Received: 1 March 2022 | Revised: 26 April 2022 | Accepted: 29 April 2022

DOI: 10.1111/bcp.15392

SHORT COMMUNICATION

Human pharmacokinetics of XBD173 and etifoxine distinguish their potential for pharmacodynamic effects mediated by translocator protein

David R. Owen[1] | Alexandra Phillips[1] | Desmond O'Connor[2] | Gabrielle Grey[1] | Lina Aimola[1] | Richard Nicholas[1] | Paul M. Matthews[1,3]

[1]Department of Brain Sciences, Imperial College London, London

[2]Pharmidex Pharmaceutical Services, London, UK

[3]UK Dementia Research Institute Centre, Imperial College London, London, UK

Correspondence
Dr David R. Owen, Department of Brain Sciences, Imperial College, Hammersmith Hospital, London W12 0NN, UK.
Email: d.owen@imperial.ac.uk

Funding information
MRC Clinician Scientist Fellowship, Grant/Award Number: MR/N008219/1; NIHR Biomedical Research Centre, Grant/Award Number: MR/N016343/1; UK Dementia Research Institute; National Institute for Health Research (NIHR) Senior Investigator Award; Lily Safra; Edmond J Safra Foundation

XBD173 and etifoxine are translocator protein (TSPO) ligands that modulate inflammatory responses in preclinical models. Limited human pharmacokinetic data is available for either molecule, and the binding affinity of etifoxine for human TSPO is unknown. To allow for design of human challenge experiments, we derived pharmacokinetic data for orally administered etifoxine (50 mg 3 times daily) and XBD173 (90 mg once daily) and determined the binding affinity of etifoxine for TSPO. For XBD173, maximum plasma concentration and free fraction measurements predicted a maximal free concentration of 1.0 nM, which is similar to XBD173 binding affinity. For etifoxine, maximum plasma concentration and free fraction measurements predicted a maximal free concentration of 0.31 nM, substantially lower than the K_i for etifoxine in human brain derived here (7.8 μM, 95% CI 4.5–14.6 μM). We conclude that oral XBD173 dosing at 90 mg once daily will achieve pharmacologically relevant TSPO occupancy. However, the occupancy is too low for TSPO mediated effects after oral dosing of etifoxine at 50 mg 3 times daily.

KEYWORDS
anti-inflammatory drugs, brain, etifoxine, translocator protein, XBD173

1 | INTRODUCTION

Pharmacological and genetic modulation of the translocator protein (TSPO) is immunomodulatory and biases microglia towards expression of immunosuppressive phenotypes in vitro.[1–6] Preclinical in vivo evidence for an anti-inflammatory effect of TSPO ligands also is compelling; in a range of neurodegenerative and inflammatory mouse models, TSPO ligands inhibit proinflammatory activation and improve clinical scores.[1,6–15] These experiments suggest that TSPO may be a novel target for central nervous systems disorders which are partly driven by neuroinflammatory mechanisms. TSPO ligands have also been investigated as tools to enhance neurosteroid synthesis and hence as potential treatments for disorders driven partly by reduced neurosteroid concentrations.[16]

XBD173 and etifoxine are TSPO ligands that have been explored extensively in preclinical models[14,17–28] and clinical studies.[16] Etifoxine is licensed for the treatment of anxiety in France. XBD173 has previously been administered to humans in an experimental medicine study.[16] However, while both have been believed to be acting through modulation of TSPO, differences in pharmacodynamics have been reported. For

David R Owen and Alexandra Phillips contributed equally to the manuscript

The authors confirm that the Principal Investigator for this paper is Professor Paul Matthews and that he had direct clinical responsibility for participants.

example, etifoxine improved clinical scores in an experimental autoimmune encephalomyelitis mouse model, whereas XBD173 did not.[20]

Here we sought to gather appropriate pharmacokinetic and binding affinity data to enable use of these molecules in challenge studies in humans for exploration of effects of TSPO modulation. Although etifoxine is used clinically, there are no publicly available pharmacokinetic data. The binding affinity of etifoxine for TSPO in the rodent brain is approximately 12 μM,[29] but the affinity in the human brain has not been reported publicly. The influence of differences in TSPO structure with the common rs6971 polymorphism, which changes the affinity of TSPO for many ligands,[30] is also unknown. To test whether the current clinically approved dosing schedule of etifoxine produces plasma concentrations which are consistent with occupancy of TSPO, we measured etifoxine binding affinity for TSPO in the human brain in subjects having different rs6971 genotypes and performed pharmacokinetic studies at the approved clinical dose of 50 mg 3 times daily (TDS) in healthy volunteers.

XBD173 was developed for the treatment of anxiety disorders. Pharmacokinetic studies with XBD173 suggested that a 90-mg dose produces peak plasma concentrations in the micromolar range.[16] However, positron emission tomography (PET) data subsequently showed only 80% TSPO peak occupancy in people with the rs6971 common variant (*high affinity binders*, HAB)[30] following a 90-mg dose.[31] This occupancy figure is substantially lower than would be predicted, as the affinity of XBD173 for TSPO in the human brain is 2–3 nM and hence micromolar concentrations would be expected to saturate it.[32] To better understand this dose occupancy relationship, we repeated pharmacokinetic analyses of XBD173 in healthy volunteers and conducted measurements of the plasma-free fraction.

What is already known about this subject

- Pharmacological and genetic modulation of the translocator protein (TSPO) is immunomodulatory and biases microglia towards expression of immunosuppressive phenotypes. XBD173 and etifoxine are TSPO ligands that have been explored extensively in preclinical models and are now being used in human challenge experiments.
- There are limited data available on plasma concentrations and plasma free fraction following administration to humans, and on the TSPO binding affinity of etifoxine. These are required to best design these experiments.

What does this study add

- The pharmacokinetic and brain-binding affinity data provided here are consistent with potential anti-inflammatory activity of orally administered XBD173 that is mediated by TSPO.
- The free plasma concentration of etifoxine is too low for pharmacologically relevant TSPO occupancy by etifoxine at the standard clinical dose

2 | METHODS

2.1 | Clinical study design

This was a single centre, open-label study enrolling 4 adult healthy volunteers (aged 35–65 y) irrespective of rs6971 genotype. Detailed inclusion and exclusion criteria are shown in the supplementary information. Eligible participants were randomised via an online system hosted by a specialist company (www.sealedenevelope.com) to receive either once daily oral XBD173 (90 mg) continuously for 7 days, a minimum 28-day washout, and then 3 times daily oral etifoxine (50 mg) continuously for 7 days, or vice versa. Although both drugs have short plasma half-lives, the kinetics of the biological effects are unknown. As a precaution, therefore, a long washout period was chosen. The first dose was taken at the clinical research facility and the participant was monitored for 4 hours. Subsequent doses were taken at home by the participant. On day 7, the participant returned for a final assessment. The study protocol was approved by West London & GTAC Research Ethics Committee (ref 17/LO/0566), and all participants provided written informed consent. The PI of the study was Paul M. Matthews (DPhil, FRCP, FMedSci) who is an author on the manuscript.

2.2 | Pharmacokinetics

Plasma samples for pharmacokinetic analysis were obtained on day 1 at 0.5, 1, 2, 3, 4 hours following the first dose. As there are no data on whether etifoxine accumulates following repeated dosing, a further sample was taken on the final day of etifoxine dosing, approximately 2–3 hours following the final dose of the drug. This analysis was not performed for XBD173 as this molecule does not accumulate.[16] Plasma samples were stored at −20°C or lower until analysis. Plasma samples (25 μL) were prepared for analysis by protein precipitation with acetonitrile containing internal standard (tolbutamide; 200 μL) followed by mixing (150 rpm, 15 min) and centrifugation (1500 g, 15 min). The supernatant (50) μL was diluted with water (100 μL) and mixed (100 rpm, 15 min). XBD173 samples were analysed by liquid chromatography–tandem mass spectrometry (LC–MSMS) (Shimadzu Nexera X2 UHPLC/Shimadzu LCMS 8060) with Phenomenex Kinetex Biphenyl (50 × 2.1 mm), 1.7-μm column and mobile phase components water/0.1% formic acid (A) and acetonitrile/0.1% formic acid (B). Mobile phase gradient was 0–0.3 minutes 2% B; 0.3–1.1 minutes increase to 95% B; 1.1–1.75 minutes 95% B, 1.75–1.8 minutes decrease to 2% B; 1.8–2.5 minutes 2% B. Flow rate was 0.4 mL/min. Injection volume was 1 μL. Etifoxine was analysed in the same way except column was Waters Aquity BEH C18 (50 × 2.1 mm), 1.7 μm. MSMS transitions for XBD173, etifoxine and tolbutamide were 401.9 > 227.1, 300.9 > 230.1 and 271.0 > 91.0 respectively. Calibration standards were prepared by spiking XDB173 or etifoxine into

control plasma over the ranges 2–10 000 ng/mL (XDB173) or 1–5000 ng/mL (etifoxine), then preparing and analysing as for the study samples. Lower limit of detection was 2 ng/mL for XDB173 and 1 ng/mL for etifoxine.

2.3 | Plasma protein binding determination

Plasma protein binding was measured by equilibrium dialysis using 96-well plate blocks with 2 compartments separated by a vertical cylinder of dialysis membrane (molecular weight cut off ~8000 Da; ThermoFisher Scientific). Plasma spiked with either etifoxine or XBD173 at 1 μM final concentration was placed in the first compartment, in the other was placed phosphate buffered saline, pH 7.4. The unit was covered and incubated at 37°C on an orbital shaker for 4 hours. After incubation, samples from both plasma (bound) chamber and the buffer (free) chamber were taken and matrix-matched by addition of the alternative blank matrix. Acetonitrile containing internal standard was added to all samples. The samples were centrifuged, and the supernatant was analysed by LC–MS (Agilent 1290 UHPLC/Agilent 6550 QToF/Waters Acquity BEH Phenyl (50 x 2.1 mm) 1.7-μm column/0.1% formic acid, acetonitrile 0.1% formic acid mobile phase gradient with flow 0.4 mL/min). The ratio of responses in free to bound samples equates to the free fraction.

2.4 | Materials

XBD173 was manufactured according to good manufacturing practice as a custom preparation by Pharmasynth, Estonia. Etifoxine was obtained from the hospital pharmacy. [^{3}H]PK11195(1-(2-Chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide specific activity = 80 Ci/mmol; radioactive concentration = 1.0 mCi/mL) was purchased from Perkin Elmer, UK. Unlabelled PK11195 and etifoxine was obtained from Sigma, UK. Nomenclature related to drugs and molecular targets conforms to the IUPHAR/BPS Guide.[33]

2.5 | Radioligand binding experiments

Details of the radioligand binding experiments are in the supplementary methods. In brief, brain tissue was obtained from 4 HABs and 4 low affinity binders (LABs) from the UK Multiple Sclerosis Tissue Bank at Imperial College and stored at −80°C until use. Binding affinity status was determined previously by radioligand binding experiments using [3H]PK11195 and unlabelled PBR28.[34] Aliquots of membrane suspension were incubated with [3H]PK11195 (0.3 nM) and 1 of 8 concentrations of etifoxine ranging from 150 nM to 30 μM. The assay was terminated via filtration through Whatman GF/B filters. Scintillation fluid (4 mL/vial, Perkin Elmer Ultima-Gold MV) was added and vials counted on a Perkin Elmer Tricarb 2900 liquid scintillation counter. A dissociation constant for [3H]PK11195 of 29.25 nM[6] was used to generate the inhibition constant (*Ki*) for etifoxine according to the Cheng and Prusoff Equation[12] using GraphPad Prism 8.1 software (GraphPad Software Inc, USA).

3 | RESULTS

3.1 | Participant characteristics

Four participants (1 female, 3 male) with mean age 51.5 years were separately dosed with XBD173 and etifoxine. Three participants were HABs and 1 was a mixed affinity binder. The washout period in between the dosing periods ranged from 28 to 42 days. All participants completed all visits. No adverse events were reported.

3.2 | Pharmacokinetics

Following oral administration of 90 mg XBD173, the median plasma maximum plasma concentration (Cmax) and time to Cmax were 114 ng/mL and 2.5 hours, respectively (Table 1). Following oral administration of 50 mg etifoxine, the median plasma Cmax and time to Cmax were 32 ng/mL and 2.0 hours, respectively. We did not find evidence for significant etifoxine accumulation after multiple doses: plasma etifoxine concentrations following the final dose on the morning of day 7 were not higher than on day 1 (Supplementary Table 2).

3.3 | Plasma free fraction

We assessed plasma free fractions using equilibrium dialysis and found that 99.66% (standard error of the mean, SEM 0.03) of the XBD173 in plasma was bound and 0.34% (SEM 0.03) free and that 99.71% (SEM 0.02) of the etifoxine was bound and 0.29% (SEM 0.02) free.

3.4 | Estimation of etifoxine K_i in brain tissue

Competition assays with unlabelled etifoxine were performed with brain tissue from 8 donors (4 HABs, 4 LABs). The mean K_i value for the HABs (7.6 ± 2.2 μM, $n = 4$) was similar to that of the LABs (7.6 ± 1.7 μM, $n = 4$; $P = .99$). The estimated K_i value for the whole population when fitted to a single site binding curve was 7.8 μM (95% CI 4.5–14.6 μM; Figure 1). Due to the limitation on etifoxine solubility, the competition curve did not fully plateau.

3.5 | Estimates of TSPO occupancy

The median Cmax of 114 ng/mL and free fraction of 0.34%, suggests a predicted free plasma XBD173 concentration of approximately 1.0 nM and a TSPO occupancy of ~30% in HABs.

For etifoxine, with an average Cmax was 32 ng/mL and free fraction of 0.29%, the predicted free etifoxine concentration was

TABLE 1 Pharmacokinetic parameters following oral administration of a single dose of 90 mg XBD173 or 50 mg etifoxine

	Participant number						
	1	2	3	4	Min	Max	Median
90 mg XBD173 single dose							
Tmax (h)	2.0	4.0	2.0	3.0	2	4	2.5
Cmax (ng/mL)	56	25	263	171	25	263	114
AUClast (ng h/mL)	148	74	556	345	74	556	247
50 mg Etifoxine single dose							
Tmax (h)	3.0	2.0	2.0	2.0	2	3	2.0
Cmax (ng/mL)	24	33	39	31	24	39	32
$AUC_{0\text{-}4\ h}$ (ng h/mL)	59	64	83	71	59	83	68

AUC, area under the time–plasma concentration curve; Cmax, maximum plasma concentration; Tmax, time to Cmax.

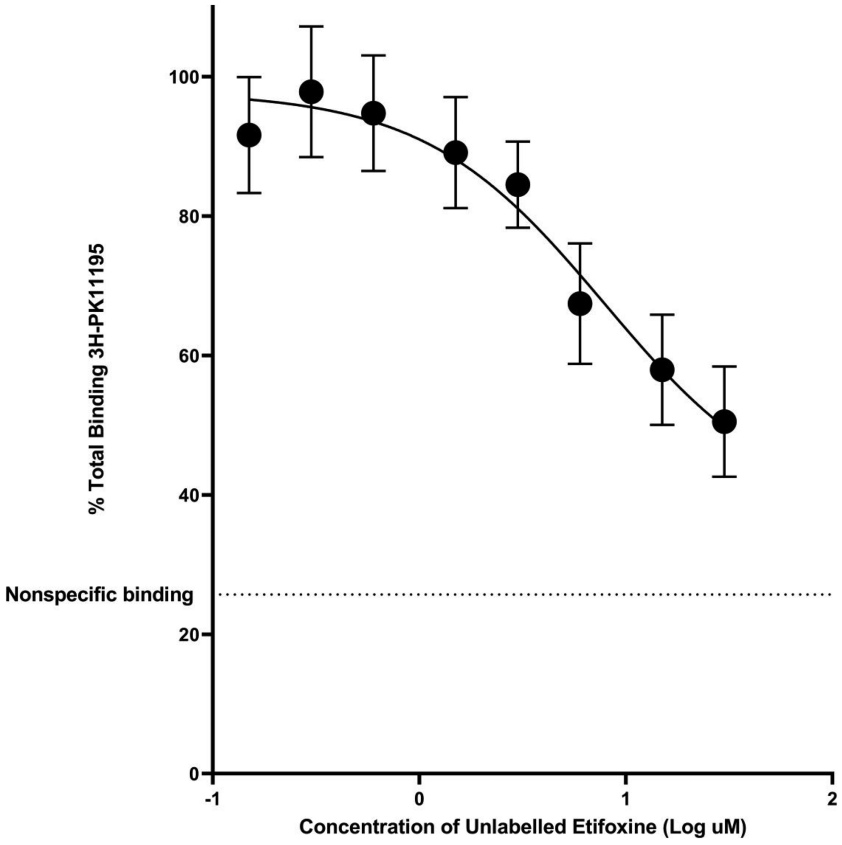

FIGURE 1 Competition assay with [^{3}H] PK11195 and unlabelled etifoxine, using human brain tissue. Each data point represents the mean value of all subjects, and the error bars represent standard error of the mean. Dotted line represents nonspecific binding determined by unlabelled PK11195 (10 μM). Data from high affinity and low affinity binders are plotted in 1 curve as etifoxine affinity was independent of rs6971 genotype.

estimated to be approximately 0.31 nM. Given the affinity of etifoxine for TSPO estimated here (7.6 μM), this implies that TSPO occupancy of etifoxine at peak concentration will be <0.01%.

4 | DISCUSSION

XBD173 and etifoxine have been explored as possible immunomodulatory TSPO ligands in preclinical and in vitro models. Here we sought to gather appropriate pharmacokinetic and binding affinity data to enable design of TSPO challenge studies in humans for future pharmacodynamic investigations.

We found that the affinity of etifoxine for TSPO in the human brain is approximately 7.8 μM, irrespective of rs6971 genotype. This estimate is similar to measures previously reported for the rodent brain (12.5 μM), heart (22.5 μM) and kidney (14 and 9 μM).[29,35,36] However, following a 50-mg oral dose, we also estimated a sub-nanomolar free plasma concentration from our pharmacokinetic studies. Assuming equilibrium between free plasma concentration and brain tissue concentrations, this would equate to TSPO occupancy of approximately 0.01%. We also did not find evidence for drug accumulation after dosing at 50 mg TDS for 7 days. Indeed, plasma etifoxine concentration on day 7 was lower than on day 1, which may reflect variability or that a participant took the day 7 morning dose later than

directed. We therefore conclude that any pharmacodynamic effects of etifoxine with a dose regimen of 50 mg TDS are not mediated by its interaction with TSPO. These low free plasma concentrations of etifoxine also appear inconsistent with pharmacological activity at the γ-aminobutyric acid-a receptor, the presumed target for anxiety.[37,38] Although the affinity has not been estimated in human tissue, in the rodent brain, etifoxine binds the γ-aminobutyric acid-a receptor with an IC50 of approximately 6.7 μM, and pharmacodynamic actions at the receptor are elicited only above 1 μM.[35] If plasma free concentration reflects concentration in the brain, any pharmacodynamic effect due to etifoxine at the standard dose is likely to be independent of this target.

PET data with XBD173 showed that a 90-mg dose is associated with only approximately 80% occupancy of TSPO in the brain,[31] despite plasma concentrations reaching 1 μM.[16] Our estimates of plasma concentration were lower (∼320 nM) but would still predict saturation (>99% occupancy) of TSPO by XBD173. Here we also have shown that if free fraction is used for estimation of TSPO occupancy, assuming equilibrium between plasma concentration and brain tissue concentration, the previously observed in vivo TSPO occupancy can be predicted from XBD173 pharmacokinetic data. However, we also show there may be substantial intersubject variability in XBD173 plasma concentration, implying that 90 mg may be insufficient to achieve high TSPO occupancy in some participants.

Our study has important limitations. First, the sample size for the pharmacokinetic study was small. Second, we took only 5 samples over 4 hours and may therefore have underestimated Cmax. Third, due to limited solubility of etifoxine, the competition curve for the radioligand binding experiment with etifoxine did not plateau. The result of these limitations is that our estimates for the plasma concentrations and etifoxine binding affinity lack precision. However, the aim of this study was to determine whether the plasma concentrations were consistent with TSPO occupancy. The disparity between our estimates of etifoxine binding affinity and free plasma concentration is substantial. It is therefore unlikely that more precise estimates would materially alter the conclusions. When estimating TSPO occupancy of etifoxine in the brain, we made the assumption that free plasma and free brain concentrations of etifoxine are in equilibrium. This may not be the case. To definitively determine whether etifoxine binds TSPO in the brain at the administered doses, a PET occupancy study would be required. Finally, we did not formally assess compliance, beyond measuring etifoxine plasma concentration on day 7. It is therefore possible that day 7 etifoxine concentrations were no higher than day 1 concentrations because the participants did not take the drug as directed.

5 | CONCLUSION

The pharmacokinetic and brain binding affinity data are consistent with potential anti-inflammatory activity of orally administered XBD173 that is mediated by TSPO. However, the free plasma concentration of etifoxine is too low for pharmacologically relevant TSPO occupancy by etifoxine at the approved clinical dose of 50 mg TDS.

ACKNOWLEDGEMENTS

The authors thank The Multiple Sclerosis and Parkinson's Tissue Bank for providing the brain tissue, the NIHR Imperial Clinical Research Facility where the clinical study was conducted, Anil Misra at Pharmidex for performing the plasma protein binding incubations, and Sandra Gueddes for performing the radioligand binding incubations.

P.M.M. acknowledges generous personal and research support from the Edmond J Safra Foundation and Lily Safra, a National Institute for Health Research (NIHR) Senior Investigator Award, the UK Dementia Research Institute and the NIHR Biomedical Research Centre at Imperial College London. The direct funding for this project was MR/N016343/1.

D.R.O. is funded by an MRC Clinician Scientist Fellowship MR/N008219/1. Open access funding enabled and organized by Projekt DEAL.

COMPETING INTERESTS

P.M.M. notes consultancy fees from Novartis and Biogen. He has received honoraria or speakers' honoraria from Novartis, Biogen and Roche and has received research or educational funds from Biogen, Novartis and GlaxoSmithKline.

CONTRIBUTORS

D.R.O. wrote manuscript, designed research, performed research, analysed data.
A.P. wrote manuscript, performed research, analysed data.
D.O.C. designed research, analysed data.
G.G. wrote manuscript, performed research.
L.A. wrote manuscript, performed research.
R.N. designed research, performed research.
P.M. wrote manuscript, designed research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

ORCID

David R. Owen https://orcid.org/0000-0002-1198-7563

REFERENCES

1. Bae KR, Shim HJ, Balu D, Kim SR, Yu SW. Translocator protein 18 kDa negatively regulates inflammation in microglia. *J Neuroimmune Pharmacol*. 2014;9(3):424-437. doi:10.1007/s11481-014-9540-6
2. Choi HB, Khoo C, Ryu JK, van Breemen E, Kim SU, McLarnon JG. Inhibition of lipopolysaccharide-induced cyclooxygenase-2, tumor necrosis factor-alpha and [Ca2+]i responses in human microglia by the peripheral benzodiazepine receptor ligand PK11195. *J Neurochem*. 2002;83(3):546-555. doi:10.1046/j.1471-4159.2002.01122.x
3. Hong SH, Choi HB, Kim SU, McLarnon JG. Mitochondrial ligand inhibits store-operated calcium influx and COX-2 production in human microglia. *J Neurosci Res*. 2006;83(7):1293-1298. doi:10.1002/jnr.20829
4. Karlstetter M, Nothdurfter C, Aslanidis A, et al. Translocator protein (18 kDa) (TSPO) is expressed in reactive retinal microglia and

modulates microglial inflammation and phagocytosis. *J Neuroinflammation*. 2014;11(1):3. doi:10.1186/1742-2094-11-3

5. Lokensgard JR, Hu S, Hegg CC, Thayer SA, Gekker G, Peterson PK. Diazepam inhibits HIV-1 Tat-induced migration of human microglia. *J Neurovirol*. 2001;7(5):481-486. doi:10.1080/135502801753170345
6. Wang M, Wang X, Zhao L, et al. Macroglia-microglia interactions via TSPO signaling regulates microglial activation in the mouse retina. *J Neurosci*. 2014;34(10):3793-3806. doi:10.1523/JNEUROSCI.3153-13.2014
7. Barron AM, Garcia-Segura LM, Caruso D, et al. Ligand for translocator protein reverses pathology in a mouse model of Alzheimer's disease. *J Neurosci*. 2013;33(20):8891-8897. doi:10.1523/JNEUROSCI.1350-13.2013
8. Zavala F, Taupin V, Descamps-Latscha B. In vivo treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of interleukin 1, tumor necrosis factor and interleukin-6. *J Pharmacol Exp Ther*. 1990;255(2):442-450.
9. Veiga S, Carrero P, Pernia O, Azcoitia I, Garcia-Segura LM. Translocator protein 18 kDa is involved in the regulation of reactive gliosis. *Glia*. 2007;55(14):1426-1436. doi:10.1002/glia.20558
10. Waterfield JD, McGeer EG, McGeer PL. The peripheral benzodiazepine receptor ligand PK 11195 inhibits arthritis in the MRL-lpr mouse model. *Rheumatology (Oxford)*. 1999;38(11):1068-1073. doi:10.1093/rheumatology/38.11.1068
11. Torres SR, Frode TS, Nardi GM, et al. Anti-inflammatory effects of peripheral benzodiazepine receptor ligands in two mouse models of inflammation. *Eur J Pharmacol*. 2000;408(2):199-211. doi:10.1016/S0014-2999(00)00760-3
12. Ryu JK, Choi HB, McLarnon JG. Peripheral benzodiazepine receptor ligand PK11195 reduces microglial activation and neuronal death in quinolinic acid-injected rat striatum. *Neurobiol Dis*. 2005;20(2):550-561. doi:10.1016/j.nbd.2005.04.010
13. Bribes E, Bourrie B, Casellas P. Ligands of the peripheral benzodiazepine receptor have therapeutic effects in pneumopathies in vivo. *Immunol Lett*. 2003;88(3):241-247. doi:10.1016/S0165-2478(03)00083-X
14. Daugherty DJ, Selvaraj V, Chechneva OV, Liu XB, Pleasure DE, Deng W. A TSPO ligand is protective in a mouse model of multiple sclerosis. *EMBO Mol Med*. 2013;5(6):891-903. doi:10.1002/emmm.201202124
15. Ma L, Zhang H, Liu N, et al. TSPO ligand PK11195 alleviates neuroinflammation and beta-amyloid generation induced by systemic LPS administration. *Brain Res Bull*. 2016;121:192-200. doi:10.1016/j.brainresbull.2016.02.001
16. Rupprecht R, Rammes G, Eser D, et al. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science*. 2009;325(5939):490-493. doi:10.1126/science.1175055
17. Mages K, Grassmann F, Jagle H, et al. The agonistic TSPO ligand XBD173 attenuates the glial response thereby protecting inner retinal neurons in a murine model of retinal ischemia. *J Neuroinflammation*. 2019;16(1):43. doi:10.1186/s12974-019-1424-5
18. Zhang H, Ma L, Guo WZ, et al. TSPO ligand etifoxine attenuates LPS-induced cognitive dysfunction in mice. *Brain Res Bull*. 2020;165:178-184. doi:10.1016/j.brainresbull.2020.10.013
19. Shehadeh M, Palzur E, Apel L, Soustiel JF. Reduction of Traumatic Brain Damage by Tspo Ligand Etifoxine. *Int J Mol Sci*. 2019;20(11):2639. doi:10.3390/ijms20112639
20. Ravikumar B, Crawford D, Dellovade T, et al. Differential efficacy of the TSPO ligands etifoxine and XBD-173 in two rodent models of Multiple Sclerosis. *Neuropharmacology*. 2016;108:229-237. doi:10.1016/j.neuropharm.2016.03.053
21. Lin YM, Sun HY, Chiu WT, et al. Etifoxine, a TSPO Ligand, Worsens Hepatitis C-Related Insulin Resistance but Relieves Lipid Accumulation. *Biomed Res Int*. 2019;2019:3102414. doi:10.1155/2019/3102414
22. Liere P, Pianos A, Oudinet JP, Schumacher M, Akwa Y. Differential effects of the 18-kDa translocator protein (TSPO) ligand etifoxine on steroidogenesis in rat brain, plasma and steroidogenic glands: Pharmacodynamic studies. *Psychoneuroendocrinology*. 2017;83:122-134. doi:10.1016/j.psyneuen.2017.05.022
23. Bahr LM, Maurer F, Weigl J, et al. Dissociation of endocrine responses to the Trier Social Stress Test in Virtual Reality (VR-TSST) by the benzodiazepine alprazolam and the translocator protein 18 kDa (TSPO) ligand etifoxine. *Psychoneuroendocrinology*. 2021;124:105100. doi:10.1016/j.psyneuen.2020.105100
24. Scholz R, Caramoy A, Bhuckory MB, et al. Targeting translocator protein (18 kDa) (TSPO) dampens pro-inflammatory microglia reactivity in the retina and protects from degeneration. *J Neuroinflammation*. 2015;12(1):201. doi:10.1186/s12974-015-0422-5
25. Leva G, Klein C, Benyounes J, et al. The translocator protein ligand XBD173 improves clinical symptoms and neuropathological markers in the SJL/J mouse model of multiple sclerosis. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(12):3016-3027. doi:10.1016/j.bbadis.2017.09.007
26. Gong J, Szego EM, Leonov A, et al. Translocator Protein Ligand Protects against Neurodegeneration in the MPTP Mouse Model of Parkinsonism. *J Neurosci*. 2019;39(19):3752-3769. doi:10.1523/JNEUROSCI.2070-18.2019
27. Crombie GK, Palliser HK, Shaw JC, Hodgson DM, Walker DW, Hirst JJ. Neurosteroid-based intervention using Ganaxolone and Emapunil for improving stress-induced myelination deficits and neurobehavioural disorders. *Psychoneuroendocrinology*. 2021;133:105423. doi:10.1016/j.psyneuen.2021.105423
28. Barron AM, Higuchi M, Hattori S, Kito S, Suhara T, Ji B. Regulation of Anxiety and Depression by Mitochondrial Translocator Protein-Mediated Steroidogenesis: the Role of Neurons. *Mol Neurobiol*. 2021;58(2):550-563. doi:10.1007/s12035-020-02136-5
29. Verleye M, Akwa Y, Liere P, et al. The anxiolytic etifoxine activates the peripheral benzodiazepine receptor and increases the neurosteroid levels in rat brain. *Pharmacol Biochem Behav*. 2005;82(4):712-720. doi:10.1016/j.pbb.2005.11.013
30. Owen DR, Yeo AJ, Gunn RN, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab*. 2012;32(1):1-5. doi:10.1038/jcbfm.2011.147
31. Owen DR, Guo Q, Kalk NJ, et al. Determination of [(11)C]PBR28 binding potential in vivo: a first human TSPO blocking study. *J Cereb Blood Flow Metab*. 2014;34(6):989-994. doi:10.1038/jcbfm.2014.46
32. Owen DR, Lewis AJ, Reynolds R, et al. Variation in binding affinity of the novel anxiolytic XBD173 for the 18 kDa translocator protein in human brain. *Synapse*. 2011;65(3):257-259. doi:10.1002/syn.20884
33. Alexander SP, Kelly E, Marrion NV, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Overview. *Br J Pharmacol*. 2017;174-(Suppl 1):S1-S16. doi:10.1111/bph.13882
34. Owen DR, Howell OW, Tang SP, et al. Two binding sites for [3H]PBR28 in human brain: implications for TSPO PET imaging of neuroinflammation. *J Cereb Blood Flow Metab*. 2010;30(9):1608-1618. doi:10.1038/jcbfm.2010.63
35. Schlichter R, Rybalchenko V, Poisbeau P, Verleye M, Gillardin J. Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etifoxine. *Neuropharmacology*. 2000;39(9):1523-1535. doi:10.1016/S0028-3908(99)00253-1
36. Costa B, Cavallini C, Da Pozzo E, Taliani S, Da Settimo F, Martini C. The Anxiolytic Etifoxine Binds to TSPO Ro5-4864 Binding Site with Long Residence Time Showing a High Neurosteroidogenic Activity. *ACS Chem Neurosci*. 2017;8(7):1448-1454. doi:10.1021/acschemneuro.7b00027
37. Mattei C, Taly A, Soualah Z, et al. Involvement of the GABAA receptor alpha subunit in the mode of action of etifoxine. *Pharmacol Res*. 2019;145:104250. doi:10.1016/j.phrs.2019.04.034

38. Hamon A, Morel A, Hue B, Verleye M, Gillardin JM. The modulatory effects of the anxiolytic etifoxine on GABA(A) receptors are mediated by the beta subunit. *Neuropharmacology*. 2003;45(3):293-303. doi:10.1016/S0028-3908(03)00187-4

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Owen DR, Phillips A, O'Connor D, et al. Human pharmacokinetics of XBD173 and etifoxine distinguish their potential for pharmacodynamic effects mediated by translocator protein. *Br J Clin Pharmacol*. 2022; 88(9):4230-4236. doi:10.1111/bcp.15392