Mapping Neural Activities of Area Postrema and Nucleus Tractus Solitarius in Awake Rats Using Pharmacological MRI: Relevance as A Potential Biomarker for Detecting Drug-Induced Emesis

C-L. Chin[1], G. B. Fox[1], V. P. Hradil[1], M. A. Osinski[1], S. P. McGaraughty[1], P. D. Skoubis[1], B. F. Cox[1], Y. Luo[1]

[1]Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL, United States

Introduction

Drug-induced emesis is a major concern in patient care and a significant hurdle in drug development. The emetic reflex is orchestrated by the "vomiting center" located in the brain stem, which is composed of three main structures: area postrema (AP), nucleus tractus solitarius (NTS), and the dorsal motor nucleus of the vagus [1]. Further advances in emesis research have, however, been hampered by limited *in vivo* animal models. The widely used ferret model provides behavioral outcomes; however, knowledge of underlying emetic neuronal activities cannot be easily obtained. In addition, the commonly used laboratory animals for drug efficacy studies, such as mice and rats, do not have an emetic reflex; it is desirable to develop a rodent surrogate marker, so that both efficacy and emetic liability can be determined in the same species. Here we aimed to image the brain activity initiated by the administration of emetic compounds in *awake* rats using rCBV-based phMRI [2-4]. We hypothesized that drugs known to induce emesis in ferrets and other species capable of vomiting would activate the AP and NTS in rats at relevant plasma concentrations. Toward this goal, apomorphine (APO), a D_2-like dopaminergic agonist, was used since it induces emesis and is well characterized in various animal models [5,6]. Additionally, ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine], a selective α4β2 neuronal nicotinic acetylcoline receptor (nAChR) agonist, was chosen since its behavioral profile is well known [7]. Finally, to correlate phMRI findings with behavioral effects, ABT-594-induced rCBV changes in AP and NTS were compared to the incidence of emesis derived from behavioral measures using a ferret model.

Materials and Methods

Animal Preparation and Behavioral Study

Male SD rats (~300 g) were studied under awake conditions. Prior to actual experiments, rats were acclimated to a dedicated animal restrainer used in imaging, following a training protocol [4]. The behavioral study was performed as previously described [6]. Briefly, castrated male ferrets (~1.3 kg) were used, and emetic activities were recorded over a 90-min period after infusing ABT-594 (0.03 or 0.1 µmol/kg, i.p.).

fMRI Experiments and *Data Analysis*

Animals were secured in a rat restrainer integrated with a dual RF coil system (INSL, Worcester MA). Experiments were carried out on a 4.7 T Varian scanner using a FLASH sequence with imaging parameters: TR/TE = 200/20 ms, FA = 30°, pixel size = 156×156 µm^2, slice thickness = 1.25 mm, 5 continuous sagittal slices, and NA = 4. The phMRI protocol included (i) 7-min baseline acquisition, (ii) a bolus injection of ultrasmall super paramagnetic iron oxide contrast agent (CA) (SH U 555 C, 10 mg Fe/kg, i.v., Schering AG, Berlin, Germany), (iii) 20-min post-CA baseline acquisition (pre-drug), (iv) a 5-min drug infusion (saline, APO 0.1, 0.3 µmol/kg, i.v. or ABT-594 0.03, 0.1, 0.3 µmol/kg, i.v.), and (v) 30-min post-drug acquisition. Data analysis was performed using AFNI [8]. The cross-correlation coefficients (*cc*) between time-course rCBV data and a step function were first calculated. Statistical parameter (z-score) was derived from *cc* and later used to create averaged activation maps (n = 5 per group).

Results and Discussion

Figure 1 shows the averaged activity maps obtained from rats treated with saline (A), APO 0.1 µmol/kg (B) or 0.3 µmol/kg (C). No activation was observed in AP and NTS from the saline group (Fig. 1A). At low doses, the administration of APO only activated a few pixels in NTS (Fig. 1B), whilst a higher dose produced a stronger and more extensive activation patterns, specifically in AP and NTS (Fig. 1C). It was reported that, when animals were infused with APO, enhanced *c-fos* expression was observed in AP and NTS in cats [9] and emetic response was not seen behaviorally in AP-ablated dogs [10]. Our phMRI data support their finding and imply neural activations in AP and NTS as detected by phMRI could be a predictor for drug-induced emetic responses. Figure 2 shows the averaged activity maps obtained from rats treated with ABT-594 at 0.03 µmol/kg (A), 0.1 µmol/kg (B), or 0.3 µmol/kg (C). Neural activities in AP and NTS and cortical regions were modulated by ABT-594 in a dose-related manner, which is consistent with our previous study [4]. There was good agreement between ABT-594 plasma levels that produced emesis in ferrets and the incidence of increased rCBV (> 10 %) in AP and NTS of rats (Table 1). In summary, our behavioral, pharmacokinetic, and imaging data indicate that phMRI in awake rats may prove valuable for elucidating neural pathways related to emesis and predicting the emetic potential of new drugs.

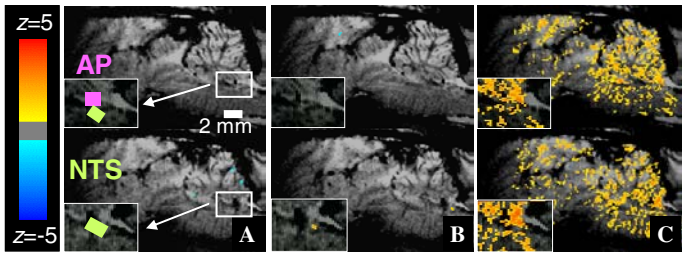

Fig 1. Saline Vehicle | APO 0.1 µmol/kg | APO 0.3µmol/kg

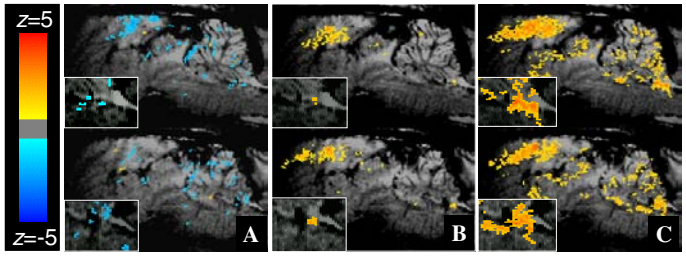

Fig 2. ABT-594 0.03µmol/kg | ABT-594 0.1µmol/kg | ABT-594 0. 3µmol/kg

Table 1 Comparison of ABT-594 behavioral (ferrets) and phMRI (rats) data .

Model Species	Doses [µmol/kg]	Plasma Conc. [C_{max}, ng/mL]	Incidence of Emesis [%]	Incidence of increased rCBV in AP/NTS [%]
Behavioral	0.03 i.p.	3.78	33	-
Ferrets	0.1 i.p.	10.85	83	-
phMRI	0.03 i.v.	2.91	-	20
Rats	0.1 i.v.	9.69	-	80
	0.3 i.v.	29.07	-	100

References **1**. Hornby PJ, Am J Med 111 Suppl 8A, 106S (2001). **2**. Chen YC *et al* Mag Res Med 38, p389 (1997). **3.** Schwartz A *et al*, Synapse, 54, p1 (2004). **4**. Skoubis PD *et al*, Neuroscience, (2005, in press). **5**. King GL, Can J Physiol Pharmacol 68, p260 (1990). **6**. Osinski MA *et al* Pharmacol Biochem Behav 81, p211 (2005). **7**. Bannon AW, *et al*, Science 5341, p77 (1998). **8**. Cox RW *et al*, NMR Biomed 10, p171, (1997). **9**. Miller AD *et al*, J. Neurosci , 14, p871 (1994). **10**. Andrews PL *et al*, Proc. Neurosci Lett, 314, p102, (2001)