responses whereas female mice displayed enhanced pain sensitivity.