Assessment of GABA<sub>A</sub> Benzodiazepine Receptor (GBzR) Sensitivity in Patients with Alcohol Dependence

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Abstract — Aim: The aim of this study was to measure GABA<sub>A</sub> benzodiazepine receptor (GBzR) sensitivity in alcohol-dependent patients and compare with matched non-dependent drinkers. Methods: Nine abstinent alcohol-dependent male patients, age matched with nine male non-dependent social drinkers, received an intravenous infusion of midazolam. Objective (saccadic eye movement slowing) and subjective (visual analogue scales) measurements were recorded at 15-min intervals for 2 h. Results: There were no differences in objective or subjective measures. Conclusions: Our hypothesis that patients with alcohol dependence would have less slowing of their eye movements in response to this challenge, reflecting reduced GBzR sensitivity, was not confirmed. The reasons for this could mean that GBzR function returns to normal with abstinence, or that this paradigm is unable to measure the subtle subtype-specific changes in GBzR sensitivity that occur following dependent alcohol use.

INTRODUCTION

There is much evidence suggesting that alcohol tolerance develops, at least in part, via its effects on the γ-aminobutyric acid type A benzodiazepine receptor (GBzR) (Follesa et al., 2006). The GBzR is a pentameric macromolecular complex that spans the neuronal membrane with binding sites for many ligands, including benzodiazepines, alcohol, barbiturates and neurosteroids as well as the principle inhibitory amino acid GABA (Barnard et al., 1998; Nutt and Malizia, 2001).

Animal models show that chronic alcohol exposure results in decreased sensitivity to alcohol and other benzodiazepine agonists, i.e. tolerance (Buck and Harris, 1990). In humans, neuroimaging studies using [11C]flumazenil positron emission tomography (PET) or [123I]iomazenil and single photon emission computed tomography (SPECT) to label the GBzR in abstinent alcohol-dependent patients generally demonstrate reduced GBzR binding, particularly in the frontal cortex (Gilman et al., 1996; Abi-Dargham et al., 1998; Lingford-Hughes et al., 1998). Genetic factors are likely to be important in partially explaining why some people become alcohol dependent and recent studies in different populations have suggested a role for the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor alpha 2 subunit gene in mediating both acute alcohol sensitivity and dependence (Covault et al., 2004; Lappalainen et al., 2005; Haughey et al., 2008).

One of the major problems in exploring tolerance has been the lack of reliable objective measures. Methods used include challenge tests, such as growth hormone response to intravenous (IV) diazepam (Shur et al., 1983; Roy-Byrne et al., 1991; Cowley et al., 1995), psychometric tests (Malpas et al., 1974; Bond and Lader, 1983) and electroencephalography (Herman and Schaerer, 1986). However, the reliability of these methods is not high with variability of responses across gender, time and between individuals (Potokar et al., 1999).

We have used a paradigm for assessing GBzR sensitivity by the technique of saccadic eye movement (SEM) analysis. SEMs are jerky eye movements made towards a visual target, which are initiated by brain stem nuclei under GABA-ergic control. Once they are initiated they are outside of voluntary control. They are one of the most sensitive physiological measures of benzodiazepine effects (Van Steveninck et al., 1991) and importantly can be used as a measure of responsiveness that is independent of motivational factors (Ball et al., 1991).

The purpose of this study was to assess GBzR sensitivity in abstinent patients with a history of alcohol dependence compared with matched non-dependent drinkers by measuring slowing of saccadic eye movement peak velocity (SEMV) in response to an IV infusion of the benzodiazepine agonist midazolam. The latter was chosen because it is available in IV formulation, has a short terminal half-life, allows effects to be determined over a range of concentrations in a short experiment and we have experience of its use in previous studies (Potokar et al., 1999; Lingford-Hughes et al., 2005). Our hypotheses were that patients with a history of alcohol dependence would have less slowing of their eye movements in response to this challenge and would experience less subjective sedation, reflecting reduced GBzR sensitivity.

METHODS

Nine male patients who fulfilled Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 1994) for alcohol dependence were recruited from local alcohol treatment services. They all had been abstinent for at least 2 months (see Table 2). They were matched for age with nine non-dependent drinkers who were recruited through colleagues and advertisements. All subjects gave written informed consent to the study, which was approved by the local ethics committee (approval number: E3823).

At a screening visit, a medical and psychiatric history was taken by an experienced psychiatrist and diagnosis made according to DSM-IV. All subjects underwent a physical examination, electrocardiogram (ECG), routine bloods (urea and electrolytes, full blood count and liver function tests) and a urine sample was obtained for a toxicology screen. Exclusion criteria for patients and non-dependent drinkers were...
significant physical illness, ECG or blood abnormalities including elevated serum transaminase levels, and cocaine or opioid use in the past 6 months. Non-dependent drinkers were excluded for excessive alcohol intake (>28 units/week). None of the non-dependent drinkers had a current or past history of Axis 1 disorder. In the patients, additional information was obtained by recording their history of alcohol consumption. Subjects were told of the nature of the study, and that an attempt was being made to understand the effect of alcohol dependence on brain receptors. They were told that midazolam might make them feel sleepy, heavy eyed and might reduce coordination. Subjects were also told that they should not drive until the following day, and that they could withdraw from the study at any time. They were asked to fill in a diary of their alcohol consumption for the week prior to the study. They were asked to follow a normal diet and had their usual breakfast on the day of the study. They were not allowed to consume caffeine-containing drinks during the test period.

Procedure
Subjects attended our testing room at the Bristol Royal Infirmary at 09:00 h. They were rested in a semi-supine position on a comfortable couch and IV cannulae were inserted (one in each arm) for the midazolam administration and blood sampling.

At the baseline, anxiety using the Spielberger trait and state anxiety inventories (STAI/SSAI; Spielberger 1983) was recorded. Subjective sedation using a visual analogue scale (VAS) was also assessed. The VAS was a 100 mm scale in intervals of 10 mm, where 0 represented ‘not at all’ and 100 represented ‘the worst ever’. SEMs and VAS were recorded at intervals of 10 mm, where 0 represented ‘not at all’ and 100 represented ‘the worst ever’. SEMs and VAS were recorded at 15-min intervals for 120 min and blood was drawn at these times for the midazolam assay. Systolic and diastolic blood pressure as well as heart rate was recorded using Dinamapp automated equipment.

Eye movements in response to a light moving across a screen were recorded, analysed and stored using the Cardiff Saccade Generator And Analysis System (CSGAAS)—see below. Baseline SEM ratings were recorded at $t = -30$ min and $t = -10$ min. At $t = 0$, the subject received midazolam 50 µg/kg made up to 10 ml with normal saline that was infused via a syringe driver over 10 min.

Measure of eye movements
A silver/silver chloride disposable electrode (Medicotest, Denmark), together with a small amount of electrode gel was placed 1 cm laterally to the outer canthus of each eye and on the glabella, after scarification with abrasive cream (Skinpure, Niloh Kohnh). Electrode impedances were measured and confirmed to be <5 kΩ. The electrodes were connected to a DC amplifier with a gain of ×1000; output from the amplifier was then sampled 256 times per second via an analogue to digital converter. The resulting digital information was then analysed by an IBM-compatible PC. Since vertical eye movement significantly alters EOG amplitude in a non-linear way (Barry and Mellville-Jones, 1965), only lateral saccades were studied. Forty-eight saccade trials were recorded at each time point, at target displacements of 10°−40°. Peak velocity for each saccade was plotted against the angle of displacement, to produce a main sequence curve. The saccade peak velocity value for each time point was produced by interpolation into the main sequence curve of 35°. For a fuller description of the methodology used, see Wilson et al. (1993).

Midazolam assay
Blood samples were placed in lithium heparin tubes and centrifuged within 30 min. Plasma was then stored at $-20^\circ$C until analysed. Midazolam was measured in plasma by a gas liquid chromatographic (GLC) method with nitrogen/phosphorus endpoint detection utilizing a three-stage extraction process, together with an internal standard to monitor recovery.

Standards (0−200 ng/ml) were extracted from the drug-free plasma obtained from non-dependent drinkers in the same manner. The inter- and intra-assay coefficients of variant were both within 10% and the assay limit of detection (defined as three times baseline noise) was 0.5 ng on column. The analytical (actual) recovery was 70%. A number of psychoactive compounds were tested for interference in the assay; none of these were found to cause problems.

Data analysis
Unpaired Student t-tests were used to determine differences in the means of variables, including baseline variables, demographic details and midazolam levels. Repeated measures mixed ANOVA was used to examine group effects, time effect and group × time interaction. Heterogeneity of covariance was tested with the Mauchly sphericity test and degrees of freedom modified using the Greenhouse–Geisser adjustment, where appropriate. Area under the curve (AUC) was used to estimate both total pharmacodynamic effect (reduction in SEMV) and total pharmacokinetic effect (concentration of midazolam) from $t = 0$ min to $t = 120$ min. The non-parametric Spearman R was used to determine whether there was a correlation of response to midazolam with duration of abstinence.

RESULTS

 Demographic and baseline clinical variables (Tables 1 and 2)
The alcohol-dependent and non-dependent groups were well matched with regard to age. The average number of years of heavy drinking in the alcohol-dependent group was $14.75 \pm 8.29$ years. There was a trend towards the patients having higher scores on trait anxiety as measured by the STAI but this was not statistically significant. The non-dependent drinkers showed a significant higher baseline anxiety as measured by VAS on the

<table>
<thead>
<tr>
<th>Table 1. Demographic data and baseline variables</th>
<th>Alcohol-dependent group</th>
<th>Non-dependent drinkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.1 (8.9) SD</td>
<td>40 (6.4) SD</td>
</tr>
<tr>
<td>Sex</td>
<td>9 males</td>
<td>9 males</td>
</tr>
<tr>
<td>STAI</td>
<td>47.8 (8.7)</td>
<td>39.0 (11.4)</td>
</tr>
<tr>
<td>SSAI</td>
<td>32.1 (7.6)</td>
<td>36.9 (9.8)</td>
</tr>
<tr>
<td>VAS anxiety</td>
<td>10 (9.0)</td>
<td>20.1 (9.4)</td>
</tr>
<tr>
<td>Units of alcohol per week</td>
<td>250 (104)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Family history</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Family history was ‘positive’ if there was a first-degree relative with a history of alcohol dependence. STAI = Spielberger trait anxiety inventory; SSAI = Spielberger state anxiety inventory. One unit of alcohol is equivalent to 8 g of alcohol.
Table 2. Clinical details

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Years of heavy drinking</th>
<th>Length of abstinence (weeks)</th>
<th>Typical units of alcohol per week</th>
<th>Family history of alcoholism</th>
<th>Previous psychiatric history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>12</td>
<td>No</td>
<td>Depression secondary to alcohol dependence</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>103</td>
<td>100+</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>9</td>
<td>182</td>
<td>Yes (sister)</td>
<td>Alcohol-related depression. Diazepam dependency in 1976</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>38</td>
<td>420</td>
<td>No</td>
<td>Social anxiety</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>58</td>
<td>224</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>26</td>
<td>250</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>15</td>
<td>112</td>
<td>Yes (father)</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>24</td>
<td>350</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>29</td>
<td>210</td>
<td>Yes (father)</td>
<td>Depression</td>
</tr>
<tr>
<td>Mean</td>
<td>14</td>
<td>35</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>8</td>
<td>30</td>
<td>104</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

One unit of alcohol is equivalent to 8 g of alcohol. Heavy drinking was defined as >50 units per week.

... day of testing compared with the alcohol-dependent patients. There was also a trend towards higher baseline state anxiety as measured by the SSAI, though this did not reach statistical significance. One possible explanation is the habituation of hospital-type appointment stress in the patients, whereas the non-dependent drinkers were not used to a medical environment and intervention.

**Plasma midazolam**

No difference was seen in the plasma concentration of midazolam between the two groups. Repeated measures-mixed ANOVA showed a time effect \( F = 55.3, df = 1.681, P = 0.000 \) but no group effect \( F = 0.327, df = 1, P = 0.576 \) or group time interaction.

**Effects of midazolam on SEM parameters**

There was no significant difference in baseline onset latencies (non-dependent drinkers 193.0, SD 45.5; alcohol-dependent patients 181.2 ms, SD 31.8, NS) or baseline SEMV (non-dependent drinkers 517.8, SD 93.6; alcohol-dependent patients 536.2, SD 59.3, NS).

Midazolam produced a reduction in velocity that peaked in both groups at \( t = 15 \) min. Many of the subjects were too drowsy at \( t = 15 \) min to complete the task (missing results for four non-dependent drinkers and three alcohol-dependent drinkers). At \( t = 105 \) min, velocities in both groups were similar to baseline and to each other (Fig. 1).

Repeated-measures ANOVA demonstrated a time effect \( F = 5.949, df = 2.5; P = 0.003 \) but no group effect \( F = 0.42, df = 1; P = 0.526 \). For onset latencies, there was neither a time effect \( F = 2.355, df = 2.8; P = 0.088 \) nor a group effect \( F = 0.61, df = 1; P = 0.446 \).

In order to get a measure of both pharmacodynamic and pharmacokinetic effect, we adapted the method used by Roy-Byrne and colleagues to assess GBR2 sensitivity in patients with panic disorder and OCD (Roy-Byrne et al., 1996). AUC was calculated for both SEMVs and plasma midazolam concentration from \( t = 0 \) min to \( t = 120 \) min. To obtain a measure of SEMV effect, we subtracted the values of AUC from that obtained by extrapolating mean baseline SEMVs to \( t = 120 \) min, for each individual. Ratios of AUC to SEMV and plasma midazolam concentration were then compared to give a measure of receptor sensitivity (Fig. 2). There was no difference in the ratios of pharmacodynamic/pharmacokinetic effect between the two groups \( t = 0.5520, df = 16, P = 0.5886 \).

In the alcohol-dependent patients, the SEMV response to midazolam as measured by the ratio of the pharmacodynamic/pharmacokinetic effects correlated with the duration of abstinence, i.e. those with a shorter duration of abstinence were
GBzR Sensitivity in Alcohol Dependence

Fig. 3. Mean plasma midazolam levels.

less sensitive to midazolam effects [Spearman’s rho = 0.717 significant at the 0.05 level (1-tailed)].

One of the patients’ (subject number 6) results are very difficult to explain. His SEMV increased after midazolam despite subjectively rating himself as drowsy. He had been abstinent for 26 weeks and was actively engaged in a rehabilitation programme with no evidence of having relapsed to drinking alcohol. He was taking disulfiram twice weekly. A literature search found no evidence to suggest that disulfiram interferes with SEMs. His midazolam level was not significantly different from the other patients. There was no change to the results when the data were analysed excluding his results.

Sedation
After the midazolam infusion, the majority of the subjects were unable to respond to the request for a rating about how sedated or anxious they were feeling. For those who did respond, their responses did not necessarily reflect the actual experience, since the ratings were often inaccurate. For example, the subjects having been woken by the investigator asking for a response would rate themselves very low for sleepiness.

There was, however, an increase in sedation in both groups following midazolam, although there was a wide variation in response, which emphasises that the ratings may not be reliable. There was a time effect ($F = 13.446$, df = 3.3; $P = 0.0001$) but no group effect ($F = 0.015$, df = 1; $P = 0.904$) (Fig. 3).

DISCUSSION

Our hypotheses that alcohol-dependent patients who were abstinent would have reduced GBzR sensitivity as measured by slowing of SEMV in response to midazolam and less subjective sedation were not confirmed. We found little difference in these parameters between the non-dependent drinkers and the alcohol-dependent patients.

There are several possible explanations for these findings. Firstly, our results could indicate that GBzR function is normal in alcohol dependence. This is not supported by animal data (Buck and Harris 1990) or in vivo neuroimaging studies (Gilman et al., 1996; Abi-Dargham et al., 1998; Lingford-Hughes et al., 1998).

An alternative explanation is that GBzR function normalizes with abstinence. A SPECT study using $^{123}$Iiomazenil to label the GBzR in subjects with alcohol dependence over the first month of sobriety showed elevated $^{123}$Iiomazenil uptake in several cortical regions at 1 week, but no significant differences were observed at 4 weeks of abstinence (Staley et al., 2005). This may reflect that configuration of GBzR subunits changes with alcohol exposure. In the basal state, synaptic receptors consisting of $\alpha_1$–3, $\beta_2$–3 and $\gamma_2$ subunits dominate, while in alcohol dependence a less functional array of subunits exist (Krystal et al., 2006). Reverting to the basal configuration may, however, not equate to normalization of function.

Lingford-Hughes et al. (2005) demonstrated subtle altered functioning of the GBzR in abstinent alcohol-dependent patients following a similar midazolam challenge performed in the PET scanner with concurrent $^{11}$C-flumazenil imaging to exclude any possible differences in the brain pK of midazolam as an explanation for differences. Midazolam similarly slowed SEMs and increased beta-electroencephalography (EEG) activity in both patients and controls. However, total EEG sleep time was significantly lower in the alcohol-dependent group, consistent with reduced sensitivity of this measure of GBzR function in alcohol dependence. One explanation for this is that only certain subtypes of the GBzR change in alcohol dependence. Those that mediate the benzodiazepine-induced slowing of SEMs in the brain stem may not, whereas those responsible for sleep induction (probably the $\alpha_1$—see Rudolph et al., 1999) may change in humans as a consequence of alcohol exposure as they do in rats (Papadeas et al., 2001). It is not yet known which subtype might mediate SEM slowing induced by benzodiazepines.

A further explanation for our results was that our sample size was relatively small and the patients had a wide range of time abstinent. Difficulty in recruiting meant that it took a number
of years to complete the study, which is not uncommon in studies of this nature. Our findings that the length of abstinence significantly correlates with GBzR sensitivity suggest that an earlier phase of abstinence should have been examined. However, our findings are in keeping with a study by Bauer et al. (1997) where the length of abstinence was 2–3 weeks.

In conclusion, our study suggests that GBzR function as tested by the midazolam paradigm is not subsensitive after 2 months of abstinence in alcohol-dependent patients. Further work to assess GBzR functioning when patients acutely relapse as well as the time course of GBzR function in the early detoxification period is needed. Future studies exploring the changes in expression and function of GBzR subtypes will hopefully clarify further the role of the GBzR in tolerance and help in the development of new treatments to improve the treatment of alcohol withdrawal and reduce relapse in alcohol-dependent patients.

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REFERENCES


