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Brief Report

BRIEF REPORT

Serotonin and Norepinephrine Transporter Occupancy of Tramadol in Nonhuman Primate Using Positron Emission Tomography

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Abstract

Background: Tramadol, a centrally acting analgesic drug, has relatively high affinity to serotonin transporter and norepinephrine transporter in addition to μ-opioid receptor. Based on this characteristic, tramadol is expected to have an antidepressant effect.

Methods: Positron emission tomography measurements with [^{11}C]MADAM and [^{18}F]FMeNER-D_2 were performed at baseline and after i.v. administration of 3 different doses (1, 2, and 4 mg/kg) of tramadol using 6 cynomolgus monkeys. The relationship between dose and occupancy for serotonin transporter and norepinephrine transporter was estimated.

Results: Tramadol occupied similarly both serotonin transporter (40%–72%) and norepinephrine transporter (7%–73%) in a dose-dependent manner. The K_d was 2.2 mg/kg and 2.0 mg/kg for serotonin transporter and norepinephrine transporter, respectively.

Conclusions: Both serotonin transporter and norepinephrine transporter of in vivo brain were blocked at >70% at a clinically relevant high dose of tramadol. This study suggests tramadol has potential antidepressant effects through the inhibition of serotonin transporter and norepinephrine transporter in the brain.

Keywords: norepinephrine transporter, occupancy, positron emission tomography, serotonin transporter, tramadol

Introduction

Tramadol is a centrally acting analgesic drug for treatment of postoperative, cancer-related, and inflammatory pain, etc. (Bravo et al., 2017). Activation of the μ-opioid receptor is generally considered to lead to the analgesic effects of tramadol. The affinity for the μ-opioid receptor of tramadol is considerably lower than that of morphine (Ki: 2.1 and 0.00034 μM, respectively) (Raffa et al., 1992), although the equivalent dose ratio of tramadol compared with morphine is reported as only 4:1 (Grond and Sablotzki, 2004). Additionally, low dependence associated with tramadol in clinical trials was reported (Bravo et al., 2017). Tramadol also has relatively high affinity to serotonin transporter (5-HTT) (Ki: 0.99 μM) and norepinephrine transporter (NET) (Ki: 0.79 μM) (Raffa et al., 1992). In addition to the activation of μ-opioid receptor, the inhibitions of both 5-HTT and NET would contribute the analgesic effect because 5-HT and NE are considered to have the antinociceptive effects via activation of descending inhibitory pathways (Grond and Sablotzki, 2004).

The main mechanism of action of antidepressants such as tricyclic antidepressant or serotonin norepinephrine reuptake inhibitor (SNRI) is thought to be the blockade of 5-HTT and NET. Based on the inhibitory effects of 5-HTT and NET, tramadol is expected to have an antidepressant effect for patients with

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depression (Barber, 2011). A previous positron emission tomography (PET) study reported that the oral administration of tramadol showed 5-HTT occupancy in a dose-dependent manner in the brain of healthy subjects (Ogawa et al., 2014). This study confirmed that tramadol blocked 5-HTT in the in vivo human brain, but 5-HTT occupancy showed only up to 60%, which is much lower than the reported 5-HTT occupancy (>70%–80%) by clinical doses of selective serotonin reuptake inhibitor (SSRI) / SNRI (Suhara et al., 2003; Meyer et al., 2004; Takano et al., 2006; Lundberg et al., 2012; Arakawa et al., 2016). This would be related to the doses examined in the study, which were relatively low (50 and 100 mg) compared with the clinical doses (100–400 mg/d). Additionally, NET occupancy by tramadol of the in vivo brain has not yet been reported.

Further investigation of in vivo target engagement for both 5-HTT and NET of tramadol would be helpful to understand the mechanism of the action of the drug and potentially expand the clinical application of the drug. The measurement of in vivo occupancy of 5-HTT and NET using nonhuman primate (NHP) is the useful method for this purpose (Takano et al., 2013). In this study, we examined both 5-HTT and NET occupancy of tramadol, including relatively high doses in NHP brain using PET.

Methods

Subjects

Six cynomolgus monkeys (4 females and 2 males, body weight 5540–9725 g) were included in this study. The NHPs were housed in the Astrid Fagraeus Laboratory of the Swedish Institute for Infectious Disease Control, Solna, Sweden. The study was approved by the Animal Ethics Committee of the Swedish Animal Welfare Agency and was performed according to "Guidelines for planning, conducting and documenting experimental research" (Dnr 4820/06-600) of Karolinska Institutet.

PET Measurements

Anesthesia was induced by i.m. injection of ketamine hydrochloride (approximately 10 mg/kg) at Astrid Fagraeus Laboratory, and then the NHPs were transported to Karolinska Institutet PET center. Inhalation anesthesia by administration of a mixture of sevoflurane, oxygen, and medical air was maintained with endotracheal intubation. The head was immobilized with a fixation device. Body temperature was maintained by a Bair Hugger model 505 (Arizant Healthcare) and monitored by an esophageal thermometer. ECG, heart rate, blood pressure, respiratory rate, and oxygen saturation were continuously monitored throughout the experiments. Fluid balance was maintained by a continuous infusion of saline.

Two PET radioligands, $[^{11}C]$MADAM for 5-HTT and $[^{18}F]$FMeNER-D_2 for NET, were used for the quantification of transporter availability in NHP brain (Schou et al., 2004; Halldin et al., 2005; Takano et al., 2013). $[^{11}C]$MADAM was used for 3 NHPs and $[^{18}F]$FMeNER-D_2 for the other 3 NHPs. One PET measurement as a baseline and another PET measurement after the i.v. administration of tramadol at 15 minutes before radioligand injection were performed for each experiment. Two PET measurements were performed in 1 day for $[^{11}C]$MADAM and on 2 separate days for $[^{18}F]$FMeNER-D_2. For NHP1, 2 sets of baseline and pretreatment conditions were performed. For NHP4, 1 baseline and 2 pretreatment conditions were performed. For the remaining 4 NHPs, a single set of both conditions was performed. Three different doses (1, 2, and 4 mg/kg) were tested for both radioligands.

PET measurements were conducted using the High Resolution Research Tomograph (Siemens Molecular Imaging) (Varrone et al., 2009). A transmission scan of 6 minutes using a single ^{137}Cs source was performed before the emission scan. List mode data were acquired immediately after i.v. injection of $[^{11}C]$MADAM for 93 minutes and $[^{18}F]$FMeNER-D_2 for 180 minutes. Images were reconstructed to 29 frames (20 seconds × 9, 1 minute × 3, 3 minutes × 5, and 6 minutes × 12) for $[^{11}C]$MADAM and 28 frames (1 minute × 5, 3 minutes × 5, 6 minutes × 5, and 10 minutes × 13) for $[^{18}F]$FMeNER-D_2.

Data Analysis

Regions of interest were delineated on MRI for putamen (target) and cerebellum (reference) for $[^{11}C]$MADAM, and thalamus (target) and caudate (reference) for $[^{18}F]$FMeNER-D_2. The summed PET images of whole scanning were co-registered to the MRI of the individual NHP. After applying the co-registration parameters to the dynamic PET data, the time-activity curves of brain regions were generated for each PET measurement. Binding potential (BP_{ND}) was quantified by the simplified reference tissue model. Occupancy (%) was calculated using the following equation: occupancy (%) = $(BP_{ND,baseline} - BP_{ND,tramadol}) / BP_{ND,baseline} \times 100$. The relationship between dose of tramadol and occupancy was estimated by the following equation: occupancy (%) = dose / (K_d + dose) × 100. K_d is the dose to occupy 50% of the target transporter. The analyses of imaging data were performed using PMOD (version 3.6; PMOD Technologies, Zurich, Switzerland).

Results

The injected radioactivity was 156.0 ± 5.8 (mean ± SD) (range, 146–165) MBq (n = 8) and 153.9 ± 5.4 (146–163) MBq (n = 7) for $[^{11}C]$MADAM and $[^{18}F]$FMeNER-D_2, respectively. No obvious change in physiological parameters was observed after administration of tramadol. The dose of tramadol, BP_{ND} at baseline and drug conditions, and occupancy for both $[^{11}C]$MADAM and $[^{18}F]$FMeNER-D_2 are shown in Table 1. Tramadol occupied similarly both 5-HTT (40%–72%) and NET (7%–73%) in a dose-dependent manner. The K_d was 2.2 mg/kg for 5-HTT and 2.0 mg/kg for NET (Figure 1).

Table 1. Dose, BP_{ND} at Baseline, and Tramadol Conditions and Occupancy

A) $[^{11}C]$MADAM

NHP	Dose (mg/kg)	BP_{ND} Baseline	BP_{ND} Tramadol	5-HTT occupancy (%)
NHP1	4.0	1.16	0.33	72
	2.0	1.01	0.50	51
NHP2	4.0	1.05	0.56	46
NHP3	1.0	1.18	0.71	40

B) $[^{18}F]$FMeNER-D_2

NHP	Dose (mg/kg)	BP_{ND} Baseline	BP_{ND} Tramadol	NET occupancy (%)
NHP4	4.0	0.72	0.19	73
	2.0		0.28	62
NHP5	4.0	0.73	0.21	71
NHP6	1.0	0.69	0.64	7

Abbreviations: BP_{ND}, binding potential; NHP, nonhuman primate.

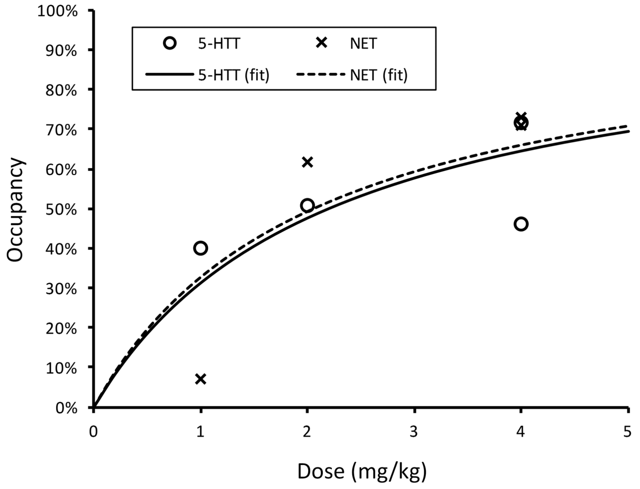

Figure 1. The relation between dose and occupancy for serotonin transporter (5-HTT) and norepinephrine transporter (NET). K_d was 2.2 mg/kg for 5-HTT and 2.0 mg/kg for NET.

Discussion

This study demonstrates that 5-HTT and NET of in vivo NHP brain were blocked by the administration of tramadol in a dose-dependent manner. Both 5-HTT and NET occupancies reached >70% at 4 mg/kg of tramadol, and the in vivo affinity (K_d) was almost identical between 5-HTT and NET.

Several PET studies have demonstrated that >70%–80% of 5-HTT occupancy by antidepressant showed the clinical effect of treatment for depression (Suhara et al., 2003; Meyer et al., 2004; Lundberg et al., 2012). Maximal clinical dose of tramadol is reported as 400 mg (Grond and Sablotzki, 2004). If the K_d for 5-HTT obtained in the current study (2.2 mg/kg) is applied to 60 kg weight of human subjects, 400 mg of tramadol would correspond to 75% 5-HTT occupancy, although direct application of NHP data to human subjects may oversimplify the different conditions of both species. However, previous human PET study reported that the K_d for 5-HTT by tramadol is 98.1 mg (Ogawa et al., 2014), which is relatively close to our result of 2.2 mg/kg (132 mg as a 60 kg human). This supports that our results from NHP would be able to extend to the human. Further evaluation of 5-HTT occupancy for higher doses of tramadol in patients will be helpful to confirm it.

This study also showed that tramadol occupied the NET in the NHP brain. Although previous studies reported the NET occupancy by antidepressants in patients with depression (Nogami et al., 2013; Takano et al., 2014), the threshold of NET inhibition for the antidepressant effect remains unclear compared with the evidence for 5-HTT occupancy. One study of them reported that the NET occupancy by clinical doses of nortriptyline, which has higher NET selective affinity than 5-HTT, was 50%–70% for patients with depression (Takano et al., 2014). Our present results also showed 70% of NET occupancy, expecting that the similar antidepressant effect as nortriptyline through the NET inhibition can be achieved by clinical doses of tramadol.

The degree of 5-HTT and NET occupancy was comparative in this study. The reported value of in vitro affinity (K_i) for 5-HTT and NET was 0.99 and 0.79 μM, respectively (Raffa et al., 1992). This ratio of 5-HTT and NET (0.99/0.79 = 1.3) is similar to that of in vivo affinity (K_d) of our study (2.2/2.0 = 1.1). A previous microdialysis study also reported a similar dose dependency of increase of 5-HT and NE by administration of tramadol (Bloms-Funke et al., 2011). All these studies showed that tramadol has almost the same potency for 5-HTT and NET.

In this study, maximal occupancy was set to 100%. This assumption would be reasonable because both radioligands showed almost full blockade in a previous study (Takano 2013). When the maximal occupancy is also fitted as occupancy (%) = dose / (K_d + dose) × O_{max}, O_{max} is estimated as 70% and 86% for 5-HTT and NET, respectively. Possible reasons why fitted maximal occupancies of 5-HTT and NET do not reach 100% are small sample size and no data of high doses, which can induce higher occupancy than the estimated maximal occupancy.

The clinical studies about evaluating tramadol for the treatment of depression are limited. Only some case studies were reported (Spencer, 2000; Shapira et al., 2001; Reeves and Cox, 2008; Rougemont-Bücking et al., 2017), although a large study such as randomized controlled trial has not been reported. Clinical trials with tramadol for treatment of depression will be needed to confirm our finding.

There are some limitations in this study. First, we did not measure the plasma concentration of tramadol. It should be performed in the future to estimate the relationship between the drug exposure and brain occupancy more accurately. Second, tramadol has an active metabolite, desmethyl-tramadol, which reportedly showed the effect for μ-opioid receptor as well as 5-HTT and NET (Grond and Sablotzki, 2004). Desmethyl-tramadol was detected in rodent brain after oral administration of tramadol (Tao et al., 2002). The present study could not evaluate the contribution of desmethyl-tramadol to the brain occupancy. Third, the i.v. injection was used as the administration route of tramadol in this study. The difference of metabolism and bioavailability between oral and i.v. administration of tramadol using NHP was reported (Kelly et al., 2015). Further study of oral administration of high dose of tramadol in the human subjects will be needed to confirm the present results, especially of NET occupancy. Fourth, we used i.m. administration of ketamine for the induction of anesthesia. The previous PET study reported that 5-HTT binding decreased after i.v. ketamine administration in conscious NHP (Yamamoto et al., 2013). Although the dose and administration route of ketamine and the anesthesia state of NHP were different from the previous report, the change of 5-HTT binding by ketamine could not be excluded, especially in the baseline condition. Fifth, the specific binding of [^{18}F]FMeNER-D_2 was relatively low compared with other PET radioligands such as [^{11}C]raclopride. The one NET occupancy showed only 7%, although other values were >60%. When this low value is excluded as outlier, K_d for NET will increase to 1.4 mg/kg. The maximum likelihood estimation with arterial input function is a useful method for high noise data and/or low occupancy (Naganawa et al., 2017), although the method is not applied in this study due to the lack of arterial blood sampling.

In conclusion, this study demonstrates that both 5-HTT and NET of in vivo NHP brain were blocked at >70% at a clinically relevant high dose of tramadol. The present result suggests that treatment by tramadol has potential antidepressant effects through the inhibition of 5-HTT and NET in the brain.

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Statement of Interest

None.

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