Table 1
Fatty acid amide hydrolase (FAAH) genotype distributions and allele frequency in patients with methamphetamine (METH) dependence/psychosis

Group	N	Genotype				Allele		
		Pro/Pro (%)	Pro/Thr (%)	Thr/Thr (%)	P-value	Pro (%)	Thr (%)	P-value
Control-1	200	139 (69.5)	58 (29.0)	3 (1.5)		336 (84.0)	64 (16.0)	
METH dependence/psychosis	153	105 (68.6)	43 (28.1)	5 (3.3)	0.57	253 (82.7)	53 (17.3)	0.68
Age of first use								
<20 years	76	49 (64.5)	25 (32.9)	2 (2.6)		123 (81.0)	29 (19.1)	
≥20 years	77	56 (72.7)	18 (23.4)	3 (3.9)	0.45	130 (84.4)	24 (15.6)	0.45
Multi-substance abuse								
No	48	30 (62.5)	17 (35.5)	1 (2.1)		77 (80.2)	19 (19.8)	
Yes	105	75 (71.4)	26 (24.8)	4 (3.8)	0.49	176 (83.8)	34 (16.2)	0.52
Latency of psychosis								
<3 years	60	43 (71.7)	16 (26.7)	1 (1.7)		102 (85.0)	18 (15.0)	
≥3 years	81	54 (66.7)	24 (29.6)	3 (3.7)	0.75	132 (81.5)	30 (18.5)	0.52
Prognosis of psychosis								
Transient	85	53 (62.4)	29 (34.1)	3 (3.5)		135 (79.4)	35 (20.6)	
Prolonged	56	43 (76.8)	12 (21.4)	1 (1.8)	0.19	98 (87.5)	14 (12.5)	0.11
Spontaneous relapse of pyschotic symptoms								
No	76	47 (61.8)	26 (34.2)	3 (3.9)		120 (78.9)	32 (21.1)	
Yes	65	50 (76.9)	14 (21.5)	1 (1.5)	0.16	114 (87.7)	16 (12.3)	0.06

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

Table 2
FAAH genotype distributions and allele frequency in schizophrenia patients

Group	N	Genotype				Allele		
		Pro/Pro (%)	Pro/Thr (%)	Thr/Thr (%)	P-value	Pro (%)	Thr (%)	P-value
Control-2	337	233 (69.1)	99 (29.4)	5 (1.5)		565 (84.0)	109 (16.0)	
Schizophrenia	260	180 (69.2)	75 (28.8)	5 (1.9)	0.91	435 (83.7)	85 (16.3)	0.94
Paranoid type	127	90 (70.9)	34 (26.8)	3 (2.4)	0.63	214 (84.3)	40 (15.7)	0.92
Hebephrenic type	127	83 (65.4)	43 (33.9)	1 (0.0)	0.61	209 (82.3)	45 (17.7)	0.62

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

consumption, latency to onset of psychosis, prognosis, spontaneous relapse, and multi-substance abuse status but found no significant association with any clinical feature. The discrepancy between the previous and present findings may result from differences of substance class. The present study analyzed methamphetamine abusers, however, Spine et al. analyzed "street-drug users". Although, they did not specify the kinds of drugs in their paper, marijuana use has been epidemic in the US, followed by cocaine and morphine. It is possible that the majority of patients examined in the previous study abuse marijuana and that is why the "street-drug use" of the previous study was associated with a mutant allele of the FAAH gene. This hypothesis should be addressed in future study. Alternatively, the ethnicity of the subjects must be considered. Our subjects were Japanese, and theirs were Caucasian. Japanese controls showed the 129Thr allele at 14.6–16.0%, compared to Caucasians at 28.3–30.6%. Homozygosity of the mutant allele was observed in only 1.5% of Japanese subjects. The rarity of the mutant homozygote in our Japanese population may result in a lack of genetic risk of the FAAH gene for Japanese "street-drug users".

No significant association with the Pro129Thr nonsynonymous polymorphism of the FAAH gene with schizophrenia was revealed. This is consistent with Sipe's study of a Caucasian population. Previously, we reported that a triplet repeat polymorphism of the CNR1 gene, which encodes the human CB1 cannabinoid receptor, was significantly associated with patients with schizophrenia, especially the hebephrenic subtype [36]. Several clinical studies have shown that exogenous cannabinoid ligands could precipitate schizophrenia, worsen prognosis, and induce relapse. Our present and previous genetic findings of the endocannabinoid system indicated that variants of cannabinoid receptors, rather than an altered endogenous agonist produced by the FAAH variant, may be important in the etiology of schizophrenia.

The power analysis showed that the present sample size had a power of 0.89 and 0.98 to detect a small effect size (w = 0.12) at an alpha value of 0.05 to detect significant allelic associations between Control-1 and total methamphetamine patients and between Conrol-2 and total schizophrenic patients, respectively. The present total sample size can be therefore considered to be large enough statistically. However, the

statistical power deteriorated in the analysis with regard to the subgroups of patients, and our results must be verified with a larger sample to conclude.

In conclusion, the cannabinoid pathway may be implicated in drug abuse, addiction, and also the pathophysiology of schizophrenia, but the Pro129Thr nonsynonymous polymorphism of the FAAH gene is not significantly associated with either methamphetamine dependence/psychosis or schizophrenia, at least in a Japanese population.

Acknowledgements

The authors are grateful to the Zikei Institute of Psychiatry (Okayama, Japan), the Ministry of Health, Labour and Welfare of Japan, and the Ministry of Education, Culture, Sports, Science and Technology of Japan for support in part by grants.

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Neuropharmacology 48 (2005) 391–397
NEURO PHARMACOLOGY
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The immunophilin ligand FK506 protects against methamphetamine-induced dopaminergic neurotoxicity in mouse striatum

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Received 3 February 2004; received in revised form 13 September 2004; accepted 29 October 2004

Abstract

Repeated use of methamphetamine (MAP) is known to cause neurotoxicity in the dopaminergic neurons of the striatum. Recently, we reported that FK506, a calcineurin inhibitor and immunosuppressive agent, could attenuate acute behavioral changes and the development of sensitization after administration of MAP. In this study, we investigated the effects of FK506 on the neurotoxicity in the dopaminergic neurons induced by repeated administration of MAP. BALB/c mice were injected subcutaneously (s.c.) with vehicle (10 ml/kg) or MAP (4 mg/kg) four times every 2 h. Vehicle (10 ml/kg) or FK506 (0.1, 0.3, 1 or 3 mg/kg i.p.) was administered 15 min before the first MAP administration. Three days later, we assessed the contents of dopamine (DA) and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in the mouse striatum using high-performance liquid chromatography (HPLC). We also examined the immunohistochemistry of dopamine transporter (DAT) and tyrosine hydroxylase (TH) in the mouse brain. Repeated administration of MAP decreased significantly the contents of DA and DOPAC in the mouse striatum, and pretreatment with FK506 inhibited significantly the reduction of DA and DOPAC in the mouse brain by repeated administration of MAP. Furthermore, repeated administration of MAP decreased significantly the immunoreactivity of DAT and TH in the striatum as compared to controls. Pretreatment with FK506 (3 mg/kg) attenuated significantly the reduction of DAT and TH immunoreactivity after repeated administration of MAP. These results suggest that FK506 shows protective effects on the MAP-induced neurotoxicity in the dopaminergic neurons of the mouse striatum.

Keywords: Methamphetamine; FK506 (tacrolimus); Dopamine; Neurotoxicity; Striatum

1. Introduction

Abuse of methamphetamine (MAP) and amphetamine is increasing worldwide. It is well known that repeated administration of MAP causes neurotoxicity in rodents and nonhuman primates by producing long-term depletion of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA), decreasing DA transporter (DAT) binding and decreasing the activity of tyrosine hydroxylase (TH) in the striatum (Davidson et al., 2001; Cadet et al., 2003). Furthermore, levels of DA and densities of TH and DAT are reduced in the

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0028-3908/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2004.10.015

post-mortem striatum of chronic MAP users (Davidson et al., 2001; Imam et al., 2001). Recent positron emission tomography (PET) studies have demonstrated that density of DAT in the caudate/putamen and nucleus accumbens of MAP users was significantly decreased as compared with normal controls (Sekine et al., 2001; Volkow et al., 2001).

Although the detailed mechanisms underlying MAP-induced dopaminergic neurotoxicity are not completely understood, several lines of evidence have suggested that the process involves excitatory amino acids, reactive oxygen species (ROS) and the neuronal nitric oxide (NO) synthase (nNOS) (Davidson et al., 2001; Cadet et al., 2003). Administration of MAP can lead to increased extracellular glutamate concentration, and the consequent *N*-methyl-D-aspartate (NMDA) receptor activation will lead to increased production of ROS, in particular through the production of NO via the coordinated action of nNOS (Davidson et al., 2001). It has been reported that glutamate receptor antagonists including (+)-MK-801 (dizocilpine) could attenuate MAP-induced neurotoxicity (Sonsalla et al., 1991; Ohmori et al., 1996). Furthermore, inhibition of nNOS by 7-nitroindazole (7-NI) prevents MAP-induced DA neurotoxicity, and mice lacking the nNOS gene are protected against MAP-induced DA neurotoxicity (Ali and Itzhak, 1998; Davidson et al., 2001).

FK506 (tacrolimus), an immunosuppressive agent, is widely used in organ transplantation. It binds to a family of proteins, including FK506 binding protein (FKBP), and then inhibits calcineurin, the Ca^{2+}-activated phosphatase, activity, resulting in the inhibition of calcium-dependent intracellular processes (Snyder et al., 1998; Klettner and Herdegen, 2003). These proteins are abundant in the brain, with localization similar to that of calcineurin (Dawson et al., 1994). Beyond immunosuppression, FK506 exerts substantial neuroprotective properties in the models of transient cerebral ischemia, excitotoxic insults, serum withdrawal, nerve fiber damage or in the presence of hydrogen peroxide (Dawson et al., 1993; Sharkey and Butcher, 1994; Furuichi et al., 2003; Klettner and Herdegen, 2003). In vitro, FK506 is also protective against NMDA excitotoxicity in cortical cultures and glutamate toxicity in retinal cultures. Additionally, FK506 provides protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-neurotoxicity in the striatum (Kitamura et al., 1994; Guo et al., 2001b). The neuroprotective effect of FK506 may be partially involved in the inhibition of calcineurin, preventing the dephosphorylation of nNOS and its subsequent activation (Guo et al., 2001b; Klettner and Herdegen, 2003). Furthermore, we reported that FK506 could suppress MAP-induced acute behavioral changes and the development of sensitization in rats, suggesting the possible role of calcineurin in MAP-induced behavioral changes and sensitization (Tsukamoto et al., 2001). However, to date nothing has been published regarding the effects of FK506 on MAP-induced dopaminergic neurotoxicity.

The present study was undertaken to investigate the effects of FK506 on the dopaminergic neurotoxicity in the mouse striatum induced by repeated administration of MAP. Because of the protective effect of hypothermia against MAP neurotoxicity, body temperature was also investigated (Albers and Sonsalla, 1995; Ali et al., 1996).

2. Methods

2.1. Animals

Male BALB/cAnNcrj (BALB/c) mice (8 weeks old, 23–29 g body weight at the beginning of the experiment; Charles River Japan Inc., Tokyo, Japan) were housed under a 12-h light–12-h dark cycle with free access to food and water. In this study, BALB/c mice were used, as they have a higher susceptibility to MAP-induced dopaminergic neurotoxicity as compared to C57BL mice (Kita et al., 1998). All experiments were performed in accordance with the Guide for Animal Experimentation, Chiba University Graduate School of Medicine.

2.2. Drugs

FK506 (Fujisawa Pharmaceutical Ltd., Osaka, Japan) was dissolved in 5% Tween 80 (polyoxyethylene sorbitan monostearate) diluted with distilled water. MAP hydrochloride (Dainippon Pharmaceutical Ltd, Osaka, Japan) was dissolved in saline. FK506 and vehicle (5% Tween 80) were injected intraperitoneally (i.p.) in a volume of 10 ml/g body weight, and MAP and saline were injected subcutaneously (s.c.) in a volume of 10 ml/kg. Other chemicals were purchased from commercial sources. The MAP dose (4 mg/kg) was expressed as a hydrochloride salt.

2.3. Drug treatment

Fifteen minutes after injection of FK506 (0.1, 0.3, 1 and 3 mg/kg) or vehicle (5% Tween 80), mice received four injections of MAP (4 mg/kg) or vehicle (saline) at 2-h intervals. Rectal temperatures were recorded 30 min before the first injection and 1 h after injection of MAP. Rectal temperature was measured using a TD-320 thermometer coupled to a rectal probe (Shibaura Electronics Co., Ltd., Saitama, Japan). We examined the effects of FK506 (1 and 3 mg/kg) alone on rectal temperature as described above.

2.4. Measurement of DA and DOPAC

The animals were sacrificed 3 days after drug treatment. Depletion of DA occurs more than 6 h after MA treatment and the depletion level remains the same as that seen 72 h after treatment for at least 10 days (Cappon et al., 2000). The brains were quickly removed and dissected on an ice-cold glass plate. The striata were isolated, weighed, frozen on dry ice and stored at −80 °C until assay. Tissue samples were homogenized by sonication in 0.2 M perchloric acid ($HClO_4$) containing 100 μM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, disodiumsalt, dihydrate (EDTA·2Na), and 100 ng/ml isoproterenol (internal standard), and were then centrifuged at 20,000 × g for 15 min at 4 °C. The supernatants were filtered through a 0.45-μm pore membrane (Millex-LH, 4 mm; Millipore, Tokyo, Japan) and analyzed for DA and DOPAC by high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD). The HPLC system consisted of a liquid chromatograph pump (EP-300, Eicom, Kyoto, Japan), a degasser (DG-300, Eicom, Kyoto, Japan), a reversed-phase column: Eicompak SC-5ODS 150 × 3.0 mm (Eicom, Kyoto, Japan), an ECD-300 electrochemical detector (Eicom, Kyoto, Japan) and a data processor (EPC-300, Eicom, Kyoto, Japan). The mobile phase was 0.1 M acetate–citric acid buffer (pH 3.5) containing 17% methanol, 5 mg/l EDTA·2Na and 190 mg/l sodium octyl sulfate.

2.5. Immunohistochemistry

Three days after the administration of drugs, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 10 ml of isotonic saline, followed by 40 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed from skulls and postfixed overnight at 4 °C in the same fixative. For DAT or TH immunohistochemistry in the striatum, serial 50-μm-thick coronal sections of brains were cut in the ice-cold 0.1 M Tris–HCl buffer saline (TBS; pH 7.5) using a vibrating blade microtome (VT1000S, Leica Microsystems AG, Wetzlar, Germany). Free-floating sections were treated with 0.3% H_2O_2 in TBS for 30 min and then blocked in TBS containing 0.2% Triton X-100, 1.5% normal goat serum (for DAT) or rabbit serum (for TH) and 0.1% bovine serum albumin (TBS–NBS) for 30 min at room temperature. The samples were incubated for 36 h at 4 °C with a primary antibody to DAT or TH. The rat anti-DAT antibodies diluted 1:10,000 (Cat. No: MAB369, Chemicon International Inc., Temecula, CA, USA) or the rabbit anti-TH antibodies diluted 1:500 (Cat. No: AB152, Chemicon International Inc., Temecula, CA, USA) in TBS–NBS were used. The sections were washed twice in TBS and then processed with the avidin–biotin–horseradish peroxidase method (Vectastain *Elite* ABC, Vector Laboratories Inc., Burlingame, CA, USA). The sections were incubated in biotinylated anti-rat or anti-rabbit secondary antisera diluted 1:200 in TBS–NBS for 1 h, incubated in avidin–biotinylated peroxidase substrate in TBS for 1 h and reacted in the solution of 0.25 mg/ml 3,3′-diaminobendine (DAB) containing 0.01% H_2O_2 for 5 min. Sections were mounted on gelatinized slides, dehydrated, cleared and coverslipped under Permount® (Fisher Scientific, Fair Lawn, NJ, USA). Sections were imaged, and the staining intensity was analyzed using the SCION IMAGE software package. To quantify the density of TH and DAT immunoreactivity, we selected the anterior regions of the striatum, since these regions contain high densities of TH and DAT (Jakowec et al., 2004).

2.6. Statistical analysis

The data were presented as the mean ± standard error of mean (S.E.M). Results of rectal temperature were analyzed by two-way analysis of variance (ANOVA), for repeated measures with treatment as the between-subjects factor and time as the within-subjects factor. When appropriate, group means at individual time points were compared by one-way ANOVA, and post hoc comparisons were performed using the Bonferroni/Dunn test. Striatal levels of DA and DOPAC and densities of DAT- and TH-immunoreactive staining intensity were analyzed by one-way ANOVA followed by the Bonferroni/Dunn test for multiple comparisons. The p values <0.05 were considered statistically significant.

3. Results

3.1. Effects of FK506 on hyperthermia induced by the administration of MAP

Repeated injections of MAP (4 mg/kg, i.p., ×4, 2-h intervals) produced hyperthermia in mice. Two-way ANOVA revealed significant differences among the six test groups [$F(20,240) = 5.51$, $p < 0.0001$]. Pretreatment with FK506 (3 mg/kg) blocked significantly ($p = 0.004$) the hyperthermia produced by MAP. Pretreatment with low-dose FK506 (0.1, 0.3 and 1 mg/kg) did not block significantly ($p = 0.092$, 0.96 and 0.34) the hyperthermia produced by MAP (Fig. 1). One-way ANOVA revealed significant differences among three groups (vehicle + saline, vehicle + MAP and FK506 (3 mg/kg) + MAP) in 60 min [$F(2,39) = 7.67$, $p = 0.016$], 180 min [$F(2,39) = 6.87$, $p = 0.0028$], 300 min [$F(2,39)$

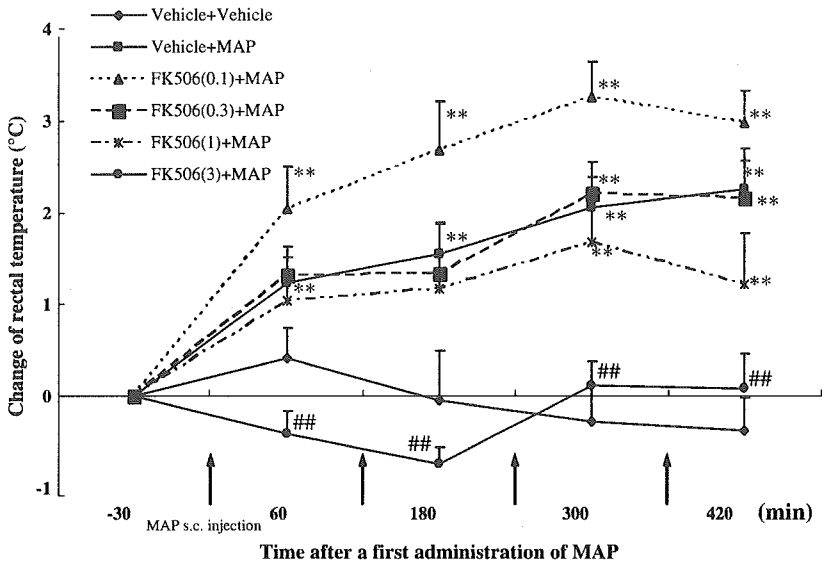

Fig. 1. Effects of FK506 on MAP-induced hyperthermia in mice. Mice received four injections of MAP (4 mg/kg, s.c.) every 2 h alone or in combination with FK506 (0.1, 0.3, 1, 3 mg/kg). FK506 was injected i.p. 15 min before the first injection of MAP. Rectal temperatures were recorded 30 min before the first injection of MAP or saline and 1 h after each MAP injection. Each value is the mean ± S.E.M. (n = 7–18 per group). $^{*}p < 0.05$, $^{**}p < 0.01$ versus saline-treated animals; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus MAP-treated animals (Bonferroni/Dunn method).

= 13.59, $p < 0.0001$] and 420 min [$F(2,39) = 18.64$, $p < 0.0001$] after the first injection of MAP. In contrast, a single administration of FK506 (1 or 3 mg/kg) alone did not alter [$F(8,56) = 0.34$, $p = 0.95$] rectal temperatures of mice (Fig. 2A).

3.2. Effects of FK506 on the reduction of striatal DA and DOPAC by administration of MAP

One-way ANOVA revealed that striatal DA [$F(5,47) = 50.44$, $p < 0.0001$] and DOPAC [$F(5,47) = 20.00$, $p < 0.0001$] levels were significantly different among saline-treated, MAP-treated and FK506-pretreated mice. Striatal DA and DOPAC levels were reduced by 82% ($p < 0.0001$) and 68% ($p < 0.0001$), respectively, in MAP-treated mice as compared to those in the saline-treated mice. Pretreatment with FK506 (1 mg/kg) attenuated significantly the reduction of DA and DOPAC to 51% ($p < 0.0001$) and 42% ($p = 0.0058$), respectively. Pretreatment with FK506 (3 mg/kg) attenuated significantly the reduction of DA and DOPAC to 50% ($p < 0.0001$) and 4% ($p < 0.0001$), respectively. The levels of DA and DOPAC in mice pretreated with FK506 (0.1 and 0.3 mg/kg) were not significantly different from those of MAP-treated mice (Fig. 3). Administration with FK506 (1 or 3 mg/kg) alone did not alter levels of DA [$F(2,14) = 1.66$, $p = 0.22$] or DOPAC [$F(2,14) = 0.10$, $p = 0.39$], or ratios of DOPAC/DA [$F(2,14) = 2.73$, $p = 0.10$] in the mouse striatum (Fig. 2B).

3.3. Immunohistochemistry

Representative microphotographs of DAT and TH immunostaining in the striatum are shown in Fig. 4. One-way ANOVA revealed significant differences of DAT [$F(2,17) = 17.26$, $p < 0.0001$] and TH [$F(2,17) = 6.47$, $p = 0.0082$] immunostaining among three groups. Three days after MAP administration, immunoreactivities of DAT and TH in the striatum were significantly reduced to 38% ($p < 0.0001$) and 54% ($p = 0.0026$) of control groups (Figs. 4B, E and 5), respectively. Pretreatment with FK506 (3 mg/kg) attenuated significantly the reduction of DAT and TH immunoreactivities to 78% ($p = 0.0021$) and 85% ($p = 0.034$) of the levels in control groups, respectively (Figs. 4C, F and 5).

4. Discussion

The results of the present study suggest that MAP-induced dopaminergic neurotoxicity in mouse striatum could be attenuated by pretreatment with FK506. A number of studies indicate that neurotoxic doses of MAP cause hyperthermia and that hypothermia could suppress MAP-induced neurotoxicity, suggesting the role of body temperature in MAP-induced dopaminergic neurotoxicity (Albers and Sonsalla, 1995; Ali et al., 1996). However, we found that FK506 alone did not alter body temperature in mice. Therefore, it is likely that pretreatment with FK506 attenuated MAP-induced neurotoxicity in mice, resulting in hypothermia in the

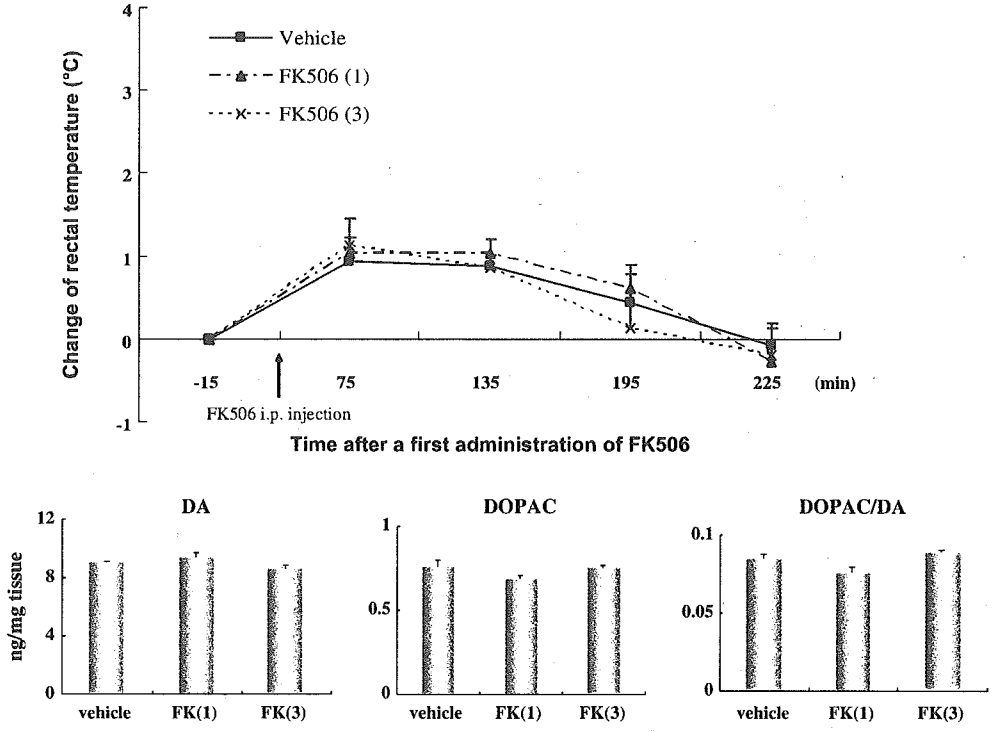

Fig. 2. (A) Effects of FK506 on body temperature in mice. Mice received vehicle (10 ml/kg) or FK506 (1 or 3 mg/kg), and body temperature in mice was measured. Each value is the mean ± S.E.M. (n = 5 or 6 per group). (B) Effects of FK506 on levels of DA, DOPAC and DOPAC/DA in the mouse striatum. Mice received vehicle (10 ml/kg) or FK506 (1 or 3 mg/kg) 3 days before decapitation. Each value is the mean ± S.E.M. (n = 5 or 6 per group).

mice pretreated with FK506. Thus, it is unlikely that the protective effect of FK506 on MAP-induced neurotoxicity may be due to the reduction of body temperature by FK506.

The immunosuppressants such as FK506 inhibit two types of enzyme, calcium/calmodulin-dependent protein phosphatase (calcineurin), and peptidyl-prolyl *cis-trans*-isomerase (PPIase: rotamase) activity such as that in the FKBP family. FK506 is protective against NMDA-induced neurotoxicity, which is partly mediated by NO, in primary neurons (Snyder et al., 1998; Klettner and Herdegen, 2003). It has been reported recently that a novel cell-permeable calcineurin autoinhibitory peptide potently inhibited calcineurin phosphatase activities and inhibited glutamate-mediated neuronal cell death (Terada et al., 2003). These findings suggest that calcineurin plays an important role in excitatory neuronal cell death and that a cell-permeable calcineurin autoinhibitory peptide could be a new drug to protect neurons from excitatory neuronal death (Terada et al., 2003). Administration of neurotoxic doses of MAP increases glutamate release in the striatum (Nash and

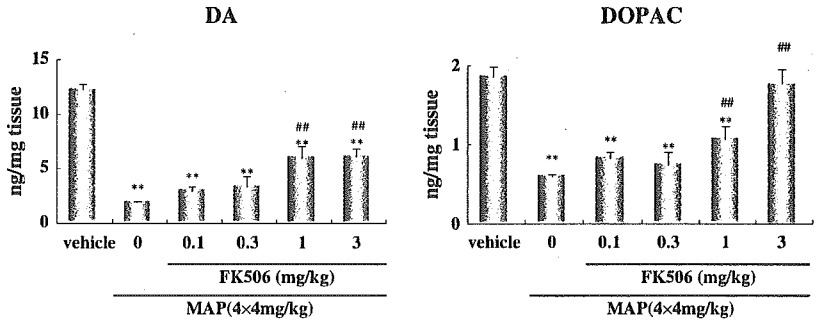

Fig. 3. Effects of FK506 on levels of striatal DA and DOPAC after administration of MAP. Mice received FK506 15 min before the first injection of MAP. Each value is the mean ± S.E.M. (n = 6–12 per group). $^{**}p < 0.01$ versus saline-treated animals; $^{\#\#}p < 0.01$ versus MAP-treated animals (Bonferroni/Dunn method).

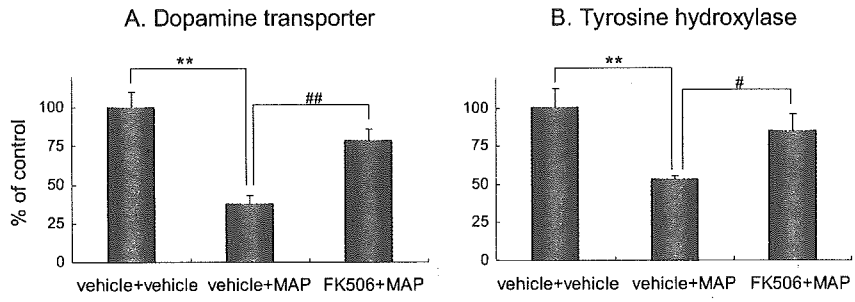

Fig. 4. Representative photomicrographs illustrating DAT (A, B and C) and TH immunoreactivity (D, E and F) in the striatum of vehicle + saline (A and D), vehicle + MAP (B and E) or FK506 + MAP (C and F). Mice received four injections of saline or MAP (4 mg/kg, s.c.) every 2 h alone or in combination with FK506 (3 mg/kg). FK506 was injected i.p. 15 min before the first injection of MAP. DAT and TH immunohistochemistry was performed 3 days after the last injections. The photographs illustrate (A) DAT-immunoreactive fibers and (D) TH-immunoreactive fibers in the striatum of the control mouse. Corresponding sections are from vehicle + MAP-treated mice (B, E) and FK506 + MAP-treated mice (C, F). Scale bar = 1 mm.

Yamamoto, 1992), and MAP-induced striatal dopaminergic toxicity could be prevented by pretreatment with NMDA antagonists (Sonsalla et al., 1991; Ohmori et al., 1996). It is also possible that the neurotoxic effects of glutamate acting through NMDA receptors involve NO, as NOS inhibitors and nNOS gene knockout block glutamate-induced toxicity (Dawson et al., 1991, 1996). Taken together, it seems that protective effects of FK506 in MAP-induced neurotoxicity could be, in part, attributed to calcineurin inhibition, since calcineurin activates NOS in the brain.

In contrast, some immunophilin ligands (e.g., GPI1046) that inhibit rotamase activity, but do not bind calcineurin, are neuroprotective against the toxic effects of MPP^+ and 6-hydroxydopamine (6-OHDA) on dopaminergic neurons in FKBP12 knockout mice (Guo et al., 2001a). Moreover, the neurotrophic action of FK506 is completely prevented by the addition of a monoclonal antibody to the immunophilin FKBP52, a component of mature steroid receptor complex (Gold et al., 1999). Considering these findings, it is likely that part of the protective action of FK506 in MAP-induced neurotoxicity may be attributed to calcineurin-independent mechanisms. Further studies using non-immunosuppressive immunophilin ligands such as GPI1046 and V10367 would be needed to define the involvement of calcineurin in the protective effects of FK506 against MAP-induced neurotoxicity.

In conclusion, these findings suggest that FK506 produces protective effects against neurotoxicity in the

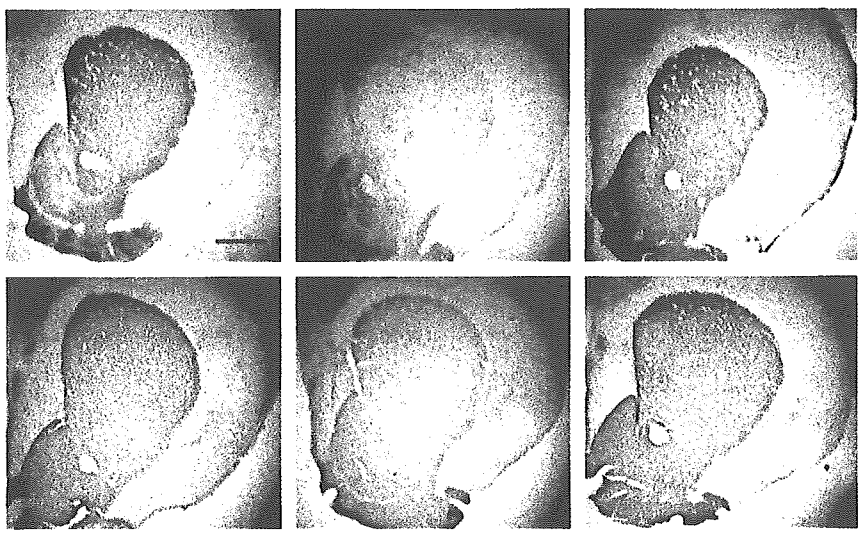

Fig. 5. Effects of FK506 on the reduction of DAT and TH immunoreactivity in the mouse striatum after administration of MAP. The mean staining of DAT and TH was determined for each experimental group and expressed as the percent of those levels in matched control mice. Each value is the mean ± S.E.M. ($n = 6$ or 7 per group). $^{**}p < 0.01$ versus vehicle + saline-treated group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus vehicle + MAP-treated group (Bonferroni/Dunn method).

mouse striatum after repeated administration of MAP, suggesting FK506 as a potential therapeutic drug for MAP-induced neurotoxicity in the brain.

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Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients

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Published: 31 January 2005

BMC Psychiatry 2005, **5**:6 doi:10.1186/1471-244X-5-6

Received: 22 September 2004

Accepted: 31 January 2005

This article is available from: http://www.biomedcentral.com/1471-244X/5/6

Abstract

Background: Recent magnetic resonance spectroscopy (MRS) studies report that glutamine is altered in the brains of schizophrenic patients. There were also conflicting findings on glutamate in cerebrospinal fluid (CSF) of schizophrenic patients, and absent for glutamine. This study aims to clarify the question of glutamine and glutamate in CSF of first episode and drug naive schizophrenic patients.

Method: Levels of glutamine and glutamate in CSF of 25 first episode and drug-naive male schizophrenic patients and 17 age-matched male healthy controls were measured by a high performance liquid chromatography.

Results: The ratio (126.1 (median), 117.7 ± 27.4 (mean ± S.D.)) of glutamine to glutamate in the CSF of patients was significantly ($z = -3.29$, $p = 0.001$) higher than that (81.01 (median), 89.1 ± 22.5 (mean ± S.D.)) of normal controls although each level of glutamine and glutamate in patients was not different from that of normal controls.

Conclusion: Our data suggests that a disfunction in glutamate-glutamine cycle in the brain may play a role in the pathophysiology of schizophrenia.

Background

Multiple lines of evidence suggest that a dysfunction in glutamatergic neurotransmission might be involved in the pathophysiology of schizophrenia [1-6]. The amino acid glutamate plays a central role in nitrogen metabolism and participates in multiple biochemical pathways. Released glutamate is taken up by glia, where it is converted to glutamine, transported back to the presynaptic neuron, and reconverted to glutamate [6,7]. Thus, it seems that glutamate-glutamine cycle plays a role in the neuron-glia communication in the synapse, and that impairment of glutamate-glutamine cycle may be implicated in the pathophysiology of schizophrenia [1-6].

By means of *in vivo* proton magnetic resonance spectroscopy (MRS), a significant increase in glutamine level was detected in the medial prefrontal cortex of never-treated schizophrenic patients compared with controls [8]. In

addition, a recent 4.0 T MRS study demonstrated that levels of glutamine in the left anterior cingulate cortex and thalamus of the never-treated patients with schizophrenia were significantly higher than those of healthy controls [9]. In contrast, significant lower levels of glutamine were found in the left anterior cingulate cortex of medicated patients with chronic schizophrenia than in the healthy controls, suggesting the role of chronic medication [10]. Thus, it is possible that the glutamate-glutamine cycle between neuron and glia may play a role in the glutamate hypothesis of schizophrenia.

Although Kim et al. [11] first reported reduction of cerebrospinal fluid (CSF) levels of glutamate in patients with schizophrenia, the findings of subsequent studies are inconsistent, with several report of no alteration in CSF levels of glutamate [12-14]. Furthermore, it was reported that a gradient for glutamate and glutamine in CSF was lack, and that there were significant correlations between the CSF and serum levels of glutamate ($r = 0.67$, $p < 0.05$) and glutamine ($r = 0.84$, $p < 0.01$)[15]. Moreover, sodium-dependent neutral amino acids transporters, located in the abluminal membranes of the blood brain barrier, are capable of actively removing neutral amino acids from the brain [16]. These findings suggest that concentration of neutral amino acids in the extracellular fluid of brain are maintained at ~10% of those of the blood [15,16].

In this study, we investigated whether levels of glutamate and glutamine or ratio of glutamine to glutamate in CSF of first episode and drug naive schizophrenic patients are different from those of age-matched healthy normal controls.

Methods

Twenty-five male patients with schizophrenia (mean age 26.1 years, range 18–39) and 17 age-matched male healthy subjects (mean age 27.3 years, range 22–44) with no psychiatric disease were enrolled in Uppsala University and Linkoping University Hospital, Sweden. All patients diagnosed according to the DSM-III-R were first episode and drug naive, i.e. they had never been treated with antipsychotic drugs. In the morning (8:00–9:00) from May 1997 to January 1998, CSF of subjects was obtained by lumbar puncture (L4-L5), and twelve to eighteen μL of CSF was collected with a 0.9 mm needle and the samples were immediately frozen at -80°C, as reported previously [17]. The ethics committee of each institute approved the present study, and we received the informed consent from the participants of the study.

Measurement of glutamate and glutamine levels were carried out according to established methods [18] with a slight modification using a high performance liquid chromatography (HPLC) system with fluorescence detection (Shimadzu Corporation, Kyoto, Japan). Ten μL of the human CSF was added with 10 μL of 0.1 M borate buffer (pH 8.0) and 30 μL of 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) in CH_3CN. The reaction mixture was then heated at 60°C for 2 min, and immediately supplemented with 100 μL of H_2O/CH_3CN (90/10) containing 0.1 % trifluoroacetic acid (TFA) to stop the reaction. Ten μL of the resultant solution was injected into the HPLC system. A reversed-phase ODS column (TSKgel ODS-80Ts, Tosoh Corporation, Tokyo, Japan) was used for the separation and quantification of glutamate and glutamine, and the gradient elution of the mobile phase was kept at a constant flow rate of 0.8 mL/min. Mobile phase 1a consisted of H_2O/CH_3CN (90/10) containing 0.1 % TFA, and phases 1b and 1c, of H_2O/CH_3CN (10/90) containing 0.1 % TFA and CH_3CN, respectively. The time program for gradient elution was programmed as follows: 0–50.5 min 1a: 1b : 1c = 95 : 5 : 0, 50.5–55.5 min 1a : 1b : 1c = 0 : 100 : 0, and 55.5–57 min, 1a : 1b : 1c = 0 : 0 : 100. The column temperature of all columns was maintained at 35°C. Fluorescence detection was made at 530 nm with an excitation wavelength at 470 nm.

Differences between two groups were analyzed using the Mann-Whitney U-test. The relationship between two variables was examined using Spearman correlation coefficients. A $p < 0.05$ was considered significant.

Results

The CSF levels (421.7 μM (median), 468.1 ± 146.1 μM (mean ± S.D.), 254.0–775.1 (range)) of glutamine in the patients were not different ($z = -1.038$, $p = 0.299$) from those (410.5 μM (median), 405.6 ± 108.6 μM (mean ± S.D.), 219.8–689.0 (range)) of normal controls. The CSF levels (4.17 μM (median), 4.25 ± 1.77 μM (mean ± S.D.), 2.22–8.88 (range)) of glutamate in the patients were not different ($z = -1.307$, $p = 0.191$) from those (5.26 μM (median), 4.73 ± 1.29 μM (mean ± S.D.), 2.54–6.51 (range)) of normal controls. However, the ratio (126.1 (median), 117.7 ± 27.4 (mean ± S.D.), 42.0–161.6 (range)) of glutamine to glutamate in the CSF of patients was significantly ($z = -3.29$, $p = 0.001$) higher than that (81.01 (median), 89.1 ± 22.5 (mean ± S.D.), 59.7–134.0 (range)) of controls (Table 1). Furthermore, we found significant correlations between glutamate and glutamine in normal controls ($r = 0.549$, $p = 0.022$) or patients ($r = 0.780$, $p < 0.001$).

Discussion

In this study, we found that the ratio of glutamine to glutamate in the CSF of first episode and drug naive schizophrenic patients was significantly higher than that of normal controls although each level of glutamine and

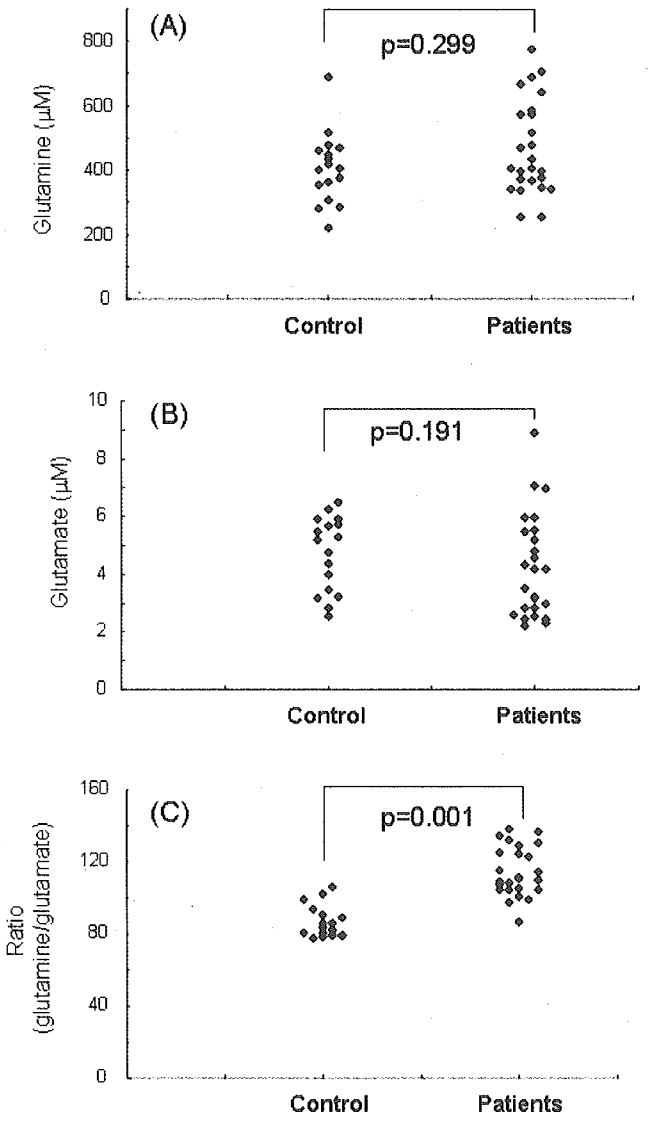

Figure 1
Levels of glutamine and glutamate, and ratio of glutamine to glutamate in CSF of normal controls, and first episode and drug naive schizophrenic patients. (A) CSF levels of glutamine in patients were not different from those of normal controls. (B) CSF levels of glutamate in patients were not different from those of normal controls. (C) Ratios of glutamine to glutamate in patients were significantly higher than those of normal controls.

glutamate in the CSF of patients was not significantly different from that of normal controls. To our knowledge, this is a first report demonstrating that the ratios of glutamine to glutamate in the first episode and drug naive patients are significantly higher than those of normal controls. In contrast, it was supposed earlier that alterations in CSF levels of glutamate are not so prominent compared with those in the brain [14]. Therefore, it is likely that a difference in glutamate (or glutamine) levels between our CSF study and MRS studies may be due to the difference between CSF samples and specific corticolimbic regions. However, it should be noted that alterations in the ratio of glutamine to glutamate are detected in the CSF samples of first episode and drug naive schizophrenic patients, suggesting an abnormality of the glia-neuronal glutamate-glutamine cycle in the brain of patients with schizophrenia.

In general, glutamine is synthesized in astrocytes from glutamate by the enzyme glutamine synthetase, found exclusively in brain glia cells. Glutamine then crosses the astrocytes to be transported into nerve cell terminals, where it is converted again into the neurotransmitter glutamate by glutaminase. It is reported that activities of glutaminase and glutamic acid decarboxylase (GAD; the rate-limiting enzyme in the synthesis of GABA by decarboxylation of glutamate) are significantly greater in the dorsolateral prefrontal cortex (DLPFC) of schizophrenia than in the control group, whereas activities of glutamate dehydrogenase, glutamine synthetase, and GABA transaminase in the DLPFC of schizophrenia are not different from the control group [19]. These findings suggest that metabolism of glutamate and GABA might be altered in the DLPFC of schizophrenic patients. Furthermore, it has been reported that activity of glutamine synthetase and glutamate dehydrogenase, the key enzymes involved in glutamate-glutamine cycle between neuron and glia, were markedly altered in the prefrontal cortex of schizophrenic patients, suggesting abnormalities in the function of glutamate-glutamine cycle in schizophrenic brain [20]. It is also well known that the glutamate-glutamine cycle between neuron and glia is tightly related to glutamate neurotransmission, glutamatergic function, and their regulation in human brain [7]. Taken together, it is likely that a dysfunction in glutamate-glutamine cycle in the brain may play a role in the pathophysiology of schizophrenia, supporting the glutamate hypothesis of schizophrenia.

As described in introduction, sodium-dependent amino acids transporters, located in the abluminal membranes of the blood brain barrier, are capable of actively removing amino acids from the brain [16,20,21]. Sodium-dependent amino acids transporter are capable of pumping both glutamine (system N) and glutamate (glutamate transporters EAAT-1, 2, and -3) from the extracellular fluid into endothelial cells [20,21]. The luminal facilitative carriers for both glutamate and glutamine can then transport them to the blood [16,20,21]. Therefore, the concentrations of naturally occurring amino acids in the CSF [presumably similar to the extracellular fluid of brain] are ~10% of those of the blood [15,16]. Taken together, it seems that alteration in the transport mechanisms regulating levels of glutamate and glutamine in CSF may be implicated in elevated glutamine/glutamate ratio in CSF of schizophrenic patients although further study is necessary.

Conclusion

Our findings suggest that a dysfunction in glutamate-glutamine cycle between neuron and glia may play a role in the pathophysiology of schizophrenia, supporting the glutamate hypothesis of schizophrenia.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contribution

KH conceived of the study, its design and coordination, and edited the manuscript. GE participated in the design of the study. CN and LHL recruited subjects and collected CSF samples. ES and MI assisted HPLC analysis and data analyses. All authors read and approved the final manuscript.

Acknowledgement

This study was supported in part by Grant-in Aid (A03) for Scientific Research on Priority Areas on "Elucidation of glia-neuron network mediated information processing systems" from Ministry of Education, Culture, Sports, Science and Technology (KH) and the Research Grant (15B-1) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare (KH).

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Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-244X/5/6/prepub

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Lithium augmentation in milnacipran-refractory depression for the prevention of relapse following electroconvulsive therapy

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Electroconvulsive therapy (ECT) is highly effective for the treatment of refractory major depression [1,2], but a high rate of relapse after discontinuation of ECT has been reported [3]. Although the effectiveness of nortriptyline and lithium combination has been reported for maintenance after ECT sessions, the relapse rate is still high, particularly during the first month of continuation therapy [4]. The development of more effective long-term relapse-prevention therapy is needed. We report here a case of refractory depression, where the addition of lithium to milnacipran after ECT sessions effectively maintained the improvements in depressive symptoms and no further ECT treatments were required.

Mrs A, a 63-year-old Japanese woman had no previous psychiatric or medical illness and no known family history of mental illness. Two months before admission, she developed a major depressive disorder. She had severe depressive mood, insomnia, and the loss of appetite, and hospitalization was needed because of her suicide attempt and mood-congruent delusions. She was resistant to treatment with various antidepressants, including amitriptyline, amoxapine, fluvoxamine, milnacipran and augmentation with risperidone, levothyroxine (thyroid hormone) or methylphenidate. ECT in conjunction with milnacipran treatment resulted in a full resolution of symptoms but she relapsed within 2 weeks, requiring a further course of ECT. Augmentation therapy of lithium carbonate (400 mg/day) was selected after the last ECT. The combined use of lithium and milnacipran has maintained her recovery from depression without adverse reactions, and no further ECT has been required.

After the discontinuation of ECT, the best choice of continuation pharmacotherapy is still unclear. Lithium augmentation of tricyclic antidepressants (TCAs) has been recommended [4,5], but our patient's tolerance of TCAs was low. Several new types of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), are now marketed. Compared with the use of milnacipran and ECT, the addition of lithium provided superior efficacy in the maintenance treatment of this case with refractory depression. SNRIs generally have high tolerability profile and also have efficacy similar to that of TCAs [6–8]. SNRIs include milnacipran, venlafaxine, and duloxetine. In Japan, only milnacipran is currently used. Although milnacipran should be considered a promising agent for the treatment of patients with major depressive disorder [7], the efficacy of its use in augmentation therapy for refractory depression is not known. To our knowledge, this is the first report that indicates the augmenting efficacy of lithium in milnacipran-resistant major depression. Although further studies are required in order to confirm its effects, lithium augmentation with milnacipran could be considered in patients with refractory major depression and in maintenance after ECT.

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Olanzapine in the treatment of resistant depression

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The combination of olanzapine and fluoxetine has been reported to treat resistant depression successfully

Acta Psychiatr Scand 2005: 111: 74–77
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DOI: 10.1111/j.1600-0447.2004.00395.x

ACTA PSYCHIATRICA SCANDINAVICA

Case report

A right orbitofrontal region and OCD symptoms: a case report

Ogai M, Iyo M, Mori N, Takei N. A right orbitofrontal region and OCD symptoms: a case report.
Acta Psychiatr Scand 2005: 111: 74–77. © Blackwell Munksgaard 2004.

Objective: To discuss the relationship between obsessive-compulsive symptoms and a right orbitofrontal lesion.
Method: Single case report.
Results: A 59-year-old man developed obsessive-compulsive disorder (OCD) symptoms after his head injury. Magnetic resonance imaging brain scans showed a small contusion in the right orbitofrontal region, and single-photon emission computed tomography revealed hypoperfusion in blood flow at the same region.
Conclusion: The OCD symptoms that developed in the present case may be attributable primarily to hypofunction in the lesion localized to the right orbitofrontal area. Although caution is needed for interpretation of the observation because of our experience of only a single case, it suggests that the right orbitofrontal region may be important in forming OCD symptoms.

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Key words: brain injuries; obsessive-compulsive disorder; prefrontal cortex

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Accepted for publication May 28, 2004

Introduction

Recent studies of the brain using magnetic resonance imaging (MRI) (1) and positron emission tomography (PET) (2) in patients with obsessive-compulsive disorder (OCD) suggest that the orbito-prefrontal cortex may be involved in the pathophysiology of the disorder. However, explicit evidence for this is lacking. We herein describe a 59-year-old man who developed OCD symptoms after a traffic accident that caused a lesion localized to the right orbitofrontal area.

Case report

The right-handed patient was involved in a traffic accident at age 52. He suffered a contusion on the right face, sprained his neck, and lost consciousness for about 30 min. He was immediately taken to the local emergency clinic at a municipal hospital and admitted, for scrutiny, to the neurosurgical department of the same hospital. No neurological deficits were detected, and no particular abnormalities were pointed out on a brain computed tomography (CT) scan. He received no active treatment during or after his stay at the hospital, and he was discharged 11 days after admission. However, his wife noticed 2 months later that the patient had lost interest in newspapers and television, and that his social activity had become restricted. She also noted several behavioral changes: he began to come home with umbrellas belonging to others and to file travel-related brochures, recording the date on which he collected each brochure; around the house, he repetitively checked for dust on the furniture and measured the length of the curtains. Half a year after the accident, he was demoted at work to a sinecure because of idleness. Eventually, he was dismissed at age 59. His wife took him to a general hospital, where he was suspected of suffering from an early stage of dementia. He was then referred to our hospital. His facial expression was limited and he showed an impoverished mental activity. He had a scar about 1 cm long above his right eyebrow. No major neurological deficit was evident. On the Hasegawa dementia scale revised version (HDS-R) (3) [almost identical to the Mini Mental State Examination, MMSE (4)], he achieved a full score. On the Wechsler Adult Intelligence scale-revision (WAIS-R) (5), his verbal IQ was 98, performance IQ 92, and total IQ 96, showing no apparent deficit in intelligence. On the Wisconsin Card Sorting test (WCST) (6), he made

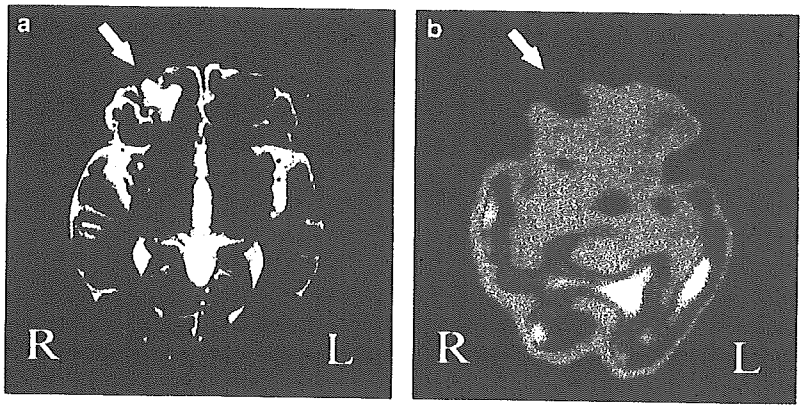

Fig. 1. (a) Magnetic resonance imaging scan showing a small contusion and cerebral atrophy in the right orbitofrontal region. (b) Single-photon emission computed tomography revealing hypoperfusion in blood flow in the same region as in (a).

poor scores (categories achieved, 4; perseverative errors, 25; total errors, 55). MRI brain scans showed a small contusion and cerebral atrophy in the right orbitofrontal region (Fig. 1a), and single-photon emission computed tomography (SPECT) with ^{123}I-iodoamphetamine revealed hypoperfusion in blood flow at the same region (Fig. 1b). There was no abnormality on an electroencephalogram. The patient was compelled to have things just right (e.g. checking for closure of curtains), and continued to bring home umbrellas belonging to others and collect travel brochures (i.e. hoarding and saving compulsions). He was also obsessive about dirt and, in fact, repetitively checked for dust on the furniture. Other repetitive behaviors and mental acts included rewriting words (e.g. writing his name, and erasing and rewriting it in a repetitive manner on simple forms of documents), and drive to measure length of the curtains. The patient reported that these repetitive behaviors had initially been aimed to ameliorate discomfort and anxiety but these behaviors had become his daily routines. His insight varied; at some time, the patient described his compulsive behaviors as excessive, but at most of time, he was overwhelmed by the compulsions. The patient's characteristics met the DSM-IV criteria (7), A to D, for OCD. In addition, when the Yale-Brown Obsessive Compulsive scale (8) (range 0–40) was applied, the score was 27, falling into the severe range.

Discussion

The present case had no medical or psychiatric history prior to his head injury. After the accident that caused a right orbitofrontal cerebral legion, he developed OCD symptoms (e.g. an urge to borrow umbrellas and checking behavior).

The accumulation of evidence from recent neuroimaging studies points to dysfunction in orbitofrontal-subcortical circuitry in patients with OCD (9). However, the underlying pathophysiological mechanism(s) still remains unknown. Some investigators have suggested that hyperperfusion in the orbitofrontal-subcortical circuits is related to OCD (10, 11, 12) but, on the contrary, others have shown cases with OCD symptoms to have decreased perfusion in the frontal lobes (13, 14, 15). Therefore, it could be that abnormality in the orbitofrontal blood flow, hypo- or hyperperfusion, leads to the formation of OCD symptoms. However, in the present case, SPECT clearly shows hypoperfusion in blood flow in the right orbitofrontal region but no hyperperfusion in other brain areas, thereby indicating that OCD symptoms observed in our patient may be attributable primarily to hypofunction in the lesion localized to the right orbitofrontal area rather than compensatory hyperfunction in other orbitofrontal-subcortical circuitry.

Because of deterioration of social functioning, 'Dementia Due to Head Trauma (DSM-IV 294.1)' might be suspected. However, he had no marked memory or attentional impairment, which is a key element for the diagnosis of dementia in the DSM-IV classification system. In effect, the patient demonstrated a full score on the MMSE- equivalent test (HDS-R). Individuals with dementia are often observed to collect useless objects. However, their behaviors are usually purposeless and inappropriate. On the contrary, the patient in our case borrowed umbrella belonging to others, collected brochures, brought them home and filed the brochures, and recorded the date when he collected them. Such complicated and persistent behaviors cannot be expected for those individuals with dementia. Furthermore, our patient did not exhibit aphasia, apraxia, or agnosia, which is frequently observed in dementia patients. As a result, OCD symptoms in the present case are not attributable to behavioral problems related to the dementia process.

Although he had no impairment of intellectual function, his general function, especially emotional

expression, interest in social issue and social function, had deteriorated strikingly. This may reflect the disturbance of the function of the frontal lobe, notably executive function, which was revealed as the result of WCST. However, there was no apparent abnormality in the left dorsolateral prefrontal cortex (DLPFC), whose function is represented by performance on WCST, on both the MRI and SPECT brain scans in our patient. The lesion of the right orbitofrontal cortex may cause the impairment of the DLPFC through paralimbic connections, which connect these two regions.

Of interest is the recently reported case of a man who suffered from a compulsive behavioral problem (an urge to borrow *cars*; in our case, an urge to borrow *umbrellas* instead) induced by a right medial orbitofrontal lesion which was related to a ruptured aneurysm of the anterior communicating artery (16). This case and ours are very alike as they were both aware of possible detrimental outcomes (Cohen et al.'s case was, in fact, arrested several times) but could not withstand the urge to conduct such misdemeanors. Although individuals with lesion-induced behavioral problems may be rare, we must pay attention to the small lesions like our case. Therefore, clinicians should be alert to the existence of such a condition and, as pointed out by Cohen et al. (1999), those afflicted with it ought to be given appropriate therapy in the early stage and protected legally to preclude them from receiving detrimental treatment from the society.

However, our interpretation that a lesion, showing the corresponding hypoperfusion on SPECT, localized to the orbitofrontal region is responsible for OCD symptoms needs caution as other subtle lesions that could not be detected on MRI may exist and SPECT applied may be insufficient to detect a small amount of hyper- or hypoperfusion within the orbitofrontal-subcortical circuitry other than the marked hypoperfusion observed in the orbitofrontal area. Nevertheless, our experience suggests that the right orbitofrontal region is important in forming OCD symptoms.

Acknowledgement

Dr Takei would like to thank the Stanley Medical Research Institute (SMRI) for financial support.

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Recent brain imaging studies have indicated that orbitofrontal cortex and striatum are involved in neuropathology of obsessive-compulsive disorder (OCD) (1, 2). However there are some contradictory results of these brain imaging studies. While some structural brain imaging studies showed that orbitofrontal and caudate nucleus volume had decreased (3) and caudate nucleus had higher signal intensity values in OCD (4), some other studies did not show any differences in caudate volume of the patients with OCD

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 132B:70–73 (2005)

Association Study Between Brain-Derived Neurotrophic Factor Gene Polymorphisms and Methamphetamine Abusers in Japan

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Several lines of evidence suggest that genetic factors might contribute to drug abuse vulnerability. Recent genomic scans for association demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene was associated with drug abuse vulnerability. In this study, we analyzed association of two *BDNF* gene single nucleotide polymorphisms (SNPs), 132C > T (C270T named formerly) in the noncoding region of exon V and 196G > A (val66met) in the coding region of exon XIIIA, with methamphetamine (MAP) abuse in Japan. No significant differences were found in the frequency of the genotype or allele in these two SNPs between MAP abusers and controls (132C > T in exon V: genotype, $P = 0.586$, allele, $P = 0.594$; 196G > A (val66met) in exon XIIIA: genotype, $P = 0.889$, allele, $P = 0.713$). Furthermore, there was no difference between clinical parameters (e.g., prognosis psychosis, spontaneous relapse, or poly-substance abuse) and the two SNPs of *BDNF* gene. These results suggest that the two SNPs (132C > T in exon V and 196G>A (val66-met) in exon XIIIA) of the *BDNF* gene may not be associated with Japanese MAP abusers. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html.

KEY WORDS: brain-derived neurotrophic factor; polymorphism; drug abuse; methamphetamine

INTRODUCTION

Family, twin, and adoption studies suggest that genetic factors are implicated in vulnerability of substance abuse [Merikangas et al., 1998; Kendler, 2001; Tsuang et al., 2001]. The genome-scanning study of poly-substance abuse vulnerability demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene might be one of the strong candidate genes to drug abuse [Uhl et al., 2001]. BDNF is a member of a neurotrophin superfamily mainly expressed within the brain. BDNF interacts with TrkB receptor tyrosine kinase, playing several important roles such as promotion of survival, differentiation, and maintenance of neurons in peripheral nervous system and central nervous system; influences to axonal growth and connectivity; participation in the local responses to various types of neuronal stress or insults [Manji et al., 2003; Mattson et al., 2003]. Furthermore, it also has been reported that the gene encoding BDNF might be an important candidate for susceptibility of neuropsychiatric disorders including bipolar disorder [Neves-Pereira et al., 2002; Sklar et al., 2002; Hashimoto et al., 2004] and schizophrenia [Krebs et al., 2000]. In the studies reporting possible association of BDNF and these disorders, two single nucleotide polymorphisms (SNPs) of *BDNF* gene has been reported. One is 196G > A (val66met) SNP in exon XIIIA (GENBANK: AF411339; at position 95422) located within the propeptide region of BDNF. The A of the ATG-translation initiation codon is denoted nucleotide +1 in exon XIIIA (GENBANK: AF411339; at position 95227). Sklar et al. [2002] reported that BDNF 196G > A (val66met) is significantly associated with bipolar disorder. Interestingly, it has been demonstrated that this SNP (val66met) is strongly suspected to influence human memory and hippocampal function [Egan et al., 2003]. Several lines of evidence demonstrated that methamphetamine (MAP) dependence may cause long-term neural damage in humans, with concomitant deleterious effects on cognitive processes such as memory and attention [Nordahl et al., 2003], suggesting the possible role of BDNF secretion in the memory deficits of MAP abusers. The other SNP frequently analyzed is 132C > T in the noncoding region of exon V (GENBANK: AF411339; at position 53620). This SNP at position 132 of exon V is numbered from the start of exon V (GENBANK: AF411339; at position 53488). It was detected and named C270T by Kunugi et al. [2001] after their searching for a novel nucleotide substitution in the

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Received 12 December 2003; Accepted 24 May 2004
DOI 10.1002/ajmg.b.30097