Protective Effects of Minocycline on the Reduction of Dopamine Transporters in the Striatum After Administration of Methamphetamine: A Positron Emission Tomography Study in Conscious Monkeys

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Background: Positron emission tomography (PET) studies of methamphetamine (METH) abusers suggest that psychotic symptoms of METH abusers may be attributable to the reduction of dopamine transporters (DAT) in the human brain. However, there are currently no particular pharmacological treatments for the wide range of symptoms associated with METH abuse.

Methods: Using a PET study in conscious monkeys, we investigated whether the second generation antibiotic minocycline could protect against the reduction of DAT in monkeys treated with METH (2 mg/kg × 3, 3-hour intervals).

Results: Pretreatment and subsequent administration of minocycline significantly attenuated the reduction of DAT in the striatum of monkeys treated with METH. Furthermore, posttreatment and subsequent administration of minocycline also significantly attenuated the reduction of DAT. In contrast, repeated administration of minocycline alone did not alter the density of DAT in the striatum of monkeys treated with METH.

Conclusions: Our findings suggest that minocycline protects against METH-induced neurotoxicity in the monkey brain. Therefore, minocycline is likely to be a promising therapeutic agent for the treatment of several symptoms associated with METH use in humans.

Key Words: Dopamine transporter, methamphetamine, minocycline, monkey brain, neurotoxicity, positron emission tomography

Methamphetamine (METH) abuse has become a major public health problem worldwide, as demonstrated by increases in the number of emergency room visits, substance abuse treatment episodes, and arrests attributable to METH manufacture and abuse. However, there are currently no particular pharmacological treatments for the wide range of symptoms associated with METH abuse (National Institute on Drug Abuse 2002). Multiple lines of evidence indicate that dopamine (DA) plays a key role in a variety of motivated behaviors associated with abused drugs, including METH (Nestler 2001, 2002; Pierce and Kumaresan 2006). In addition, it is well known that METH elevates extraneuronal DA concentrations through its actions on the plasma membrane DA transporter (DAT) (Davidson et al 2001; Hanson et al 2004; Mortensen and Amara 2003).

Positron emission tomography (PET) studies of METH users have demonstrated that the reduction of DAT in the striatum is associated with motor and cognitive impairment (Volkow et al 2001) and that the reduction of DAT is also associated with the duration of METH use and the severity of psychiatric symptoms (Sekine et al 2001, 2003). These findings suggest that psychotic symptoms of METH users may be attributable to the reduction of DAT in the brain. Furthermore, it has been demonstrated that the densities of DAT in the striatum are significantly decreased in the postmortem brains of chronic METH users (Wilson et al 1996). Thus, although METH-induced neurotoxicity in the dopaminergic terminals is well documented, the precise mechanism underlying METH-induced neurotoxicity remains unknown (Cadet et al 2003). In addition, chronic METH users show severe structural and functional deficits in areas of the brain associated with emotion, especially depression and anxiety, as well as memory (London et al 2004). From the point of view of developing novel pharmacological interventions for the treatment or prevention of METH abuse, it is necessary to develop therapeutic drugs to protect against the reduction of DAT in the brain associated with METH use.

Minocycline is a second-generation tetracycline that has been in use for over 30 years. This drug easily crosses the blood-brain barrier and has powerful neuroprotective properties in several models of neurological diseases, including Parkinson disease, Huntington disease, amyotrophic lateral sclerosis (ALS), and ischemic stroke (Blum et al 2004; Domercq and Matute 2004; Stirling et al 2005; Yong et al 2004). For example, the impressive therapeutic effects of minocycline have been demonstrated in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of Parkinson's disease (Du et al 2001; Wu et al 2002). Minocycline mitigates both the demise of nigrostriatal dopaminergic neurons and the formation of nitrotyrosine produced by MPTP (Wu et al 2002). In addition, minocycline not only prevents MPTP-induced activation of microglia but also the formation of mature interleukin-1β and the activation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and induction of nitric oxide synthase (iNOS), three key microglial-derived cytotoxic mediators (Wu et al 2002). Thus, it is likely that a blockade of microglial activation by minocycline plays a role in the neuroprotective actions of this drug (Tikka et al 2001; Wu et al 2002; Zhu et al 2002). On the other hand, direct neuronal

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protection by minocycline has been documented and probably involves the preservation of mitochondrial integrity and cytochrome c, followed by the suppression of caspase-dependent as well as caspase-independent cell death (Chen et al 2000; Wang et al 2003). Taken together, these findings indicate that minocycline holds great promise as a therapeutic drug for the treatment of human neurodegenerative diseases (Blum et al 2004; Domercq and Matute 2004; Stirling et al 2005; Yong et al 2004).

The current study was conducted to investigate whether minocycline could protect against the reduction of DAT in monkeys treated with METH. For this purpose, we used PET imaging in conscious monkeys.

Methods and Materials

Subjects

Ten young-adult male rhesus monkeys (*Macaca mulatta*) weighing from 4 kg to 6 kg were used for PET measurements. Monkeys were maintained and handled in accordance with the recommendations of the US National Institutes of Health and also the guidelines of the Central Research Laboratory, Hamamatsu Photonics (Hamamatsu, Shizuoka, Japan). The monkeys were trained to sit on a chair by means of twice-weekly training sessions over the course of 3 months. The magnetic resonance images (MRI) of all monkeys were obtained with a Toshiba MRT-50A/II (.5T) (Toshiba Medical Systems Corporation, Tokyo, Japan) under anesthesia with pentobarbital. The stereotactic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line with monkeys secured in a specially designed head holder (Takechi et al 1994). At least 1 month before the PET study, an acrylic plate, with which the monkey was fixed to the monkey chair, was attached to the head under pentobarbital anesthesia as described previously (Onoe et al 1994).

Drug Administration

Methamphetamine hydrochloride (Dainippon Pharmaceuticals Ltd., Osaka, Japan) was administered intramuscularly (2 mg/kg as a salt, three times at 3-hour intervals) into each monkey using the previously reported method (Hashimoto et al 2004) with slight modifications. This dose regimen of METH closely approximates the binge use of METH by some humans (20 to 40 mg every 2 to 3 hours) (Konuma 1994).

In the first trial, which consisted of minocycline pretreatment, METH administration, and subsequent minocycline administration, subjects ($n = 3$) received minocycline (Wako Pure Chemicals Ltd., Tokyo, Japan; 200 mg, subcutaneous [SC], 0800 hours) or vehicle (physiological saline 1 mL, SC, 0800 hours) as a control condition 30 minutes before administration of METH and a subsequent administration of minocycline (200 mg, SC, twice daily [b.i.d.], 0800 and 2000 hours) or vehicle according to the method reported previously with slight modifications (Diguet et al 2004) (Figure 1A). In the second trial, which consisted of METH administration, minocycline posttreatment, and subsequent minocycline administration, subjects ($n = 2$) received minocycline (200 mg, SC, 2000 hours) 30 minutes after the final administration of METH, followed by subsequent administration of minocycline (200 mg, SC, b.i.d., 0800 and 2000 hours) or vehicle. In both experiments, the subsequent administration of minocycline was performed for 6 consecutive days (Figure 1B; day 2 to day 7). In the third trial, to examine the effect of minocycline alone on the DAT in the monkey brain, subjects ($n = 2$) received minocycline (200 mg, SC, b.i.d, 0800 and 2000 hours) for 7 days (Figure 1C).

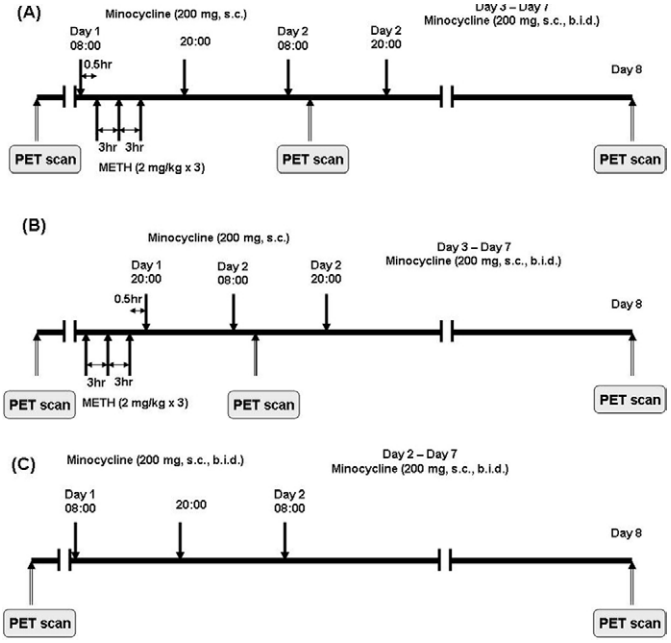

Figure 1. Treatment schedule of METH and/or minocycline in the monkeys. **(A)** Minocycline (200 mg, SC, 0800 hours) or vehicle (saline; 1 mL) was administered to monkeys ($n = 3$). Thirty minutes after injection, METH (2 mg/kg × 3, 3-hour intervals) was administered to the subjects (day 1). Then, minocycline (200 mg, SC, 2000 hours) or vehicle (saline; 1 mL) was administered to the monkeys (day 1). Minocycline (200 mg, b.i.d., 0800 and 2000 hours) was administered daily for 6 consecutive days (day 2 to day 7). **(B)** METH (2 mg/kg × 3, 3-hour intervals) was administered to the monkeys ($n = 2$). Thirty minutes after injection, minocycline (200 mg, SC, 2000 hours) was administered to the subjects (day 1). Then, minocycline (200 mg, b.i.d., SC, 0800 and 2000 hours) was administered daily for 6 consecutive days (day 2 to day 7). **(C)** Minocycline (200 mg, SC, 0800 and 2000 hours) was administered to the monkeys ($n = 2$) (day 1 to day 7). METH, methamphetamine; SC, subcutaneous; b.i.d., twice daily.

Synthesis of [^{11}C]-Labeled Compounds

Carbon-11 (^{11}C) was produced by ^{14}N (p,α)^{11}C nuclear reaction using a cyclotron (HM-18; Sumitomo Heavy Industries, Tokyo, Japan) at the Hamamatsu Photonics PET Center and obtained as [^{11}C]CO2, which was converted to [^{11}C]methyl iodide. [^{11}C]2β-carbomethoxy-3β-(4-fluorophenyl)tropane (β-CFT) (for DAT) and [^{11}C]SCH 23390 (for DA D_1 receptors) were synthesized as previously reported (Harada et al 2002; Tsukada et al 2001). The radiochemical and chemical purities of labeled compounds were greater than 98% and 99%, respectively. After analysis for identification, the solution was passed through a .22-μm pore size filter before intravenous administration to the monkeys.

PET Scans

Positron emission tomography data were collected before (control) and at 1 day (day 2) and 7 days (day 8) after the repeated administration of METH or METH/minocycline. In the trial in which minocycline was administered alone, PET data were collected before (control) and 7 days (day 8) after the repeated administration of minocycline. Data were collected on a high-resolution PET scanner (SHR-7700; Hamamatsu Photonics K. K., Hamamatsu, Japan) with transaxial resolution of 2.6-mm full-width at half-maximum (FWHM) and a center-to-center distance of 3.6 mm (Watanabe et al 1997). The PET camera allowed 31 imaging slices to be recorded simultaneously. After an overnight fast, animals were fixed to the monkey chair with