Functional neurotoxicity evaluation of noribogaine using video-EEG in cynomolgus monkeys

Simon Authier a,b,⁎, Michael V. Accardi a, Dominique Paquette c, Mylène Pouliot a, Joseph Arezzo d,e, R. John Stubbs f, Ronald J. Gerson g, Lawrence T. Friedhoff h, Holger Weiß i

a CitoxLAB North America, 445 Armand Frappier, Laval, QC H7V 4B3, Canada
b Faculty of Veterinary Medicine, University of Montreal, P.O. box 5000, St-Hyacinthe, QC J2S 7C6, Canada
c Centre Vétérinaire DMV, 2300 54e Av, Lachine, QC H8T 3R2, Canada
d Department of Neuroscience, Albert Einstein College of Medicine, USA
e Department of Neurology, Albert Einstein College of Medicine, USA
f Stubbs & Henzel Pharma Consulting, LLC, PO Box 935, Blue Bell, PA 19422, USA
g Gerson Pharma Solutions, LLC, USA
h Pharmaceutical Special Projects Group, LLC, USA
i DemeRx, Inc., Fort Lauderdale, Florida, USA

⁎ Corresponding author at: CIToxLAB North America, 445 Armand Frappier, Laval, QC H7V 4B3, Canada
E-mail address: authiers@ca.citoxlab.com (S. Authier).

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A B S T R A C T

Introduction: Continuous video-electroencephalographic (EEG) monitoring remains the gold standard for seizure liability assessments in preclinical drug safety assessments. EEG monitored by telemetry was used to assess the behavioral and EEG effects of noribogaine hydrochloride (noribogaine) in cynomolgus monkeys. Noribogaine is an iboga alkaloid being studied for the treatment of opioid dependence.

Methods: Six cynomolgus monkeys (3 per gender) were instrumented with EEG telemetry transmitters. Noribogaine was administered to each monkey at both doses (i.e., 160 and 320 mg/kg, PO) with an interval between dosing of at least 6 days, and the resulting behavioral and EEG effects were evaluated. IV pentylenetetrazol (PTZ), served as a positive control for induced seizures.

Results: The administration of noribogaine at either of the doses evaluated was not associated with EEG evidence of seizure or with EEG signals known to be premonitory signs of increased seizure risk (e.g., sharp waves, unusual synchrony, shifts to high-frequency patterns). Noribogaine was associated with a mild reduction in activity levels, increased scratching, licking and chewing, and some degree of poor coordination and related clinical signs. A single monkey exhibited brief myoclonic movements that increased in frequency at the high dose, but which did not appear to generalize, cluster or to be linked with EEG abnormalities. Noribogaine was also associated with emesis and partial anorexia. In contrast, PTZ was associated with substantial pre-ictal EEG patterns including large amplitude, repetitive sharp waves leading to generalized seizures and to typical post-ictal EEG frequency attenuation.

Interpretation: EEG patterns were within normal limits following administration of noribogaine at doses up to 320 mg/kg with concurrent clinical signs that correlated with plasma exposures and resolved by the end of the monitoring period. PTZ was invariably associated with EEG paroxysmal activity leading to ictal EEG. In the current study, a noribogaine dose of 320 mg/kg was considered to be the EEG no observed adverse effect level (NOAEL) in conscious freely moving cynomolgus monkeys.

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1. Introduction

Noribogaine is the primary human metabolite of ibogaine, an alkaloid (Fig. 1) extracted from the root bark of the West African shrub Tabernanthe iboga (Maciulaitis, Kontrimaviciute, Bressolle, & Briedis, 2008). The benefits of ibogaine in the treatment of addiction for multiple drugs of abuse (e.g., cocaine, morphine, heroin, alcohol, and nicotine) were documented as early as the 1960’s but has since seen further confirmation in rodent and human experimentation (Goutarel, Golnhoffer, & Siddens, 1993; Alper, Lotsof, Frenken, Luciano, & Bastiaans, 1999; Baumann, Pablo, Ali, Rothman & Mash, 2001; Maciulaitis et al., 2008). However, despite the apparent anti-addictive properties of ibogaine, its use in addiction therapy is limited due to its associated severe side effects, which include hallucination, bradycardia, whole-body tremors and ataxia (Alper, Lotsof, & Kaplan, 2008; Glick, Maisonneuve, & Szumilinski, 2000; Maas & Strubelt, 2006; Maciulaitis et al., 2008).
et al., 2008). Upon administration, ibogaine can be metabolized by cytochrome P4502D6 (CYP2D6) into noribogaine (Obach, Pablo, & Mash, 1998) a metabolite that, unlike ibogaine, does not produce tremors and/or ataxia (Baumann, Rothman, Pablo & Mash, 2001). Investigations using rats have shown that noribogaine mediates ibogaine-like anti-addictive effects inducing long-lasting decreases in morphine, nicotine, cocaine and alcohol self-administration (Carnicella, He, Yowell, Glick, & Ron, 2010; Chang, Hanania, Mash, & Maillet, 2015; Glick, Kuehne, Maisonneuve, Bandarage, & Molinari, 1996) as well as possessing high drug permeability across the blood–brain barrier (Pearl, Hough, Boyd, & Glick, 1997). A greater understanding of the effects of noribogaine activity on central nervous system (CNS) function in higher order mammalian species, which possesses greater comparative anatomical and pharmacological value to humans, is considered useful during drug development.

CNS safety pharmacology studies in large animals (e.g., dogs, nonhuman primates) often use electroencephalographic (EEG) monitoring systems. Technological advances have paired EEG recordings with telemetry (Authier et al., 2014; Kramer & Kinter, 2003) and/or integrated video systems (Authier et al., 2009) during drug safety testing. To this end, we assessed the neuro-behavioral effects of noribogaine in cynomolgus monkeys using video-EEG monitoring.

2. Methods

2.1. Statement on use and care of animals

During this investigation, care and use of animals were conducted in accordance with principles outlined in the current Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources. CitToxLAB’s facility is AAALAC accredited and the procedures were reviewed and approved by the Institutional Animal Care and Use Committee prior to conduct. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animals and environment

All animals were housed under standard laboratory conditions with controlled temperature (21 ± 3 °C), humidity (30%–70%), 12 h light/dark cycle and 10–15 air changes per hour. Temperature and relative humidity were monitored continuously. The animals were provided a standard certified commercial primate chow (Certified Hi-Fiber Primate Diet 7195CTM, Harlan Teklad, Madison, WI, USA) twice daily and municipal tap water (which has been exposed to ultraviolet light and purified by reverse osmosis) via an automatic watering system or water bottles ad libitum. On dosing days, feeding was done 1–3 h prior to and 4–6 h after noribogaine administration. Six (6; 3 males/3 females) cynomolgus monkeys (Macaca fascicularis), aged approximately 2 years and weighing approximately 2.6–2.9 kg at the beginning of the study were used.

2.3. Surgical instrumentation

Cynomolgus monkeys were previously surgically cannulated with a catheter inserted into the femoral vein and were surgically prepared with telemetry transmitters (TL11M2-D70-EEE, DSI, St-Paul, MN, USA) as described previously (Authier et al., 2009). A prophylactic antibiotic (Cefazolin, Novopharm, Toronto, ON, Canada, 30 mg/kg) was administered by intravenous (IV) injection at least 15–20 min prior to surgery and every 2 h post-injection for a maximum of 3 doses. Pre-emptive analgesia was attained via a transdermal Fentanyl patch (Sandov, Boucherville, QC, Canada, 12.5 μg/h) placed on the animal the day prior to surgery and removed 2 days post-surgery. A non-steroidal anti-inflammatory (Meloxicam, Boehringer Ingelheim, Burlington, ON, Canada, 0.1 mg/kg, SID, SC) was administered for 3 days after surgery. A local anesthetic (Marcaine, Hospira, Montreal, QC, Canada, 0.4 ml; Lidocaine, Vetoquinol, Lavaltrie, QC, Canada; 20 mg/ml, 0.4 ml) was injected subcutaneously in 6–10 sites distributed over the surgical sites during the surgery. Animals were placed on a heating pad and inhaled a mixture of oxygen (O2) and isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, Canada) with the O2 flow meter and the vaporizer set at 1.0 L/min and 2.0%, respectively. Respiratory rate was maintained between 12 and 15 breaths/min with an inspiratory airflow pressure between 18 and 20 cm H2O using a mechanical ventilator (Hallowell EMC, Pittsfield, MA, USA). During anesthesia, body temperature and end-tidal CO2 were monitored continuously. A longitudinal incision was performed lateral, but close, to the linea alba, and the internal abdominal oblique muscle was separated from the aponeurosis of the transverse abdominus. The telemetry transmitter was placed between the internal abdominal oblique muscle and the aponeurosis of the transversus abdominis muscle. The rectus abdominis was sutured with a simple continuous suture and electroencephalographic (EEG)
electrodes were tunneled subcutaneously to a small skin incision in the neck. The abdominal skin incision was closed with interrupted buried sutures and the animal was placed in sternal recumbency to expose the cranium for the remainder of the surgery. EEG leads were placed in the 10–20 system to monitor standard bipolar derivations (C3-O1, C4-O2 and Cz-Oz). A linear groove was made in the cranial cortical bone to secure the electrodes with surgical glue (Vetbond™, 3M, St-Paul, MN, USA) and acrylic. Electromyogram (EMG) recordings were obtained using electrodes sutured to longitudinal muscles in the neck area and recorded continuously with the telemetry transmitter. A period of 4 weeks was allowed between the surgery and the start of experimental procedures. Both the EEG and EMG electrodes were well tolerated in all animals.

2.4. EEG analysis methods and behavioral monitoring

EEG data were obtained from animals using telemetry transmitter leads using bipolar derivations (C3-O1, C4-O2 and Cz-Oz). The EEG was recorded continuously from at least 24 h prior to dosing to at least 24 h post-dosing completion (Dataquest ART, Data Science International, St-Paul, MN, USA). The EEGs were subjected to computer analysis from at least 30 min pre-dosing to at least 24 h post-dosing ( NeuroScore, Data Science International, St-Paul, MN, USA). Spectral analysis was performed on 60-s epochs to quantify the absolute and relative amplitude of EEG frequency bands (delta [0.5–4 Hz], theta [4–8 Hz], alpha [8–12 Hz], sigma [12–16 Hz], beta [16–24 Hz] and gamma [24–50 Hz]) and individual frequencies [0.5–127 Hz]. Digital color cameras (Geovision, Irvine, CA, USA), with daylight and infrared night vision connected to a computerized system (IBM Intellistation Z pro, Cary, 3.8 Ghz, 3.5 TB hard drive, New Orchard Road Armonk, NY, USA), were used for behavioral video monitoring. Animals were continuously monitored (except when animals were outside of their home cage for blood collection and/or during data backup). A standard lexicon was used for description of behavioral observations.

2.5. Statistical analysis

For each parameter, a one-way analysis-of-variance (ANOVA) was conducted and the residuals were saved. Gaussian distributions were evaluated using the Shapiro–Wilk test on residuals. Whenever the Shapiro–Wilk test was found to be significant (p ≤ 0.001) then the data were transformed and re-submitted to the analysis. The Levene test was used to examine the homogeneity of group variances. For the ANOVA model, if the overall group differences were shown significant (F-Test), then pair-wise comparisons were conducted using Tukey’s test. Data are presented as mean (SD).

2.6. Blood collection for pharmacokinetic analysis

Blood samples (a target of 1.6 ml each) were collected from all animals at various time points. The pharmacokinetic parameters of noribogaine were assessed following oral administration of 320 mg/kg in male and female monkeys at the following time points: pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 5, 9, 14 and 24 h after dosing. Following single oral administration at a dose level of 160 mg/kg, blood samples were collected at pre-dose and 1 h post-dose. Blood samples were collected using the implanted catheter into tubes containing sodium heparin as an anticoagulant and kept on wet ice pending centrifugation. The animals were not restrained and were not taken out of the cage for blood collection, unless deemed necessary. Samples were centrifuged under refrigeration (set to 4 °C at 1500g RCF) and placed on dry ice pending storage in a freezer set to −70 °C until analysis. Non-compartmental pharmacokinetic analysis was performed on the plasma concentration data using the WinNonlin version 5.2 software (Pharsight, Corporation, Cary, NC, USA), and consisted of the assessment of standard parameters including T_{max}, T_{last}, C_{max}, C_{last}, AUC_{last}, AUC_{0–∞} and t_{1/2}.

2.7. Pharmacological treatments

Noriibogaine hydrochloride (noriibogaine; Dose level: 160 and 320 mg/kg; DemeRx Inc., Fort Lauderdale, FL, USA) and the control (vehicle) were administered by oral gavage administration in a dose escalation design. Animals had a wash-out period of at least seven days between each treatment based on the reported half-life of noribogaine. The dose volume was 5 ml/kg for all animals, including vehicle controls. The last treatment was for positive control, pentylenetetrazol (PTZ, dose level: 38–65 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) administered by IV infusion at a dose rate of 10 ml/kg/h via a surgically implanted catheter.
implanted catheter. Upon observation of PTZ induced seizure, diazepam (1.0 mg/kg, IV; Sandoz, Boucherville, QC, Canada) was administered.

3. Results

3.1. Behavioral video monitoring and clinical observations

Following administration of noribogaine at 160 mg/kg, 3 out of 6 animals presented clinical signs including excessive scratching and licking, chewing motion, emesis, partial anorexia (i.e., decreased appetite), slightly decreased activity level, and brief periods of poor coordination. A single monkey also exhibited mild and infrequent twitches that resembled myoclonic jerks as well as periods of muscle tremor. Similar clinical signs were observed at 320 mg/kg but with higher incidence and/or severity. The EEG signals associated with these events were carefully reviewed by the boarded certified neurologist on our team (D. Paquette) and by an outside pre-clinical EEG expert (J. Arezzo). Both experts agreed that the observed movements were not related to cortically-generated sharp waves and had no EEG correlate. At the high dose, retching, salivation, moderately decreased activity level, and tremors were also observed. IV infusion of PTZ was associated with retching, excessive scratching, repeated yawning, excessive vocalization, salivation, emesis, uncoordination, slightly to severely decreased activity level, lying on cage floor, myoclonic jerks, tremors and/or clonic or tonic convulsions. PTZ induced seizures were self-limited or successfully controlled with a single dose of diazepam. Body weights did not present any variations that could be attributed to the administration of noribogaine at any dose in this study.

3.2. EEG monitoring by telemetry recordings

Animals were evaluated prior to study initiation to confirm normal patterns of EEG. (Figs. 2 and 3). EEG following noribogaine treatment at 160 or 320 mg/kg remained within normal limits (Figs. 4 and 5). As expected, EEG recorded by telemetry in awake moving and sometimes sleeping monkeys is characterized by a rich mixture of frequencies and amplitudes and by occasional movement artifacts. The key finding in the present study is that there was no evidence either by computer scanning or by review by experts of significantly altered EEG associated with noribogaine.

EEG paroxysmal activity was invariably noted after PTZ infusion with an average of 72:30 min post-infusion onset (range from 45:46 to 93:29; Table 1; Fig. 6) corresponding to a mean cumulative dose of 48.33 mg/kg. PTZ infusion led to generalized seizures (Fig. 7) confirmed by behavioral signs (i.e., recumbency, tonic–clonic movements and evidences of loss of consciousness) and ictal EEG activity (i.e., spike trains) in all cynomolgus monkeys. Review of EEG traces revealed the presence of generalized seizure activity at an average of 81:24 min into the PTZ infusion (range from 55:25 to 97:41; Table 1) representing a cumulative dose of 54.27 mg/kg. The doses of PTZ required to induce paroxysmal EEG activity and generalized seizure were comparable to previously reported data in this nonhuman primate model (Authier et al., 2009). Continuous infusion of PTZ was associated with episodes of synchronous neuronal depolarization with increasing amplitudes leading to generalized convulsions in all animals. PTZ induced seizures were successfully controlled with a single IV diazepam injection except for one female animal in which the seizure was self-limiting (Fig. 8). The post-ictal period was characterized by an immediate and transient attenuation of all power bands (delta [0.5–4 Hz], theta [4–8 Hz], alpha [8–12 Hz], sigma [12–16 Hz], beta [16–24 Hz] and gamma [24–50 Hz]). This attenuation may be attributed, at least partially, to IV administration of diazepam but also to typical postictal EEG changes.

3.3. Pharmacokinetic analysis

Plasma concentrations at 1 h following oral administration of noribogaine at 160 and 320 mg/kg were similar in both genders. The plasma concentrations were generally variable between animals as well within the same time points. After the administration of the high dose (320 mg/kg), the maximum plasma concentrations of noribogaine were achieved between 2.5 and 24 h post-dosing in males and between 1 to 9 h in females with mean plasma concentrations ($C_{\text{max}}$) of 1133 ± 820 and of 1003 ± 423 ng/ml in males and females, respectively. Plasma concentrations declined slowly thereafter, where the last concentrations ($C_{\text{last}}$) correspond to the last time point collected (24 h). Mean plasma concentrations were similar amongst the two genders (Fig. 9) with the concentration observed at $C_{\text{last}}$ ($C_{\text{last}}$) noted as 615 ± 921 and 300 ± 111 ng/ml in males and females, respectively. The systemic exposure to noribogaine was similar in both genders as shown by the male to female $C_{\text{max}}$ and $AUC_{\text{last}}$ ratios of 1.13 and 1.15, respectively.

4. Discussion

This study aimed to assess the CNS safety of noribogaine in cynomolgus monkeys by addressing associated behavioral and EEG effects. Accordingly, this investigation showed that noribogaine administration (at doses of up to 320 mg/kg) was associated with a range of clinical signs in cynomolgus monkeys but these clinical signs did not correlate with abnormal EEG findings suggestive of seizure activity. Clinical signs such as tremors, myoclonic jerks or uncoordination/ataxia often raise concerns related to potential seizurogenic effects of the drug candidate. Enhanced physiological tremors (Daneault et al., 2011) or myoclonus (Kojovic et al., 2011) may be considered as pre-ictal signs but these changes are not specific to seizure and may also be observed in normal healthy individuals. As part of the preclinical safety testing program, EEG is used to characterize brain activity post-dosing and during clinical signs suggestive of a seizure risk. As noted in the current study, a variety of clinical signs are not correlated to abnormal EEG

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time to paroxysmal activity (min:s)</th>
<th>Time to seizure (min:s)</th>
<th>Seizure duration (min:s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53:40</td>
<td>60:19</td>
<td>3:32</td>
</tr>
<tr>
<td>2</td>
<td>76:36</td>
<td>83:31</td>
<td>3:10</td>
</tr>
<tr>
<td>3</td>
<td>93:29</td>
<td>97:41</td>
<td>3:04</td>
</tr>
<tr>
<td>4</td>
<td>81:52</td>
<td>95:20</td>
<td>0:51</td>
</tr>
<tr>
<td>5</td>
<td>45:46</td>
<td>55:25</td>
<td>0:38</td>
</tr>
<tr>
<td>6</td>
<td>83:36</td>
<td>96:10</td>
<td>0:45</td>
</tr>
<tr>
<td>Mean</td>
<td>72:30</td>
<td>81:24</td>
<td>2:00</td>
</tr>
</tbody>
</table>
activity. While EEG is useful in drug development, certain limitations need to be considered. Partial seizure may not be identified at EEG if the focal ictal discharge is distant or deep, or involves too small a neuronal aggregate for synchronized depolarization activity to be detected on the EEG (Smith, 2005). Despite these potential limitations, EEG remains a valuable tool in preclinical drug safety assessments with the ability to characterize brain activity during safety pharmacology studies (Bassett et al., 2014; Metea, Litwak, & Arezzo, 2015; Rachalski et al., 2014).

Recent studies in humans have shown that a single oral dose of noribogaine (between 3 to 180 mg) was well tolerated but demonstrated a concentration-dependent increase in the QT interval (Glue et al., 2016; Glue et al., 2015). Compared to ibogaine, noribogaine displays a slow pharmacokinetic clearance rate in humans (Glue et al., 2016; Glue et al., 2015; Maciulaitis et al., 2008; Mash et al., 2001; Mash et al., 2000) which may be beneficial to its clinical use. Pharmacokinetics of noribogaine in cynomolgus monkeys in the current study also revealed a relatively long exposure profile in this species. Noribogaine is a compound with known poly-pharmacology that partially overlaps with ibogaine. Principally, both compounds have affinity for serotonin and dopamine transporters and opioid receptors. However, there are differences between the two compounds. Noribogaine has marginal affinities to NMDA, sigma 2 and 5-HT2 receptors compared to ibogaine (Baumann et al., 2001a; Bowen et al., 1995; Mash, Staley, Baumann, Rothman, & Hearn, 1995a; Mash et al., 1995b; Staley et al., 1996). These differences could explain the differences in pharmacology and toxicology findings that were reported with these two molecules.

While positive control drugs such as PTZ are not mandatory in non-clinical EEG studies, their inclusion can be used to confirm sensitivity and specificity of the model to accurately identify abnormal EEG traces. Interpretation of EEG traces is known to be a complex task and electroencephalographers sometimes disagree about whether a particular pattern is epileptiform and representative of seizures (Fisher, Scharfman, & deCurtis, 2014). The use of a positive control agent in seizure liability testing studies can help to mitigate ambiguities in the EEG interpretation. But the use of a positive control drug, such as PTZ, a noncompetitive antagonist of the γ-aminobutyric acid (GABA)A receptor complex (Hansen, Sperling, & Sánchez, 2004; Huang et al., 2001) also presents potential limitations. Animals exposed to positive control agent cannot be subjected to histopathological evaluations to assess for neuropathological effects of the drug due to potential confounding changes induced by the proconvulsive agent (Jandová, Riljak, Pokorný, & Langmeier, 2007). Furthermore, the use of a positive control may involve considerations related to application of the 3R (i.e. reduction, replacement and refinement). It remains that the value of a non-clinical EEG study to assess drug safety relies on data interpretation and the inclusion of a positive control can be important to characterize EEG morphologies in the electrode montage that is used.

When conducting EEG studies, the presence of CNS related clinical signs such as tremors, ataxia, myoclonus, decreased activity or emesis can be valuable as early signs of toxicity. While the sequence and exposures at which these clinical signs occur in clinical investigations may differ from results obtained in non-clinical studies, it remains that...
those clinical signs can be monitored in patients and used as a potential stopping rule when appropriate. In addition, the presence of CNS related clinical signs might weigh into the decision to select the high dose in a non-clinical EEG study. When clear and significant CNS related clinical signs are present in the animal model, it may be justified to stop the drug dose level escalation prior to attaining a dose inducing seizure.

In conclusion, the current study assessed the functional neurotoxicity of noribogaine when administered orally at doses of 160 and 320 mg/kg to conscious freely moving cynomolgus monkeys with continuous EEG monitoring by telemetry. While CNS related clinical signs were observed at both dose levels investigated, no ictal activity was present in any of the animals. This study illustrates an application of EEG monitoring in non-human primates for functional neurotoxicity assessments in noribogaine clinical trials.

Conflicts of interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: JA, JS, RjG, LTF and HW were financially supported directly or indirectly by DemeRx, Inc. MVA, MP, RK and SA have no conflict of interest, other than their employment in a contract research organization.

Fig. 8. End of EEG seizure activity (i.e. a polyspike–wave pattern) induced by the positive control PTZ in a male cynomolgus monkey with post-ictal attenuation of higher EEG frequencies.

Fig. 9. Time profile of the mean plasma concentrations post-noribogaine administration (320 mg/kg) for male and female cynomolgus monkeys (n = 3 per gender, ± SD).

References


