Occupancy of serotonin transporter by tramadol: a positron emission tomography study with \([^{11}\text{C}]\)DASB

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Abstract

Tramadol is used for the treatment of pain, and it is generally believed to activate the \(\mu\)-opioid receptor and inhibit serotonin (5-HT) and norepinephrine (NE) transporters. Recent findings from animal experiments suggest that 5-HT reuptake inhibition in brain is related to pain reduction. However, there has been no report of 5-HT transporter (5-HTT) occupancy by tramadol at clinical doses in humans. In the present study, we investigated 5-HTT occupancy by tramadol in five subjects receiving various doses of tramadol by using positron emission tomography (PET) scanning with the radioligand \([^{11}\text{C}]\)DASB. Our data showed that mean 5-HTT occupancies in the thalamus by single doses of tramadol were 34.7\% at 50 mg and 50.2\% at 100 mg. The estimated median effective dose (ED\(_{50}\)) of tramadol was 98.1 mg, and the plasma concentration was 0.33 \(\mu\)g/ml 2 h after its administration; 5-HTT occupancy by tramadol was dose-dependent. We estimated 5-HTT occupancy at 78.7\% upon taking an upper limit dose (400 mg) of tramadol. The results of the present study support the finding that 5-HTT inhibition is involved in the mechanism underlying the analgesic effect of tramadol in humans, and a clinical dose of tramadol sufficiently inhibits 5-HTT reuptake; this inhibition is similar to that shown by selective serotonin reuptake inhibitors (SSRIs).

Received 13 September 2013; Reviewed 8 November 2013; Revised 1 December 2013; Accepted 16 December 2013; First published online 15 January 2014

Key words: Occupancy, pain, positron emission tomography, serotonin transporter, tramadol.

Introduction

Tramadol, ((1R,5S,2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol), a centrally acting analgesic with lower dependence liability than that associated with morphine, was developed by Grünenthal Pharma GmbH & Co. (Germany) in 1977. This compound gained approval and has been marketed as injections and oral preparations in more than 100 countries around the world. Tramadol is used extensively for relief and palliation of cancer pain (Tassinari et al., 2011) and for postoperative pain management (Ridgway, 2004).

Tramadol is generally believed to exert analgesic effects via activation of the \(\mu\)-opioid receptor and inhibition of serotonin (5-HT) and norepinephrine (NE) reuptake (Driessen and Reimann, 1992; Raffa et al., 1992; Lee et al., 1993; Frink et al., 1996; Bamigbade et al., 1997; Oliva et al., 2002). Tramadol and its major metabolite M1 (O-desmethyl-tramadol) have the strongest affinity for the \(\mu\)-opioid receptor (Raffa et al., 1993). Tramadol’s affinity for the \(\mu\)-opioid receptor (Ki value=2.1 \(\mu\)mol/l) is several hundred-fold lower than that of morphine (Frink et al., 1996), but the equipotent dose ratio of tramadol compared with morphine is expected to be 4:1 (Lehmann, 1994; Grond and Sablotzki, 2004). Thus, the potency ratio is higher than would be predicted if tramadol acted only through the \(\mu\)-opioid receptor, implying that tramadol exerts its analgesic effect via additional pathways. In addition to the opioid receptor-mediated analgesic effect, tramadol may act synergistically via activation of the descending pain inhibitory system and diminution of afferent nociceptive stimuli by inhibition of 5-HT (Ki value=0.99 \(\mu\)mol/l) and NE (Ki value=0.79 \(\mu\)mol/l) reuptake (Driessen and Reimann, 1992; Raffa et al., 1993; Frink et al., 1996; Bamigbade et al., 1997; Leventhal et al., 2007).

Recent findings from animal experiments suggest that the 5-HT reuptake inhibition in the brain is related to a reduction of pain (Matsuzawa-Yanagida et al., 2008; Hayashi et al., 2009; Hache et al., 2012). However, there
Table 1. Sex, age, administered dose of tramadol; Conc., plasma concentration of tramadol (μg/ml); BP
ND, binding potential relative to non-displaceable binding; Occu., serotonin transporter (5-HTT) occupancy (%).

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Dose of tramadol (mg)</th>
<th>Conc. (μg/ml)</th>
<th>BPND</th>
<th>Occu. (%)</th>
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</tbody>
</table>

is no report of in vivo human brain 5-HT transporter (5-HTT) occupancy by tramadol. We investigated 5-HTT occupancy and the pharmacological mechanisms of tramadol in healthy subjects by using positron emission tomography (PET) with the radioligand [11C]DASB.

**Materials and methods**

**Subjects**

Five volunteers participated (three females, two males; mean age ± S.D., 29.6 ± 4.6; Table 1). All subjects were free of any somatic, neurological or psychiatric disorders and they had no history of current or previous drug abuse according to examinations by physicians (KO and AT). After complete description of this study, written informed consent was obtained from all subjects. The study was approved by the institutional review board of Nippon Medical School and was accomplished in compliance with the latest revision of the Declaration of Helsinki.

**Study design**

The subjects underwent three PET scans, two on the 1st day of the schedule, and the 3rd ≥ 2 wk later. Single doses of tramadol immediate release capsules at 50 mg (50 mg × 1 capsule) and 100 mg (50 mg × 2 capsules) were administered alternately to all subjects orally. The 1st PET scan was performed before drug administration. The subjects were randomly assigned to receive 50 or 100 mg of tramadol, and the 2nd PET scan was performed 4 h after the beginning of the 1st scan. Then the subjects were crossed over to the other dose level, i.e. those who had received 50 mg of tramadol for the 2nd PET scan were now assigned to receive 100 mg of tramadol and vice versa. The 2nd and 3rd PET scans were performed 2 h after drug administration because the time-to-maximum blood concentration (Tmax) of tramadol is about 2 h (Grond and Sablotzki, 2004).

**PET procedure**

A PET scanner system, Eminence SET-3000GCT/X (Shimadzu Corp., Japan), was used to measure regional brain radioactivity. Each scan was preceded by a 4 min transmission scan for attenuation correction using a 137Cs source. After an i.v. bolus injection of [11C]DASB, brain radioactivities were measured up to 90 min (1 min × 4, 2 min × 13, 4 min × 5, 8 min × 5). Injected radioactivity was 344.0–381.5 MBq (mean ± S.D., 365.3 ± 10.5 MBq). Specific radioactivity was 13.3–69.5 GBq/μmol (mean ± S.D., 34.8 ± 16.8 GBq/μmol) at the time of injection. Magnetic resonance imaging (MRI) of the brain was performed with a 1.5 T MR scanner, Intera 1.5 T Achieve Nova (Philips Medical Systems, The Netherlands). T1-weighted images were obtained at 1 mm slices. The MRI results revealed no apparent structural abnormalities.

**Plasma concentration of tramadol**

Venous blood samples were taken 2 h after administration of tramadol (just before the 2nd and 3rd PET scans, respectively), collected in tubes containing EDTA-2Na, and centrifuged for 10 min at 3000 r/min at 4 °C. Separated plasma samples were stored at −80 °C until analysis. The plasma concentrations of tramadol were determined by gas chromatography–mass spectrometry (6890/5973 GC/MS; Agilent Technologies, USA) with a lower limit of quantification of 0.01 μg/ml (Mitsubishi Chemical Medience Corp., Japan).

**Data analysis**

All MR images were co-registered to the PET images using the software package PMOD 3.4 (PMOD Technologies, Switzerland). Volumes of interest (VOIs) were drawn manually on summed PET images with reference to co-registered MR images and were defined for the thalamus and cerebellum. Different persons (KO, AT and RA) verified the accuracy of the VOI settings twice. Regional radioactivity was calculated for each frame, corrected for decay and plotted vs. time. [11C]DASB bindings were expressed as binding potentials relative to non-displaceable binding (BPND) (Innis et al., 2007). BPND of [11C]DASB in the thalamus was calculated using the simplified reference-tissue model method (Lammertsma and Hume, 1996). We used the cerebellum as the reference brain region because of its negligible 5-HTT density (Kish et al., 2005; Parsey et al., 2006). The occupancies of 5-HTT were calculated by the following equation:

Occu(%) = (BPbaseline − BPdrug)/BPbaseline × 100.
where Occu (%) is the occupancy of 5-HTT, BP_{baseline} is BP_{ND} before administration of tramadol, and BP_{drug} is BP_{ND} after its administration. The relationships between dose or plasma concentration and occupancies of 5-HTT were modeled by the following equation:

$$\text{Occu} (%) = \frac{D}{(\text{ED}_{50} + D)} \times 100,$$

where D is the dose or plasma concentration of tramadol, and median effective dose (ED_{50}) is the dose or plasma concentration required to induce 50% occupancy (Suhara et al., 2003; Takano et al., 2006a).

**Results**

Typical summed PET images of [11C]DASB with control subjects (no drug), tramadol at 50 mg and at 100 mg are shown in Fig. 1. Plasma concentration of tramadol (μg/ml), BP_{ND} and 5-HTT occupancy are shown Table 1.

5-HTT occupancy by tramadol in the thalamus was 29.9–38.7% (mean±s.d., 34.1±3.8%) at 50 mg and 37.1–59.8% (mean±s.d., 50.2±8.7%) at 100 mg. The relationships between administered dose or plasma concentration of tramadol and 5-HTT occupancy are shown in Fig. 2a, b. Estimated ED_{50} of tramadol was 98.1 mg in administered dose and 0.33 μg/ml in plasma concentration 2 h after drug administration.

**Discussion**

This study revealed that, on average, approximately 30 and 50% of 5-HTT were occupied by tramadol after single-dose administrations of tramadol at 50 and 100 mg, respectively (Table 1 and Fig. 2a). The plasma concentrations of tramadol and its occupancy of 5-HTT after single-dose administrations of 50 and 100 mg are shown in Fig. 2b, which illustrates a dose-dependent...
increase in 5-HTT occupancy by tramadol. Thus, in vivo experiments confirmed that tramadol inhibited 5-HTT in the human brain.

In an experiment conducted using rat brain, the Ki value of tramadol against the μ-opioid receptor was reported to be several hundred-fold lower than that of morphine, whereas, in terms of the clinical analgesic effects, the equipotent dose ratio of tramadol compared with morphine is expected to be 4:1 (Lehmann, 1994; Grond and Sablotzki, 2004). This suggests that tramadol exerts an analgesic effect through multiple mechanisms other than μ-opioid receptor agonistic activity, which would include 5-HT and NE reuptake inhibition in the descending pain inhibitory system (Driessen and Reimann, 1992; Raffa et al., 1992; Frink et al., 1996; Bamigbade et al., 1997; Oliva et al., 2002).

In the present study we showed that tramadol occupies 5-HTT in the human brain. Although there is a study that reported the relationship between pain and 5-HTT in the human brain using PET (Kupers et al., 2011), it is not clear how 5-HTT is related to the analgesic effect. More recently, it was reported that emotion and pain are closely linked through the 5-HT system (Bushnell et al., 2013). Selective serotonin reuptake inhibitors (SSRIs), one category of antidepressants, have been reported to exert an analgesic effect by influencing emotion (Keefe et al., 2011). Like SSRIs, occupying 5-HTT in human brain with tramadol would have some kind of influence on emotion via the 5-HT system. In this regard, tramadol would exercise an analgesic effect via a mechanism of action other than opioid receptors. To clarify the direct relationship between the analgesic effect of tramadol and the occupancy of 5-HTT in human brain, it will be necessary to investigate the degree of 5-HTT occupancy required for an analgesic effect to be exerted.

Another interesting finding of this study is that a therapeutic dose of tramadol occupied 5-HTT at a level similar to that shown by milnacipran, which is used as a serotonin norepinephrine reuptake inhibitor (SNRI). 5-HTT occupancy by 25–200 mg of milnacipran, within clinical dosage, in the thalamus is 33.0–61.5% (Nogami et al., 2012). On the other hand, we showed that 5-HTT occupancy by an initial dose of 50 or 100 mg tramadol was 29.9–59.8%, similar to that of a clinical dose of milnacipran. Several case reports have described tramadol as exhibiting antidepressant effects in patients with major depression (Pinto et al., 1996; Spencer, 2000; Shapira et al., 2001; Reeves and Cox, 2008). In previous studies which investigated 5-HTT occupancy by SSRIs, 5-HTT occupancy exceeded 80% in clinically responsive patients with major depression (Meyer et al., 2001, 2004; Suhara et al., 2003; Takano et al., 2006a, b). It has been reported that the average plasma concentration of tramadol in three healthy subjects at the time of taking 400 mg was 1.22 μg/ml (Grond and Sablotzki, 2004). On the basis of these data and the ED₅₀ obtained in the present study, we estimated the 5-HTT occupancy to be 78.7%.

Our result suggests that tramadol at an upper limit dose per day exhibits a level of 5-HTT inhibition similar to that exhibited by SSRIs, and that tramadol possesses a potent 5-HTT inhibitory action comparable to that of known SSRIs.

There are several limitations to our study. Meyer et al., reported a relationship between 5-HTT occupancy and the antidepressant effect of SSRI in the striatum (Meyer et al., 2001, 2004). However, in this study, we measured 5-HTT occupancy in the thalamus in order to compare the effects of tramadol and milnacipran. Thalamus has the highest specific binding of 5-HTT (Houle et al., 2000). Takano et al., reported no significant regional differences in 5-HTT occupancy by fluvoxamine (Takano et al., 2006a, b). In addition, previous PET studies have reported 5-HTT occupancy in the thalamus to be >80% in clinically responded patients with SSRIs, tricyclic antidepressants (TCAs) and SNRIs (Suhara et al., 2003; Takano et al., 2006a, b). Regional differences of 5-HTT occupancy by tramadol should be discussed in the future. Compounds that exert analgesic effects via mechanisms involving 5-HTT and NE transporter (NET) inhibitory activities, such as tricyclic antidepressants and SNRIs, have been shown to be clinically effective as adjuvant analgesics (Magni et al., 1987; Ventafridda et al., 1987; Kalso et al., 1996), and they have been widely used. However, we did not investigate the role of NE in this study. Previous animal experiments have shown that tramadol also has high affinity for NET (Frink et al., 1996; Trivedi et al., 2008). In humans, NE is considered to play a role in one of the mechanisms of the action of antidepressants, and thus NET occupancy in human brains was investigated using PET (Sekine et al., 2010; Nogami et al., 2012). However, the relationship between antidepressant effects and NET occupancy has not yet been clarified.

The relationship between NET inhibition and analgesic effect in human brains also remains to be studied. Investigation of NET occupancy by tramadol will provide clues for elucidating the role of NE in its analgesic and antidepressant effects. Furthermore, we did not evaluate the relationship between pain and the effect of tramadol administration. It is difficult to evaluate pain objectively and quantitatively because it is subjective in nature. Therefore, the development of appropriate evaluation methods, such as a combination of PET and functional MRI, for patients experiencing pain or subjects experiencing quantitative pain stimulation is also necessary. Several studies concerning 5-HTT radioligand binding possibly depending on biological factors such as the hormone status of females and 5-HTT polymorphisms were reported recently (Praschak-Rieder et al., 2007; Jovanovic et al., 2009). However, in this study, we did not evaluate those factors.

In conclusion, in vivo experiments confirmed the inhibition of 5-HTT by tramadol in the human brain, suggesting that tramadol exerts an analgesic effect through mechanisms other than opioid receptor agonistic activity.
Our results suggest that tramadol possesses potent 5-HTT inhibitory activity comparable to that of SSRIs, and it may be effective against depression when administered within a clinical-dose range. Further investigation of 5-HTT occupancy by tramadol will provide clues concerning the role of 5-HTT in tramadol’s analgesic and antidepressant effects.

Acknowledgments

This work was partially supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology (MEXT, Japan). We thank Mr Koji Nagaya, Mr Koji Kanaya, Mr Masaya Suda, Ms Megumi Takei, Mr Kazuyoshi Honjo and Mr Minoru Sakurai (Clinical Imaging Center for Healthcare, Nippon Medical School, Tokyo, Japan) for their assistance in performing the PET experiments and MRI scanning, Ms Michiyo Tamura (Nippon Medical School Hospital, Tokyo, Japan) for her help as a clinical research coordinator, Dr Tsuyoshi Nogami (Department of Neuropsychiatry, Nippon Medical School, Tokyo, Japan) for technical advice, and Dr Shinji Kageyama for the measurement of plasma concentrations of tramadol (Mitsubishi Chemical Medience Corp., Tokyo, Japan).

Statement of Interest

This work was partially supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology (MEXT, Japan). Dr Suzuki has received speaker’s honoraria from Pfizer and Eisai within the past 3 yr. Dr Okubo has received grants or speaker’s honoraria from Dainippon Sumitomo Pharma, GlaxoSmithKline, Janssen Pharmaceutical, Otsuka, Pfizer, Eli Lilly, Astellas, Yoshitomi and Meiji within the past 3 yr. For the remaining authors none were declared.

References


