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Effective Reduction of Acute Ethanol Withdrawal by the Tetracycline Derivative, Tigecycline, in Female and Male DBA/2J Mice

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Abstract

Background—Alcohol Use Disorder (AUD) is a spectrum disorder characterized by mild to severe symptoms, including potential withdrawal signs upon cessation of consumption. Approximately five hundred thousand patients with AUD undergo clinically relevant episodes of withdrawal annually (Kosten and O'Connor, 2003). Recent evidence indicates potential for drugs that alter neuroimmune pathways as new AUD therapies. We have previously shown the immunomodulatory drugs, minocycline and tigecycline, were effective in reducing ethanol consumption in both the two-bottle choice and drinking-in-the-dark paradigms. Here, we test the hypothesis that tigecycline, a tetracycline derivative, will reduce the severity of ethanol withdrawal symptoms in a common acute model of alcohol withdrawal (AWD) using a single anesthetic dose of ethanol in seizure sensitive DBA/2J (DBA) mice.

Methods—Naive adult female and male DBA mice were given separate injections of 4 g/kg i.p. ethanol with vehicle or tigecycline (0, 20, 40 or 80 mg/kg i.p.). The 80 mg/kg dose was tested at three time-points (0, 4, 7 hrs) post-ethanol treatment. Handling-induced convulsions (HIC) were measured before, and then over 12 hours following ethanol injection. HIC scores and areas under the curve were tabulated. In separate mice, blood ethanol concentrations (BEC) were measured at 2, 4, and 7 hours post injection of 4 g/kg i.p. ethanol in mice treated with 0 and 80 mg/kg i.p. tigecycline.

Results—AWD symptom onset, peak magnitude, and overall HIC severity were reduced by tigecycline drug treatment compared to controls. Tigecycline treatment was effective regardless of

timing throughout AWD, with earlier treatment showing greater efficacy. Tigecycline showed a dose responsive reduction in acute alcohol withdrawal convulsions, with no sex-differences in efficacy. Importantly, tigecycline did not affect BECs over a time course of elimination.

Conclusions—Tigecycline effectively reduced alcohol withdrawal symptoms in DBA/2J mice at all times and dosages tested, making it a promising lead compound for development of a novel pharmacotherapy for AWD. Further studies are needed to determine the mechanism of tigecycline action.

Keywords

Tigecycline; Minocycline; Handling-Induced Convulsions; Alcohol Withdrawal; Alcoholism

INTRODUCTION

Alcohol use disorder (AUD) is a chronic disease with genetic and environmental influences, and is characterized by excessive craving, inability to control drinking, tolerance, and other alcohol-related problems (O'Brien, 2014). Individuals with more severe AUD can experience withdrawal symptoms when they reduce or stop alcohol consumption (Parthasarathy et al., 2015). Alcohol withdrawal (AWD) symptoms can include, among others, anxiety, delirium, hallucinations, insomnia, nausea, tachycardia, tremors, and seizures (Kosten and O'Connor, 2003). The exact mechanism of withdrawal pathophysiology is not completely understood. The most widely accepted explanation of alcohol withdrawal is increased excitatory glutamatergic and reduced inhibitory GABAergic neurotransmission in the central nervous system (McKeon et al., 2008). Reports vary widely with onset of symptoms between 2 to 24 hours following cessation, even in the presence of significant BEC, making AWD a common problem for emergency department and inpatient hospital physicians. In the last 30 years, treatments for AWD have improved (Carlson et al., 2012, Cooper and Vernon, 2013). However, despite the increased availability of withdrawal treatment, AWD continues to be a significant clinical problem as the majority of people afflicted with AUD in the United States go untreated (NIAAA, 2010). New and safer, as well as more effective, treatments are needed to address this issue.

Currently, the clinical standard of care for AWD varies by severity of symptoms, which range from mild tremors to severe convulsive seizures. In general, it is prudent to address severe symptoms and prevent kindling effects (Myrick and Anton, 2000). In a select group of patients, mild AWD symptoms can be treated on an outpatient basis using long-acting benzodiazepines and a support system of a quiet, lowly lit environment in the home (Blondell, 2005). Outpatient treatment is the most cost-effective, but does not allow for careful monitoring. Tonic-clonic withdrawal seizures, hallucinations and autonomic instability are generally treated on an inpatient basis with short-acting benzodiazepines or anti-seizure medications such as phenobarbital, carbamazepine or valproic acid. The development of delirium tremens (DT) is a medical emergency, which requires immediate treatment and close monitoring, often delivered in an intensive care unit setting. Nutrition and pharmacotherapeutics are given intravenously until symptoms subside, usually within one week. Benzodiazepines are administered in a symptom-triggered and/or scheduled fixed dosage to prevent seizures and psychomotor agitation. In refractory cases, phenobarbital,

propofol or dexmedetomidine can be used adjunctively in the short-term and often require mechanical intubation for respiratory depression (Perry, 2014). Unfortunately, of the numerous medications used to treat AWD, the most effective also have an abuse potential and many carry a significant risk of toxicity, in particular, through respiratory depression, especially with concurrent drinking.

Demonstration of the quantitative similarity of AWD signs between humans and rodents (Goldstein and Pal, 1971, Goldstein, 1973) led to the use of inbred mouse strains and the development of models in which to study the mechanisms of AWD action and test novel treatments (Freund, 1969, Goldstein, 1972a, Crabbe et al., 1980, Kosobud and Crabbe, 1986, Crabbe, 2014). Early studies also showed that genetics and the environment influenced AWD traits (Crabbe, 1987, Buck et al., 1997, Bergeson et al., 2003, Metten and Crabbe, 2005), including a dose-related component to AWD severity (Goldstein, 1972b). The DBA/2J mouse strain has been an invaluable laboratory setting resource toward understanding the development of AWD as measured by handling-induced convulsions (HICs) following acute or chronic ethanol administration. The DBA/2J mouse is commercially available and its AWD has been well characterized (Buck et al., 1997, Kozell et al., 2005, Reilly et al., 2008). We chose to use the acute DBA model, which includes a single administration of an anesthetic dose of ethanol, is not complicated by concurrent treatment of pyrazole used in the chronic model, and because it also maintains a quantitative similarity to human withdrawal (Buck et al., 1997).

Minocycline, which has been found to be effective in reducing alcohol consumption, was the last of the second generation tetracycline antibiotics developed (Chopra and Roberts, 2001) and has been shown to have a number of neuroprotective and anti-inflammatory properties (Garrido-Mesa et al., 2013). Tigecycline is a third generation glycylcycline tetracycline derivative developed 30 years after minocycline (Chopra and Roberts, 2001). The immunomodulatory effects of tigecycline have not been fully characterized, although it has been suggested that tetracyclines, as a drug class, are immunomodulatory in addition to their anti-microbial actions (Domercq and Matute, 2004, Garrido-Mesa et al., 2013). Along with their antibiotic properties, tetracyclines have been shown to be effective anti-convulsants (Wang et al., 2012).

As mentioned, the DBA/2J mouse strain has been well-characterized for both behavioral and genetic contributions during acute AWD (Buck et al., 1997). Using a simple, single dose, acute model involving HIC scoring of AWD in DBA mice, we tested the hypothesis that tigecycline, a drug without known or likely abuse potential, would reduce withdrawal seizures caused by a bolus injection of a sedative-hypnotic concentration of ethanol. Given there are sex differences in general seizures (van Luijtelaar et al., 2014), and sex is an important factor in many ethanol actions, including for withdrawal effects (Wiren et al., 2006, Gorin-Meyer et al., 2007), we used both female and male mice in our studies.

MATERIALS AND METHODS

Animal Husbandry

Animal procedures were performed in accordance with NIH “Guide for the Care and Use of Laboratory Animals” and were approved by the IACUC at the Texas Tech University Health Sciences Center. Adult DBA/2J female and male mice (purchased at 8–10 weeks from The Jackson Laboratory, Bar Harbor, ME) were individually housed, prior to and during testing, under 12:12 lighting in clear plastic mouse cages (Techniplast, Italy) with cedar bedding and *ad libitum* food and water. Mice were habituated to their new environment for at least one week and were used when approximately 70–80 days of age. The mice were weighed prior to the start of experiments. The weights were used to place the mice into balanced groups and to determine drug and ethanol dosages. Separate mice were used for each dose as well as for the pharmacokinetic study.

Drug Administration

Ethanol (95% v/v) was obtained from Pharmco-Aaper (Brookfield, CT) and used to prepare a 20% v/v solution in 0.9% sterile saline. Tigecycline was obtained from Cayman Chemical Company (Ann Arbor, MI; Item #15026). BD 1 mL tuberculin syringes were obtained from ThermoFisher Scientific (Waltham, MA; Item #309625). Intraperitoneal (i.p.) injections were used to administer all tigecycline or vehicle (0.01 cc/kg) and ethanol (0.04 cc/kg) to naive DBA/2J animals. Mice were given an i.p. injection of drug/vehicle right of midline while the ethanol injection was given left of midline. Ethanol was administered at a 4 g/kg dose with the tigecycline drug dosages at 0, 20, 40 and 80 mg/kg in saline.

Handling-Induced Convulsions (HIC)

HIC scores were measured prior to concurrent administration of 4 g/kg i.p. ethanol in saline and tigecycline (0, 20, 40, 80 mg/kg i.p.) and thereafter hourly for 12 hours total. The 80 mg/kg dose was also administered as separate experiments at 4 and 7 hours after ethanol administration. The scoring system was similar to that previously described (Finn and Crabbe, 1999). Briefly, mice were picked up from their home cage by the tail, observed for ~1 second and then gently spun 180° bi-directionally, or in other words, a full 360°. One to five observers, some of whom were blinded to treatment, scored each mouse’s convulsions using the scale described: 0, no convulsion; 1, only a facial grimace after 360° spin; 2, tonic convulsion after 360° spin; 3, tonic-clonic convulsion after 360° spin; 4, tonic convulsion elicited when lifted by the tail; 5, tonic-clonic convulsions when lifted by the tail; 6, tonic-clonic convulsion in cage; 7, spontaneous severe tonic-clonic convulsion without handling. The scores were tabulated and then averaged for each time-point. For the dose response curve, the pre-HIC score was subtracted and a simple AUC was calculated by adding scores across the 12-hr time-course.

Pharmacokinetics of EtOH

To test the effect of tigecycline treatment on the metabolism of ethanol in vivo, pharmacokinetic profiling was completed. Mice were concurrently injected with a single dose of tigecycline (80 mg/kg i.p.) or saline and with 4 g/kg i.p. EtOH (20% in saline).

Submandibular blood samples (47.5 μ l) were taken at 120, 240 minutes after injection and at 420 minutes by sacrificing the mice. The blood samples were processed and analyzed as described below for BEC analysis.

Blood Ethanol Concentration Determinations

Blood ethanol concentration (BEC) was determined using headspace gas chromatography (GC) from 45 μ l blood samples in 150 μ l of water in crimp top GC vials as previously described (Agrawal et al., 2014). BECs were quantified using known concentrations of ethanol (0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml) in an Agilent 7683 automatic liquid sampler GS (Agilent Technologies, Palo Alto, CA).

Statistics

Numerical HIC scores were calculated by subtracting baseline scored (with zero as the lowest possible score) from post-injection scored and summed across the twelve-hour time course to calculate a simple area under the curve. 3-way analysis of variance (ANOVA) was examined to determine the effect of treatment (saline vs tigecycline) and sex (female vs male) with time as a repeated measure, when appropriate. Significant interactions were further analyzed with 1-, 2-way ANOVAs with Dunnett's and Tukey's post hoc tests. With an *a priori* hypothesis that ethanol-induced withdrawal would differ in response to tigecycline, each sex was analyzed separately, and together, in some cases, in the absence of a significant interaction. Differences in BECs were analyzed by 2-way ANOVA in the same manner with an additional linear regression analysis for detection of differences in slope. Data are presented as mean $\pm$ SEM, with statistical significance set at $p < 0.05$. Statistical analyses were conducted using Prism 6 Software (Graphpad, Inc., San Diego, CA) and Systat (version 11, SYSTAT software, Inc., Richmond, CA).

RESULTS

Tigecycline significantly reduced the severity of acute ethanol-induced withdrawal convulsions in both female and male DBA mice

Since our companion study results showed that tigecycline reduced binge alcohol consumption without a negative effect on water consumption (Syapin, companion paper 2016), the drug was used first at an 80 mg/kg dose to test the general hypothesis that the tetracycline derivative would reduce acute AWD convulsions. Our hypothesis was supported; tigecycline given at the time of ethanol injection delayed the onset, reduced the peak magnitude of effect, and reduced the overall severity of withdrawal related convulsions in both female and male DBA mice as shown in Figure 1A and 1B respectively. Due to the complete suppression in HICs by intoxication at hrs 1 – 3 (all zero values), an initial repeated measures ANOVA (time $\times$ sex $\times$ 0 hr tigecycline treatment) was conducted across hrs 4 – 12. Tigecycline treatment significantly suppressed HICs ($F(1,20) = 52.81$, $p < 0.001$), but there was no main effect of sex and no significant interaction of sex $\times$ treatment. HIC scores changed significantly across time ($F(8,160) = 30.294$, $p < 0.001$), and there was a significant interaction between time and treatment ($F(8,160) = 5.27$, $p < 0.001$). There was no interaction of time with sex and no 3-way interaction of sex, time or treatment. Onset of AWD, as defined by an increase above baseline, occurred at hour 5 for control and at 7

hours for tigecycline condition suggesting drug treatment delayed onset of AWD.. Peak magnitude was offset and also differed with treatment. Controls showed an average 3.1 HIC score at hour 7 and for tigecycline treatment the highest average was 1.8 HIC at hours 8–9. As our original hypothesis was that sex differences would be apparent, we followed our initial analysis by separating the sexes and completing 2-way ANOVAs for (treatment $\times$ time) individually for the male and female mice. Treatment was defined as saline treatment at 0 hr or administration of an 80 mg/kg i.p. dose of tigecycline at 0, 4 and 7 hrs following a single dose of 4 g/kg ethanol exposure. Significant differences in HIC scores between saline and tigecycline treatments given at 0, 4 and 7 hrs were seen for both female and male experiments.

In females, a significant treatment $\times$ time interaction was detected by 2-way ANOVA ($F(36, 325) = 2.21, p = 0.0002$) with significance for treatment ($F(3,325) = 13.61, p < 0.0001$) and time ($F(12, 325) = 38.14, p < 0.0001$). Tukey's posthoc *t*-test analyses indicated tigecycline was efficacious to reduce AWD symptoms regardless of time of administration, but that the earlier treatment was initiated, the better the outcome (Figure 1A). Significant reduction of AWD was as follows: Tigecycline given concurrent with ethanol reduced HICs over hours 5–8 and at 10 hours ($5 - p < 0.007$; $6 - p < 1.6 \times 10^{-12}$; $7 - p < 2.8 \times 10^{-6}$; $8 - p < 0.02$; and $10 - p < 0.01$). When tigecycline was administered 4 hours after ethanol, a similarly strong response was seen over hours 6 to 10: ($6 - p < 0.01$; $7 - p < 0.04$; $8 - p < 0.005$; $9 - p < 0.02$; and $10 - p < 0.001$). As was predicted, treatment with tigecycline given at the peak of AWD (7 hrs) was effective, but to a lesser extent, reaching significance only at the 9 ($p < 0.05$) and 10 ($p < 0.04$) hour time points.

Efficacy of tigecycline to reduce AWD in the male mice showed a similar pattern. When tigecycline treatment was given concurrent with ethanol, HICs were reduced between 6 and 11 hours: ($6 - p < 2.4 \times 10^{-6}$; $7 - p < 3.5 \times 10^{-7}$; $8 - p < 0.02$; $9 - p < 0.02$; $10 - p < 0.001$; and $11 - p < 0.02$). Given at 4 hours into AWD, tigecycline significantly reduced HICs at hours 6 and 7 compared to controls: ($6 - 2.6 \times 10^{-5}$; $7 - p < 0.005$). Similar to the female mice, when treatment was later (at 7 hrs), it was less effective with only hour 9 scores meeting significance ($9 - p < 0.04$).

Tigecycline significantly reduced the severity of acute ethanol-induced withdrawal convulsions in a dose-responsive manner

Following the determination that 80 mg/kg tigecycline effectively reduced acute AWD HIC, a dose response including additional saline control animals as well as 20 and 40 mg/kg tigecycline doses were added to the 80 mg/kg dose, as shown in Figure 2A. Analysis of AUC revealed that dose had a significant effect ($F(3, 46) = 11.61, p < 0.001$), but there was no significant effect of sex and no interaction between treatment and sex. Dunnett's multiple comparison post hoc analysis revealed a statistically significant difference between the saline control and the 80 mg/kg tigecycline dose for both females and males ($p < 0.05$), while all other doses did not meet significance. Because the main effect of sex and interaction were not significant, data from females and males were collapsed together and reanalyzed (Figure 2B). Tigecycline produced a significant and dose-dependent reduction in AUC ($F(3,50) = 12.545, p < 0.001$) (Figure 2B). Post-hoc testing indicated that the 20, 40

and 80 mg/kg doses were significantly different from control ($20 = p < 0.004$; $40 = p < 0.0002$; $80 = p < 0.000000009$). These data confirm that tigecycline was equally effective at reducing acute AWD severity in male and female mice.

Tigecycline did not affect the pharmacokinetic elimination of ethanol in either female or male mice

Analysis of blood for ethanol content at 2, 4 and 7 hours after initial ethanol administration and treatment revealed no effect of the tigecycline on BEC levels (Figure 3). Linear regression analyses followed by 2-way ANOVA analysis were performed on the BEC curve created from the 3 time-point sample for all groups. There was no statistically significant effect of treatment, no differences by sex, and no interaction ($F(3, 51) = 1.00$, $p = 0.4$). There were statistically significant differences between elevations of the lines formed by linear regression analyses, measured by the y-intercept ($F(3, 54) = 3.5$, $p = 0.02$). This finding suggests that, while overall elimination of ethanol does not differ between animals, there may be a difference in blood ethanol levels reached. Linear regression analyses of female and male groups separately revealed no significant differences in elevation within each sex ($F(1,27) = 1.64$, $p = 0.21$ and $F(1,26) = 0.0003$, $p = 0.99$) indicating that differences in overall BEC occurred only initially between female and male groups, regardless of treatment. Importantly, our companion paper also shows no effect of tigecycline on the pharmacokinetic elimination of ethanol in male and female C57BL/6J mice (Syapin, companion paper 2016).

DISCUSSION

Neuroimmune pathways have been implicated in alcohol use disorders in studies examining rodent models and humans [For a review, see: (Robinson et al., 2014)]. Our own studies have shown that some, but not all, tetracycline derivatives with potential neuroimmune modulatory action reduced alcohol consumption (Agrawal et al., 2011, Agrawal et al., 2014, Syapin, companion paper 2016). Minocycline and other tetracyclines have additionally been shown to reduce several types of seizure activity (Wang et al., 2012). In fact, minocycline was effective in a patient with astrocytoma-related seizures (Nowak et al., 2012). In maximal electric shock and subcutaneous Metrazol (pentylenetetrazol; PTZ) models of epilepsy, doxycycline, minocycline and tetracycline were effective in reducing seizure activity (Wang et al., 2012). PTZ chemical kindled seizures changed gene expression of the $\gamma 2$ subunit of the γ -aminobutyric acid A ($GABA_A$) receptor and N-methyl-D-aspartate receptor (NMDA) 2A (NR2A) of the NMDA receptor, as well as tumor necrosis factor α (TNF α); all were normalized with minocycline treatment, which significantly reduced PTZ seizures (Ahmadi et al., 2014). Lipopolysaccharide- (LPS) and viral-mediated seizures were also reduced by minocycline; with viral seizures reduced primarily by blocking the generation of seizures (Gullo et al., 2014, Libbey et al., 2011). Evidence that brain inflammation mediated by glial cells leads to seizures rather than seizures mediating inflammation is accumulating and was tested in a model of pilocarpine-induced status epilepticus for which minocycline was an effective treatment (Wang et al., 2015). Whether alcohol withdrawal leads to neuroinflammation is unknown.

Based on alcohol's known neuroimmune action and the literature indicating potential for tetracycline class anti-seizure activity, we tested the hypothesis that tigecycline would reduce acute AWD convulsions. Our results confirmed our hypothesis with a strong, tigecycline-mediated, dose-dependent reduction in AWD-induced HIC over 12 hours, measured by AUC. Detailed analysis of the HIC scores across 12 hours indicated that the 80 mg/kg tigecycline dose produced a strong delay in onset, reduction in peak magnitude and decrease in overall severity of acute AWD. There was no effect of tigecycline on blood alcohol elimination, which was expected as we had not previously detected a change in the pharmacokinetic elimination of minocycline or tigecycline in C57BL/6J mice (Agrawal et al., 2014, Syapin, companion paper 2016). Interestingly, while we did see sex differences in the anti-drinking and anti-nociceptive actions of tigecycline in our companion studies (Bergeson et al., companion paper, 2016, Syapin, companion paper 2016), we saw no sex differences or interactions in its ability to reduce alcohol-withdrawal related seizure activity. Given that sex differences are known in general seizures (van Luijckelaar et al., 2014), coupled with the long-known important role of sex as a factor in alcohol actions, including for withdrawal effects (Wiren et al., 2006), this was surprising.

Doxycycline, minocycline, tetracycline and now tigecycline have been shown to reduce seizures (Libbey et al., 2011, Nowak et al., 2012, Wang et al., 2012, Ahmadi et al., 2014, Gullo et al., 2014, Wang et al., 2015). The active tetracycline action to reduce convulsive properties is likely similar to the known actions of minocycline, which has been the best-characterized tetracycline. These properties include the ability to block microglial activation and consequent pro-inflammatory cytokine release as well as inhibition of matrix metalloproteinases and actions on GABA_A and NMDA receptors (Lee et al., 2006, Ahmadi et al., 2014, Wang et al., 2015, Yan et al., 2015). Tigecycline, unlike other tetracyclines, has calcium ionophore activity, which could also potentially mediate a suppression of convulsions (Cockeran et al., 2012).

Identification of new treatments of alcohol withdrawal tremors and seizures is an important endeavor. The current standard of care has several limitations. Long-acting benzodiazepines are effective against AWD seizures, but non-compliance with alcohol consumption (benzodiazepines × alcohol) can be dangerous, even deadly (Cooper and Vernon, 2013). As a consequence, benzodiazepine treatment is limited to in-patient service. Tigecycline is a reasonable alternative given its likely safety with alcohol consumption. One drawback is that the FDA approved formulation is via intravenous administration (Meagher et al., 2005). As a consequence, it would be important to study the half-life of tigecycline's anti-convulsive effect in an oral formulation. Minocycline, which can be given orally, could also be a potential new treatment and those studies are ongoing. Having said that, in general, patient populations presenting to clinics and emergency departments are often not stable enough for current outpatient treatment, which requires: mild symptoms, no underlying organ dysfunction or psychosis, no history of previous DTs or previous seizures, presence of a caregiver for 24 hr monitoring for at least 3–5 days at home, daily medical evaluation, absence of pregnancy, and ability to tolerate oral medications and hydration (among other criteria).

In conclusion, our results indicate that tigecycline has potential as a novel pharmacotherapeutic treatment of AUD, including ethanol withdrawal-induced convulsions. Importantly, tigecycline suppresses consumption and inflammatory pain while reducing withdrawal and has not shown abuse potential, which is a complication of benzodiazepines, the current standard of care. Use of tigecycline might allow increased out-patient treatment as it also does not have the dangerous sedation and respiratory depressing effects of benzodiazepines. These three positive aspects of tigecycline (no abuse potential, multifaceted AUD treatment, reduced respiratory side-effects) are not met in any one current treatment. Future studies in chronically dependent animals are needed to confirm the importance of our results. In addition, determination of potential harmful interactions with ethanol (e.g. sedation) needs to be comprehensively evaluated. Comparative studies with benzodiazepines for efficacy and bioavailability is also warranted. Since several tetracycline analogs, including tigecycline, are approved by the FDA, moving to human studies will have limited barriers.

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Figure 1. Tigecycline reduced acute alcohol (4 g/kg) withdrawal-mediated Handling Induced Convulsions (HIC)

Overall withdrawal severity from a single dose of 4 g/kg i.p. 20% ethanol in saline did not differ by sex ($F(3,88) = 1.21, p = 0.31$). However, A) female and B) male mice are shown and were each analyzed separately by 2-way ANOVA for treatment $\times$ time. The interaction of treatment $\times$ time was significant for both experiments completed in female ($F(36, 325) = 2.21, p = 0.0002$) and male ($F(36, 325) = 1.78, p = 0.0005$) DBA/2J mice. Both time and treatment reached significance of $p < 0.0001$ in both experiments. Regardless of the time administered, 80 mg/kg i.p. tigecycline reduced ethanol withdrawal symptoms. When treatment began concurrent with intoxication, the onset of withdrawal symptoms was delayed and the magnitude and intensity were significantly reduced. When treatment was delayed by 4 or 7 hrs, the treatment was effective, but with reduced positive outcomes. Significance is shown below the graph as * $p < 0.05$, with exact details given in the results section. Saline and 80 mg/kg i.p. tigecycline treated animals are showed by solid black versus gray dashed and dotted lines respectively. Data points are presented as mean $\pm$ SEM, $n = 6-10$ /group.

Figure 2. Tigecycline showed a dose-response reduction of HIC

The average area under the curve (AUC) with subtracted baseline for the 12 hour period was calculated for females and males and is shown in (A). When data is collapsed for sex (B), each dose is significant (20, $p < 0.004$; 40, $p < 0.0002$; 80, $p < 0.000000009$). Data points are presented as mean AUC $\pm$ SEM, $n = 10$ for saline groups and $n = 5-7$ for treatment groups.

DBA/2J Pharmacokinetic Elimination

Figure 3.

Tigecycline treatment did not change the pharmacokinetic elimination of ethanol in either female or male mice. Data points are presented as mean $\pm$ SEM, $n = 4-5$. Lines represent the linear regression slope, with triangles and boxes representing females and males respectively, with dashed and dotted lines representing control and treatment respectively.