Sigma-1 Receptor Target Occupancy Study with Dynamic PET Scan Analysis of ANAVEX®2-73, a Clinical Candidate for Neurodegenerative and Neurodevelopmental Diseases

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BACKGROUND

ANAVEX®2-73, a selective sigma-1 receptor (S1R) agonist, has undergone testing in a Phase 2a trial of Alzheimer's disease patients. Over a period of 57 weeks, ANAVEX®2-73 demonstrated a favorable safety profile and a concentration-dependent improvement against exploratory endpoints MMSE and ADCS-ADL. Positron emission tomography (PET) with [^{18}F]FTC146, a novel S1R radiotracer, was used in the current study to substantiate target engagement in mice.

MATERIALS & METHODS

Radiochemistry: [^{18}F]FTC-146 was prepared with an automated synthesis module.

Animals: Seven week old wild type male mice (FVB.129P2-*Pde6b*$^{+}$ *Tyr*$^{c\text{-}ch}$/AntJ) (N=4-5) were used to evaluate target engagement of ANAVEX®2-73 in the brain.

PET Scanning and Autoradiography Procedure: ANAVEX®2-73 was administered orally in 4 dose groups (0, 1, 10, 30 mg/kg) with N=4-5 per group. Mice were anesthetized using isoflurane 20 minutes after administration of ANAVEX®2-73 and tail vein catheters were inserted. After 60 minutes post-drug delivery, [^{18}F]FTC-146 (210±22 µCi, 7.77±0.81 MBq) was injected intravenously and a dynamic PET scan (Siemens Inveon D-PET, 60 minutes) was performed (**Figure 1**). Following the PET scan, the mice were perfused and the brains were collected and sectioned at 20 µm for *ex vivo* autoradiography (ARG). The brain regions collected were: frontal cortex, caudate, hippocampus, thalamus, amygdalar cortex, pons, cerebellum and muscle for normalization.

Metabolism Correction Procedure: Separate mice (N=6, 0 mg/kg ANAVEX®2-73) were used to derive the whole blood to plasma ratio and parent fraction for metabolite correction. Tail vein catheters were similarly implanted and the mice were injected intravenously with a bolus of [^{18}F]FTC-146 (780±214 µCi, 28.86±7.92 MBq). Arterial blood was collected from the left ventricle of the heart at 1, 5, 15, 30 and 60 minutes. [^{18}F]FTC-146 parent and metabolite compounds were separated using a radio-HPLC and the fractions were counted with a gamma counter.

Analysis: Two-tissue compartment kinetic (2TCM) modeling was performed in the whole brain using PMOD 3.7. To measure arterial whole blood input function, an imaged-derived input function (IDIF) was gathered from the left ventricle of the heart. The hottest pixels were used to define IDIF. For receptor occupancy calculations[1], the k_3/k_4 macro parameter was used for binding potential. SUV was also used to assess target engagement of ANAVEX®2-73 with the S1R. To analyze *ex vivo* ARG, ImageJ was used to define regions of interest and all structures were normalized to muscle.

RESULTS

For [^{18}F]FTC-146 production[2], the molar radioactivity was 12.8 ± 5.7 Ci/µmol (474 ± 211 GBq/µmol) and radiochemical purity was 91-94% at the end of synthesis. The two-tissue compartment model was successfully fitted to the 60-minute dynamic mouse brain data (**Figure 2**). The whole blood IDIF was described by a 3-exponential model. After 5 minutes post-injection, 16% of counts in plasma could be attributed to the parent radioligand. The data suggested a fixed correction of 1:1.14 for whole blood counts to plasma. Model macro parameters and receptor occupancy calculations are summarized in **Table 1**. PET data shows S1R occupancy is dose-dependent for ANAVEX®2-73 with a plateau at 68% ± 11% in whole brain **(Figure 3**). For analysis of *ex vivo* ARG, selected brain regions (frontal cortex, caudate, hippocampus, thalamus, amygdala, pons, cerebellum) were analyzed and receptor occupancy ranged from 30-60% (**Figure 4**). These results were compared with PRE-084, a well-known S1R agonist.

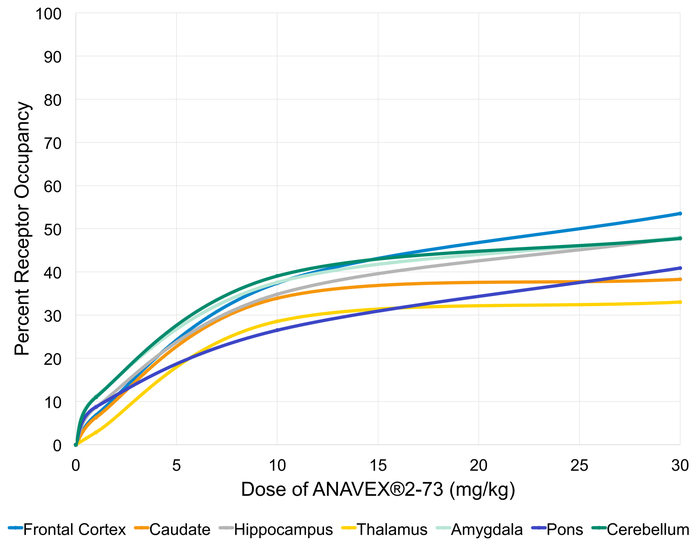

Figure 4. This chart represents the percent receptor occupancy calculated using *ex vivo* ARG with [^{18}F]FTC-146 throughout the brain.

RESULTS (continued)

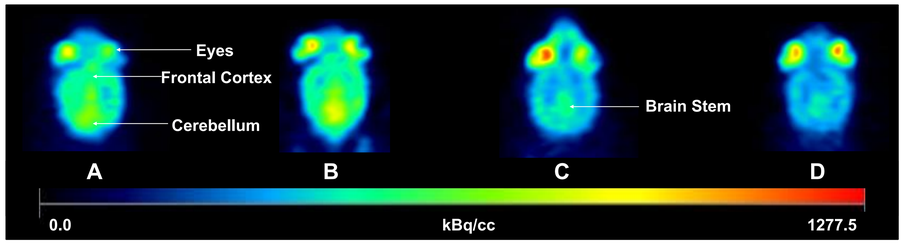

Figure 1. Static image of PET scan of ANAVEX®2-73 in mouse at time 15-20 minutes at the following drug doses: A = WT control, B = 1 mg/kg ANAVEX®2-73, C = 10 mg/kg ANAVEX®2-73 and D = 30 mg/kg ANAVEX®2-73.

Table 1. This table summarizes binding potential and distribution volume values calculated using 2TCM and the calculated percent receptor occupancy.

Drug Category	Average Binding Potential (k_3/k_4)	Average Volume Distribution (V_T)	Percent Receptor Occupancy
WT Control	1.02 ± 0.17	15.3 ± 3.5	N/A
1 mg/kg ANAVEX®2-73	0.81 ± 0.11	13.6 ± 2.1	20.8 ± 10.8
10 mg/kg ANAVEX®2-73	0.34 ± 0.09	7.9 ± 1.8	66.7 ± 9.1
30 mg/kg ANAVEX®2-73	0.32 ± 0.11	8.2 ± 1.8	68.3 ± 10.6
1 mg/kg PRE-084	0.93 ± 0.19	14.6 ± 1.5	8.4 ± 18.5

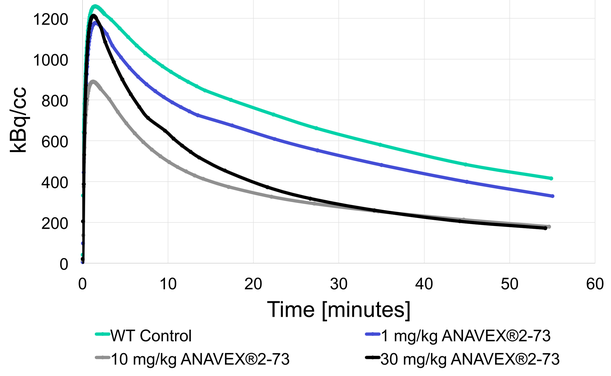

Figure 2. Above are the average 2TCM model curves (n=4-5 per dose category) in whole brain using ANAVEX®2-73 to block [^{18}F]FTC-146 during a 60-minute dynamic PET scan.

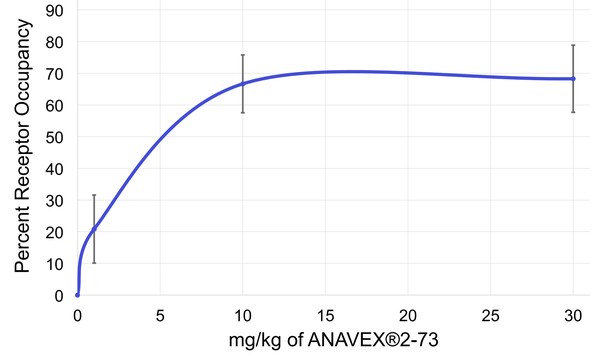

Figure 3. This graph represents the percent receptor occupancy in whole brain with ANAVEX®2-73 using the binding potentials calculated from a 60-minute dynamic PET scan with [^{18}F]FTC-146 and metabolite corrected two-tissue compartment modeling.

DISCUSSION & CONCLUSION

This is the first imaging study of ANAVEX®2-73 and the results confirm dose-dependent S1R target engagement via *ex vivo* ARG and PET using [^{18}F]FTC-146 as the radiotracer. The range of doses employed here (1-30 mg/kg PO) have been used in numerous animal disease models with improvements observed in both behavioral and biochemical readouts. Since the mice equivalent human plasma exposures correspond to human doses of 10-50 mg PO, which have been assessed in previous ANAVEX®2-73 clinical studies, these results pose the possibility that S1R receptor occupancy in humans is similar to that found in mice.

ACKNOWLEDGEMENTS

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REFERENCES

1. Zhang Y, Fox GB. [2012], J Biomed Res, 26(2):69-76
2. James ML, Shen B, et al. [2012], J Med Chem, 55(19):8272-8282

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